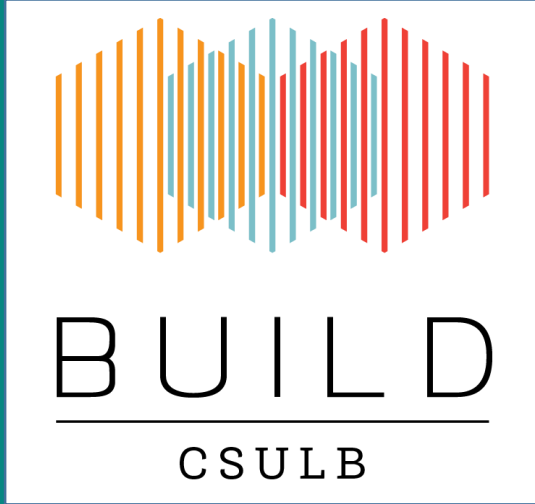


Interactions between the *18-Wheeler* gene and X-chromosome linked genes affect in salivary gland development in *Drosophila melanogaster*

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INTRODUCTION

- Drosophila salivary glands are ideal to study because their development is analogous to organs in mammals (Andrew and Ewald, 2010).
- The Toll-like receptor 18-wheeler was implicated in salivary gland morphogenesis (Kolesnikov and Beckendorf, 2007) and it was shown to affect embryonic salivary gland invagination.
- To detect which genes on the X-chromosome interact with 18-wheeler there are two different stocks that we cross with the deletion stocks. Both contain a *forkhead*-Green Fluorescent Protein (*fkh-GFP*) reporter that express GFP in the developing salivary glands.
- One stock is called 15-1 that has the normal functioning 18-wheeler gene, meaning normal glands.
- Another stocked called 84-1 that contains the 18-wheeler gene mutation, meaning abnormal glands.

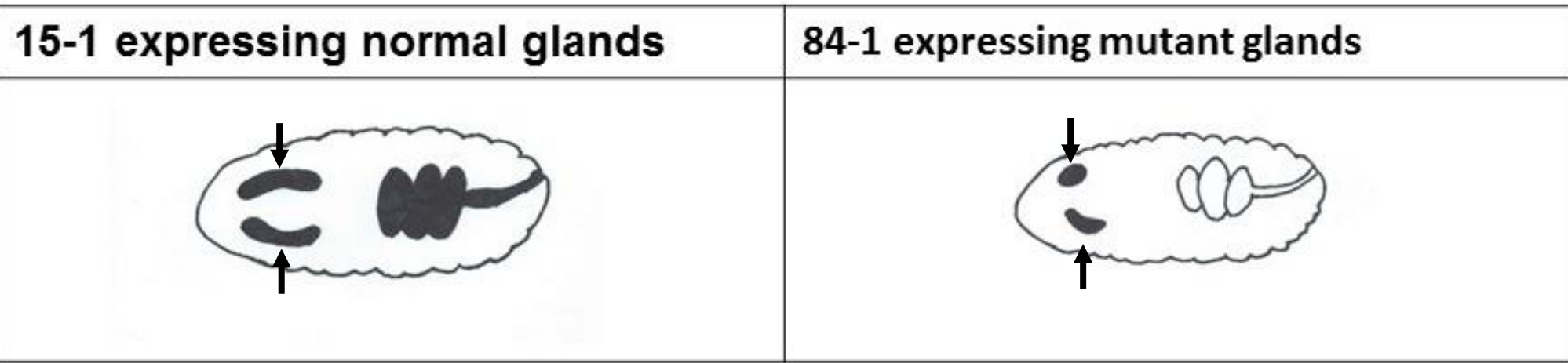


Figure 1. Picture of fruit fly embryo with the black arrows pointing to the salivary gland. (A) Embryo A expresses normal salivary glands because it has two functional copies of 18-wheeler. Embryo B expresses abnormal gland as it has a mutant copy of 18-wheeler.

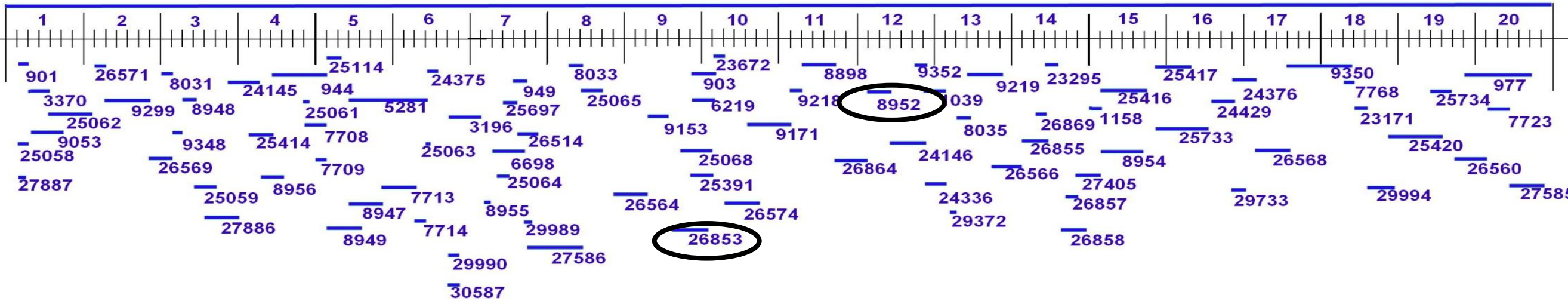


Figure 2. Visual display of X-chromosome deletions. Each bar represents a region of the X-chromosome that has been removed. The number indicates the fly stock associated with that deletion. The numbers circled in black are the deficiency fly stocks discussed in the results.

HYPOTHESIS

If there is gene interaction between the 18-wheeler mutation and the deficiency (df) then cross-breeding the 84-1 stock, which expresses the 18-wheeler mutation, with the deletion stocks will result in progeny with abnormal salivary glands. Cross-breeding the 15-1 stock with deletion stock will not result in normal salivary glands.

METHODS

Step 1: Rebalance deficiency stocks with GFP Tag

- Collect Virgin df flies and cross them to 6873 males to tag df stocks with GFP
- Once the expected progeny emerge, we collect the adult virgins flies that express kidney bean shaped eyes

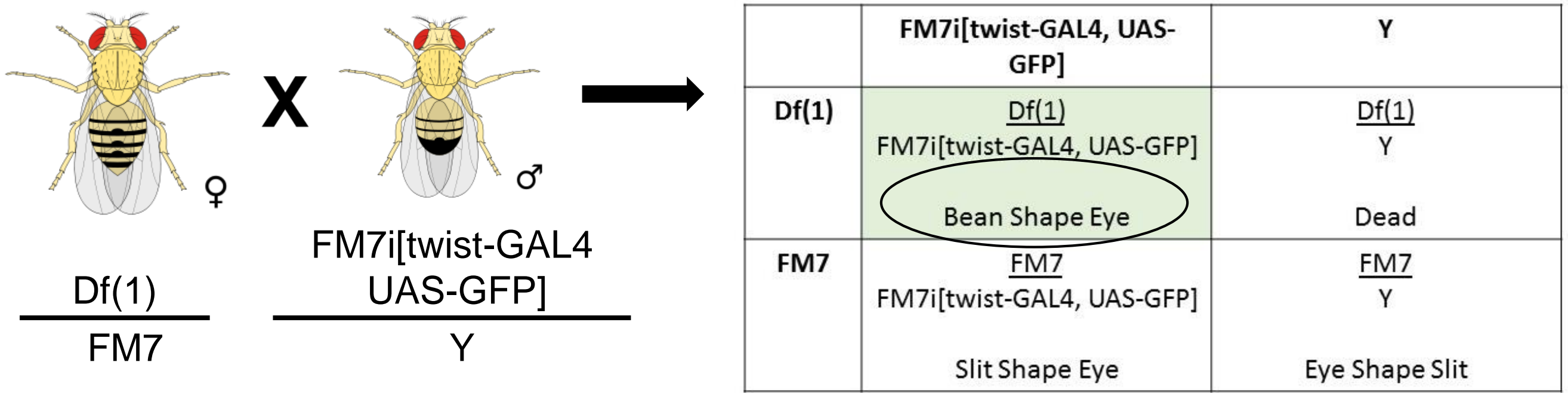


Figure 3 . The fly to the left is female carrying the deficiency (df) on her X-chromosome and fly to the right is male with GFP. Once the flies are mated, the adult progeny with GFP and the df will have kidney bean eyes. A Punnett Square of expected progeny to the right. The green box is highlighted because it's the progeny collected for step 2. The other flies are discarded.

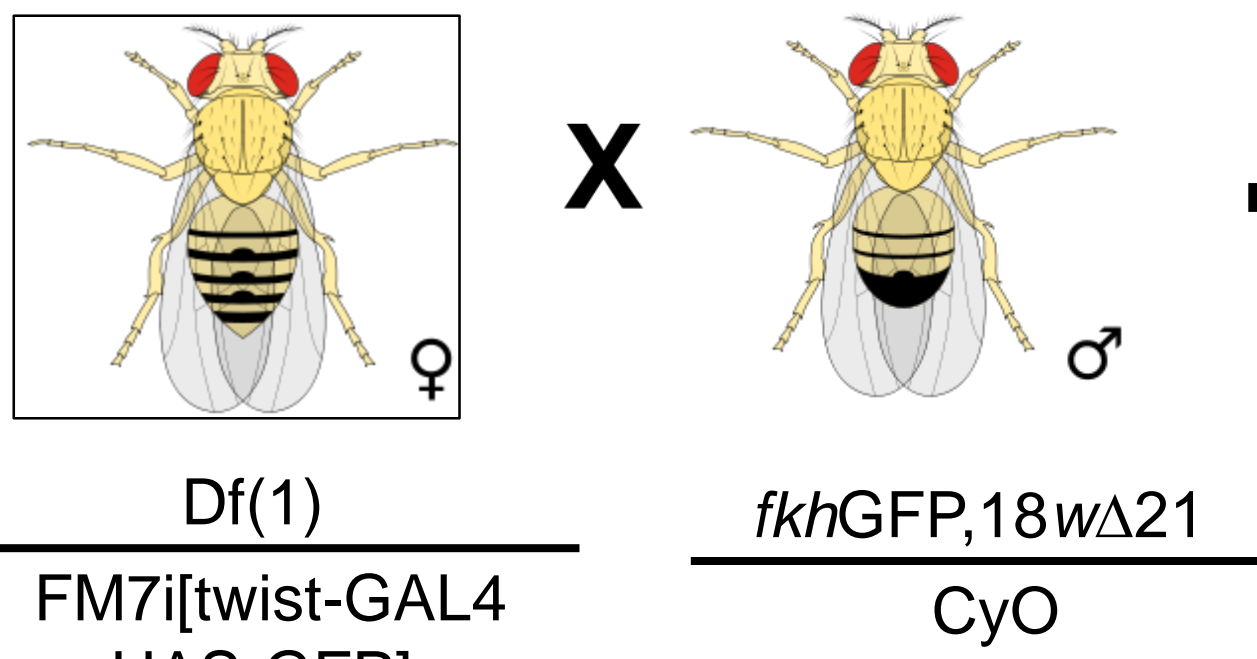
Table 1. Abbreviations of genes.

Df(1)	Name of deficiency	FM7i[twist-GAL4, UAS-GFP]	Marked balancer chromosome to show who carries the balancer
Y	Male chromosome	FM7i	Balancer chromosome, order of genes rearranged
fkh	Forkhead, transcription factor expressed in salivary glands	18w	Toll-like receptor, gene of interest

METHODS

Step 2: Mate rebalanced deficiency (df fly stock) virgin female with 18-wheeler mutant male (84-1 fly stock)

- Mate rebalanced deficiency (df fly stock) virgin female with 18-wheeler wildtype male (15-1 fly stock)
- Age, collect, and fix fly embryos from a grape juice plate.



Step 3: Immunostaining of embryos against GFP expressed in salivary glands and gut

- Approximately 25% of embryos stained have the correct genotype and will stain the way predicted
- Embryos are stored in methyl salicylate and mounted in GMM
- Light microscopy is used to record and analyze results

	X; <i>fkhGFP</i> , 18w	Y; <i>fkhGFP</i> , 18w	X;CyO	Y;CyO
DF(1)				
FM7i[twist-GAL4, UAS-GFP]				

Table 3. Punnett Square of expected progeny of cross and results of the stain. The green highlighted embryos are the ones carrying 18-wheeler and the df. The embryos in the bottom row stain black, embryos in the top right corner do not stain.

RESULTS

Figure 4. 26853 x 15-1. The black boxed embryos represents mutations. Unboxed embryos represent the wildtype embryos. Embryos are presented from youngest (stage 11) to oldest (stage 17).

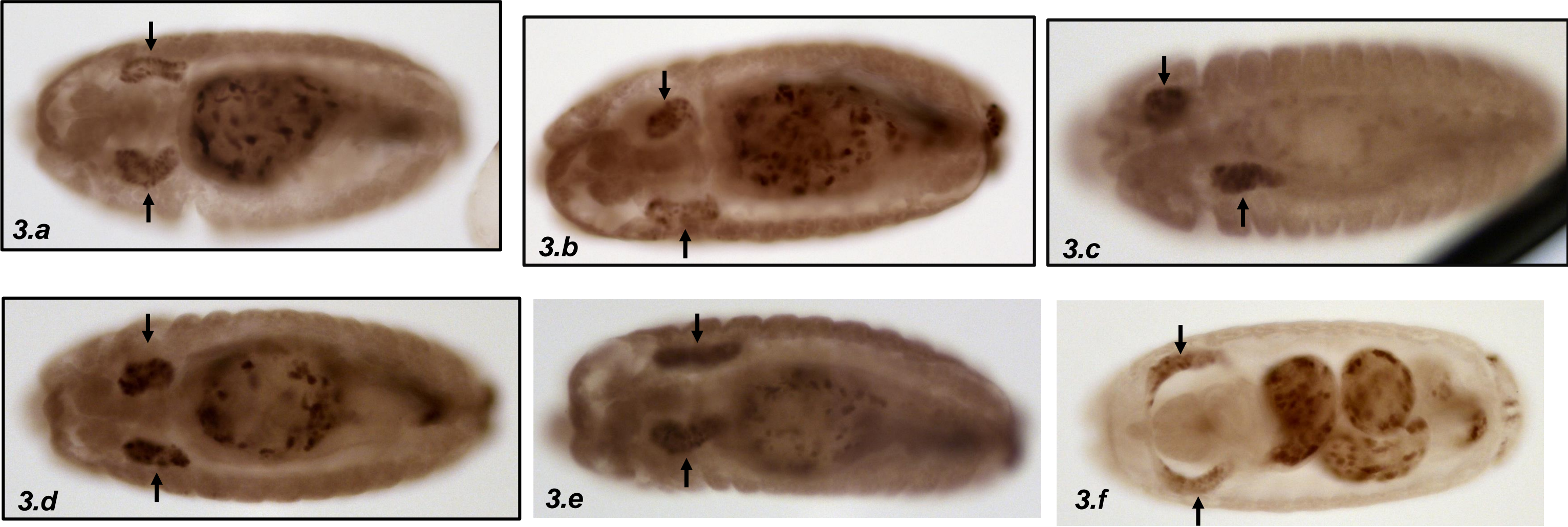
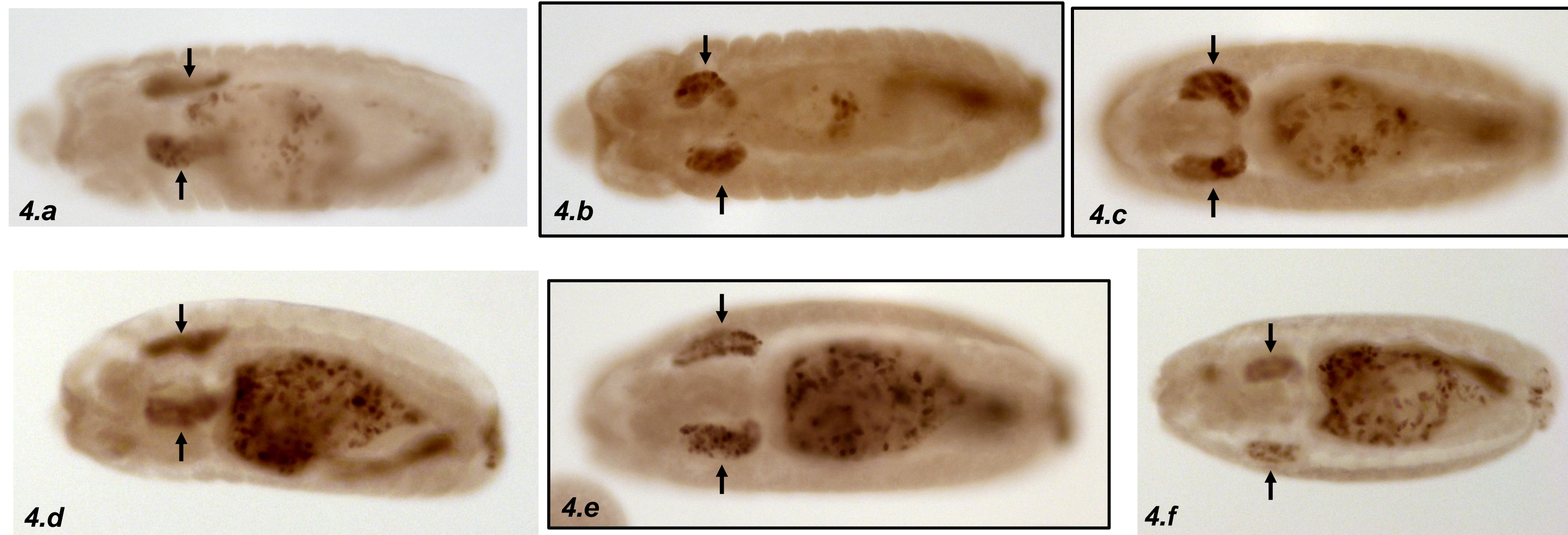


Figure 5. 26853 x 84-1 The black boxed embryos represents mutations. Unboxed embryos represent wildtype embryos. Embryos are presented from youngest (stage 11) to oldest (stage 12).



RESULTS

Figure 6. 8898 x 15-1. Boxed embryos represent the mutations expressed. Unboxed embryos present wildtype embryos. The youngest embryo is stage 11 and the oldest is stage 17 and over.

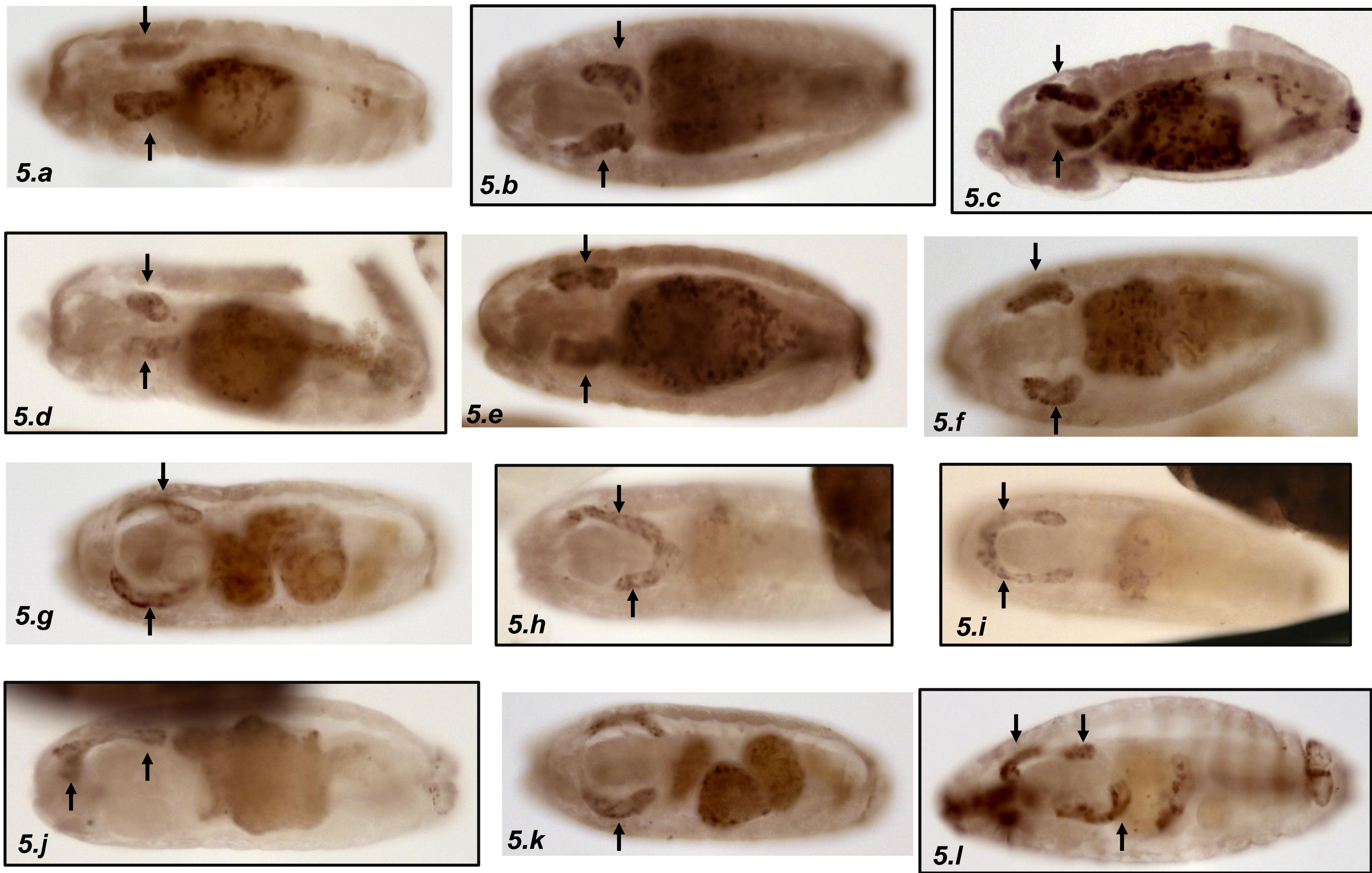
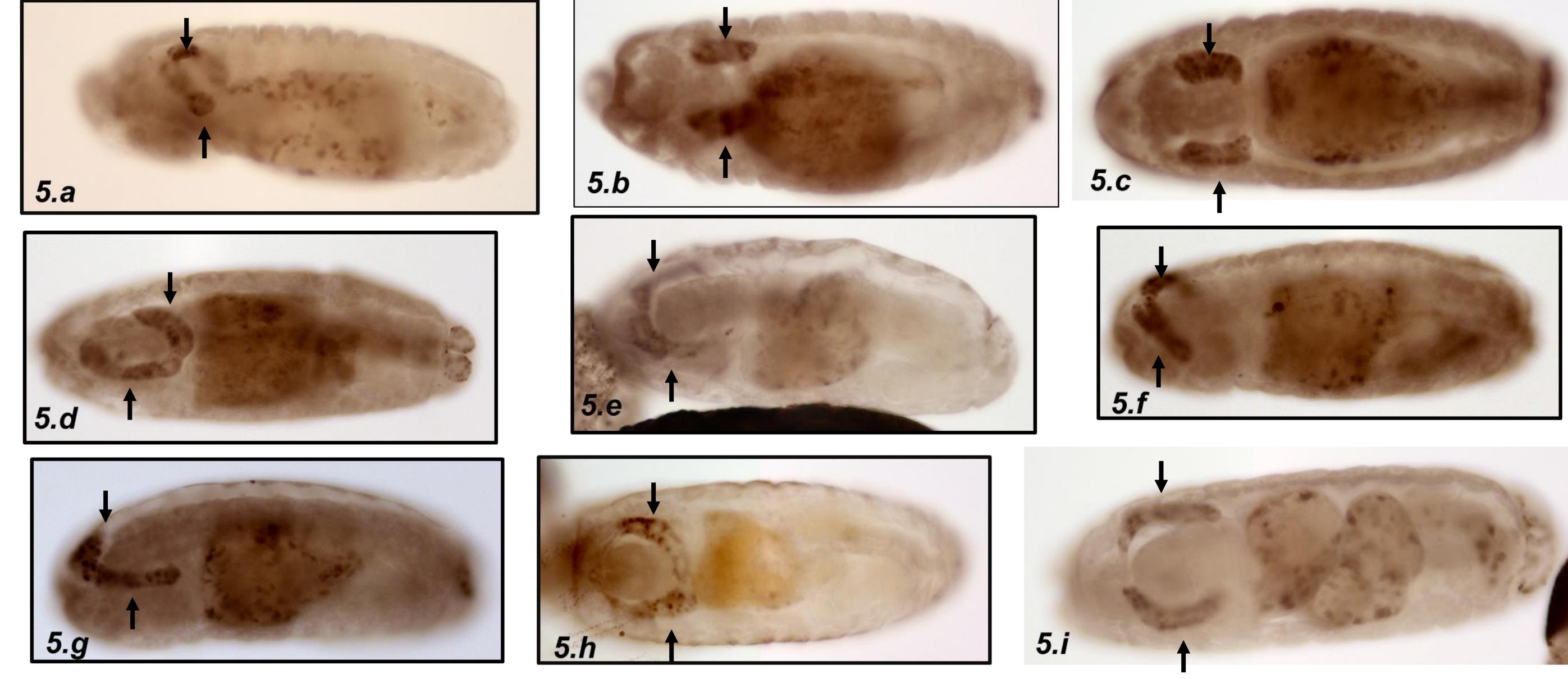


Figure 7. 8898 x 84-1. Boxed embryos express a mutation and unboxed embryos express wildtype salivary gland. The youngest embryo is stage 11 and the oldest is stage 17.



CONCLUSION AND FUTURE WORK

- In the deficiencies 8898 and 26853 there is an interaction shown between the deleted region and the 18-wheeler mutation.
- In these regions, there are possible candidate genes causing this interaction, meaning the gene(s) may play a role in development.
 - 26853: The deficiency by itself causes a defect in salivary gland morphogenesis, which is partially recused by reducing the dose of 18-wheeler.
 - 8898: In contrast, the 8898 deficiency causes a modest defect that is enhanced by the reducing the dose of 18-wheeler.
- Our next steps include ordering fly stocks carrying smaller deletions mapping within the 8898 and 26853 deletions to narrow down the number of candidate genes.

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