

## Supplementary Figure Legends

**Figure S1.** A close-up of fine wing pigmentation differences between *S. leonensis* and *S. ocellaris*. The pupal expression of Engrailed exactly prefigures the sites of future white spots in the adult wings. This correlation between the expression of Engrailed and the absence of black pigmentation reinforces the putative role of En in making the white spots on *Samoaia* species' wings.

**Figure S2.** *In situ* hybridization of *yellow* and *engrailed* in *S. leonensis*. (A) Spotted pharate wing and expression of the transcript *y* in late pupal wing. (B) Time course of *en* expression over pupal wing development.

**Figure S3.** Molecular interactions between proteins of the hedgehog loop in case of depletion and overexpression of engrailed in *D. melanogaster*. We followed the expression of the different players by immunostaining in the third instar larvae wing disc and the late pupal wing. Our results confirm the existence of an active hedgehog loop in the wing disc, and show for the first time that this loop becomes at least partially inactive during late pupal wing development.

**Figure S4.** Immunostainings of Dll in the Hawaiian drosophilids and the species *Chymomyza amoena*. The expression pattern of Dll (right panel) prefigures the black domains in the adult wing (left panel).

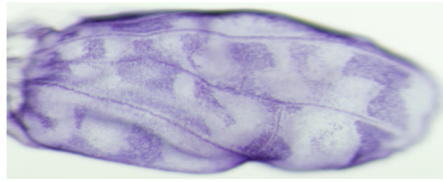
**Figure S5.** Time course experiments for additional genes. (A) Critical time points were identified for loss-of-function and gain-of-function contexts. The phenotypes range from severe A-P polarity defects to weak vein malformation for gain-of-function (B) and loss-of-function (C) contexts.

**Figure S6.** Overview of time course experiments. Loss-of-function experiments for the whole set of genes investigated define a time window (black domain) during which genes cannot be recruited for a novel function. Gain-of-function experiments define a time window (dark grey domain) during which co-option is possible, but unlikely in regard to morphological alteration of the wing such recruitment might create. Gene co-option is more likely to occur beyond the dark grey domain (see pale grey domain).

**Table S1.** List of primers used in this study.

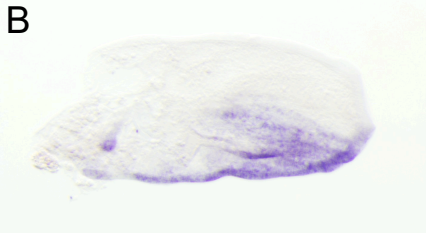
**Table S2.** Gene accession numbers.

A

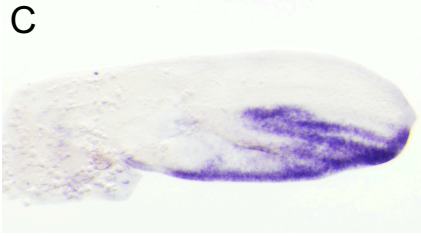


yellow

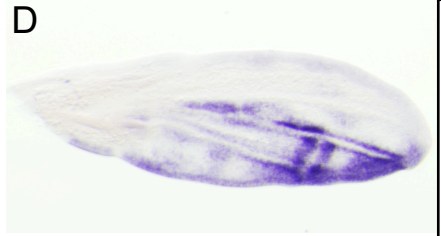
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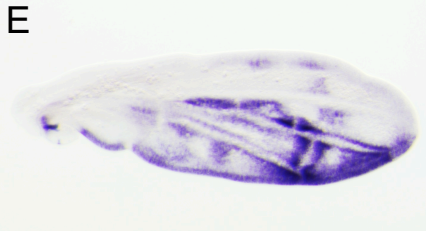
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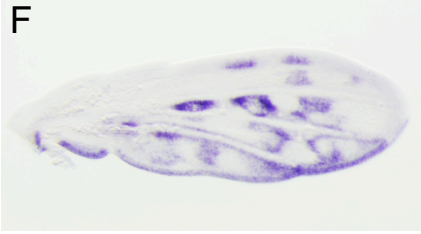
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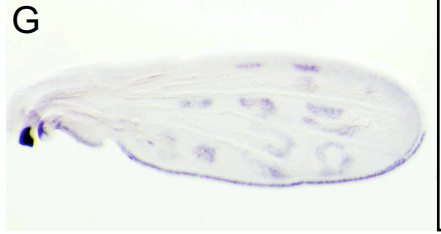
E



F

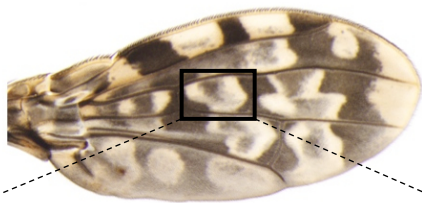


G

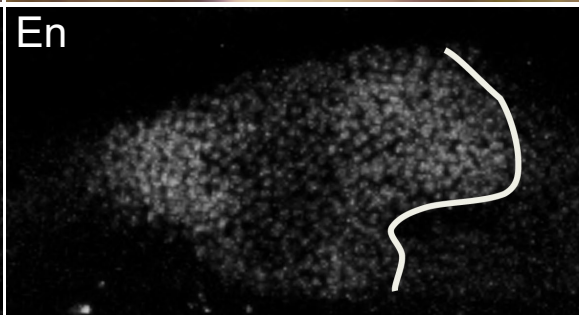
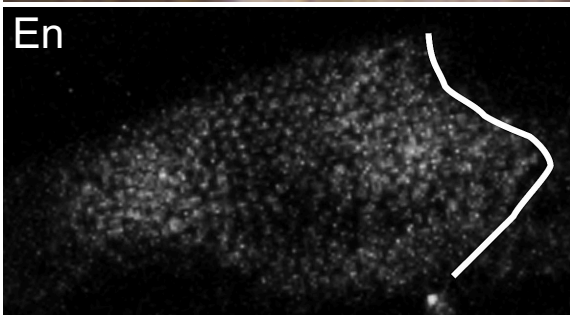
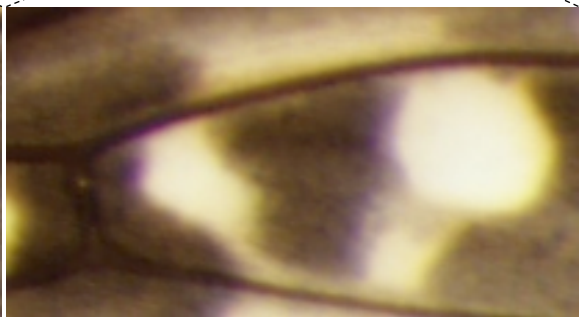
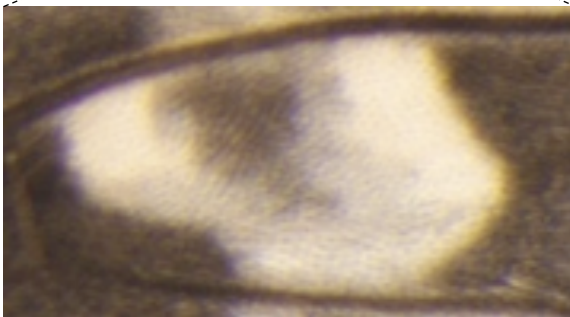
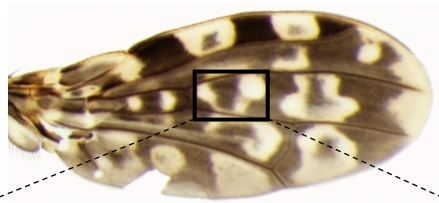


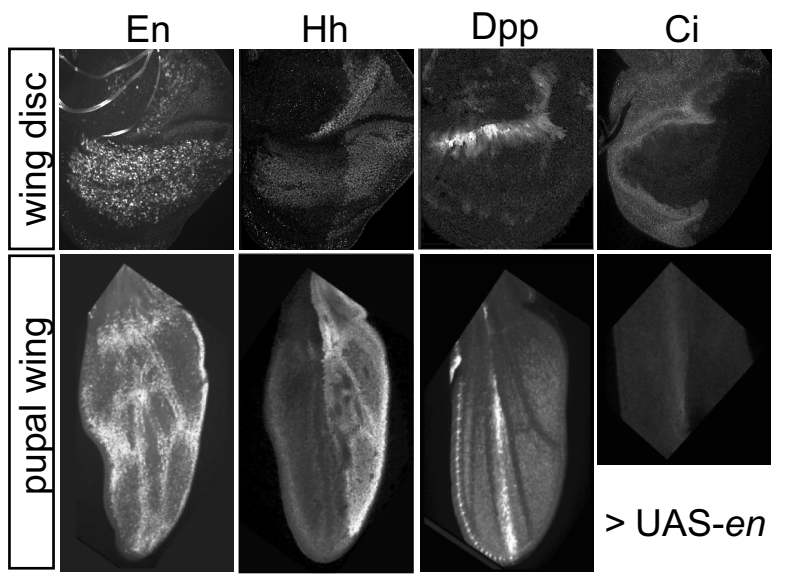
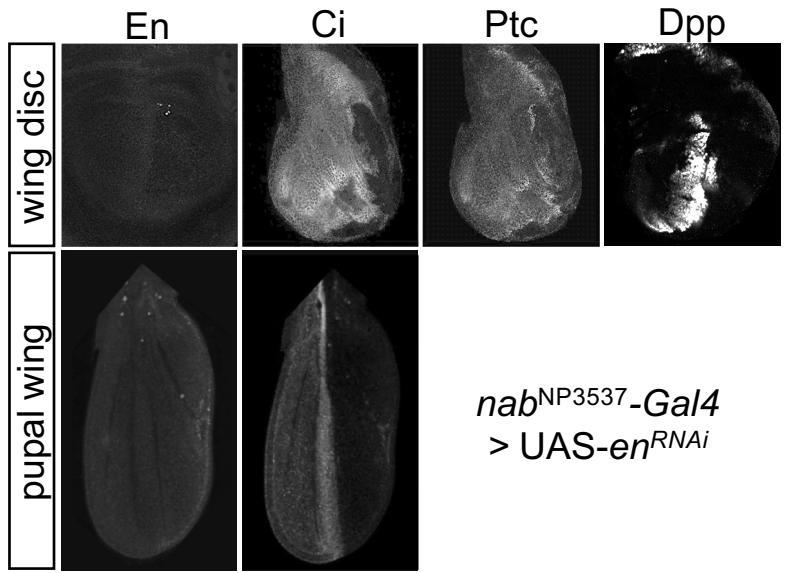
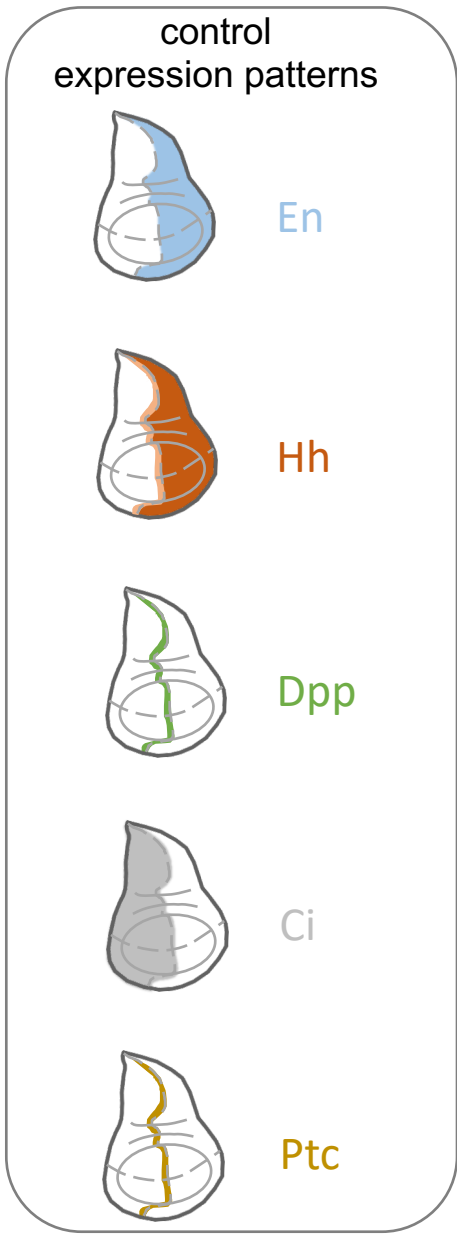
engrailed

*S. leonensis*

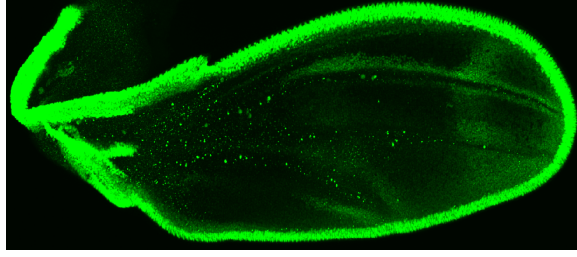
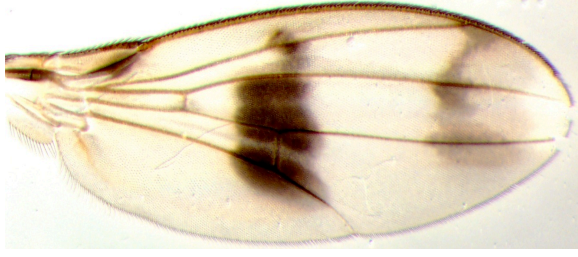


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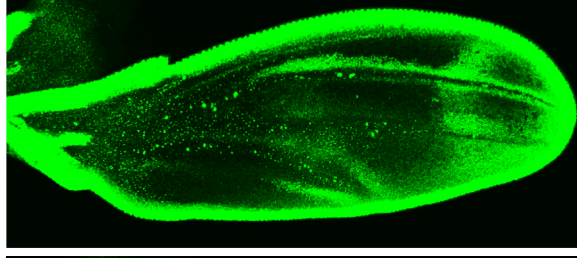




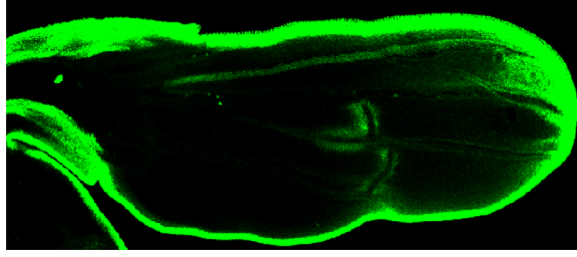
*C. amoena*



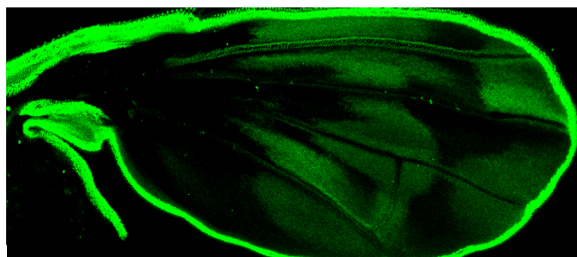
*D. hawaiiensis*



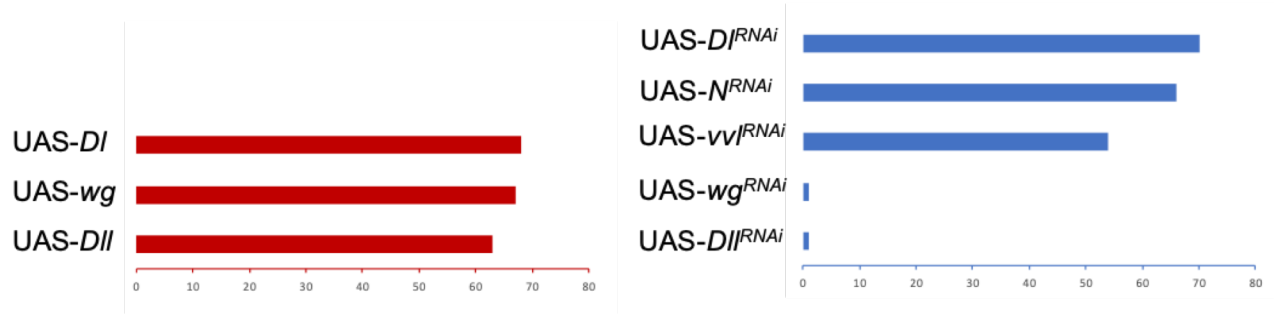
*D. silvestris*



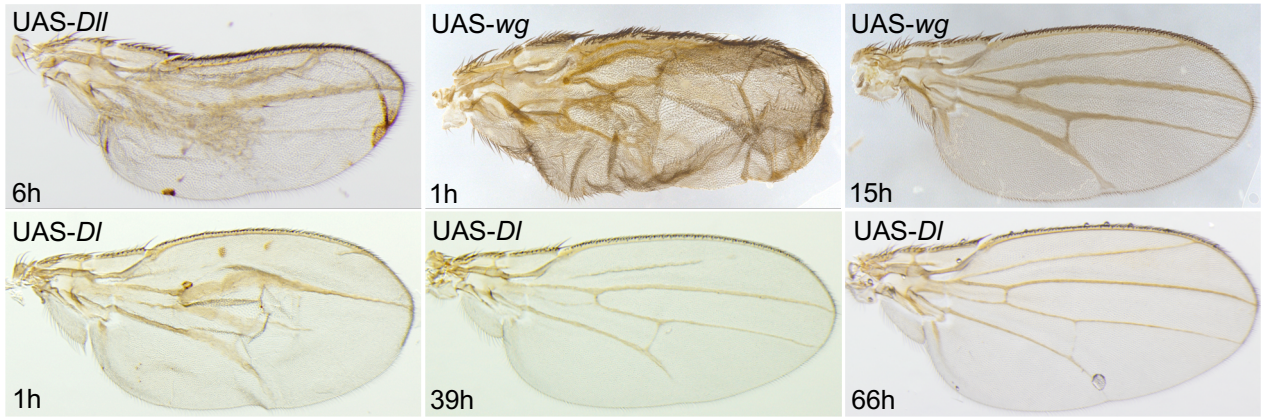
*D. grimshawi*



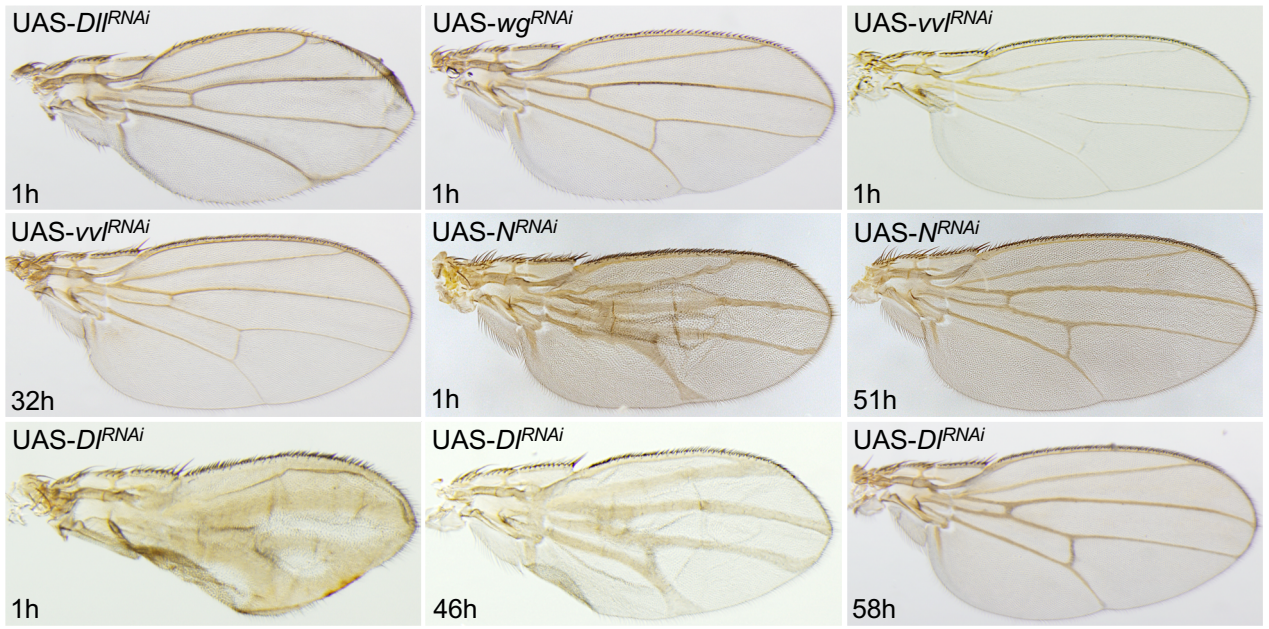
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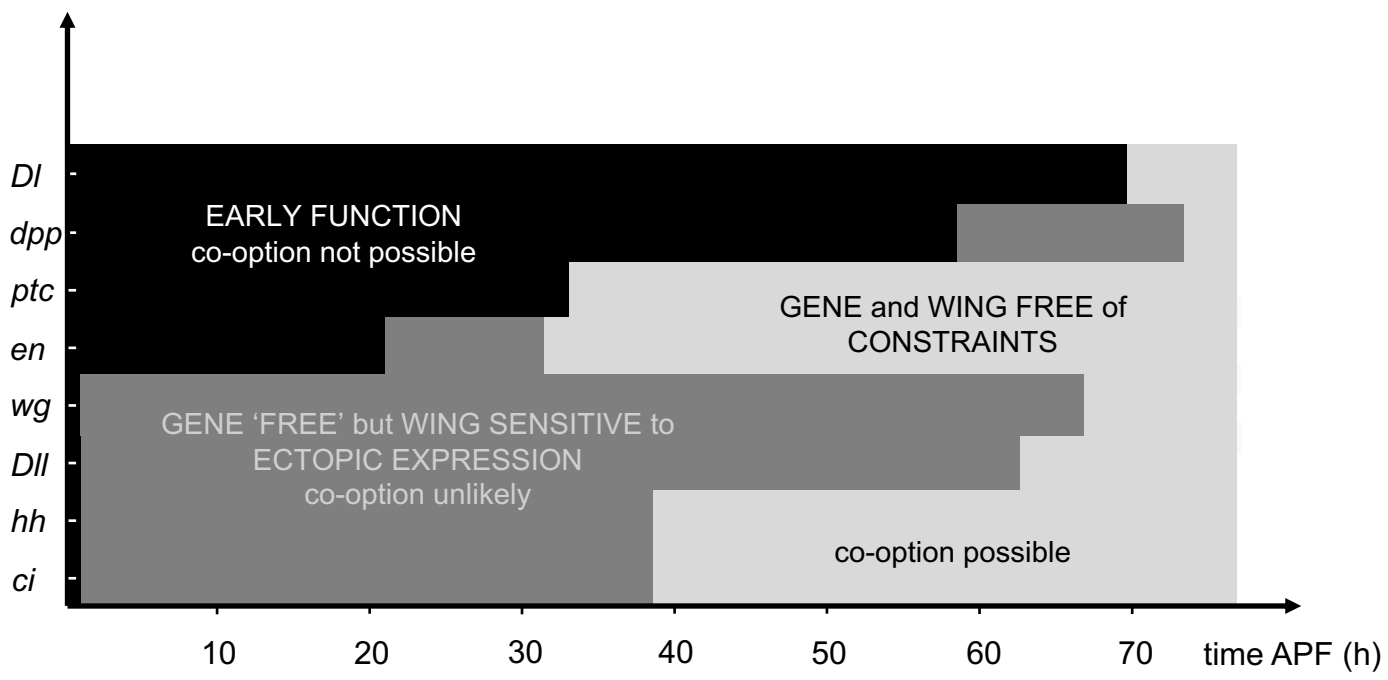


B



C





Gene	Primer name	Sequence 5'-3'	References
Amyrel	zone2bis	GTAAATNGGNCCACGCGAAG	Da Lage et al. (2007)
	relrev+	GTTCCCCAGCTCTGCAGCC	
	reludir	TGGATGCNGCCAAGCACATGGC	
cubitus interruptus	relavbis	GCATTTGTACCGTTTGTGTCGTTATCG	Holland et al. (1991)
	deg-ci -Fwd	GAGAGGATCCNTTYAARGCNCARTAYATG	
decapentaplegic	deg-ci -Rev	GAGAAGCTTRTGACNGTYTTNACRTGYTT	this study
	deg-dpp -Fwd	CTGAAHAGCACIGAIACGGTSAG	
Delta	deg-dpp -Rev	GTMCTTTGGTCRTTGAGRTASAGCAT	this study
	deg-Dl -Fwd	SCKBGCCTCSGCRTCGTCCTT	
Distal-less	deg-Dl -Rev	TGYCAYAAAYGGMGGMACCTGCATGAA	this study
	deg-Dll -Fwd	TGATACCAATACTGSGGCACATA	
Dopa decarboxylase	deg-Dll -Rev	ATGATGAARGCMGCTCAGGG	this study
	deg-Ddc -Fwd	TTCCASGAGTACTCCATGTCCTCG	
ebony	deg-Ddc -Rev	GGCAGGATGKATGAAGGACATTGAG	this study
	deg-e -Fwd	CCCATSACCTCKGTGGAGCCGTA	
engrailed	deg-e -Rev	CTGCATCGCATCTTYGAGGAGCA	this study
	deg-en -Fwd	GRTCGCTGTASCGSGTGACAGTA	
even skipped	deg-en -Rev	AATCAGCGCCAGTCCACCAG	this study
	deg-eve -Fwd	TGCCTVTCCAGTCCRGAYAACTC	
	deg-eve -Rev	TACGCCTCAGTCTTGATAGGG	
	deg-hh -Fwd	ACCTTGABARGGCATTGGCATACCA	
hedgehog	deg-hh -Rev	ATCGGWGATCGDGTGCTRAGCATG	this study
	Dme-hh -Fwd	TGCTTCACGCCGAAAGCACA	
	Dme-hh -Rev-T7	<b>TAATACGACTCACTATAGG</b> ACCACAATGGTGCCCTCGCGGGTCA	
	Sle-hh -Fwd	TCATGCCGGAGAGCACCCGCGC	
Notch	Sle-hh -Rev-T7	<b>TAATACGACTCACTATAGG</b> AGTTGACTACAATGGTCCCCTCT	this study
	deg-N -Fwd	CATCCVTGCCAGAAYGAGGG	
Notum	deg-N -Rev	GIGGRCAITCGCAYTTGTAGCC	this study
	deg-Notum -Fwd	TGGAAYATHCAYGADATGGGCGG	
	deg-Notum -Rev	GAGCAGYTCVAGRAADCATCTC	
	deg-ptc -Fwd1	ACCCAGCTGCGCATSAGRAAGG	
patched	deg-ptc -Rev1	GCTGACGGCSGCSTATGCGG	this study
	deg-ptc -Fwd2	ACCCAGCTGCGCATSAGRAACG	
	Dme-ptc -Fwd	AGCACCCAGGTGGTTCCGTTTTTG	
	Dme-ptc -Rev-T7	<b>TAATACGACTCACTATAGG</b> CTGTTGTTGTTGCAGCTCTTCGGAT	
tantalus	Sle-ptc -Fwd	GTATGCCGGAGAGCAGTCGCAAGGA	this study
	deg-tant -Fwd	TCRCAGCWAAGCCGMGGCGA	
	deg-tant -Rev	TGAGGCCATCRTTRAAGGARATGGC	
wingless	deg-wg -Fwd	AGCACGYCARGCRGAGATGCG	this study
	deg-wg -Rev	TTACCTRTTRTYTTGCGHCCC	
yellow	deg-y -Fwd	TATCCGGAYTGCGMAGCAATACGG	this study
	deg-y -Rev	GTCGCGATCSACAATGCCATGGAA	



gene	species	accession	
Amyrel	<i>Drosophila deflecta</i>	LS992514	
	<i>Drosophila guttifer</i>	LS992515	
	<i>Drosophila deflecta</i>	LS992508	
	<i>Drosophila funebris</i>	LS992505	
	<i>Drosophila guttifer</i>	LS992504	
Distal-less	<i>Drosophila quadrilineata</i>	LS992507	
	<i>Samoaia attenuata</i>	LS992664	
	<i>Samoaia hirta</i>	LS992666	
	<i>Samoaia leonensis</i>	LS992506	
	<i>Samoaia ocellaris</i>	LS992665	
	<i>Drosophila deflecta</i>	LS992509	
	<i>Drosophila guttifer</i>	LS992513	
	<i>Drosophila quadrilineata</i>	LS992512	
	Dopa decarboxylase	<i>Samoaia attenuata</i>	LS992668
		<i>Samoaia hirta</i>	LS992669
<i>Samoaia leonensis</i>		LS992510	
<i>Samoaia ocellaris</i>		LS992667	
<i>Zaprionus ghesquierei</i>		LS992511	
<i>Drosophila deflecta</i>		LS992462	
<i>Drosophila funebris</i>		LS992463	
<i>Drosophila guttifer</i>		LS992464	
<i>Drosophila quadrilineata</i>		LS992465	
ebony		<i>Samoaia attenuata</i>	LS992671
	<i>Samoaia hirta</i>	LS992672	
	<i>Samoaia leonensis</i>	LS992466	
	<i>Samoaia ocellaris</i>	LS992670	
	<i>Zaprionus ghesquierei</i>	LS992467	
	<i>Drosophila deflecta</i>	LS992516	
	<i>Drosophila funebris</i>	LS992519	
	<i>Drosophila guttifer</i>	LS992517	
engrailed	<i>Drosophila quadrilineata</i>	LS992520	
	<i>Samoaia attenuata</i>	LS992674	
	<i>Samoaia hirta</i>	LS992675	
	<i>Samoaia leonensis</i>	LS992518	
	<i>Samoaia ocellaris</i>	LS992673	
	<i>Drosophila deflecta</i>	LS992280	
	<i>Drosophila funebris</i>	LS992281	
	<i>Drosophila guttifer</i>	LS992282	
even skipped	<i>Drosophila quadrilineata</i>	LS992283	
	<i>Samoaia attenuata</i>	LS992677	
	<i>Samoaia hirta</i>	LS992678	
	<i>Samoaia leonensis</i>	LS992284	
	<i>Samoaia ocellaris</i>	LS992676	
	<i>Zaprionus ghesquierei</i>	LS992285	
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	<i>Drosophila guttifer</i>	LS992526
	<i>Drosophila quadrilineata</i>	LS992523
hedgehog	<i>Samoaia attenuata</i>	LS997505
	<i>Samoaia hirta</i>	LS997506
	<i>Samoaia leonensis</i>	LS992525
	<i>Samoaia ocellaris</i>	LS997504
	<i>Zaprionus ghesquierei</i>	LS992524
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	<i>Drosophila guttifer</i>	LS992531
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	<i>Samoaia hirta</i>	LS997500
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	<i>Zaprionus ghesquierei</i>	LS992533
	<i>Drosophila deflecta</i>	LS992536
	<i>Drosophila funebris</i>	LS992540
	<i>Drosophila guttifer</i>	LS992537
	<i>Drosophila quadrilineata</i>	LS992541
tantalus	<i>Samoaia attenuata</i>	LS997493
	<i>Samoaia hirta</i>	LS997494
	<i>Samoaia leonensis</i>	LS992538
	<i>Samoaia ocellaris</i>	LS997492
	<i>Zaprionus ghesquierei</i>	LS992539
	<i>Drosophila deflecta</i>	LS992558
	<i>Drosophila funebris</i>	LS992560
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	<i>Drosophila quadrilineata</i>	LS992561
yellow	<i>Samoaia attenuata</i>	LS997496
	<i>Samoaia hirta</i>	LS997497
	<i>Samoaia leonensis</i>	LS992559
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