Development 140, 2997-3007 (2013) doi:10.1242/dev.096719

Iridophores and their interactions with other chromatophores are required for stripe formation in zebrafish

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SUMMARY

Colour patterns of adult fish are produced by several types of pigment cells that distribute in the dermis during juvenile development. The zebrafish, *Danio rerio*, displays a striking pattern of dark stripes of melanophores interspersed by light stripes of xanthophores. Mutants lacking either cell type do not form proper stripes, indicating that interactions between these two chromatophore types are required for stripe formation. A third cell type, silvery iridophores, participates to render a shiny appearance to the pattern, but its role in stripe formation has been unclear. Mutations in *rose* (*rse*) or *shady* (*shd*) cause a lack or strong reduction of iridophores in adult fish; in addition, the melanophore number is drastically reduced and stripes are broken up into spots. We show that *rse* and *shd* are autonomously required in iridophores, as mutant melanophores form normal sized stripes when confronted with wild-type iridophores in chimeric animals. We describe stripe formation in mutants missing one or two of the three chromatophore types. None of the chromatophore types alone is able to create a pattern but residual stripe formation occurs with two cell types. Our analysis shows that iridophores promote and sustain melanophores. Furthermore, iridophores attract xanthophores, whereas xanthophores repel melanophores. We present a model for the interactions between the three chromatophore types underlying stripe formation. Stripe formation is initiated by iridophores appearing at the horizontal myoseptum, which serves as a morphological landmark for stripe orientation, but is subsequently a self-organising process.

KEY WORDS: Iridophores, Pigment pattern formation, *shady*, *rose*, Chimeras

INTRODUCTION

Colour patterns are prominent features of many animals; they have important functions in protection against UV irradiation, camouflage, kin recognition, shoaling and sexual selection. Colour patterns in birds and mammals are generated by melanocytes, which produce melanin and transfer it to the tissues of fur or plumage. In fish, amphibia and reptiles, chromatophores retain their pigment and it is their distribution in the dermis that determines the pattern (Kelsh, 2004).

The characteristic stripe pattern of zebrafish is composed of black melanophores, yellow xanthophores and silvery iridophores containing light-reflective purine platelets; these are responsible for the shiny appearance of the pattern. All chromatophores, except the retinal melanocytes, originate from the neural crest, a transient pluripotent embryonic cell population. In the zebrafish embryo, neural crest cells and their progeny differentiate directly into chromatophores that form the larval pattern (Eisen and Weston, 1993; Raible and Eisen, 1994). During metamorphosis, the adult pattern is generated by new chromatophores that emerge in the dermis. There is increasing evidence that these cells are produced by neural crest-derived stem cells that have been set aside in distinct niches, such as the ganglia of the peripheral nervous system or the base of the fins (Budi et al., 2008; Budi et al., 2011; Hultman et al., 2009; Hultman and Johnson, 2010; Tryon et al., 2011; Tu and Johnson, 2011; Dooley et al., 2013).

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Accepted 10 May 2013

Adult chromatophores localise in different tissue layers. At the most superficial level, chromatophores are organised in the dorsal epidermis on scales and around scale pockets (Kirschbaum, 1975). Other pigment cells lie deep in the body, e.g. the dense sheet of iridophores covering the viscera and the melanophores associated with blood vessels. The chromatophores that form the horizontal pigment stripes characteristic for zebrafish distribute in the dermis. This pattern starts to develop with a light stripe (interstripe) in the region of the horizontal myoseptum. Subsequently, dark stripes appear dorsally and ventrally to this interstripe, and more interstripes and stripes are added as the fish grows (Kirschbaum, 1975; Takahashi and Kondo, 2008; Parichy et al., 2009). Melanophores are restricted to the dark stripes, whereas a dense sheet of iridophores covered by xanthophores form the light interstripes. A thin layer of iridophores spreads over the melanophores (Fig. 1A). These are S-iridophores, whereas another iridophore type, L-iridophores, is located underneath the melanophore stripes (Hirata et al., 2003).

Several genes involved in the formation of the adult pigment pattern have been identified by mutations causing a strong reduction in number or the complete absence of one or more of the chromatophore types (Johnson et al., 1995; Haffter et al., 1996; Kelsh et al., 1996; Lister et al., 1999; Parichy et al., 1999). The *nacre* (*nac*) gene encodes the transcription factor Mitfa (Lister et al., 1999). *nac* mutants lack both larval and adult melanophores. In larvae, the pattern of iridophores and xanthophores is normal. In adult fish, however, iridophores and xanthophores do not form proper stripes (Maderspacher and Nüsslein-Volhard, 2003). The *pfeffer/panther* (*pfe*) gene encodes the receptor tyrosine kinase Csf1ra/Fms (Parichy et al., 2000a). In *pfe* mutants, the development of xanthophores is strongly suppressed in larvae and abolished in adults (Haffter et al., 1996; Odenthal et al., 1996). Additionally, in adults the number of melanophores is reduced (Parichy et al., 2000a). Iridophores and melanophores are normal in larvae, but are

Fig. 1. Adult phenotypes of iridophore mutants. (**A**) Wild-type fish. To denote individual stripes, we follow the nomenclature of Parichy et al. (Parichy et al., 2009), which we have extended by naming the interstripes, the central interstripe being X0. Additional stripes and interstripes added dorsally and ventrally during development are numbered according to their sequence of appearance. (**B**,**C**) *rse* mutants. Weak (B) and strong (C) *rse* alleles show a reduction of iridophores and melanophores. In strong *rse* mutants (Fig. 1C), the dense S-iridophore zone in X0 is lacking, and S-iridophores spread thinly from X0 dorsally and ventrally over the melanophores. In the weaker allele, a small ridge of dense S-iridopores persists (B). There are at most four melanophore stripes compared with five in a typical wild-type adult. Stripe 2D is reduced or absent, 2V is better preserved and 1V stays more strictly in a wild-type position parallel to X0 in *rse* compared with *shd* (D). (**D**,**E**) *shd* mutants. The white arrow in E displays an escaper S-iridophore patch (Lopes et al., 2008). In close proximity, the number of melanophores is significantly increased. (**F**) Double mutant *rse;shd*. The phenotype is indistinguishable from that of *shd* mutants.

not able to maintain the striped organisation in mutant adults. In both *nac* and *pfe* mutants, introduction of the missing cell type by blastula transplantation restores normal stripe formation, thus indicating that *nac* and *pfe* act cell-autonomously in melanophores and xanthophores, respectively (Maderspacher and Nüsslein-Volhard, 2003; Parichy and Turner, 2003). These results show that the striped pattern depends strongly on the interactions between melanophores and xanthophores.

The process of stripe formation displays properties of self-organising systems based on Turing-type interactions (Turing, 1952; Gierer and Meinhardt, 1972; Meinhardt and Gierer, 1980). In zebrafish, stripe formation has been modelled based on interactions between melanophores and xanthophores observed in normal development and during regeneration (Asai et al., 1999; Yamaguchi et al., 2007; Nakamasu et al., 2009).

Several mutations are known to cause a reduction of iridophores (Johnson et al., 1995; Haffter et al., 1996; Kelsh et al., 1996; Lang et al., 2009). *shady* (encoding leukocyte tyrosine kinase) mutants lack iridophores both in larvae and in adults whereas in *rose* (encoding endothelin receptor b1a) mutants only the adult pattern is affected (Parichy et al., 2000b; Lopes et al., 2008). In both mutants, strikingly, the melanophore numbers are also strongly reduced and the stripes are broken up into spots. A similar phenotype is displayed in *transparent* (*tra*) mutants (Walker and Streisinger, 1983; Krauss et al., 2013), *bnc2* (Lang et al., 2009) and *roy* (White et al., 2008). Strikingly, mutants lacking iridophores display a normal striped pattern in the anal and tail fins. Therefore, iridophores have been regarded not to be active players in the process of stripe formation (Maderspacher and Nüsslein-Volhard, 2003; Parichy and Turner, 2003; Nakamasu et al., 2009). Alternatively, stripe formation in the fins may share some but not all properties of the mechanism working in the dermis of the trunk.

In this study, we show that iridophores play a crucial role in melanophore stripe formation in the dermis. We present the phenotypes and the development of the pigment pattern of *rse* and *shd* mutants. By creating chimeric animals we show that both act cell-autonomously in iridophores, whereas the reduction in melanophore number and stripe formation is caused by the absence of iridophores. To elucidate the interactions between iridophores and the other two chromatophore types, we analyse the development of the pigment pattern in mutants lacking either melanophores or xanthophores, *nac* and *pfe*, as well as in double mutants, in which two types of chromatophores are absent. We deduce several short- and long-range interactions between iridophores, xanthophores and melanophores and present a model for the interactions underlying stripe formation in zebrafish. Finally, we identify the horizontal myoseptum, which is lacking in the *choker* mutants, as a prepattern for the first interstripe, whereas the subsequent formation of the pattern of alternating stripes and interstripes depends on the mutual interaction between all three chromatophores.

MATERIALS AND METHODS

Zebrafish maintenance and genetics

We investigated zebrafish (*Danio rerio*) of the following genotypes: *nac*^{w2}, *pfe*^{tm236}, *cho*^{tm26} (ZFIN database), *shd*^{j9s1} (Lopes et al., 2008) and *Tg(TDL358:gfp)* (Levesque et al., 2013). We describe two new N-ethyl-N-nitrosourea (ENU)-induced *rse* alleles, *rse*^{tAN17X} (weak) and *rse*^{tLF802} (strong), which we will refer to as *rse*^{w} and *rse*^{s} or simply *rse*. The phenotype of the stronger allele resembles that of the published amorphic allele (Parichy et al., 2000b). Zebrafish were maintained as described by Brand et al. (Brand et al., 2002). Metamorphic fish were staged [AR, anal fin ray/6.2 mm standardised standard length (SSL); PB, pelvic fin bud/7.2 mm SSL; PR, pelvic fin ray/8.6 mm SSL; SP, squamation onset posterior/9.6 mm SSL; J, juvenile/11.0 mm SSL; J+, juvenile+/13 mm

SSL; and J++, juvenile++/16 mm SSL] according to Parichy et al. (Parichy et al., 2009).

Cell transplantation

Chimeric animals were generated by transplantation of blastula cells into embryos of the same stage essentially as described by Kane and Kishimoto (Kane and Kishimoto, 2002). The number of transplanted cells was estimated to be in the range of 30-50. Animals were raised to adulthood and analysed for donor-derived chromatophores.

Image acquisition and analysis

Adult fish were briefly anaesthetised with 0.004% MS-222 (Sigma) and imaged with Canon D5MarkII/MACRO 100 (Figs 1, 3). Fish fixed in 4% paraformaldehyde/0.08% glutaraldehyde (Sigma) (Fig. 2) were photographed under a Leica MZ1 stereomicroscope. Metamorphic fish were anaesthetised, embedded in low melting point agarose with 4.5 mg/ml ± epinephrine (Sigma) for melanosome contraction, and photographed under a Leica M205 FA stereomicroscope with a Leica DCF300 FX camera using the software LAS V4.1 to allow multifocus images. An illumination was chosen to display iridophores optimally while xanthophore visibility is poor. Photographs were processed in Adobe Photoshop.

RESULTS

Melanophore stripe formation is affected in the iridophore mutants *rse* and *shd*

In zebrafish, melanophores are restricted to the stripes whereas iridophores and xanthophores are present in both stripes and interstripes (Fig. 1A; Fig. 2A). Two types of iridophores can be distinguished in the trunk. Superficial S-iridophores form a dense zone in the interstripe (‘dense S-iridophores’) and spread as a thin net-like layer (‘blue S-iridophores’) over the melanophores of the stripe. Xanthophores are located on top of S-iridophores. L-iridophores form a homogeneous silvery sheet below melanophores (Hirata et al., 2003). The colour of the iridophores may appear silvery, golden, brownish or blue, depending on the illumination (Fig. 1A; Fig. 2).

Mutations in the genes *rse* and *shd* result in similar recessive adult phenotypes of varying strengths, displaying (1) a reduction (*rse*) or absence (*shd*) of S-iridophores, (2) a reduced number of melanophores and (3) fewer stripes (Fig. 1B-D). With increasing loss of iridophores, the stripes dissolve into a series of spots. The central stripes 1D and 1V, adjacent to the first interstripe X0, remain most prominent, whereas the stripes added later are more strongly reduced. The interstripe regions become wider as S-iridophores are lost from the interstripes. In the weak *rse* allele (Fig. 1B) a thin ridge of S-iridophores is present in X0, and the adjacent stripes are fairly well preserved. By contrast, in the absence of dense S-iridophores (Fig. 1C,D; Fig. 2B,C) the melanophores are reduced and broken up into spots. Xanthophores cover the regions between melanophores (Fig. 2B′,C′). In strong mutants (*shd*), neither S- nor L-iridophores develop in the dermal trunk, both 2D and 2V stripes are strongly reduced or absent (Fig. 2C) and the horizontal alignment of 1V spots may be lost (Fig. 2C, grey arrows).

Iridophores are also reduced in other regions of the body: *shd* mutants show a strong reduction of iridophores in the eye, whereas in *rse* mutants this phenotype is more subtle (Fig. 1C,D). The operculum and gut are sparingly covered by iridophores, rendering the gills, the intestine and the melanophore-covered blood vessels along the myosepta visible through the skin (Fig. 1B-E; Fig. 2B,C). Along the dorsal aspect of the fish, the numbers of iridophores and melanophores are reduced, and the melanophores appear less aggregated (supplementary material Fig. S1). The iridophores in the anal and tail fins are reduced but the striped pattern of the fins is not affected (Fig. 1B-F).

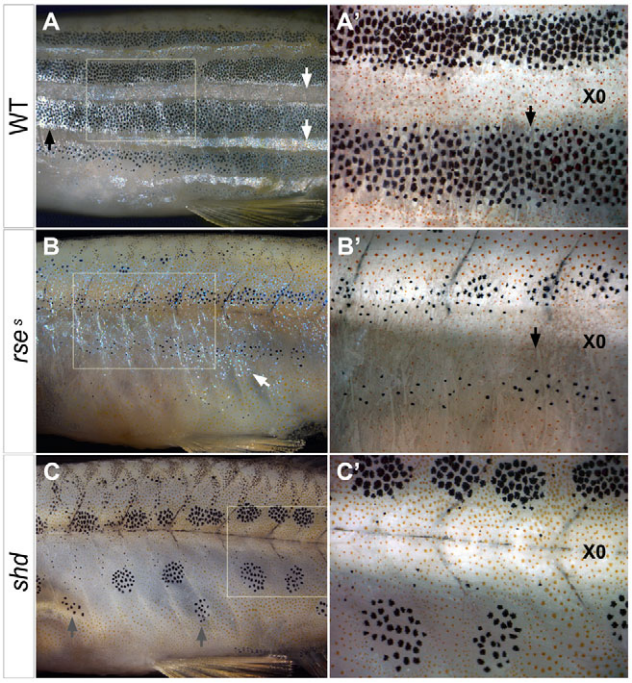

Fig. 2. Details of adult phenotypes in iridophore mutants. Images of the anterior trunk of fixed animals photographed under white light. L-iridophores (black arrows) and dense S-iridophores (white arrows) reflect maximally at different angles of light and therefore shine up in different anteroposterior regions. Insets in A-C demarcate the magnified views in A′-C′ that were taken under UV light to avoid reflections from iridophores and to highlight xanthophores. Under this illumination, L-iridophores are visible as bundles of greyish vertically oriented fibres (black arrows). (**A**,**A′**) Wild-type fish. (**B**,**B′**) *rse* mutant fish. An illumination was chosen to avoid reflection of L-iridophores, whereas S-iridophores are visible (white arrow). L-iridophores, which are largely restricted to melanophore regions in wild type (A,A′, black arrows), expand into interstripe regions in *rse* mutants (B,B′). The light appearance in stripe 1D in B′ is due to the absence of L-iridophores. (**C**,**C′**) *shd* mutant fish. Ventrally, some of the melanophore patches (grey arrows) appear to be located between 1V and 2V. The light area in X0 in C′ is probably due to reflective properties of musculature.

The double mutant *rse;shd* (Fig. 1F) does not show a significant increase in the strength of the phenotype when compared with the strongest single mutant, i.e. a mutation in *rse* does not enhance the mutant effect of *shd*. This indicates that in both mutants the same cell type(s) are primarily affected.

Mutants lacking xanthophores and melanophores display abnormal iridophore distribution

To elucidate the dependencies between iridophores, xanthophores and melanophores, we analysed the adult phenotypes of *pfe* and *nac* mutants, as well as those of the double mutants *nac;pfe*, *shd;pfe* and *shd;nac*.

No xanthophores

In *pfe* mutants (Fig. 3A,A′), melanophore stripes are broken up into spots, separated by dense S-iridophore regions extending into the stripes. Melanophores also appear ectopically as single cells within the interstripes. Adult *pfe* mutant fish show an increase in S-iridophores and a reduction of melanophore number although stripes appear to be of normal width, at least in anterior regions where the striped organisation is better preserved (Fig. 3A).

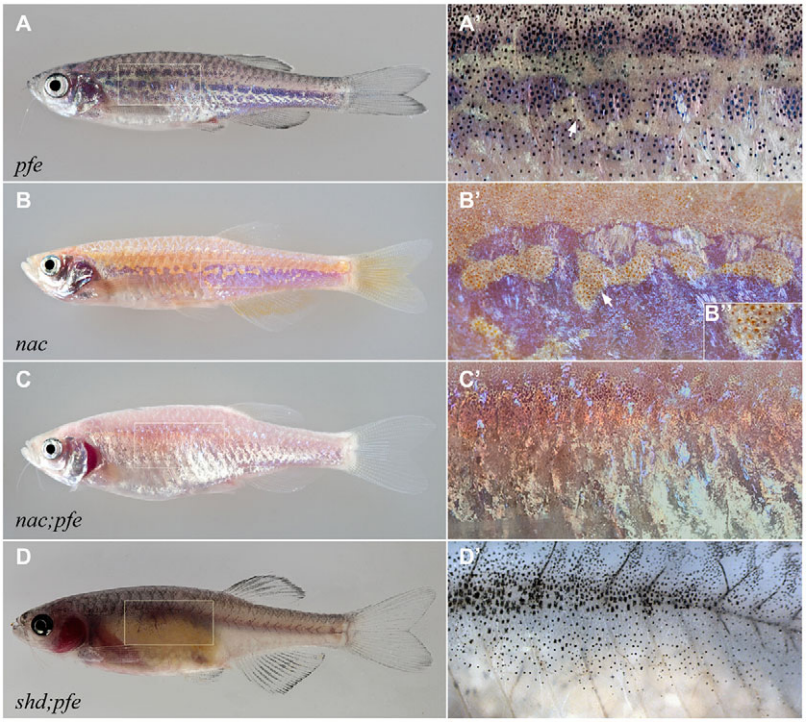

Fig. 3. Adult phenotypes of xanthophore, melanophore and double mutants. *pfe* (**A**,**A′**), *nac* (**B-B″**), *nac;pfe* (**C**,**C′**) and *shd;pfe* (**D**,**D′**) mutant fish. The boxed areas in A-D are enlarged in A′-D′. Arrow in A′ marks X1V in the *pfe* mutant. In the *nac* mutant, xanthophores strictly colocalise with dense S-iridophores (B′); the arrow points to a region of X1V that is enlarged in B″. S-iridophores spread over the entire flank in *nac;pfe* (C′). Melanophores associated with the blood vessels are visible along myosepta in *shd;pfe* (D,D′).

No melanophores

nac mutants lack melanophores completely. In addition, the number of xanthophores is variable. A prominent interstripe of xanthophores and dense S-iridophores with irregular borders forms in the region of X0, accompanied by spots ventrally (Fig. 3B,B′). Xanthophores strictly colocalise with the dense S-iridophores. X0 interstripe and X1V spots are separated from regions composed of a thin net of blue S-iridophores, as well as L-iridophores (Fig. 3B′). We regard these regions as rudimentary 1V stripes (lacking melanophores) and interpret this pattern as a form of residual stripe formation. In those *nac* individuals that display a reduced number of xanthophores, the dense iridophores of the first interstripe may expand dorsally and ventrally, like in *nac;pfe* mutants (see below) (Lister et al., 1999; Maderspacher and Nüsslein-Volhard, 2003).

No melanophores and xanthophores

In the double mutant *nac;pfe* (Fig. 3C,C′), a dense layer of S-iridophores covers the entire trunk region, which is replaced towards the anterior area by L-iridophores.

No iridophores and xanthophores

In *shd;pfe* double mutants, melanophores are dispersed over the flank and they also cover the region of the first interstripe. Their density gets lower with age, in particular towards the ventral aspect of the fish (Fig. 3D′).

No melanophores and iridophores

Xanthophores homogeneously fill most of the flanks in a dense layer in *shd;nac* fish (data not shown).

In conclusion, none of the three chromatophore types alone is capable of forming a pattern. In the presence of two of the three chromatophore types, irregular and incomplete stripes are formed. Lack of iridophores, and to a lesser extent of xanthophores, is correlated with a strong reduction of melanophore numbers (10-15% reduction in *rse* and *shd* mutants, 50% reduction in *pfe* mutants, compared with wild type; see Fig. 7).

shd and *rse* are required in iridophores but not in melanophores

As both iridophores and melanophores are affected in *rse* and *shd* mutants, we investigated in which cell types the gene products of *rse* and *shd* are required. We generated chimeric animals of various mutant combinations by transplantations of blastula donor cells into host embryos. Resulting adult fish were analysed for regions displaying a striped pattern. First, we used wild-type donor embryos, which carried the transgenic GFP marker *Tg(TDL358:gfp)* (Levesque et al., 2013) to label iridophores, for transplantation into *rse* mutant hosts. The resulting chimaeras displayed reconstituted stripe patterns in regions where GFP-positive iridophores developed (Fig. 4A,A′). This result shows that *rse* is required in iridophores.

In transplantations of *nac;pfe* cells into *rse* or *shd* mutant hosts, the resulting chimeric fish display large regions of restored stripe formation. Iridophores derived from the *nac;pfe* donor form interstripes of normal width, and the stripes contain normal numbers of melanophores that must be mutant for *rse* or *shd* as the *nac;pfe* donors are unable to produce melanophores and xanthophores (Fig. 4B,C). In the reciprocal experiment, we transplanted cells from *rse* or *shd* mutants into *nac;pfe* double mutants. These chimaeras develop regions containing donor-derived melanophores and xanthophores organised with host iridophores into stripes of wild-type pattern (Fig. 4D; data not shown). These results demonstrate that melanophores and xanthophores mutant for *rse* and *shd* restore stripe formation when confronted with iridophores provided by the *nac;pfe* host. Thus, *rse* and *shd* are not required in melanophores or xanthophores. When we transplanted cells reciprocally between *rse* and *shd* mutants, we did not observe rescue of stripe formation (0/124 with *rse* hosts, 0/87 with *shd* hosts), confirming that both genes are autonomously required in the same cell type, iridophores.

To conclude, the complex phenotypes, including the strong reduction in melanophore numbers in *shd* and *rse* mutants, are caused by the absence or reduction of iridophores.

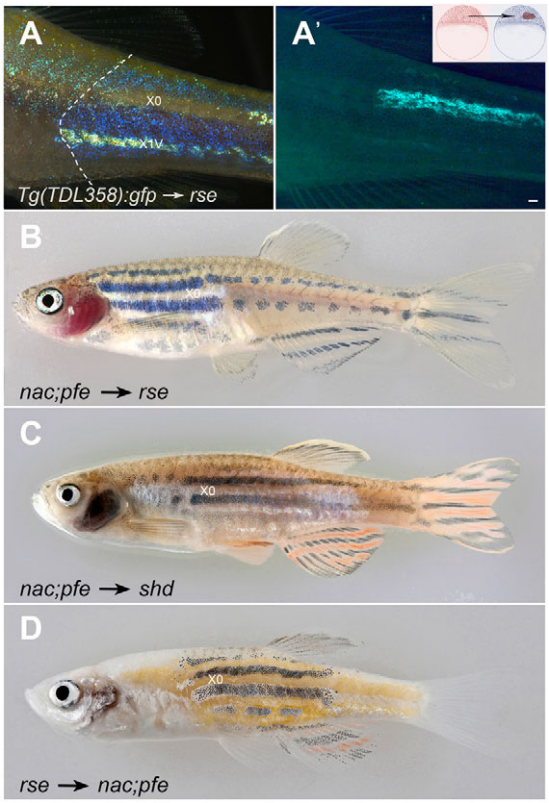

Fig. 4. Phenotypes of chimeric animals. Pictures show stripes of wild-type pattern in chimeric animals produced by blastula cell transplantation of the indicated genotypes (A′, inset). The position of X0 is indicated. (**A**,**A′**) *Tg(TDL358:gfp)* → *rse*. Because GFP expression reduces with age, this animal displays weak labelling in X1V. The left boundary of the clone is marked with a white dashed line. (A) incident light, dark background, (A′) UV light. (**B-D**) The frequency of successful transplantations is highly variable, ranging from 10% (2/25, *shd* → *nac;pfe;* 7/41; *rse* → *nac;pfe*) to ~50% (21/44 and 11/19 in transplantations of *nac;pfe* donors into *rse and shd* hosts, respectively). The size of the clones is also variable, but the quality does not depend on size, e.g. even in very small clones we observe the effect of iridophores on melanophore number.

Development of the adult pattern in iridophore mutants

To obtain a better understanding of the role of iridophores in adult pigment pattern formation, we analysed stripe formation through metamorphic stages in wild type and iridophore mutants. We monitored fish from the late larval stage CR [caudal fin ray, 4.9 mm standard length (SSL), 16 days post-fertilisation (dpf)] to young adults J++ (juvenile++, 16 mm SSL, 3 months post-fertilisation), through selected stages (see Materials and methods) (Parichy et al., 2009).

Iridophores

In wild-type larvae, the first metamorphic chromatophores becoming visible in the dermis of the lateral trunk are S-iridophores at stage CR (Fig. 5A,A′, arrows). S-iridophores are marked with the transgene *Tg(TDL358:gfp)* (Fig. 5A,A′, arrows; note that at this stage the iridophores on top of the swim bladder are much more prominent). Individual iridophores appear in the anterior trunk in a segmental fashion. During stage PR, iridophores spread outside X0 over the melanophores of 1D and 1V (Fig. 5C; Fig. 6B). Iridophores continue to increase in number and the first ventral interstripe is formed during stage SP by a dense accumulation of S-iridophores (Fig. 6B-D).

In *rse* mutants, the initial development of iridophores resembles wild type except that their number is strongly reduced (Fig. 5E,F). During stage PR, iridophores spread over the neighbouring melanophore regions in a similar manner to wild type (Fig. 6F) and the iridophore density of X0 is reduced suggesting a migration of cells from X0 into the periphery. This results in a rather homogeneous distribution of bluish iridophores over interstripes and stripes (Fig. 6F-H) and the loss of the dense ridge of iridophores in X0. *shd* mutants are completely devoid of reflective iridophores on their lateral flanks (Fig. 6I-L) and *Tg (TDL358:gfp)* labelling of dermal iridophores is absent (data not shown).

L-iridophores

During juvenile development at around stage J, L-iridophores begin to appear underneath the ventral melanophore stripes. They form first anteriorly behind the head and extend into more posterior and dorsal regions (data not shown). *shd* mutants lack this cell type.

Melanophores

In the posterior trunk of wild-type fish, melanophores begin to appear during stage PB. The first metamorphic melanophores arise dorsally and ventrally to X0 (Fig. 6A). During subsequent stages of metamorphic development, melanophores increase in number and localise closer to X0, i.e. in the region of the prospective 1V and 1D (Fig. 6B).

In *rse* and *shd* mutants, metamorphic melanophores become first visible during stage PB, comparable to wild type (Fig. 6E,I). During the following stages, however, fewer melanophores emerge and they remain more homogeneously dispersed over the entire flank, i.e. there is not much accumulation towards the prospective stripe regions (Fig. 6F,G,J,K). We describe the melanophores of 1V, ventral to X0, because in this region no epidermal scale melanophores complicate the observation of stripe formation in the dermis. Homogeneously distributed melanophores start to aggregate into patches that are now located in the region of the prospective 1V stripe (Fig. 6H,L). In *shd* mutants, fewer melanophores emerge and they remain more homogeneously dispersed over the entire flank, i.e. there is not much accumulation toward the prospective stripe regions (Fig. 6F,G; supplementary material Fig. S2). Melanophores can form patches anywhere in the space ventral to X0 (Fig. 6K,L). Melanophores located in rather ventral positions often associate with melanophores of the larval ventral stripe (Fig. 6L). These aggregates can give rise to vertical stripe-like arrangements surrounded by a layer of xanthophores.

Xanthophores

In wild type, metamorphic xanthophores become visible in interstripe X0 during stage PB, and the intensity of pigmentation and density of cells increases in subsequent stages. Appearance of xanthophores in *rse* and *shd* mutants is delayed in X0. In the ventral region of wild type, xanthophores follow the appearance of iridophores in interstripes. In *rse* and *shd* mutants, they fill the space between melanophore aggregates (data not shown).

In the mutants, there is a remarkable variation between individuals. One of five *rse* mutant fish had formed stripes already at stage PR, comparable to wild type. One of five *shd* mutant fish displayed a homogeneous distribution of melanophores ventrally to X0 until stage J++.

The number of melanophores per ventral hemisegment in wild type, *rse* and *shd* mutants was calculated by counting cells in the ventral stripes of the eight hemisegments above the anal fin. In *shd* and *rse* mutants during metamorphosis we observe ~30%, and in the adults only 10-20% of the normal melanophore number (Fig. 7).

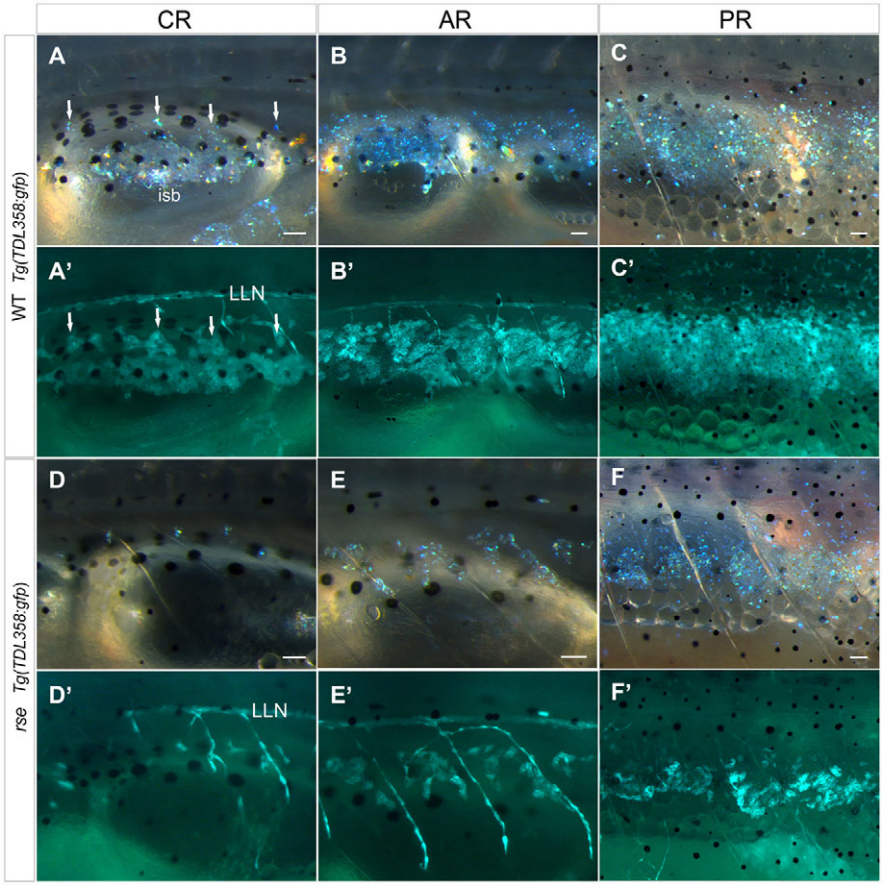

Fig. 5. Iridophore development in the anterior trunk. Control (*TDL358:gfp*) (**A-C′**) and *rse* (*TDL358:gfp*) (**D-F′**) fish. Expression of GFP is shown in A′-F′. In A, the first appearance of individual iridophores is marked by arrows. The majority of iridophores visible at this time point are interior ones on top of the swimbladder (isb). GFP expression is detectable slightly before iridophores become visibly pigmented (A,A′). GFP is also seen in the glia of the lateral line system. The lateral line nerve (LLN) marks the horizontal myoseptum, which at the anterior of the embryo is located dorsally to the first interstripe. Iridophores expand and form oval patches, which coalesce during stage AR (B,B′), though remnants of segmental boundaries do persist until stage PR (C,C′). Stages: CR, caudal fin ray; AR, anal fin ray; PR, pelvic fin ray. Scale bars: 50 μm.

Development of the adult pattern in xanthophore and melanophore mutants

We analysed the development of the pigment pattern of *pfe* and *nac* mutants, as well as double mutants missing two of the three chromatophore types.

No xanthophores

In *pfe* mutants, iridophores start to appear in the X0 region, similar to wild type (Fig. 8A-D). Occasionally, iridophores arise even slightly earlier than in wild type, during stage CR (data not shown), and the iridophore region is expanded. The accumulation of melanophores in 1D and 1V during the early metamorphic stages is less pronounced compared with wild type (supplementary material Fig. S2). During further development, S-iridophores ingress into the territory of 1D and 1V, thereby splitting the melanophore field into patches (Fig. 8B,C). From stage SP onwards, melanophore and iridophore patches mix in the ventral half of the flank (Fig. 8C).

No melanophores

In *nac* mutants, a prominent interstripe of xanthophores and iridophores with irregular borders forms in the region of X0 (Fig. 8E-H). During SP, the ventral region is covered by a thin blue iridophore sheet. In juvenile stages, dense iridophore patches covered with xanthophores mark the appearance of X1V.

No melanophores, no xanthophores

In *nac;pfe* double mutants, iridophores initially form a X0 interstripe between stages AR and PB (Fig. 8I), as in wild type. However, even more pronounced than in *nac* and *pfe* single mutants, S-iridophores increase in number and expand into dorsal and ventral regions in subsequent stages (Fig. 8J,K). Finally, they cover the entire lateral region (Fig. 8L).

No iridophores, no xanthophores

In the double mutant *shd;pfe*, melanophores appear rather evenly distributed in numbers that are slightly increased compared with *shd* or *pfe* single mutants (Fig. 8M-P). Whereas the melanophores in *shd* aggregate during the juvenile stages, a homogeneous distribution which decreases in density towards the ventral aspect of the fish persists in *shd;pfe* (Fig. 8P).

No iridophores, no melanophores

shd;nac double mutants lack melanophores as well as iridophores. Xanthophores homogeneously cover most of the flanks in a dense layer (data not shown).

A prepattern for self-organising stripe formation

The mutant *choker* (*cho*) was identified for its defects in pigmentation and somite formation. Mutant larvae lack the horizontal myoseptum and the stripe of larval melanophores associated with it (Kelsh et al., 1996; van Eeden et al., 1996; Svetic et al., 2007).

We found that *cho* mutant animals occasionally survive to adulthood. *cho* fish develop a peculiar pigment pattern. Stripes and interstripes of normal width are formed in a parallel arrangement, but they are heavily curved, sometimes branched and often interrupted, and they also may run in a vertical rather than horizontal orientation, unique in each mutant fish (Fig. 9A,B). During metamorphosis, the appearance of iridophores is considerably delayed. Anteriorly they are first visible during stage AR, rather

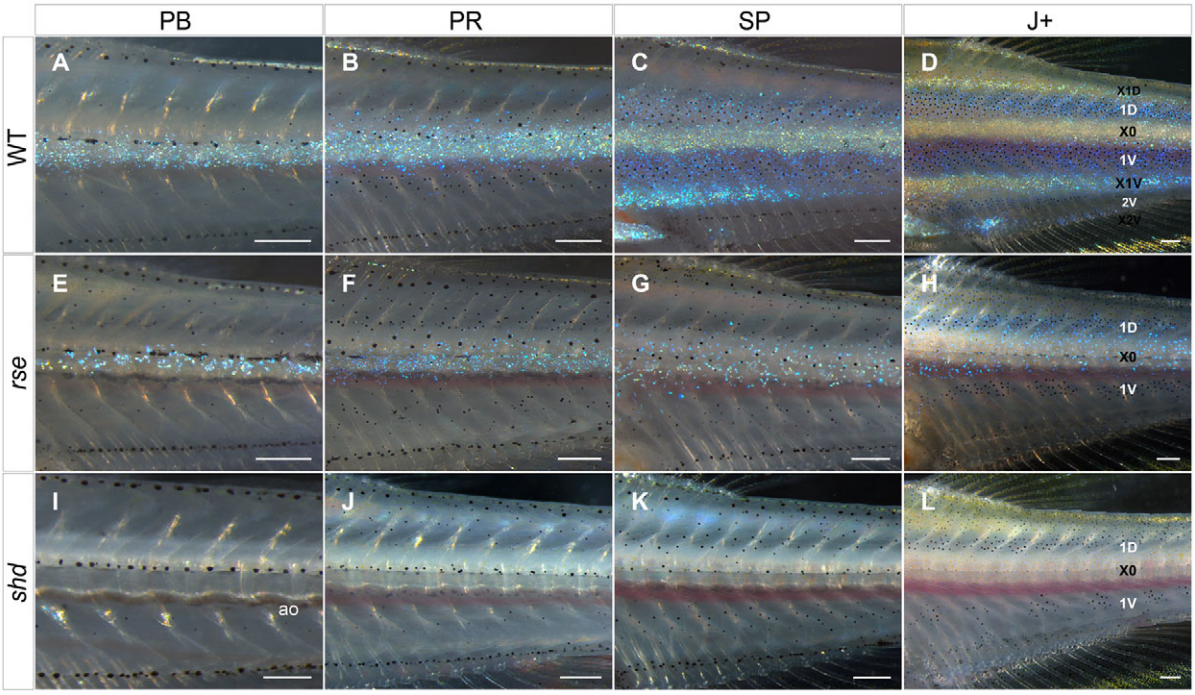

Fig. 6. Iridophore development in the posterior trunk. (**A-D**) Wild type. Iridophores appear first at the horizontal myoseptum and grow in density. In the melanophore region, they maintain their bluish reflection, whereas their colour turns golden in the dense interstripe regions. Melanophores of the larval lateral stripe that are still present along the horizontal myoseptum (A) are cleared from this area during stages PR and SP (B,C). (**E-H**) *rse* mutant. Iridophores grow in number but remain always below the level of wild type. (**I-L**) *shd* mutant. No iridophores develop. Metamorphic melanophores avoid the region of X0, but the clearance of larval melanophores from this area is considerably delayed compared with wild type. ao aorta. Stages: PB, pelvic fin bud. PR, pelvic fin ray. SP, squamation onset posterior. J+, juvenile+. Scale bars: 250 µm.

than in CR (data not shown). In the posterior trunk, the first iridophores become apparent during stage PR (Fig. 9D). Melanophores, however, are not delayed; they populate the medium and posterior trunk in a rather homogeneous fashion in stage PB (Fig. 9C). During stage PR, more melanophores are added, initially intermingled with newly arising iridophores (Fig. 9D). The iridophores accumulate in patches with arbitrary positions and orientations (Fig. 9E). In the centre of the new iridophore patches, xanthophores start to appear during stage PR, and melanophores aggregate into stripes and disappear from the iridophore patches. At stage J+, melanophore and iridophore areas are largely separated. The arbitrary nature of the orientation of stripes found in adults indicates that during normal development the appearance of iridophores at the horizontal myoseptum serves as a morphological landmark for stripe orientation, but subsequent stripe formation as well as stripe width are determined in an autonomous manner.

DISCUSSION

Cell-autonomous requirement of *shd* and *rse* in iridophores

In this article, we investigate zebrafish mutants defective in individual chromatophores with the aim of elucidating interactions between the pigment cell types during stripe pattern formation. Regarding melanophores and xanthophores, chimeric animals have shown that *nac* and *pfe* are required autonomously in the two cell types, respectively (Maderspacher and Nüsslein-Volhard, 2003; Parichy and Turner, 2003) (data not shown). We conclude that the mutants are primarily affected in a single cell type and that phenotypic consequences observed in the patterning of the remaining cell types indicate chromatophore interactions.

To determine the role of iridophores in pattern formation, we investigated the cell-type specificity of the mutants *rse* and *shd*. Cell transplantations show that *rse* and *shd* cells can give rise to normally behaving melanophores and xanthophores, which form regular stripes when confronted with wild-type iridophores. This indicates that *rse* and *shd* are cell-autonomously required only in iridophores and that the adult melanophore phenotype is caused by the lack of iridophores. The same result has been obtained for *tra* (Krauss et al., 2013). *shd* may be required for the specification of iridophores (Lopes et al., 2008), *rse* possibly for the expansion of the iridophore population (J.K., unpublished), and in *tra* mutants iridophores do not survive (Krauss et al., 2013). Despite different functions in iridophore development, these three genes have similar effects on melanophores. We speculate that a positive signal emanates from iridophores in the dermis sustaining melanophores during stripe pattern formation and maintenance.

Iridophores differentiate into two distinct forms. We consider L-iridophores that are located under the melanophore stripes to be dispensable for stripe formation, as they appear late in juvenile development after the basic striped pattern has been established. Further, they are absent in *shd* and *tra*, but present in *rse* mutants, with very similar consequences on melanophore numbers (Fig. 7). L-iridophores might, however, play a role in stripe maintenance. S-iridophores differentiate into a dense form in the interstripes, spreading into a thin, bluish layer covering the melanophores in the striped regions. These ‘blue iridophores’ are present in *rse* but absent in *shd* mutants. A subtle difference we observe between the *rse* and *shd* phenotypes is that in *shd* (and aged *tra*) adults the stripes almost always break up into spots, whereas in *rse* mutants more frequently a coherent thin stripe is maintained (Fig. 1). This may be attributed to

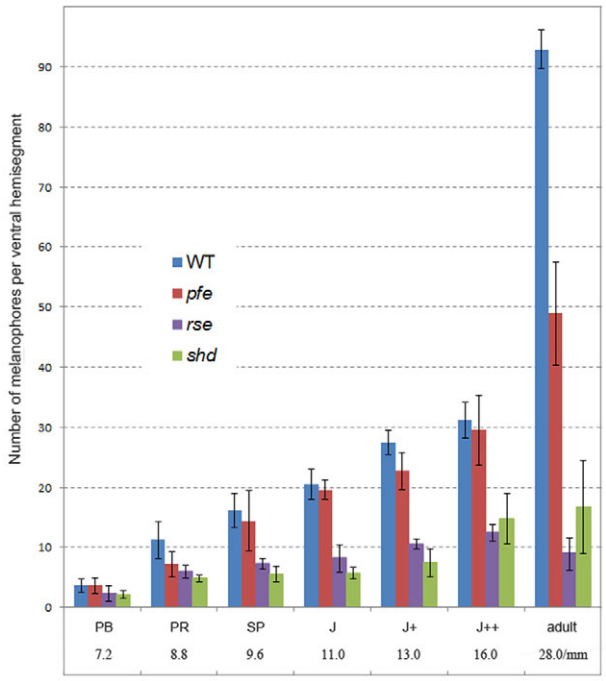

Fig. 7. Quantification of melanophores. Melanophores were counted in the ventral portion of eight segments above the anal fin and averaged per ventral hemisegment. Melanophores of the larval ventral melanophore stripe were not included. Error bars indicate the standard deviation in a random sample. The number of animals used for counting was at least five for each measurement point.

the blue S-iridophores, as they are absent in *tra* and *shd* mutant fish. Because in both *rse* and *shd* mutants the melanophore numbers are reduced to the same extent (Fig. 7), it seems that the dense S-iridophores of the interstripes exert a long-range effect on the aggregation and support of melanophores in the neighbouring stripes.

We conclude that stripe formation is predominantly based on interactions between S-iridophores, xanthophores and melanophores, which are eliminated in the mutants *shd*, *pfe* and *nac*, respectively.

Stripes do not form with only one chromatophore type

Because *shd*, *pfe* and *nac* act cell-autonomously in the respective cell types, the mutants allowed us to investigate the behaviour of any two chromatophore types in the absence of the third, and the potential of each cell type left on its own in double mutants.

In double mutants, each cell type is capable of filling the entire space; in other words, neither of them needs another cell type to expand. However none of them is able to form a pattern in the absence of the other two. In the double mutant *nac;pfe*, iridophores cover the flank of the fish in a dense silvery layer (Fig. 3C); likewise in *shd;nac* the xanthophores spread evenly in the dermis. This is remarkable because in wild type, xanthophores are always resting on top of iridophores. In the absence of both iridophores and xanthophores (*shd;pfe*), melanophores are also capable of distributing evenly, but they do so at a lower density than in wild type. This indicates that, in contrast to iridophores and xanthophores, melanophores have a tendency to avoid each other and depend on iridophores or xanthophores in their neighbourhood to aggregate into stripes or spots. In support of this idea, Takahashi and Kondo (Takahashi and Kondo, 2008) describe in regeneration experiments that melanophores in the absence of xanthophores do not aggregate but spread out into the available space, maximising their distance from each other.

These observations indicate that pigment pattern formation requires the interaction of at least two cell types. We observe residual stripe formation in each of the single mutants lacking only one of the three chromatophore types. The phenotypes of single and double mutants are schematically illustrated in Fig. 10.

Defective stripe formation with two pigment cell types

In *pfe* mutants, devoid of xanthophores, iridophores display a strong tendency to spread and invade melanophore regions. Melanophores fail to maintain a coherent stripe organisation, which is interrupted by invasions of iridophores. Whereas in wild type melanophores are separated from dense iridophores by a transitional zone, in *pfe* the melanophores appear immediately adjacent to dense S-iridophore regions. Single melanophores are even observed on top of the interstripe iridophores. However, a horizontal alignment of melanophore spots maintaining approximately normal width of stripes is preserved in *pfe* mutants and in many individuals three stripe-like melanophore arrangements are discernible.

In *nac* mutants, in the absence of melanophores, a prominent X0 interstripe region composed of dense S-iridophores and xanthophores occupies the lateral side of the fish. Ventrally, ‘stripe’ regions composed of blue S-iridophores but devoid of melanophores, and interstripe regions are added. The boundary between these regions is ragged, and the width of both ‘stripe’ and interstripe is narrow. We note that xanthophores and dense S-iridophores attract each other as they strictly colocalise; furthermore, the presence of xanthophores prevents the spreading of dense S-iridophores into ventral regions, which occurs in the double mutant *nac;pfe*.

The lack of iridophores causes a severe reduction of melanophore numbers, as observed in the *rse* and *shd* phenotypes (Fig. 7). Nevertheless, a pattern composed of dense melanophore spots or thin stripes is maintained in which xanthophores fill the space left by the melanophores. In comparison to *shd;pfe*, it appears that the presence of xanthophores is required for melanophores to aggregate into stripes or spots. Melanophores in *shd* mutants show a weaker tendency to aggregate into a stripe region close to X0, compared with *rse* mutants (Fig. 2B,C). The distance between dorsal and ventral melanophore spots or stripes is considerably enlarged. Thus, although xanthophores and melanophores are perfectly capable of segregating into different regions, the alignment into stripes requires blue S-iridophores present in *rse*, but absent in *shd* and *tra* mutants.

Model of the interactions between the three chromatophore types

Based on the analysis of single and double mutants, we deduce the following interactions between different pigment cell types (Fig. 11).

S-iridophores support the production and maintenance of high numbers of melanophores in their neighbourhood and cause their aggregation into stripe regions. As locally melanophores are strictly separated from dense S-iridophores, this strong positive interaction occurs over a distance of several cell diameters. This support is apparent in the melanophore reduction observed in *shd* and *rse* mutants, as well as in transplantation experiments in which melanophore stripes of normal width appear in these mutants when supplied with wild-type iridophores (Fig. 4B,C). Further, the spontaneous iridophore patches that are sometimes present in *shd* mutants are always surrounded by aggregations of melanophores (Fig. 1E). One might ask whether the effect of iridophores on melanophores is exerted via xanthophores. But the accumulation of

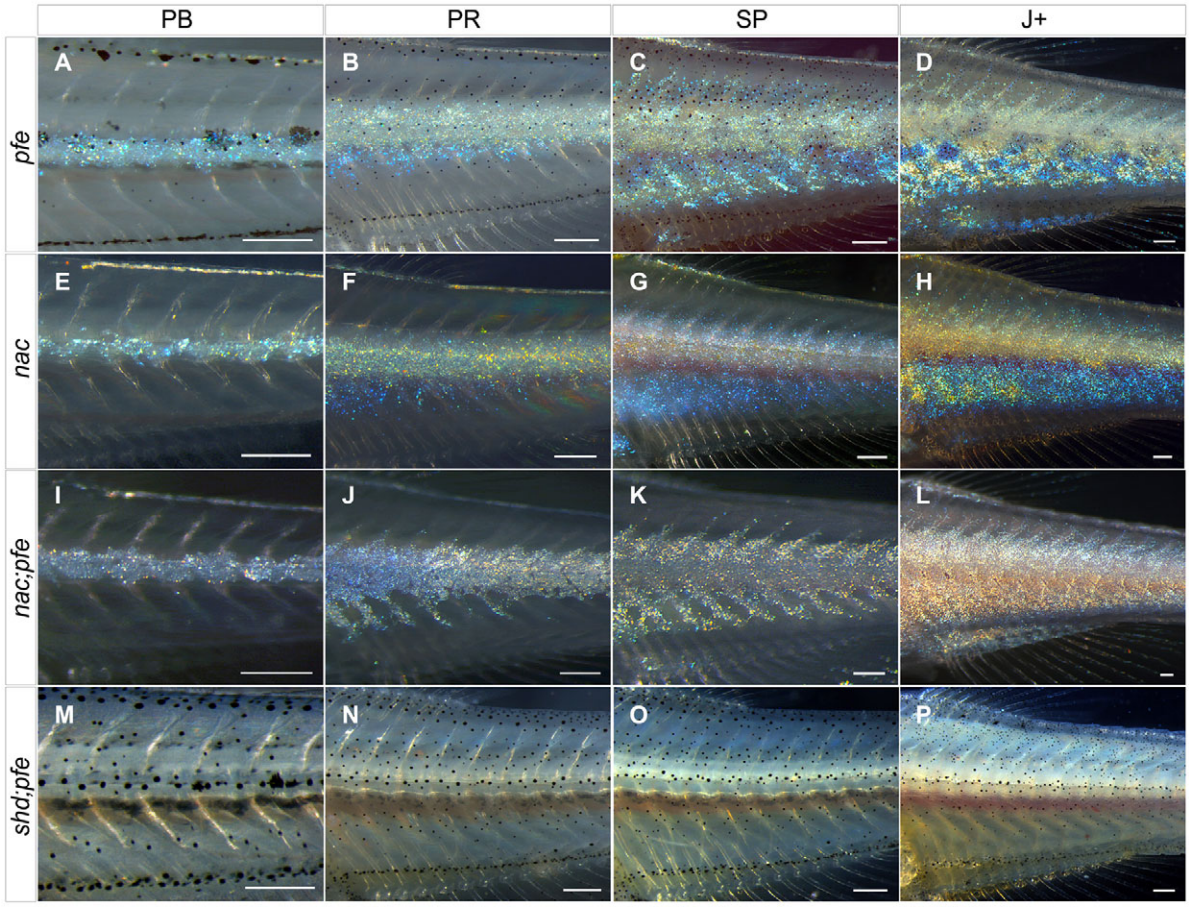

Fig. 8. Iridophore development in the posterior trunk of xanthophore, melanophore and double mutants. *pfe* (**A-D**), *nac* (**E-H**), *nac;pfe* (**I-L**) and *shd;pfe* (**M-P**) mutants. In *pfe*, as well as in *nac* and *nac;pfe* mutants, X0 is more prominent than in wild type; it expands to cover the entire flank in *nac;pfe* mutants. In *shd;pfe* mutants, melanophores distribute evenly also in the territory normally occupied by X0. Stages: PB, pelvic fin bud; PR, pelvic fin ray. SP, squamation onset posterior; J+, juvenile+. Scale bars: 250 µm.

melanophores in 1D and 1V in *pfe* mutants (Fig. 3A; Fig. 8C) indicates that iridophores alone can cause the aggregation of melanophores in their immediate environment. The mutual exclusion of stripe and interstripe suggests a short-range repulsion between iridophores and melanophores.

Dense S-iridophores and xanthophores exhibit mutual attraction. This is seen in wild type, with xanthophores being embedded in the sheet of S-iridophores from the beginning of stripe formation. The mutual attraction is most prominently apparent in the *nac* phenotype, in which xanthophores strictly colocalise with dense S-iridophores. We also observe that in *cho* mutants xanthophores follow the distribution of iridophores. Xanthophores, however, do not require iridophores for spreading, as seen in the free distribution in *shd* mutants, filling the space between melanophores. Xanthophores might exert a positive signal sustaining melanophores at a distance, as melanophore number is reduced in adult and to a

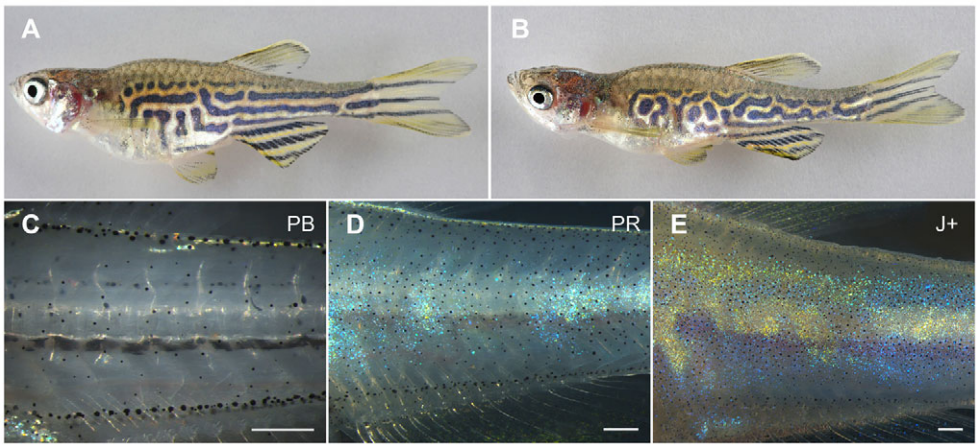

Fig. 9. The *choker* phenotype. (**A**,**B**) Adult individuals, displaying parallel stripes with arbitrary orientation. *choker* adults can reach normal sizes and are fertile. (**C-E**) Metamorphic development. In the absence of the horizontal myoseptum, melanophores appear before iridophores and xanthophores, evenly dispersed over the flank (C). Iridophores and xanthophores emerge in patches interspersed with melanophores (D), but then aggregate into stripe-like arrangements, and separate from melanophore regions (E). The aorta is visible as dark horizontal shade in C. Scale bars: 250 µm.

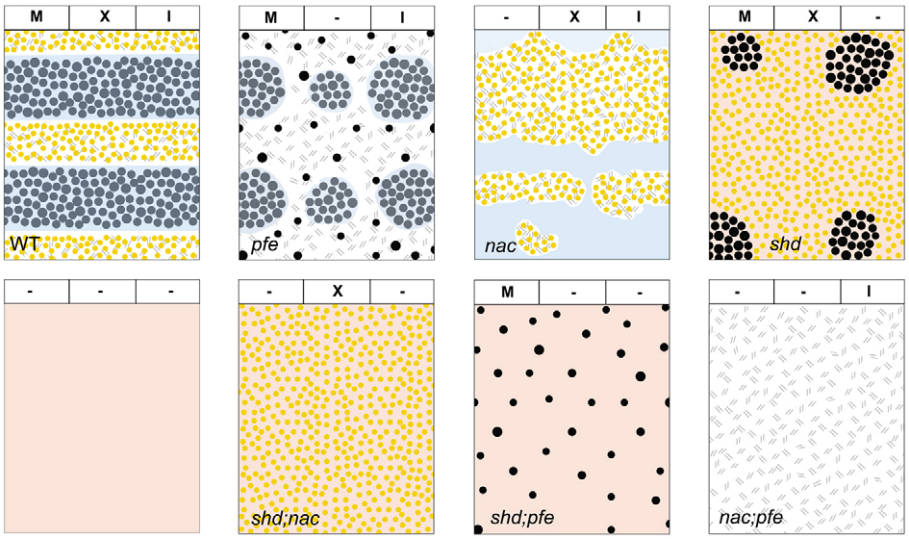

Fig. 10. Schematics of the central striped region in wild-type and mutant zebrafish. The iridophore cell bodies are not discernible in live fish. Dense S-iridophores are symbolised with grey dashes on a white background, whereas the thin blue iridophore sheet covering the melanophores in wild type and *pfe* mutants are indicated in light blue. Fish skin background devoid of pigment cells is indicated in pink. In contrast to the wild type, the mutant phenotypes are highly variable; the drawings illustrate the characteristic features. M, melanophores (black circles); I, iridophores; X, xanthophores (yellow circles).

lesser extent in juvenile *pfe* mutants. A positive long-range and a negative short-range effect of xanthophores on melanophores has also been postulated from regeneration experiments (Nakamasu et al., 2009). Xanthophores prevent iridophores from spreading, as deduced from the *pfe* mutants in which S-iridophores invade the melanophore territory, as well as in the double mutant *nac;pfe*.

Xanthophores and melanophores mutually repel each other. In *pfe* mutants, melanophores can be seen ectopically in the interstripe region, which is never observed in wild-type adults. In the absence of iridophores in *shd* or *rse* mutants, the interstripes, filled with xanthophores, may be considerably enlarged with a sharp boundary between xanthophore and melanophore regions. Iridophores may only have a minor local repulsive interaction with melanophores, by contrast; although the dense iridophore sheet is excluding melanophores, the vicinity appears to be required for dense melanophore stripe formation (see above). We therefore propose that xanthophores and iridophores support aggregation of melanophores by reducing their tendency to avoid each other.

Formation of horizontal stripes requires a prepattern

The first interstripe, X0, is formed by iridophores emerging at the horizontal myoseptum followed by xanthophores. This interstripe appears in both *pfe* and *shd* mutants, suggesting that either iridophores or xanthophores alone can form the first interstripe. We propose that this origin provides a prepattern, as has been suggested previously (Maderspacher and Nüsslein-Volhard, 2003).

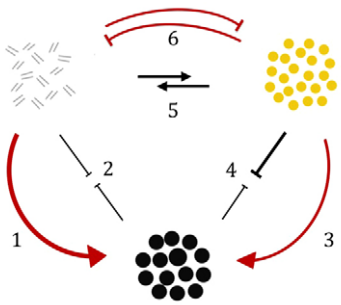

Fig. 11. Scheme of interactions between chromatophore types. Red curved arrows, long-range interactions; black arrows, short-range interactions. For further details, see text.

Melanophores appear in the dermis dispersed dorsally and ventrally to the horizontal myoseptum. While melanophores are increasing in number, they aggregate close to X0 into 1V and 1D. The dense S-iridophores of the interstripe expand dorsally and ventrally to produce a field of blue iridophores that spreads thinly over the melanophore stripes. A parallel copy of an interstripe is formed from a new accumulation of dense iridophores within the blue region (Fig. 6C; Fig. 8C,D,H). In wild type, blue iridophores are associated with melanophores and dense iridophores with xanthophores.

Consistent with the notion that the horizontal myoseptum acts as a morphological prepattern, we observe that in *cho* mutants, which lack the horizontal myoseptum, chromatophore-dependent stripe-forming processes act at arbitrary orientations. In *cho* mutants, melanophores appear during metamorphosis evenly distributed in the dermis at normal time points, whereas iridophores are delayed. This is consistent with the finding that melanophores do not emerge to the skin through the horizontal myoseptum, but migrate along spinal nerves through the myotomes and appear in the dermis at locations dorsal or ventral to the horizontal myoseptum (Dooley et al., 2013). The arbitrary position of the later-appearing iridophores suggest that they, in the absence of a horizontal myoseptum, find their way to the dermis by following paths taken by melanophores.

Conclusions

So far only melanophores and xanthophores, but not iridophores, have been proposed to play an essential role in stripe formation (Asai et al., 1999; Yamaguchi et al., 2007; Nakamasu et al., 2009). The phenotype of mutants lacking iridophores and the residual stripe/interstripe formation in *nac* and *pfe* mutants shows, however, that iridophores play an essential role in stripe formation; thus, interactions between all three chromatophore types contribute in overlapping ways (Fig. 11). Both iridophores and xanthophores exert short-range and long-range interactions with melanophores. S-iridophores attract melanophores in high numbers, induce their aggregation into the prospective stripe area (positive long-range effect) (1) and are to some extent able to exclude them from the interstripe (negative short-range effect) (2). Xanthophores have a minor effect on melanophore aggregation (positive long-range effect) (3), but keep melanophores out from interstripe regions resolutely (negative short-range effect) (4). Interactions between iridophores and xanthophores are required to confine the shape of interstripes (5,6). Although in *cho* mutants a primary signal to orient

the stripes is missing, the *cho* phenotype produces patterns of parallel stripes and interstripes, indicating that stripe width is autonomously controlled by cell-cell interactions. Melanophores, in conjunction with blue iridophores, may contribute to control the width and continuity of stripes.

Acknowledgements
We thank Brigitte Walderich for help with the transplantation experiments; Iris Koch for help with Fig. 10; and Ajeet Singh, Uwe Irion, Andrey Fadeev, Alessandro Mongera, Christian Söllner and Patrick Müller for discussions and critical reading of the manuscript.

Funding
This work was supported by the Max-Planck-Gesellschaft, FRG. Deposited in PMC for immediate release.

Competing interests statement
The authors declare no competing financial interests.

Author contributions
H.G.F. discovered the role of iridophores in stripe formation, isolated the new *rse* alleles, provided the figures of the mutants during metamorphosis and performed the melanophore counts. J.K. and H.G.F. provided the figures of adult mutants. J.K. isolated the TDL 358 transgenic line and discovered the *choker* adult phenotype. H.-M.M. performed the transplantations under the supervision of H.G.F. C.N.-V. coordinated the investigations, finalised the model (Figs 10 and 11) and wrote the manuscript together with H.G.F.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.096719/-/DC1

References
Asai, R., Taguchi, E., Kume, Y., Saito, M. and Kondo, S. (1999). Zebrafish leopard gene as a component of the putative reaction-diffusion system. *Mech. Dev.* **89**, 87-92.
Brand, M., Granato, M. and Nüsslein-Volhard, C. (2002). Keeping and raising zebrafish. In *Zebrafish: A Practical Approach* (ed. C. Nüsslein-Volhard and R. Dahm), pp. 7-37. New York, NY: Oxford University Press.
Budi, E. H., Patterson, L. B. and Parichy, D. M. (2008). Embryonic requirements for ErbB signaling in neural crest development and adult pigment pattern formation. *Development* **135**, 2603-2614.
Budi, E. H., Patterson, L. B. and Parichy, D. M. (2011). Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. *PLoS Genet.* **7**, e1002044.
Dooley, C. M., Mongera, A., Walderich, B. and Nüsslein-Volhard, C. (2013). On the embryonic origin of adult melanophores: the role of ErbB and Kit signalling in establishing melanophore stem cells in zebrafish. *Development* **140**, 1003-1013.
Eisen, J. S. and Weston, J. A. (1993). Development of the neural crest in the zebrafish. *Dev. Biol.* **159**, 50-59.
Gierer, A. and Meinhardt, H. (1972). A theory of biological pattern formation. *Kybernetik* **12**, 30-39.
Haffter, P., Odenthal, J., Mullins, M. C., Lin, S., Farrell, M. J., Vogelsang, E., Haas, F., Brand, M., van Eeden, F. J. M., Furutani-Seiki, M. et al. (1996). Mutations affecting pigmentation and shape of the adult zebrafish. *Dev. Genes Evol.* **206**, 260-276.
Hirata, M., Nakamura, K., Kanemaru, T., Shibata, Y. and Kondo, S. (2003). Pigment cell organization in the hypodermis of zebrafish. *Dev. Dyn.* **227**, 497-503.
Hultman, K. A. and Johnson, S. L. (2010). Differential contribution of direct-developing and stem cell-derived melanocytes to the zebrafish larval pigment pattern. *Dev. Biol.* **337**, 425-431.
Hultman, K. A., Budi, E. H., Teasley, D. C., Gottlieb, A. Y., Parichy, D. M. and Johnson, S. L. (2009). Defects in ErbB-dependent establishment of adult melanocyte stem cells reveal independent origins for embryonic and regeneration melanocytes. *PLoS Genet.* **5**, e1000544.
Johnson, S. L., Africa, D., Walker, C. and Weston, J. A. (1995). Genetic control of adult pigment stripe development in zebrafish. *Dev. Biol.* **167**, 27-33.
Kane, D. A. and Kishimoto, T. (2002). Cell labeling and transplantation techniques. In *Zebrafish: A Practical Approach* (ed. C. Nüsslein-Volhard and R. Dahm), pp. 95-119. New York, NY: Oxford University Press.
Kelsh, R. N. (2004). Genetics and evolution of pigment patterns in fish. *Pigment Cell Res.* **17**, 326-336.
Kelsh, R. N., Brand, M., Jiang, Y. J., Heisenberg, C. P., Lin, S., Haffter, P., Odenthal, J., Mullins, M. C., van Eeden, F. J., Furutani-Seiki, M. et al. (1996). Zebrafish pigmentation mutations and the processes of neural crest development. *Development* **123**, 369-389.
Kirschbaum, F. (1975). Untersuchungen über das Farbmuster der Zebrabarbe Brachydanio rerio (Cyprinidae, Teleostei). *Roux's Arch. Dev. Biol.* **177**, 129-152.
Krauss, J., Astrinides, P., Frohnhöfer, H. G., Walderich, B. and Nüsslein-Volhard, C. (2013). *transparent*, a gene affecting stripe formation in zebrafish, encodes the mitochondrial protein Mpv17 that is required for iridophore survival. *Biol. Open* (in press).
Lang, M. R., Patterson, L. B., Gordon, T. N., Johnson, S. L. and Parichy, D. M. (2009). Basonuclin-2 requirements for zebrafish adult pigment pattern development and female fertility. *PLoS Genet.* **5**, e1000744.
Levesque, M. P., Krauss, J., Koehler, C., Boden, C. and Harris, M. P. (2013). New tools for the identification of developmentally regulated enhancer regions in embryonic and adult zebrafish. *Zebrafish* **10**, 21-29.
Lister, J. A., Robertson, C. P., Lepage, T., Johnson, S. L. and Raible, D. W. (1999). nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development* **126**, 3757-3767.
Lopes, S. S., Yang, X., Müller, J., Carney, T. J., McAdow, A. R., Rauch, G. J., Jacoby, A. S., Hurst, L. D., Delfino-Machín, M., Haffter, P. et al. (2008). Leukocyte tyrosine kinase functions in pigment cell development. *PLoS Genet.* **4**, e1000026.
Maderspacher, F. and Nüsslein-Volhard, C. (2003). Formation of the adult pigment pattern in zebrafish requires leopard and obelix dependent cell interactions. *Development* **130**, 3447-3457.
Meinhardt, H. and Gierer, A. (1980). Generation and regeneration of sequence of structures during morphogenesis. *J. Theor. Biol.* **85**, 429-450.
Nakamasu, A., Takahashi, G., Kanbe, A. and Kondo, S. (2009). Interactions between zebrafish pigment cells responsible for the generation of Turing patterns. *Proc. Natl. Acad. Sci. USA* **106**, 8429-8434.
Odenthal, J., Rossnagel, K., Haffter, P., Kelsh, R. N., Vogelsang, E., Brand, M., van Eeden, F. J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M. et al. (1996). Mutations affecting xanthophore pigmentation in the zebrafish, Danio rerio. *Development* **123**, 391-398.
Parichy, D. M. and Turner, J. M. (2003). Temporal and cellular requirements for Fms signaling during zebrafish adult pigment pattern development. *Development* **130**, 817-833.
Parichy, D. M., Rawls, J. F., Pratt, S. J., Whitfield, T. T. and Johnson, S. L. (1999). Zebrafish sparse corresponds to an orthologue of c-kit and is required for the morphogenesis of a subpopulation of melanocytes, but is not essential for hematopoiesis or primordial germ cell development. *Development* **126**, 3425-3436.
Parichy, D. M., Ransom, D. G., Paw, B., Zon, L. I. and Johnson, S. L. (2000a). An orthologue of the kit-related gene fms is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish, Danio rerio. *Development* **127**, 3031-3044.
Parichy, D. M., Mellgren, E. M., Rawls, J. F., Lopes, S. S., Kelsh, R. N. and Johnson, S. L. (2000b). Mutational analysis of endothelin receptor b1 (rose) during neural crest and pigment pattern development in the zebrafish Danio rerio. *Dev. Biol.* **227**, 294-306.
Parichy, D. M., Elizondo, M. R., Mills, M. G., Gordon, T. N. and Engeszer, R. E. (2009). Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Dev. Dyn.* **238**, 2975-3015.
Raible, D. W. and Eisen, J. S. (1994). Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. *Development* **120**, 495-503.
Svetic, V., Hollway, G. E., Elworthy, S., Chipperfield, T. R., Davison, C., Adams, R. J., Eisen, J. S., Ingham, P. W., Currie, P. D. and Kelsh, R. N. (2007). Sdf1a patterns zebrafish melanophores and links the somite and melanophore pattern defects in choker mutants. *Development* **134**, 1011-1022.
Takahashi, G. and Kondo, S. (2008). Melanophores in the stripes of adult zebrafish do not have the nature to gather, but disperse when they have the space to move. *Pigment Cell Melanoma Res.* **21**, 677-686.
Tryon, R. C., Higdon, C. W. and Johnson, S. L. (2011). Lineage relationship of direct-developing melanocytes and melanocyte stem cells in the zebrafish. *PLoS ONE* **6**, e21010.
Tu, S. and Johnson, S. L. (2011). Fate restriction in the growing and regenerating zebrafish fin. *Dev. Cell* **20**, 725-732.
Turing, A. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. B* **237**, 37-72.
van Eeden, F. J., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C. P., Jiang, Y. J., Kane, D. A. et al. (1996). Mutations affecting somite formation and patterning in the zebrafish, Danio rerio. *Development* **123**, 153-164.
Walker, C. and Streisinger, G. (1983). Induction of Mutations by gamma-Rays in Pregonial Germ Cells of Zebrafish Embryos. *Genetics* **103**, 125-136.
White, R. M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., Bourque, C., Dovey, M., Goessling, W., Burns, C. E. et al. (2008). Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell Stem Cell* **2**, 183-189.
Yamaguchi, M., Yoshimoto, E. and Kondo, S. (2007). Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proc. Natl. Acad. Sci. USA* **104**, 4790-4793.