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Mutations affecting pigmentation and shape of the adult zebrafish

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Abstract Mutations causing a visible phenotype in the adult serve as valuable visible genetic markers in multicellular genetic model organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Arabidopsis thaliana*. In a large scale screen for mutations affecting early development of the zebrafish, we identified a number of mutations that are homozygous viable or semiviable. Here we describe viable mutations which produce visible phenotypes in the adult fish. These predominantly affect the fins and pigmentation, but also the eyes and body length of the adult. A number of dominant mutations caused visible phenotypes in the adult fish. Mutations in three genes, *long fin*, *another long fin* and *wanda* affected fin formation in the adult. Four mutations were found to cause a dominant reduction of the overall body length in the adult. The adult pigment pattern was found to be changed by dominant mutations in *wanda*, *asterix*, *obelix*, *leopard*, *salz* and *pfeffer*. Among the recessive mutations producing visible phenotypes in the homozygous adult, a group of mutations that failed to produce melanin was assayed for tyrosinase activity. Mutations in *sandy* produced embryos that failed to express tyrosinase activity. These are potentially useful for using tyrosinase as a marker for the generation of transgenic lines of zebrafish.

Key words Pigment pattern · Fin · Fish skeleton · Tyrosinase · Zebrafish

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Introduction

Visible hereditary traits such as eye or hair colour in humans are amongst the most obvious properties being passed on to offspring over generations. Well-defined visible traits like seed shape and seed colour in peas allowed Mendel to formulate the principles that form the basic foundation for understanding genetic inheritance. The discovery of a mutant strain of *Drosophila melanogaster* with white eyes instead of red eyes stimulated Morgan and his collaborators to collect a large number of mutants with visible adult phenotypes (Morgan 1910; Morgan et al. 1915). Since then, a large number of mutations causing visible phenotypes in adult *Drosophila* have been accumulated (Lindsley and Zimm 1992). The availability of such mutations has been very important for the development of *Drosophila* as a genetic model organism. Mutations causing visible adult phenotypes are particularly useful for genetic mapping experiments and as markers on balancer chromosomes. Genetically marked balancer chromosomes have enormously simplified the maintenance of lethal and sterile stocks and allow sophisticated genetic screens to be carried out in which a particular

chromosome is followed throughout a breeding scheme. These balancer chromosomes are usually marked by dominant mutations, which makes it easy to identify these chromosomes in heterozygous individuals.

In *Caenorhabditis elegans*, the availability of visible genetic markers has been similarly important for its success as a genetic model organism (Wood 1988). In *C. elegans*, visible markers are usually mutations affecting the shape or behaviour of the larvae.

Visible genetic markers have become very useful in generating transgenic animals. In *Drosophila* for example, the *rosy* and *white* genes which affect eye colour are frequently used as a visible marker for the successful generation of transgenic individuals. The inserted DNA includes the cloned gene for *rosy* or *white* leading to a change of eye colour in the otherwise homozygous mutant transgenic animal. In the mouse, more than 130 mutations at more than 50 loci affecting coat colour and pigmentation have been described (Hogan et al. 1986; Silvers 1979). Mice homozygous for mutations at the *albino* (*c*) locus have no pigment at all, neither in the skin nor in the eyes. The gene encoding tyrosinase, which is a key enzyme in melanin biosynthesis, was found to map to the *albino* (*c*) locus (Kwon et al. 1987; Ruppert et al. 1988). Expression of tyrosinase from a transgene was found to rescue the coat colour and eye pigmentation of *albino* mice (Beermann et al. 1990; Tanaka et al. 1990). In the medaka fish, *Oryzias latipes*, stable transgenes encoding the mouse tyrosinase resulted in wild-type pigmentation of an orange-coloured variant (Matsumoto et al. 1992). An albino mutant lacking tyrosinase activity in medaka was recently shown to be due to a transposable element in the tyrosinase gene (Koga et al. 1995).

A number of transgenic lines of zebrafish have been produced and recent progress has been made towards insertional mutagenesis using a pseudotyped retroviral vector (Lin et al. 1994a). For this purpose, a zebrafish mutation that could be rescued by a tyrosinase encoding transgene would be a very useful tool. It has been shown that a mutation causing the lack of melanin can be used as a visible genetic marker for the generation of germline chimeras of the zebrafish (Lin et al. 1992).

The common name of the zebrafish derives from the striped pigment pattern displayed by the adult. A handful of mutations causing a visible phenotype in the adult zebrafish are available in petshops or were fortuitously isolated in the laboratory, which indicated the mutability of the zebrafish genome. These include *golden*, *brass*, *albino*, *transparent*, *sparse*, *rose*, *leopard* and *long fin* (Johnson et al. 1995; Kirschbaum 1975; Streisinger et al. 1986; University of Oregon Zebrafish group 1993; Tresnake 1981). The *leopard* mutant was long thought to be a separate species termed *Brachydanio frankei*. Hybrids were produced between the two strains and found to produce fertile progeny which indicated that *B. frankei* is a spotted variant of *Danio rerio* (*B. rerio*; Frank and Zukal 1981; Frankel 1979; Petrovicky 1964, 1966)

In this paper, we describe a number of dominant mutations affecting pigmentation, fins or overall body length of the adult zebrafish. We also describe a number of recessive mutations that are homozygous viable and display visible phenotypes in the homozygous adults. One of these is a mutation in a gene possibly encoding the zebrafish homolog of tyrosinase.

Materials and methods

Fish were maintained as described in Mullins et al. (1994). The wild type strain was *Tü* (Haffter et al. 1996). leo^{t1} lof^{dt2} double mutants were obtained from a pet shop (Elias 1984; Johnson et al. 1995; Kirschbaum 1975) and outbred to wild type to obtain strains carrying either of the two mutations individually. gol^{b1}, alb^{b4}, spa^{b5}, brs^{b2}, rse^{b140} and tra^{b18} were kindly provided by C. Walker and C. Kimmel from the University of Oregon (The University of Oregon Zebrafish group 1993; Johnson et al. 1995; Streisinger et al. 1986).

Segregation analysis between pairs of dominant mutations was carried out by crossing double heterozygotes to wild-type and counting the frequency of wild-type and mutant progeny. If all progeny were mutant, the two mutations were assumed to be linked. If 25% wild type, 25% of either single heterozygous and 25% of transheterozygous progeny occurred, the two mutations were assumed to be unlinked. For segregation analysis involving the recessive allele leo^{t1}, the transheterozygotes were crossed to fish that were homozygous for leo^{t1} and the absence of wild-type-looking fish among the progeny was taken as evidence for linkage,

Table 1 Mutations affecting body length of the adult

Gene	Alleles	Embryonic phenotype	Dominant phenotype	References
däumling (dml)	*dtn8*	Dominant short, delayed swim bladder formation	Dominant short	This paper
liliput (lil)	*dtc232*	None	Dominant short	This paper
piggytail (pgy)	*ty40, tc227a, ta206, ti216c, tx223*	Dominant: loss of ventral tail fin[a]. Recessive: dorsalized	Dominant[a] short	Mullins et al. 1996
smurf (smf)	*dtf239*	None	Dominant short	This paper
spine-stein (sps)	*to2e, th279b*	Material collecting in neurocoel	Short, semiviable	Brand et al. 1996
swirl (swr)	*ta72a, tc24b, tc300a*	Dominant: loss of ventral tail fin. Recessive: dorsalized	Dominant short, no tail fin	Mullins et al. 1996
stöpsel (stp)	*dtl28d*	None	Dominant short	This paper

[a] Dominant phenotype in *pgy* is variable and only found if the mother is a heterozygous carrier too (Mullins et al. 1996)

Table 2 Mutations affecting the adult fins

Gene	Alleles	Embryonic phenotype	Adult phenotype	References
another long fin (alf)	*dty86d*	None	Dominant: long fins, tail hangs down	This paper; Eeden et al. 1996
blasen (bla)	*ta90*	Bubbly fins	Reduced fins, subtle	Eeden et al. 1996a
finless (fls)	*te370f*	None	No fins	This paper, Eeden et al. 1996a
fransen (fra)	*tc17, tk219a, tr206b, tm55*	Fin necrosis	Reduced fins, subtle	Eeden et al. 1996a
frayed (fyd)	*tj2b*	Fin necrosis	Reduced fins, subtle	Eeden et al. 1996a
frilly fins (frf)	*tf5, th42, tp34, ty69, tp207, tm317a, tz252*	Wavy fin edges	Reduced fins, short	Eeden et al. 1996a
ikarus (ika)	*tm127c*	No or tiny pectoral fins	No or tiny pectoral fins	Eeden et al. 1996a
krom (krm)	*tc227d*	curly pectoral fins	Reduced pectoral and pelvic fins, semiviable	Eeden et al. 1996a
long fin (lof)	*dt2*	None	Dominant: long fins	This paper, Tresnake 1981; Eeden et al. 1996a
mercedes (mes)	*tz209, tm305*	Split tailfin	Split tailfin, only in *tm305*	Hammerschmidt et al. 1996a; Kelsh et al. 1996; Eeden et al. 1996a
nagel (nag)	*ta84, tb22, tl28b, tm42b, tm51, tm68, tn3, tp41a, tr3, ty58, tc8, tu27a, tn210, tm147c, tg254b, te335, tj258a, tt231, tq207, tq274, tw234, tl246, ty124b*	Fin necrosis	Reduced fins, subtle	Eeden et al. 1996a
pinfin (pif)	*tm95b, tj16, te226d, tr274*	Bubbly fins	Reduced fins, subtle	Eeden et al. 1996a
rafels (rfl)	*tb233b, tp66, tc228, tm235b, tq266c, tr240, tc280b, tg308b, tp266, te217, tz245, te370b*	Fin necrosis	Reduced fins, subtle	Eeden et al. 1996*a*
stein und bein (sub)	*tq289a*	Reduced number of otoliths	No pelvic fins, reduced dorsal fin	Eeden et al. 1996a; Whitfield et al. 1996
wanda (wan)	*dty127*	None	Dominant: irregular pigment pattern, missing or reduced fins	This paper Eeden et al. 1996a

whereas the presence of wild-type fish was evidence for the lack of linkage.

Skeletal stainings were done as described by Eeden et al. (1996b).

Tyrosinase catalyses three reactions, the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (dopa), the oxidation of dopa to dopaquinone and oxidation of 5,6-dihydroxyindole, a later product in the pathway (Hearing 1987; Hearing and Jiménez 1989; Korner and Pawelek 1982). The subsequent reactions to form melanin can proceed spontaneously. Tyrosinase activity was measured by assaying for the conversion of dopa into melanin in fixed embryos. The assay was a modification of one described by Wakamatsu et al. (1984) as follows: approximately 48-h-old embryos were washed with phosphate buffered saline (PBS) and cooled on

Table 3 Mutations affecting the adult pigmentation

Gene	Alleles	Embryonic phenotype	Adult phenotype	References
albino (alb)	*b4, ti9, tj20e, tm83a, ti225a, tr282a, tf31a*	No melanin pigmentation	No melanin pigmentation, red eyes	This paper Kelsh et al. 1996; Streisinger et al. 1986
asterix (ase)	*dtt269*	None	Dominant: fewer and wider stripes	This paper
bedimmed (bed)	*tc240*	Dull iridophores	Pale greenish-blue stripes	Kelsh et al. 1996
brass (brs)	*b2*	Reduced melanin pigmentation	No melanin pigmentation in body	Kelsh et al. 1996; Streisinger et al. 1986
brassy (bry)	*tm111*	Reduced melanin pigmentation,	No melanin pigmentation red eyes	Kelsh et al. 1996
dropje (dro)	*tr256*	expanded melanophores	Expanded melanophores, subtle	Kelsh et al. 1996
earache (era)	*tc288c*	Abnormal ear shape	Expanded melanophores	Whitfield et al. 1996
fading vision (fdv)	*th236a*	*R*educed melanin pigmentation, degenerating retina	Reduced melanin pigmentation, small eyes	Kelsh et al. 1996; Heisenberg et al. 1996
golden (gol)	*b1, tg271, ty213*	Reduced melanin pigmentation	Reduced melanin pigmentation	Streisinger et al. 1986 Kelsh et al. 1996
leopard (leo)	*t1, dto29, dtq270, dtw28*	None	Spotted pigmentation	This paper; Kirschbaum 1975
mustard (mrd)	*ta229c, tj204, tp72i, tn215, tk68b, tv41*	No melanin pigmentation	No melanin pigmentation, red eyes	This paper; Kelsh et al. 1996
obelix (obe)	*dtd15, dty7, dtc271d*	None	Dominant: fewer stripes, wider interstripes	This paper
obscure (obs)	*to2b*	small melanophores, subtle	Pinkish colour	Kelsh et al. 1996
pfeffer (pfe)	*tc227b, tq211, tg17, te220, tm236b, tg283a*	Reduced number of xanthophores	Dominant: interrupted stripes. Recessive: spotted pigmentation	This paper; Odenthal et al. 1996b; Kelsh et al. 1996
rose (rse)	*b140*	Reduced number of iridophores and xanthophores subtle	Reduced iridophores and melanophores, fish appear pink	The University of Oregon Zebrafish group 1993; Johnson et al. 1995
salz (sal)	*tf34, tp71c, tm246b, tf238b, tt254a, tb213c, tl241 .*	Reduced number of xanthophores	Dominant: interrupted stripes. Recessive: spotted pigmentation	This paper Kelsh et al. 1996; Odenthal et al. 1996b
sandy (sdy)	*te326 ty79, tk20, tm118, to102, to1*	No melanin pigmentation	No melanin pigmentation, red eyes, semiviable	This paper Kelsh et al. 1996
shady (shd)	*ty82, tp218 tc205, te295, te300, tf238c, th219, tm46a, ti263c, ty9, ty70, tj229e*	Reduced number of iridophores	Reduced melanophores and iridophores, only in *te295b*	Kelsh et al. 1996

Table 3 (continued)

Gene	Alleles	Embryonic phenotype	Adult phenotype	References
sparse (spa)	*b5, b134, th35c, tm18b, tm63a, tp44, tm228, tm102c, tj245, te237, to75b*	Reduced number of melanophores	Mottled stripes	Kelsh et al. 1996; Streisinger et al. 1986
tartar (tar)	*td9*	Reduced xanthophore pigmentation	Reduced melanin pigmentation, semiviable	Odenthal et al. 1996b
transparent (tra)	*b18*	Reduced number of iridophores	Reduced iridophores and melanophores, fish appear transparent	The University of Oregon Zebrafish group 1993
wanda (wan)	*dty127*	None	Dominant: irregular pigment pattern, missing or reduced fins	This paper Eeden et al. 1996a
(unresolved)	*dts37*	None	Interrupted stripes	This paper

Table 4 Mutations affecting the adult eyes

Gene	Alleles	Embryonic phenotype	Adult phenotype	Reference
bumper (bum)	*to20, tm127d, tg413*	Lens degeneration	Lens degeneration, expanded melanophores	Heisenberg et al. 1996
fading vision (fdv)	*th236a*	Reduced melanin pigmentation, degenerating retina	Reduced melanin pigmentation, small eyes	Kelsh et al. 1996; Heisenberg et al. 1996
korinthe (kor)	*tm292b*	Lens degeneration	Lens degeneration, expanded melanophores	Heisenberg et al. 1996
rosine (rne)	*tm70h*	Small lens	Small lens	Heisenberg et al. 1996
yobo (yob)	*tk13, ty44d, tc251*	Reduced xanthophore pigmentation	Small eyes, maternal effect causing head and tail truncations	Odenthal et al. 1996b

ice. The embryos were then fixed for 12 min in 12.5% glutaraldehyde in PBS on ice. Subsequently, the embryos were washed five times for 2 min each with 0.3% Triton X-100 in 55 mM sodium phosphate buffer (pH 6.8) followed by three 2-min washes with 55 mM sodium phosphate buffer (pH 6.8). The embryos were then stained in 5 mM L-dopa, 5% sucrose in 55 mM sodium phosphate buffer (pH 6.8) or, as a control, with the addition of 5 mM diethyldithiocarbamic acid (a specific inhibitor of tyrosinase) at 37°C for 4–6 h.

Results

The adult zebrafish displays a collection of morphological features that distinguish the adult from the larva and that are subject to changes caused by viable mutations (Fig. 1). We do not intend to describe the morphology of the adult in detail, but instead give a brief account of the traits we found to be most useful as visible genetic markers in the zebrafish. We have performed a large scale screen for mutations affecting early development of the zebrafish (Haffter et al. 1996). In addition to mutations affecting early development, we identified a number of dominant and recessive mutations causing a visible phenotype in the adult (Tables 1–4). These mutations affect the body length, fins, eyes or pigmentation of the adult fish. Dominant mutations affecting the adult were identified in the F2 generation of our breeding scheme (Haffter et al. 1996) since the phenotype appeared in 50% of the members of an F2 family.

Mutations causing reduced body length of the adult

The skeleton of a wild-type zebrafish (*Tü*-line; Haffter et al. 1996) usually has 31 vertebrae. In some cases the number is only 30 and in rare cases the number is increased to 32. Posterior to the 4 most anterior vertebrae, with which the Weberian apparatus is associated, are 10 rib-bearing vertebrae of the trunk followed by 17 vertebrae of the tail with hemal arches protruding from the ventral side of the centra. The Weberian apparatus is a

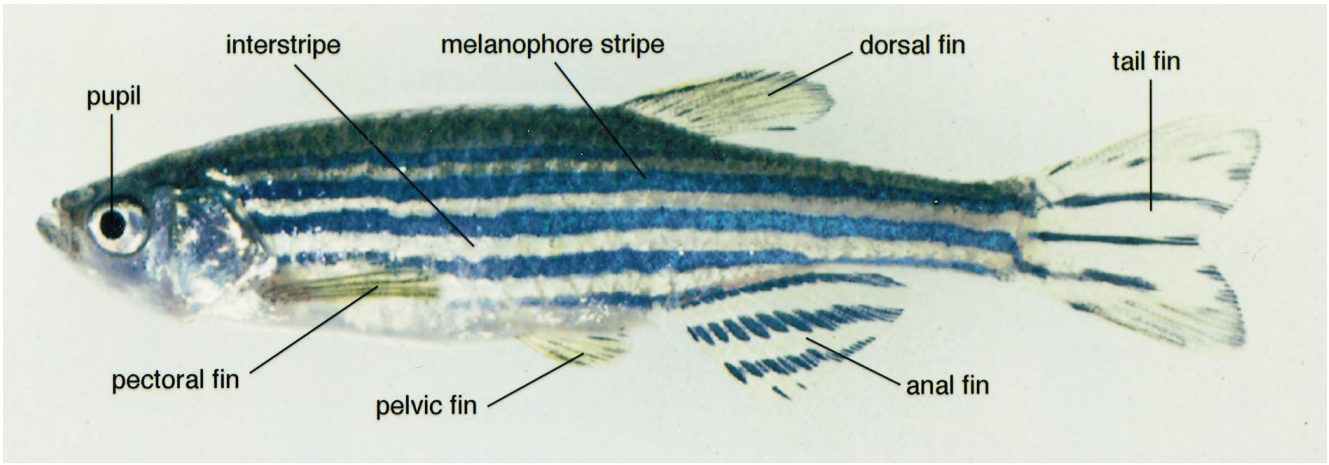

Fig. 1 Adult male zebrafish (*Tü*-line) with normal body length, fins, eyes and pigmentation

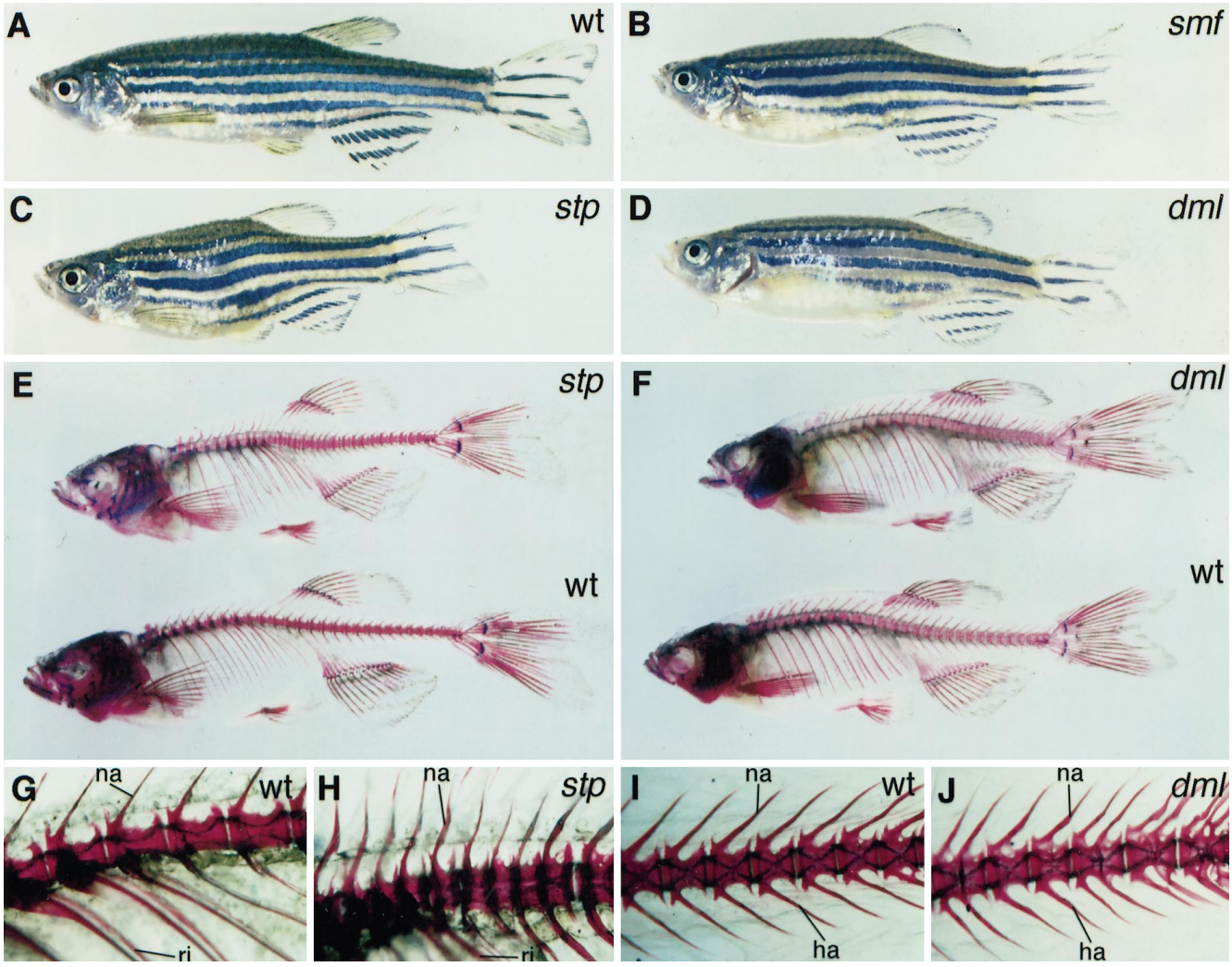

Fig. 2A–J Dominant mutations affecting body length of the adult zebrafish. **A–D** Live photographs. **E–J** Skeletal stainings. **A** Wild-type zebrafish. **B** *smurf*^{dtf239} heterozygote. **C** *stöpsel*^{dtl28d} heterozygote. **D** *däumling*^{dtn8} heterozygote. **E** skeleton of a *stöpsel*^{dtl28d} heterozygote (*top*) and wild-type sibling (*bottom*), showing the defects in the thoracic region of the vertebral column in *stöpsel*^{dtl28d} heterozygote. **F** *däumling*^{dtn8} heterozygote (*top*) and wild-type sibling (*bottom*), showing the defects in the posterior region of the vertebral column in *däumling*^{dtn8} heterozygote. **G** Detail of the trunk vertebrae of a wild-type sibling and **H** a *stöpsel*^{dtl28d} heterozygote, showing the reduction in length of the vertebrae. **I** Detail of the tail vertebrae of a wild-type sibling and **J** a *däumling*^{dtn8} heterozygote, showing the reduction in length of the vertebrae. Some of the vertebrae are fused, with multiple hemal arches and neural arches (*na* Neural arches, *ha* hemal arches, *ri* ribs)

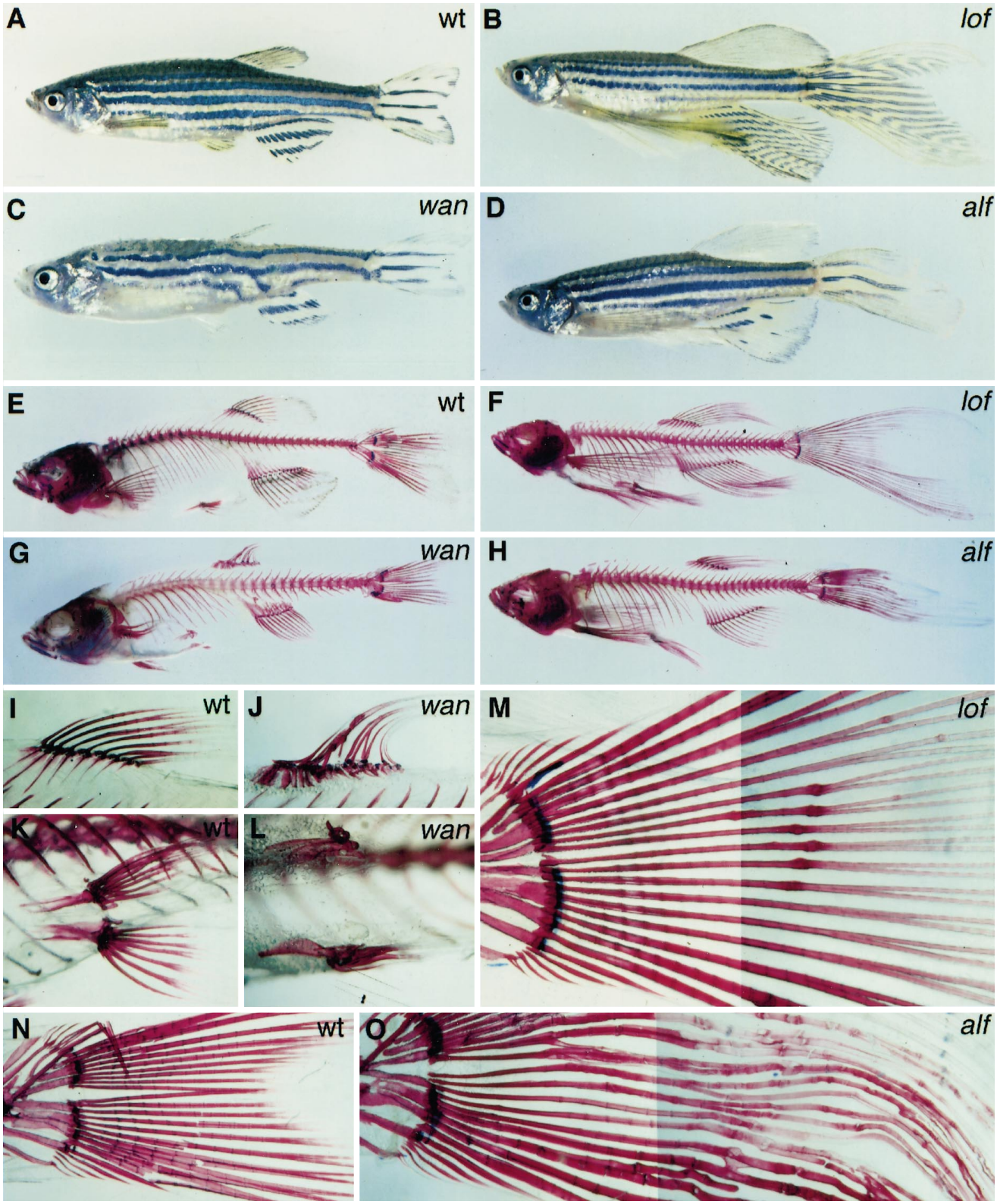

Fig. 3A–O Dominant mutations affecting the fins of the adult zebrafish. **A–D** Live photographs. **E–O** Skeletal stainings. **A** Wild-type zebrafish. **B** *long fin*^{dt2} heterozygote with long fins that have additional stripes of melanophore pigmentation. **C** *wanda*^{dty127} heterozygote, with reduction of all fins, especially the dorsal fin, and an abnormal pigment pattern. **D** *another long fin*^{dty86d} heterozygote with longer fins. **E** Wild-type zebrafish skeleton. **F** Skeleton of *long fin*^{dt2} heterozygote with long fin rays. **G** Skeleton of *wanda*^{dty127} heterozygote, with reduced pterygiophores and lepidotrichia. **H** Skeleton of *another long fin*^{dty86d} heterozygote with longer lepidotrichia. **I** Details of wild-type sibling and **J** *wanda*^{dty127} heterozygote dorsal fins, showing the reduction of the pterygiophores and lepidotrichia, which are mostly unsegmented. **K** Details of wild-type sibling and **L** *wanda*^{dty127} heterozygote pelvic fins, showing the rudimentary structures in *wanda*^{dty127} heterozygote. **M** Tailfin of *long fin*^{dt2}, which has an increased number of segments in the lepidotrichia compared to the tailfin of a wild type (**N**). In *another long fin*^{ty86d}, the lepidotrichia of the tailfin are highly irregular in length and segmentation pattern

series of small bones used by teleosts to communicate waves from the swim bladder to the ear. The 27-rib or hemal-arch-bearing vertebrae have neural arches extending from the dorsal side of the centra. We identified 4 dominant mutations, *liliput* (*lil*), *smurf* (*smf*), *stöpsel* (*stp*) and *däumling* (*dml*), which lead to a reduced adult body length (Fig. 2A–D). *lil*^{dtc232} and *smf*^{dtf239} show no obvious skeletal abnormalities and the reduced body length phenotype seems to be due to reduced growth (data not shown). Skeletal staining of *stp*^{dtl28d} and *dml*^{dtn8} adult fish revealed that the short body length of these two mutants is associated with abnormalities of the axial skeleton (Fig. 2E–J). In *stp*, the trunk vertebrae and the anterior vertebrae of the tail are most severely affected and dramatically reduced in length (Fig. 2E, H). As a result, *stp* is not only reduced in overall body length, but also displays a peculiar body shape (Fig. 2C). In *dml*, in contrast, the posterior half of the vertebral column is most severely affected. The number of centra is reduced to between 23 and 25, and the vertebrae are frequently fused or reduced in length (Fig. 2F, J). In both mutants, *stp* and *dml*, the number of ribs, neural arches and hemal arches is normal. The fusion of centra in *dml* therefore shows vertebrae with multiple arches (Fig. 2J). In addition, the number of fin rays of the anal fin is slightly reduced. *dml* also displays a subtle reduction in overall body length at larval stages and causes delayed swim bladder formation.

dml is only semiviable and up to 50% of *dml* carriers die before reaching adulthood. The other three dominant mutants with an adult short body length, *lil*, *smf* and *stp* have no dominant or recessive embryonic or larval phenotype and are fully viable. All four mutants (including *dml*) seem to have a reduced mating efficiency. Incrosses of all four mutants and intercrosses among several of these mutants have been raised, but obvious homozygous or double heterozygous individuals were not noted among the progeny of these crosses. Allelism among these four mutations has not been determined yet and their assignment to separate genes is therefore preliminary.

Mutations in two genes (*piggytail*, *swirl*) cause a dorsalization phenotype in homozygous embryos and loss of ventral tail fin structures in heterozygotes (Mullins et al. 1996). The loss of ventral tail fin structures leads to short adults, some of which lack the tail fin. Mutations in *spine-stein* cause an embryonic phenotype in the neural tube, which also leads to short adults (Brand et al. 1996).

Mutations affecting the adult fins

The adult zebrafish has two sets of paired fins and three median fins (Fig. 1). The paired fins are the pectoral and pelvic fins. The median fins are the dorsal, caudal and anal fins. All fins are supported by skeletal elements. Located in the lobes are exoskeletal lepidotrichia. These are paired rods that are segmented in a proximo-distal direction. On the proximal end, the lepidotrichia articulate with endoskeletal fin supports which are referred to as pterygiophores in the paired, dorsal and anal fins and hypuralia in the caudal fin. We have identified a number of dominant and recessive mutations resulting in defects in the adult fins (Table 2).

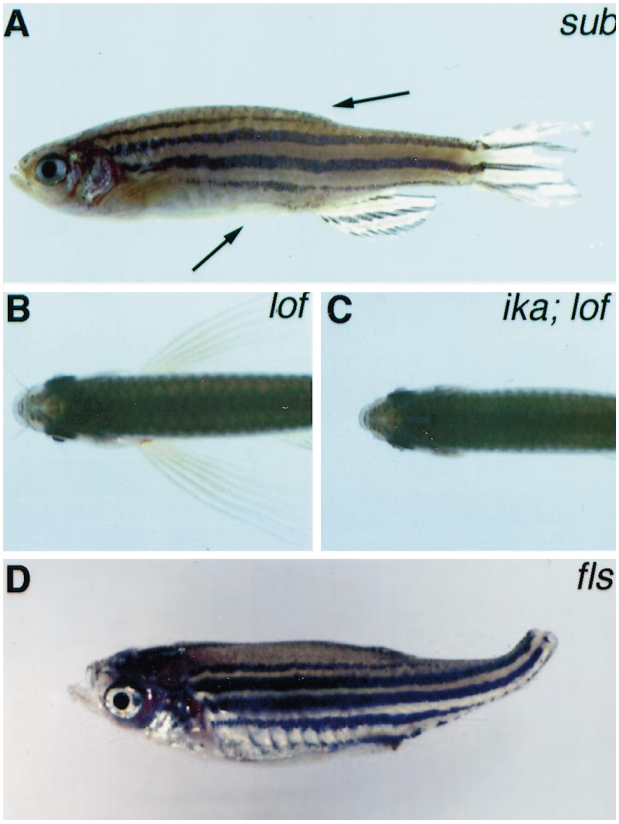

Fig. 4A–D Recessive mutation affecting the fins of the adult. **A** *stein und bein*^{tq289a} lacks the pelvic fins on the ventral side and the dorsal fins of the fish (*arrows*). **B** Dorsal view of *long fin*^{dt2} with pectoral fins for comparison with a *long fin*^{dt2}, *ikarus*^{tm127c}, double mutant, which lacks the pectoral fins (**C**). **D** *finless*^{te370f}, which lacks all the adult fins

Dominant mutations in three genes, *long fin* (*lof*), *another long fin* (*alf*) and *wanda* (*wan*) result in abnormalities of the adult fins (Fig. 3). None of these mutations af-

Fig. 5A–P Mutations affecting the adult pigmentation of the zebrafish. **A** Wild type. **B** *asterix*^{dtt269} heterozygote with fewer and broader melanophore stripes. **C** *obelix*^{dty7} heterozygote with fewer melanophore stripes and broader interstripes. **D** *obelix*^{dty7} homozygote with a further reduction in the number of melanophore stripes. The stripes are also interrupted and irregular in width. **E** *leopard*^{dto29} heterozygote with a spotted pigment pattern. **F** *leopard*^{dto29} homozygote with very few tiny spots of melanophore pigmentation. **G** *salz*^{tt254a} heterozygote with interrupted melanophore stripes. **H** *salz*^{tt254a} homozygote with an antero-posterior gradient of residual fine spotted melanophore pigmentation. **I** *sparse*^{b5} homozygote with an abnormal melanophore pigment pattern. **J** *golden*^{b1} homozygote with reduced melanophore pigmentation. **K** *albino*^{b4}, **L** *mustard*^{tv41}, and (**M**) *sandy*^{tk20} homozygotes which lack melanin pigmentation. **N** *shady*^{te295} homozygote with reduced number of iridophores and reduced melanophore pigmentation. **O** *fading vision*^{th236a} homozygote with reduced melanophore pigmentation, very small eyes and tumorous outgrowth from the eyes. **P** *yobo*^{tc251} homozygote with a small pupil

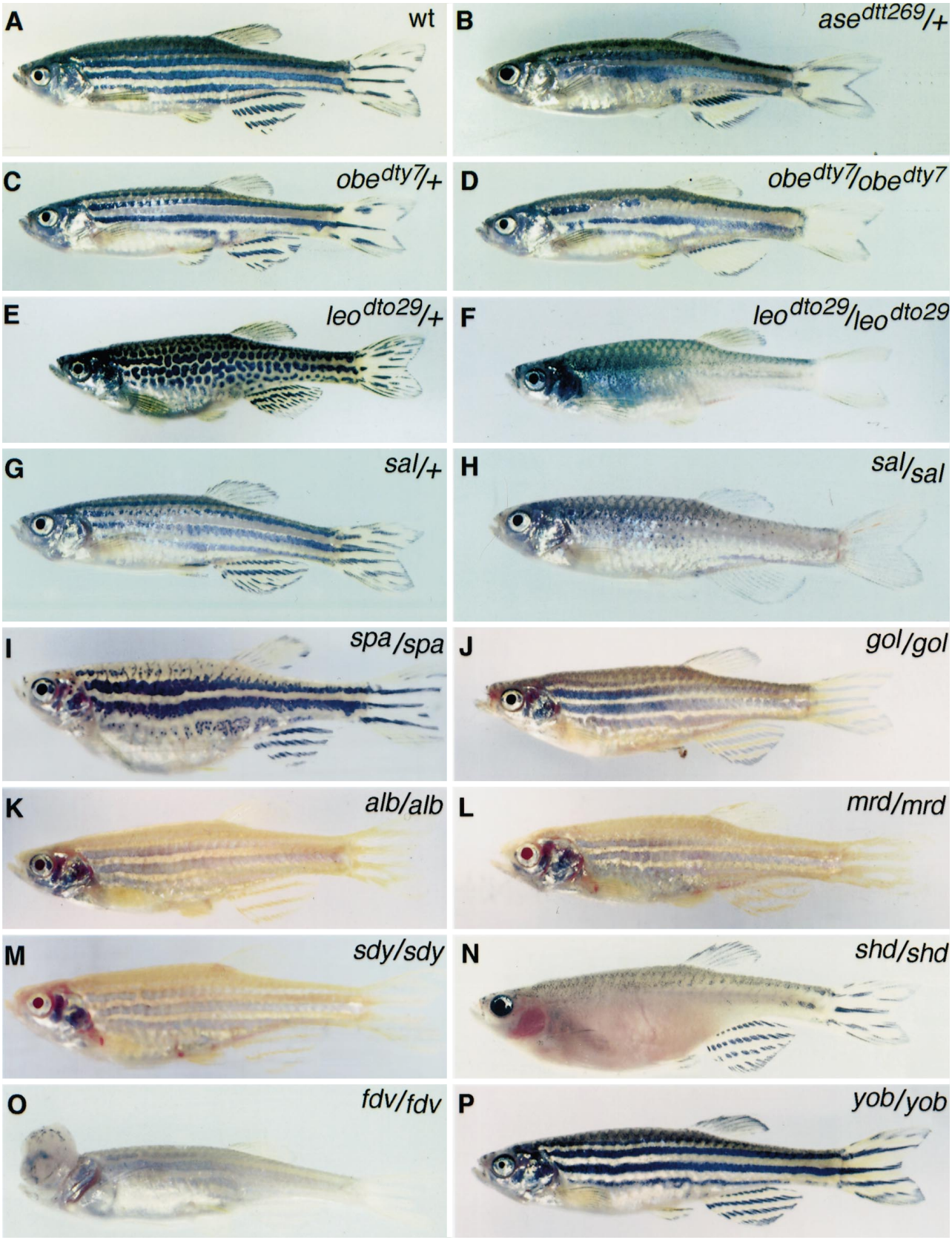

Fig. 5 For legend see page 267

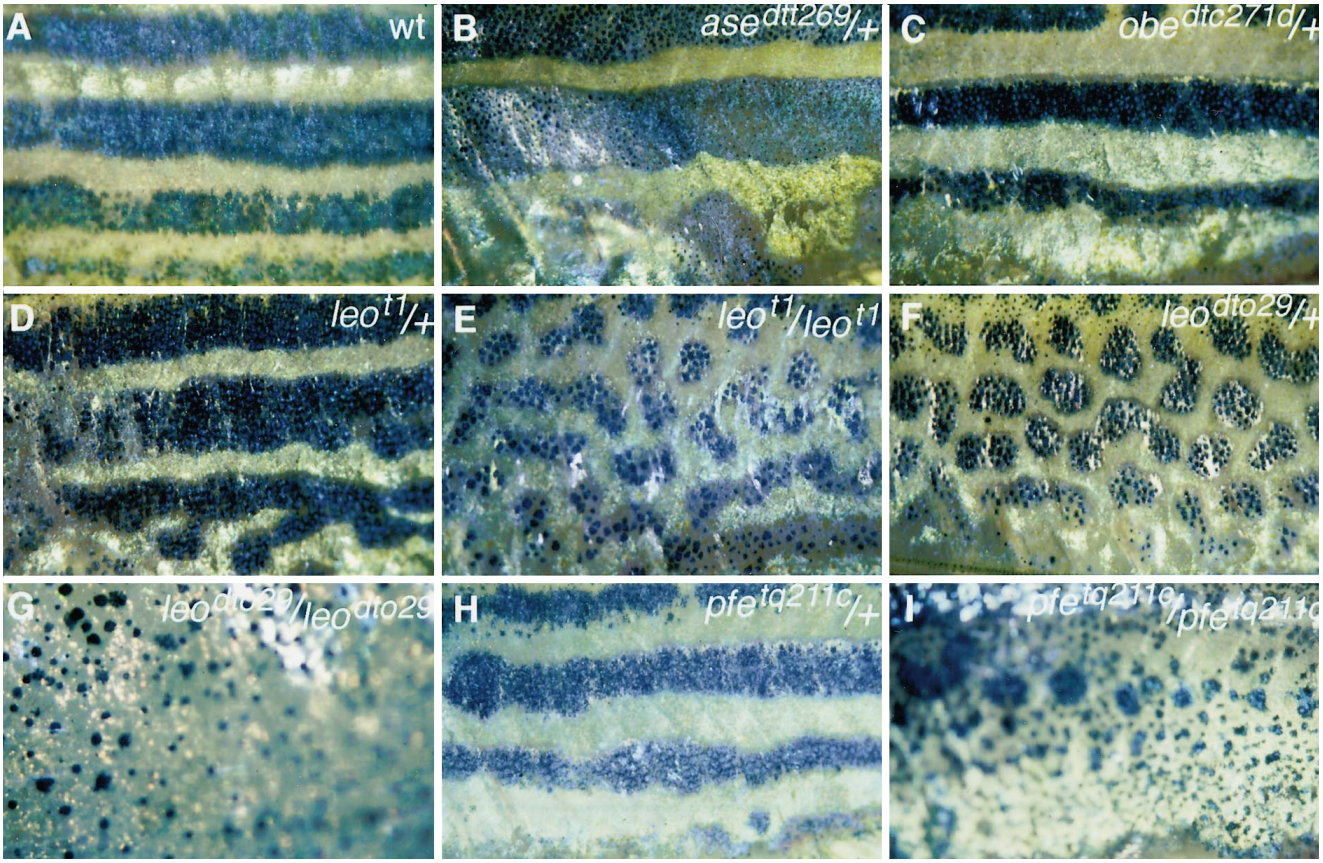

Fig. 6A–I Details of the phenotypes caused by mutations affecting the adult pigment pattern. **A** Wild type. **B** *asterix*^{dtt269} heterozygote with fewer and broader melanophore stripes. **C** *obelix*^{dtc271d} heterozygote with fewer melanophore stripes and broader interstripes. **D** *leopard*^{t1} heterozygote with slightly wavy melanophore stripes, resulting in thinner interstripes. **E** *leopard*^{t1} homozygote with a spotted pigment pattern. **F** *leopard*^{dto29} heterozygote with a spotted pigment pattern similar to *leopard*^{t1} homozygote. **G** *leopard*^{dto29} homozygote with very few tiny spots of melanophore pigmentation. **H** *pfeffer*^{tq211c} heterozygote with interrupted melanophore stripes. **I** *pfeffer*^{tq211c} homozygote with an antero-posterior gradient of residual fine spotted melanophore pigmentation

fect the fins of the embryo or early larvae. *lof*^{dt2} is a spontaneous dominant mutation, which results in lengthening of all adult fins (Fig. 3B) compared to wild type (Fig. 3A). In addition, an increased number of stripes is seen in the pigment pattern of the anal and caudal fins. Skeletal staining revealed that the lengthening of the fins is due to increased length of the lepidotrichia (Fig. 3F), whereas the pterygiophores are unaffected. The segments of the lepidotrichia are increased in number (Fig. 3M), but the length of each segment is unchanged and the arrangement of the segments is as regular as the in wild type (Fig. 3N).

In mutants heterozygous for *alf*^{dty86}, all fins are longer, but the tail fin has lost its symmetrical appearance and hangs down (Fig. 3D). The pigmentation stripes on the fins are not increased in number as in *lof*^{dt2}, but instead appear irregular and less prominent. The pterygiophores of *alf*^{dty86} heterozygotes look essentially normal, whereas the lepidotrichia are very irregular (Fig. 3H). The length of the individual segments is very variable and individual lepidotrichia are frequently interrupted and irregularly forked (Fig. 3O). *alf*^{dty86} was found to segregate independently of *lof*^{dt2}. Double heterozygotes for *lof*^{dt2} and *alf*^{dty86} look identical to *alf*^{dty86} heterozygotes and when crossed to a wild type result in 25% wild-type progeny, 25% *lof*^{dt2} heterozygotes and 50% that are either *alf*^{dty86} heterozygotes or *alf*^{dty86}, *lof*^{dt2} double heterozygotes.

The dominant mutation *wan*^{dty127} causes variable reduction or absence of adult fins and a change of the adult pigment pattern (Fig. 3C). In contrast to *lof*^{dt2} and *alf*^{dty86}, *wan*^{dty127} also affects the pterygiophores of the fins (Fig. 3G). The number of pterygiophores and lepidotrichia is reduced and, in severe cases, only malformed rudiments are seen (Fig. 3 K, L). The fin rays of *wan*^{dty127} heterozygotes, if present, are frequently unsegmented and look similar to the unbranched anterior fin rays. In addition, the number of vertebrae is in most cases reduced by one or two. The pigmentation defect in *wan*^{dty127} heterozygotes is best described as a reduction in number and width of melanophore stripes combined with abnormal branching of the stripes (Fig. 3C). Besides the visible phenotype in the adult, *wan*^{dty127} mutant fish seem to mate less efficiently than wild type, which may make it somewhat less useful as a genetic marker. No obvious additional phenotypes corresponding to homozygous individuals were found among the progeny of incrosses of *lof*^{dt2}, *alf*^{dty86} or *wan*^{dty127}. However the presence of homozygotes in these populations was never confirmed.

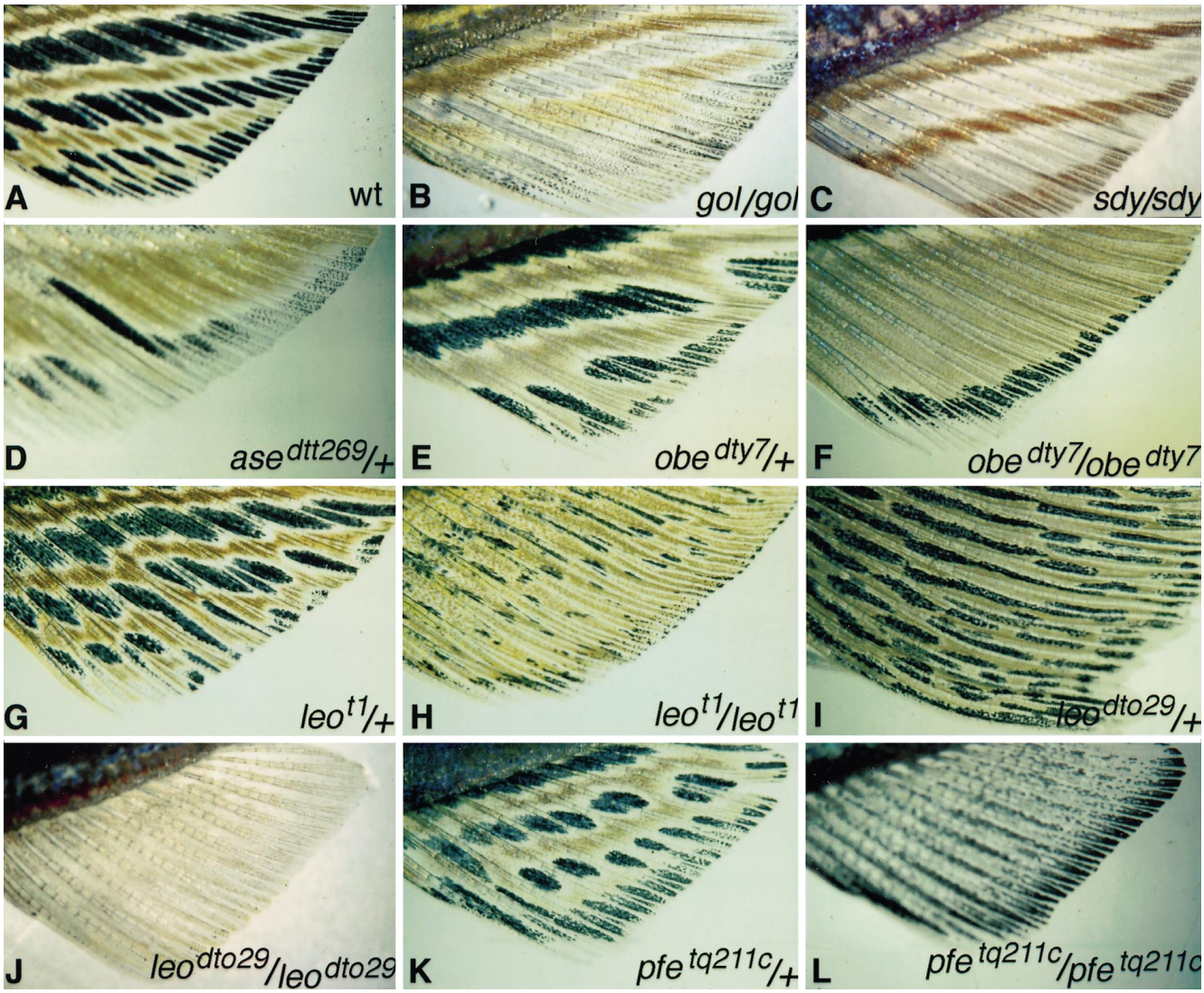

Fig. 7A–L Anal fins, showing details of the phenotypes caused by mutations affecting the adult pigmentation. **A** Wild type. **B** *golden*^{b1} homozygote with reduced melanophore pigmentation. **C** *sandy*^{tk20} homozygote which lacks melanin pigmentation. **D** *asterix*^{dtt269} heterozygote with fewer and broader melanophore stripes. **E** *obelix*^{dty7} heterozygote with fewer melanophore stripes and broader interstripes. **F** *obelix*^{dty7} homozygote with a stronger phenotype than the *obelix*^{dty7} heterozygote. **G** *leopard*^{t1}heterozygote with slightly wavy melanophore stripes, resulting in thinner interstripes. **H** *leopard*^{t1} homozygote with a spotted pigment pattern. **I** *leopard*^{dto29} heterozygote with a spotted pigment pattern similar to *leopard*^{t1} homozygote. **J** *leopard*^{dto29} homozygote with very few tiny spots of melanophore pigmentation and very little xanthophore pigmentation. **K** *pfeffer*^{tq211c} heterozygote with interrupted melanophore stripes. **L** *pfeffer*^{tq211c} homozygote with residual fine spotted melanophore pigmentation

Most recessive mutations producing defects in the adult fins were identified by the embryonic fin phenotype they cause (Eeden et al. 1996a). Surprisingly, one mutant, *stein und bein* (*sub*), which lacks the pelvic and dorsal fins was identified by the lack of otoliths in the embryo (Fig. 4A; Whitfield et al. 1996). However, since only one allele of *sub* was identified, it cannot be excluded that the different phenotypes in the larvae and the adult are due to two separate, but closely linked mutations. Mutations in seven genes (*blasen*, *fransen*, *frayed*, *frilly fins*, *nagel*, *pinfin*, *rafels*) with obvious fin phenotypes in the embryo result only in a comparatively mild reduction of the adult fin. Two mutations, *ikarus* and *krom*, which specifically affect the pectoral fins of the larvae also affect the pectoral fins in the adult (Fig. 4C). A mutation in the gene *finless* (*fls*) results in the absence of all fins in the adult (Fig. 4D; Eeden et al. 1996a). This mutant was found fortuitously in an incross performed for the maintenance of another mutation, and it is expected that more recessive mutations with visible phenotypes in the adult will be discovered in the background of our mutant stocks.

Mutations affecting the adult pigment pattern

The pigment pattern of the adult zebrafish consists of three types of pigment cells, the black melanophores, the yellow xanthophores and the iridescent iridophores (Fig. 1). The black melanophores mainly contribute to

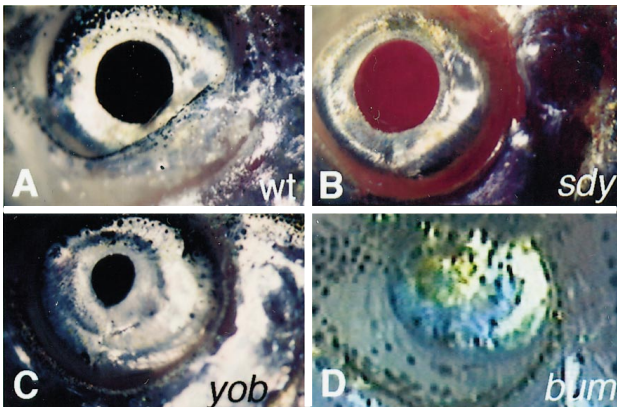

Fig. 8A–D Mutations affecting the eye of the adult. **A** Eye of a wild type. **B** Red eye of *sandy*^{tk20} which lacks melanin pigmentation. **C** Small pupil of *yobo*^{tc251}. **D** Adult eye of *bumper*^{tm127d}, which suffers from lens degeneration

the dark stripes, whereas the xanthophores mainly contribute to the lighter interstripes that separate the melanophore stripes. The iridophores contribute to both stripes and cause the iridescent reflection of the fish. A number of genetic loci, which participate in the formation of the adult pigment pattern have been described (Johnson et al. 1995; Kirschbaum 1975; Streisinger et al. 1986).

Fig. 9A–H Images of 2-day-old live embryos or fixed embryos assayed for tyrosinase activity by the conversion of the substrate 3,4-dihydroxyphenylalanine (dopa) into melanin. **A, C, E, G** Embryos incubated with dopa for tyrosinase activity. **B, D, F, H** Live images, showing the degree of melanin pigmentation. **A, B** Wild-type control, with normal tyrosinase activity and normal melanin pigmentation. **C, D** *albino*^{ti225} has tyrosinase activity, although it lacks melanin pigmentation. **E, F** *mustard*^{tp72i} shows strong tyrosinase activity in the eyes, but weak tyrosinase activity in the body. It lacks melanin pigmentation in the entire embryo. **G, H** *sandy*^{tk20} lacks melanin pigmentation and tyrosinase activity in the entire embryo

In addition to *wan*^{dty127} (see above), we identified dominant mutations in five other genes (*asterix*, *obelix*, *leopard*, *salz*, *pfeffer*), which change the striped pigment pattern of the adult (Figs. 5–7). In *asterix* (*ase*), *ase*^{dtt269}, the dominant phenotype is a broadening and a reduction in the number of melanophore stripes in the body and anal and tail fins (Figs. 5B, 6B, 7D). *ase*^{dtt269} homozygous fish show a stronger phenotype, with a further reduction in the number of melanophore stripes (data not shown). A similar phenotype is caused by mutations in *obelix* (*obe*), of which we identified three alleles of varying strength. The strongest allele is *obe*^{dtd15}, *obe*^{dtc271d} is intermediate in strength and the weakest allele is *obe*^{dty7}. The dominant phenotype of *obe* is a reduction in the number of melanophore stripes with some interruptions and a broadening of the white interstripes (Figs. 5C, 6C, 7E). Fish that are homozygous for *obe* or transheterozygous for two different alleles of *obe* display a further reduction in the number of melanophore stripes, which are irregular in width and interrupted (Figs. 5D, 7F). If strong alleles of *obe* are combined, the stripes do not extend over the entire length of the body. In a swimming group of fish, *obe* or *ase*^{dtt269} individuals are most easily identified by the lack or reduction of the middle melanophore stripe in the tail fin.

A spontaneous mutation in the gene *leopard* (*leo*), *leo*^{t1}, is commonly available in pet shops as a homozygote which displays a spotted adult pigment pattern instead of stripes (Figs. 6E, 7G). As a heterozygote, this allele also causes slightly irregular melanophore stripes which make the bright interstripes appear thinner (Figs. 6D, 7H). Howeve, since the dominant phenotype is so subtle, we generally refer to this allele as recessive. We have isolated as dominants three stronger alleles of *leo*, which all display clearly discernible dominant phenotypes. The allelic series of *leo* mutations shows increasing allele strength in the following order: *leo*^{t1}, *leo*^{dtw28}, *leo*^{dtq270}, *leo*^{dto29}. Fish that are heterozygous for the

Table 5 Mutations that are homozygous viable or semiviable without obvious phenotype in the adult

Phenotypic class	Genes	References
Yolk	*indigested, mal profit*	Hammerschmidt et al. 1996b
Fins	*tutu, microwaved, stomp,*	Eeden et al. 1996a
Hatching gland	*dirty nose*	Hammerschmidt et al. 1996b
Brain	*atlantis, big head, parachute*[a], *recover,*	Furutani-Seiki et al. 1996; Jiang et al. 1996
Ear	*earache, earplugs, einstein, half stoned, headphones, lauscher, monolith, rolling stones,*	Whitfield et al. 1996
Eye	*helderziend, sunrise, belladonna*	Heisenberg et al. 1996; Karlstrom et al. 1996
Blood	*sauternes*[a]	Ransom et al. 1996
Heart	*breakdance, scotchtape*[a], *tango*[a],	Chen et al. 1996
Pigmentation	*matt, stolen pearls, cookie, dropje, fata morgana, nickel, pewter, stars-and-stripes, union jack, parade, brie, kefir quark, ricotta, tofu, yocca, feta*	Kelsh et al. 1996; Odenthal et al. 1996b
Motility	*accordeon*[a], *bandoleon*[a], *fakir, mach two, spaced out,*	Granato et al. 1996
Notochord	*blobbed, kinks, wavy tail*	Odenthal et al. 1996a
Somites	*after eight, beamter, deadly seven, fused somites, U shaped somites*[a]	Eeden et al. 1996b

[a] Only one viable allele, otherwise essential for viability

strongest allele, *leo*^{dto29}, have a spotted pigment pattern (Figs. 5E, 6F, 7I) similar to that of *leo*^{t1} homozygotes, whereas fish that are homozygous for *leo*^{dto29} only have very tiny spots of melanophore pigmentation and a reduction in xanthophore pigmentation (Figs. 5F, 6G, 7J). Fish that are transheterozygotes for any two alleles of *leo* usually present a phenotype that is very close to the homozygous phenotype of the stronger allele (data not shown).

Combinations of mutations in *ase*, *obe* or *leo* show additive effects. For example a *leo*^{dtq270}, *obe*^{dtd15} double heterozygote has spots that are wider apart. Double heterozygotes could therefore easily be identified to test for linkage between two mutations by segregation analysis (Materials and methods). Neither *ase*, *obe* nor *leo* cause any obvious phenotype at embryonic or larval stages. One dominant mutation causing slight interruptions of the melanophore stripes in the adult (*dts37*) has not been tested in segregation analysis and is listed as unresolved among the dominant mutations in Table 3.

Heterozygous carriers for mutations in *salz* (*sal*) or *pfeffer* (*pfe*) have a subtle phenotype in the adult, in which the melanophore stripes are interrupted (Figs. 5G, 6H, 7 K). Due to the subtle character of the dominant phenotype, these mutations were given recessive allele designations (Table 3). Fish that are homozygous for mutations in *sal* or *pfe* have no or very few visible xanthophores. Furthermore, the extent of the area covered by melanophores is strongly reduced and only a few patches of melanophores are visible. The number of these melanophore patches decreases in an antero-posterior gradient (Figs. 5H, 6I, 7L). In *sal* or *pfe* homozygous larvae the number of xanthophores is specifically reduced (Kelsh et al. 1996; Odenthal et al. 1996b).

Most of the recessive mutations with adult phenotypes affect pigmentation in the embryo and/or larva (Kelsh et al. 1996; Odenthal et al. 1996b). A few examples of homozygous mutations causing adult pigmentation phenotypes are shown in Fig. 5. We have identified new alleles of the genes *sparse, golden* and *albino,* which have all been previously identified (Streisinger et al. 1986). Mutations in *sparse* (*spa*) result in mottled melanophore stripes in the adult (Fig. 5I), whereas *golden* (*gol*) affects the intensity of melanophore pigmentation in the embryo and adult (Figs. 5J, 7B; Kelsh et al. 1996; Streisinger et al. 1986). Mutations in *albino* (*alb*), *mustard* (*mrd*) or *sandy* (*sdy*) lack melanin pigmentation in the embryo and adult, resulting in yellow fish with red eyes (Figs. 5K–M, 7C, 8B; Kelsh et al. 1996; Streisinger et al. 1986). Mutations in *shady* (*shd*) cause a reduction in the number of iridophores in the larva (Kelsh et al. 1996). In the adult, *shd* also lacks iridophores and at least one allele (*shd*^{te295}) also shows a reduction of melanophore pigmentation in the body, but not in the fins (Fig. 5N).

Mutations affecting the eyes of the adult

Mutations in three genes (*bumper*, *korinthe*, *rosine*) cause defects in the eyes of the larvae and adult (Fig. 8D; Heisenberg et al. 1996). These fish are probably blind and have melanophores that appear expanded in size. A mutation in *fading vision* (*fdv*) affects melanophore pigmentation and the eyes of the larva and adult, causing small eyes and tumorous outgrowth from the adult eye (Fig. 5O; Kelsh et al. 1996). Usually, the defect in the adult affects the same organ as in the embryo or larva. The most striking exception is *yobo* (*yob*), which shows reduced xanthophore pigmentation in the embryo and a maternal effect from homozygous females (Odenthal et al. 1996b). Adult fish that are homozygous for *yob* have normal xanthophore pigmentation but the pupils appear to be smaller than in the wild type (Figs. 5P, 8C).

Candidates for the zebrafish homolog encoding tyrosinase

A class of mutants, *alb*, *mrd* and *sdy*, which show no melanin pigmentation in homozygous embryos and homozygous adults (Fig. 7; Kelsh et al. 1996), are potential can-

didates for carrying mutations in the zebrafish homolog encoding tyrosinase. We assayed tyrosinase activity in these mutants by assaying for the conversion of 3,4-dihydroxyphenylalanine (dopa) into melanin (Materials and methods; Fig. 9). Strong tyrosinase activity was found in the melanophores and eyes of *alb* embryos (Fig. 9C). Tyrosinase activity in embryos mutant for *mrd* was strong in the eyes but reduced in melanophores (Fig. 9E). No tyrosinase activity was found in *sdy* (Fig. 9G), making this the most likely candidate for a mutation in a gene required for expressing functional tyrosinase or in the gene encoding tyrosinase itself. Most alleles of *sdy* are only semiviable, but the surviving homozygous adults are fertile. The weak allele sdy^{to102a} is fully viable, but it also shows some residual melanin pigmentation.

Homozygous viable mutations without a phenotype in the adult

We have identified mutations in 44 genes, that are homozygous viable and produce a visible phenotype in the adult. Mutations in another 50 genes are homozygous viable, but fail to produce an obvious phenotype in the adult (Table 5). This may reflect a specific function of these genes for embryonic or larval development. In other cases, the embryonic or larval phenotype is rather mild, which could be compensated for during later development. We also identified homozygous viable alleles of 6 genes that are otherwise indispensable for viability. These may be useful as visible embryonic or larval genetic markers since they offer the genetic versatility of homozygous viable mutations. Another potentially useful genetic marker is *half baked*, which, although it is homozygous lethal, displays enlarged hatching glands as a dominant phenotype in heterozygous offspring from heterozygous fathers whereas offspring from female carriers show defects in epiboly (Kane et al. 1996).

Discussion

In our large-scale screen for mutations affecting early development of the zebrafish, we identified 1,200 mutants that we considered worthwhile keeping based on the uniqueness of their phenotype (Haffter et al. 1996). A large number of mutations that are homozygous viable were kept not only because of the specificity of the phenotype they caused, but also because of their potential use as genetic markers. To date 861 mutations have been assigned to 349 genes (Haffter et al. 1996), and in 100 of these genes we found at least 1 allele that is homozygous viable or semiviable. Mutations in 44 of these genes result in phenotypes that are visible in the adult (Tables 1 and 2), whereas mutations in the remaining 56 genes cause phenotypes that are visible at embryonic or larval stages only.

The adult zebrafish displays a variety of morphological features that differ in many respects from the morphology of the embryo early larva. This difference is also reflected by the large number of mutations causing a phenotype in the embryo or larvae, but not in the homozygous adult. Furthermore, a number of mutations causing visible phenotypes in the adult do not show any phenotype at embryonic or larval stages. We also found a number of examples in which the larval and embryonic phenotype differ substantially. The most striking example is *yobo*, which causes a xanthophore pigmentation phenotype in the larvae and an eye phenotype in the adult. In addition, *yobo* shows a maternal effect, resulting in truncated head and tail of the embryo (Odenthal et al. 1996b). Such a variety of phenotypes suggests that the same molecule is required separately for different purposes in the organism.

In *salz* (*sal*) or *pfeffer* (*pfe*) homozygous larvae the number of xanthophores is specifically reduced but the melanophore pattern is normal (Kelsh et al. 1996; Odenthal et al. 1996b). In homozygous adults of *sal* or *pfe*, xanthophore and melanophore pigment pattern is affected. As both genes are associated with the same phenotypic traits, this suggests that the xanthophore cells are required in the adult for generating or maintaining melanophores in a striped pattern. Alternatively, *sal* and *pfe* could be required separately by the melanophores to form the striped pattern. It has been reported previously that the number of xanthophore cells is reduced in the anal fins of $leopard^{t1}$ (*leo*) homozygous fish. In fish that are homozygous for the stronger allele leo^{dto29}, xanthophore and melanophore pigmentation is further reduced. This suggests that, in the adult (but not in the early larva), melanophores and xanthophores are interdependent on each other for the formation of a striped pattern. In *sal*, *pfe* and *leo*, this effect is seen in the pigmentation of the body and fins. A similar dependence of a striped melanophore pattern on the presence of iridophores can be seen in *shady* (*shd*), *rose* and *transparent* (see also Johnson et al. 1995). However, in *shd* for example, the lack of iridophores mostly affects the melanophore stripes in the body, whereas the stripes in the fins are essentially normal. Since only one allele of *shd* shows this effect, it cannot be excluded that the phenotype of shd^{te295} homozygous adults is due to a separate, closely linked mutation.

Most of the mutations identified in the screen cause embryonic lethality (Haffter et al. 1996). The need to reidentify carriers of lethal mutations every generation makes the maintenance of large numbers of mutant stocks a laborious task. The maintenance of a lethal mutant stock could be simplified enormously if a linked recessive mutation that is homozygous viable is identified. The lethal mutation is then kept as a transheterozygote over the viable mutation in one stock, which is maintained by outcrossing to fish that are homozygous for the viable mutation. Selecting against the visible mutation in that outcross enriches dramatically for carriers of the lethal mutation, which simplifies the identification of mutant carriers in the next generation. Using a viable mutation for balancing a lethal stock is desirable, because the

genetic background of the homozygous viable stock can easily be refreshed by alternating between outcrossing homozygous individuals to wild type in one generation and inbreeding heterozygous individuals in the next generation for raising a homozygous generation. By following such a breeding scheme, problems caused by continuous inbreeding can be avoided. Alternatively, lethal mutations could be balanced over another lethal mutation. Such a breeding scheme would require the continuous inbreeding between double heterozygotes, which in our hands up to date generally resulted in reduced fertility after a few generations.

Towards the aim of balancing mutations, mapping of the genes identified by mutation will be very important. Two efforts towards generating a genetic map of molecular markers are presently under way. An RAPD (random amplified polymorphic DNA) map using random decamer primers to amplify arbitrary DNA sequences by PCR was generated and is being refined by the group of J. Postlethwait (Postlethwait et al. 1994). In the laboratory of H. Jacob, an SSR (simple sequence repeats) map using PCR primers homologous to unique sequences flanking CA repeats is being generated (Knapik et al. 1996).

The availability of a genetic marker for the generation of transgenic lines of zebrafish is a very desirable tool. A number of transgenic lines of zebrafish have been generated by simple injection of DNA (Bayer and Campos-Ortega 1992; Culp et al. 1991; Lin et al. 1994b; Stuart et al. 1990) or by infection using a pseudotyped retroviral vector (Lin et al. 1994a). Without a dominant marker, genotyping of the potential transgenic fish is very laborious. Tyrosinase has been used as a dominant marker in the generation of transgenic animals in the mouse and medaka fish (Beermann et al. 1990; Matsumoto et al. 1992; Schedl et al. 1993; Tanaka et al. 1990). *sandy* (*sdy*), which lacks tyrosinase activity, is a candidate for encoding tyrosinase in zebrafish, which may allow the use of tyrosinase as a dominant marker for transgenes.

However, even if *sdy* does not encode tyrosinase itself, a tyrosinase transgene might produce visible pigmentation in this mutant. A reconstructed tyrosinase gene from the mouse produced wild-type pigmentation in an orange-coloured variant of medaka, which has normal melanin pigmentation in the eyes and does not seem to be due to mutations in the tyrosinase encoding gene (Matsumoto et al. 1992). Surprisingly, endogenous mRNA encoding tyrosinase was detected in amelanotic skin of this orange coloured variant of medaka (Inagaki et al. 1994). It is therefore conceivable that a similar tyrosinase transgene may produce melanophore pigmentation not only in *sdy*, but also in *mustard* or other mutants with defects in melanophore pigmentation (Kelsh et al. 1996). A potential problem is that strong alleles of *sdy* are only semiviable, which may make this mutant more difficult to use as a host for tyrosinase-expressing transgenes.

Mutations causing visible phenotypes are very useful genetic markers, because they allow the tracing of individual chromosomes during sophisticated genetic experiments. Ideally, a dominant marker expresses a different phenotype in the homozygous state than in the heterozygous. This advantageous feature is the case for *asterix*, *obelix* and *leopard*. Very useful visible genetic markers are obviously those which give a visible phenotype in the larva and adult without any effect on the viability and fertility of the homozygous adults. The most useful visible genetic markers in our collection are *salz* and *pfeffer*, which cause a recessive phenotype in the larva and a recessive and a dominant phenotype in the adult, both of which can be distinguished from each other.

Many of the mutations described here provide a valuable resource for studying differences between embryonic and adult development. Mutations affecting all fins either cause an adult phenotype only or a strong phenotype in the embryo and a rather subtle phenotype in the adult. However, mutations specifically affecting the pectoral fins result in equally strong defects in the embryo and adult. These mutations can therefore be used to distinguish shared from separate developmental pathways involved in the formation of embryonic and adult fins. Similarly, mutations specifically affecting either embryonic or adult pigmentation allow the separate study of developmental processes involved in embryonic or adult pigment pattern formation.

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References

Bayer TA, Campos-Ortega JA (1992) A transgene containing lacZ is expressed in primary sensory neurons in zebrafish. Development 115:421–426

Beermann F, Ruppert S, Hummler E, Bosch FX, Müller G, Rüther U, Schütz G (1990) Rescue of the albino phenotype by introduction of a functional tyrosinase gene into mice. EMBO J 9:2819–2826

Brand M, Heisenberg C-P, Warga RM, Pelegri F, Karlstrom RO, Beuchle D, Picker A, Jiang Y-J, Furutani-Seiki M, Eeden FJM van, Granato M, Haffter P, Hammerschmidt M, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. Development 123:129–142

Chen J-N, Haffter P, Odenthal J, Vogelsang E, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Nüsslein-Volhard C (1996) Mutations affecting the cardiovascular system and other internal organs in zebrafish. Development 123:293–302

Culp P, Nüsslein-Volhard C, Hopkins N (1991) High-frequency germ-line transmission of plasmid DNA sequences injected into fertilized zebrafish eggs. Proc Natl Acad Sci USA 88: 7953–7957

Eeden FJM van, Granato M, Schach U, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg C-P, Jiang Y-J,

Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996a) Genetic analysis of fin formation in the zebrafish, *Danio rerio*. Development 123:255–262

Eeden FJM van, Granato M, Schach U, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Warga RM, Allende ML, Weinberg ES, Nüsslein-Volhard C (1996b) Mutations affecting somite formation and patterning in the zebrafish *Danio rerio*. Development 123:153–164

Elias J (1984) *Brachydanio frankei*-Schleierform. Aquaria 34:81–85

Frank S, Zukal R (1981) The leopard *Danio*; Hybrid, mutant or valid species? Trop Fish Hobby 29:5–15

Frankel JS (1979) Inheritance of spotting in the leopard *Danio*. J Hered 70:287–288

Furutani-Seiki M, Jiang Y-J, Brand M, Heisenberg C-P, Houart C, Beuchle D, Eeden FJM van, Granato M, Haffter P, Hammerschmidt M, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Neural degeneration mutants in the zebrafish, *Danio rerio*. Development 123:229–239

Granato M, Eeden FJM van, Schach U, Trowe T, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Genes controlling and mediating locomotion behaviour of the zebrafish embryo and larva. Development 123:399–413

Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, Eeden FJM van, Jiang Y-J, Heisenberg C-P, Kelsh RN, Furutani-Seiki M, Vogelsang E, Beuchle D, Schach U, Fabian C, Nüsslein-Volhard C (1996) The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. Development 123: 1–36

Hammerschmidt M, Pelegri F, Kane DA, Mullins MC, Serbedzija GN, Eeden FJM van, Granato M, Brand M, Furutani-Seiki M, Haffter P, Heisenberg C-P, Jiang Y-J, Kelsh RN, Odenthal J, Warga RM, Nüsslein-Volhard C (1996a) Identification of *dino* and *mercedes*, two genes regulating dorsal development in the zebrafish embryo. Development 123:95–102

Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Haffter P, Heisenberg C-P, Jiang Y-J, Kelsh RN, Odenthal J, Warga RM, Nüsslein-Volhard C (1996b) Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, *Danio rerio*. Development 123:143–151

Hearing VJ (1987) Mammalian monophenol monooxygenase (tyrosinase): Purification, properties, and reactions catalyzed. Methods Enzymol 142:154–165

Hearing VJ, Jiménez M (1989) Analysis of mammalian pigmentation at the molecular levels. Pigm Cell Res 2:75–85

Heisenberg C-P, Brand M, Jiang Y-J, Warga RM, Beuchle D, Eeden FJM van, Furutani-Seiki M, Granato M, Haffter P, Hammerschmidt M, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Genes involved in forebrain development in the zebrafish, *Danio rerio*. Development 123: 191–203

Hogan B, Constantini F, Lacy E (1986) Manipulating the mouse embryo. Cold Spring Harbor Laboratory Press, New York

Inagaki H, Bessho Y, Koga A, Hori H (1994) Expression of the tyrosinase-encoding gene in a colorless melanophore mutant of the medaka fish, *Oryzias latipes*. Gene 150:319–324

Jiang Y-J, Brand M, Heisenberg C-P, Beuchle D, Furutani-Seiki M, Kelsh RN, Warga RM, Granato M, Haffter P, Hammerschmidt M, Kane DA, Mullins MC, Odenthal J, Eeden FJM van, Nüsslein-Volhard C (1996) Mutations affecting neurogenesis and brain morphology in the zebrafish, *Danio rerio*. Development 123:205–216

Johnson SL, Africa D, Walker C, Weston JA (1995) Genetic control of adult pigment stripe development in zebrafish. Dev Biol 167:27–33

Kane DA, Maischein H-M, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Haffter P, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kelsh RN, Mullins MC, Odenthal J, Warga RM, Nüsslein-Volhard C (1996) The zebrafish early arrest mutants. Development 123:57–66

Karlstrom RO, Trowe T, Klostermann S, Baier H, Brand M, Crawford AD, Grunewald B, Haffter P, Hoffman H, Meyer SU, Müller BK, Richter S, Eeden FJM van, Nüsslein-Volhard C, Bonhoeffer F (1996) Zebrafish mutations affecting retinotectal axon pathfinding. Development 123:427–438

Kelsh RN, Brand M, Jiang Y-J, Heisenberg C-P, Lin S, Haffter P, Odenthal J, Mullins MC, Eeden FJM van, Furutani-Seiki M, Granato M, Hammerschmidt M, Kane DA, Warga RM, Beuchle D, Vogelsang E, Nüsslein-Volhard C (1996) Zebrafish pigmentation mutants and the processes of neural crest development. Development 123:369–389

Kirschbaum F (1975) Investigations on the colour pattern of the zebrafish *Brachydanio rerio* (Cyprinidae, Teleostei). Roux's Arch Dev Bio 177:129–152

Knapik EW, Goodman A, Atkinson OS, Roberts CT, Shiozawa M, Sim CU, Weksler-Zangen S, Trolliet MR, Futrell C, Innes BA, Koike G, McLaughlin MG, Pierre L, Simon JS, Vilallonga E, Roy M, Chiang P-W, Fishman M, Driever W, Jacob HJ (1996) A reference cross for zebrafish (*Danio rerio*) anchored with simple sequence length polymorphisms. Development 123: 451–460

Koga A, Inagaki H, Bessho Y, Hori H (1995) Insertion of a novel transposable element in the tyrosinase gene is responsible for an albino mutation in the medaka fish, *Oryzias latipes*. Mol Gen Genet 249:400–405

Korner A, Pawelek J (1982) Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. Science 217: 1163–1165

Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. Proc Natl Acad Sci USA 84: 7473–7477

Lin S, Long W, Chen J, Hopkins N (1992) Production of germline chimeras in zebrafish by cell transplants from genetically pigmented to *albino* embryos. Proc Natl Acad Sci USA 89: 4519–4523

Lin S, Gaiano N, Culp P, Burns JC, Friedmann T, Yee J-K, Hopkins N (1994a) Integration and germ-line transmission of a pseudotyped retroviral vector in zebrafish. Science 265: 666–669

Lin S, Yang S, Hopkins N (1994b) lacZ expression in germline transgenic zebrafish can be detected in living embryos. Dev Biol 161:77–83

Lindsley DL, Zimm GG (1992) The genome of *Drosophila melanogaster*. Academic Press, San Diego

Matsumoto J, Akiyama T, Hirose E, Nakamura M, Yamamoto H, Takeuchi T (1992) Expression and transmission of wild-type pigmentation in the skin of transgenic orange-colored variants of medaka (*Oryzias latipes*) bearing the gene for mouse tyrosinase. Pigm Cell Res 5:322–327

Morgan TH (1910) Sex-linked inheritance in *Drosophila*. Science 32:120–122

Morgan TH, Sturtevant AH, Muller HJ, Bridges CB (1915) The mechanisms of mendelian heredity. Holt, Reinhart and Winston, New York

Mullins MC, Hammerschmidt M, Haffter P, Nüsslein-Volhard C (1994) Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. Curr Biol 4:189–202

Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, Brand M, Furutani-Seiki M, Granato M, Haffter P, Heisenberg C-P, Jiang Y-J, Kelsh RN, Eeden FJM van, Nüsslein-Volhard C (1996) Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral determinants. Development 123: 81–93

Odenthal J, Haffter P, Vogelsang E, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Warga RM, Allende ML, Weinberg ES, Nüsslein-Volhard C (1996a) Mutations affecting the formation of the notochord in the zebrafish, *Danio rerio*. Development 123:103–115

Odenthal J, Rossnagel K, Haffter P, Kelsh RN, Vogelsang E, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Mullins MC, Nüsslein-Volhard C (1996b) Genes affecting xanthophore pigmentation in the zebrafish, *Danio rerio*. Development 123: 391–398

Petrovicky I (1964) Krizeni druhu *Brachydanio rerio* a *Brachydanio frankei*. Akrarium a terarium 7:5–6, 66–69

Petrovicky I (1966) Hybridization between *Brachydanio rerio* (Hamilton-Buchanan) and *Brachydanio frankei* (Meinken). Ischthyol Aquar J 37:53–67

Postlethwait JH, Johnson SL, Midson CN, Talbot WS, Gates M, Ballinger EW, Africa D, Andrews R, Carl T, Eisen JS, Horne S, Kimmel CB, Hutchinson M, Johnson M, Rodriguez A (1994) A genetic linkage map for the zebrafish. Science 264:699–703

Ransom DG, Haffter P, Odenthal J, Brownlie A, Vogelsang E, Brand M, Eeden FJM van, Furutani-Seiki M, Granato M, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Nüsslein-Volhard C (1996) Characterization of zebrafish mutants with defects in embryonic hematopoiesis. Development 123:311–319

Ruppert S, Müller G, Kwon B, Schütz G (1988) Multiple transcripts of the mouse tyrosinase gene are generated by alternative splicing. EMBO J 7:2715–2722

Schedl A, Montiliu L, Kelsey G, Schütz G (1993) A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice. Nature 362: 258–261

Silvers WK (1979) The coat-colors of mice – A model for mammalian gene action and interaction. Springer, Berlin Heidelberg New York

Streisinger G, Singer F, Walker G, Knauber D, Dower N (1986) Segregation analysis and gene-centromere distances in zebrafish. Genetics 112:311–319

Stuart GW, Vielkind JR, McMurray JV, Westerfield M (1990) Stable lines of transgenic zebrafish exhibit reproducible patterns of transgene expression. Development 109:577–84

Tanaka S, Yamamoto H, Takeuchi S, Takeuchi T (1990) Melanization in albino mice transformed by introducing cloned mouse *tyrosinase* gene. Development 108:223–227

The University of Oregon Zebrafish Group (1993) Mutant strains at the University of Oregon. Zebrafish Sci Monitor 2:4–5

Tresnake I (1981) The long-finned zebra *Danio*. Trop Fish Hobby 29:43–56

Wakamatsu Y, Oikawa A, Obika M, Hirobe T, Ozato K (1984) Fish hereditary melanoma cell lines of different degrees of cell differentiation. Dev Growth Diff 26:503–513

Whitfield TT, Granato M, Eeden FJM van, Schach U, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg C-P, Jiang Y-J, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Mutations affecting development of the zebrafish inner ear and lateral line. Development 123: 241–254

Wood WB (1988) The nematode *Caenorhabditis elegans*. Cold Spring Harbor Press, New York