

**Touch of
Evolution**

Michael Ludwig 2004

Functional Evolution and Canalization of Gene Regulation

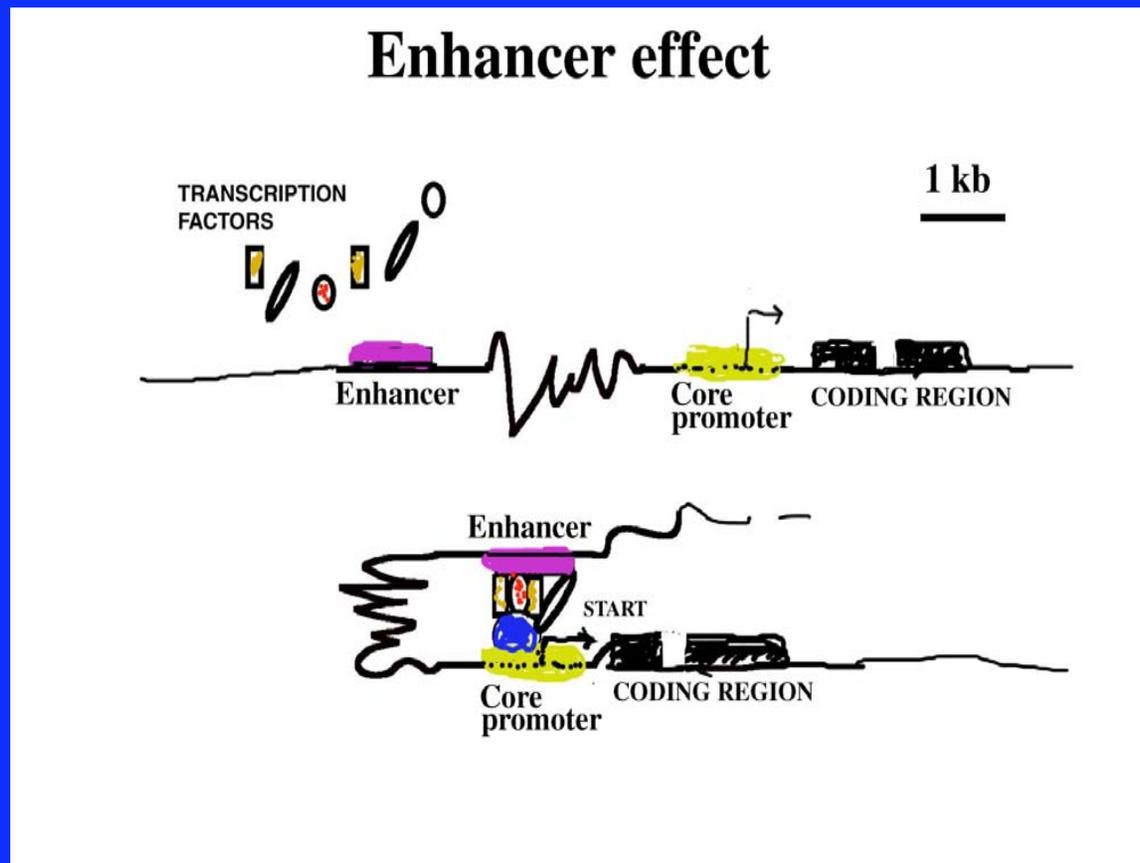
Michael Ludwig

Susan Lott

Martin Kreitman

Nanjing University, Dec. 15 2006

Enhancer increases transcription from a linked core promoter. Effect is orientation independent and shows some flexibility with respect to distance.



Molecular Evolutionist Camp

Regulatory evolution is brought about primarily by substitutions in *cis*-sequences

transcription factors are selectively constrained because they bind to many sites in the genome

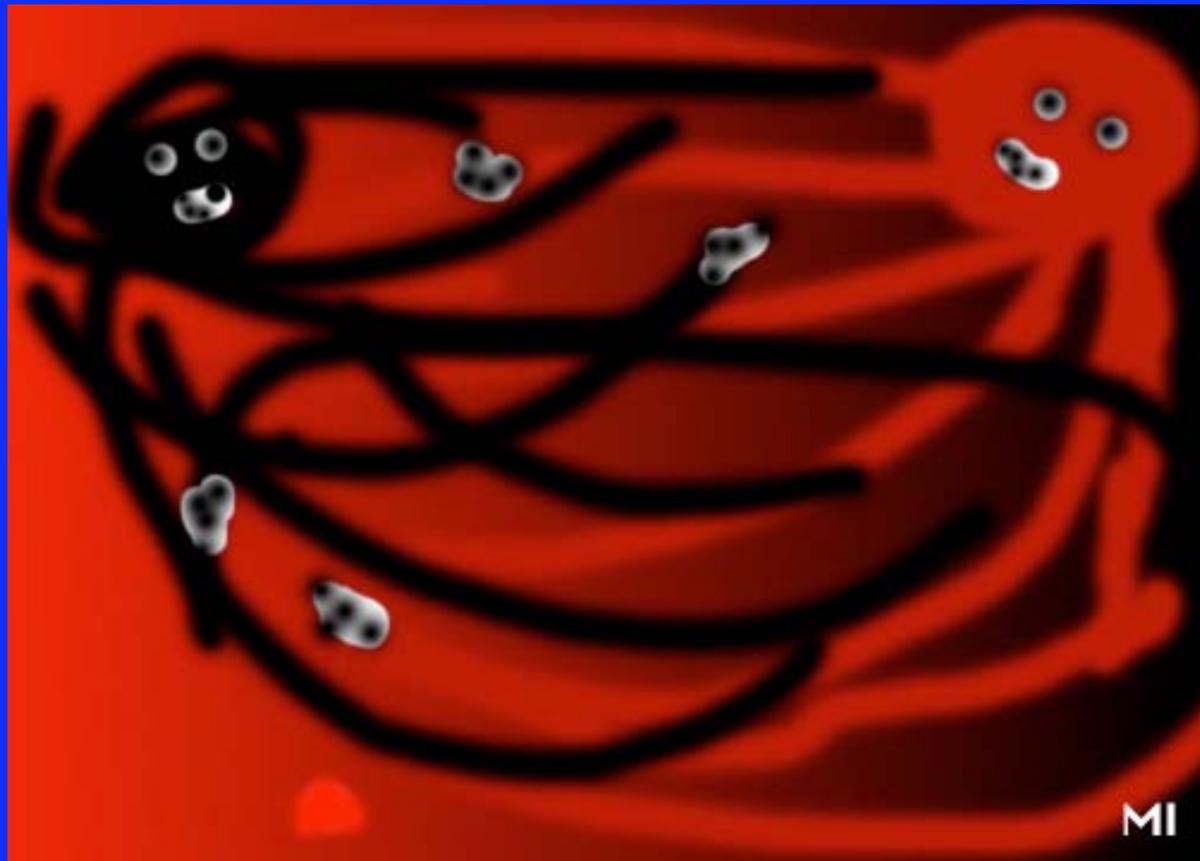
cis- modules can evolve by local fine-tuning

Developmentalist Camp

- **Evolution is brought about by shifts in expression of *trans*-acting proteins**
 - Patterns of transcription factor expression establish the coordinates specifying cell fates

Impressionist (Ludwig) Camp

“Regulatory evolution”



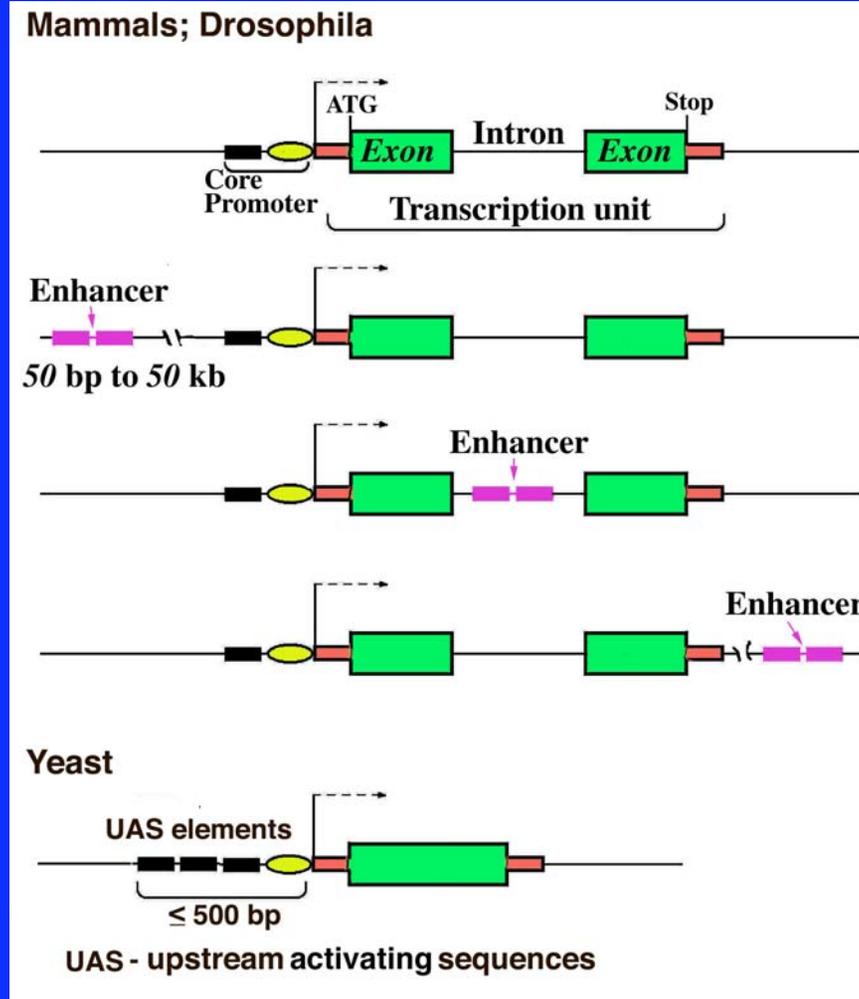
Questions

- 1. Are there general rules about how enhancers evolve and about the form(s) of natural selection governing their evolution?**
- 2. Do regulatory networks controlling development dampen noise introduced by genetic and environmental variability?**
- 3. Can traits controlled by “robust” networks evolve, and if so, at what pace?**

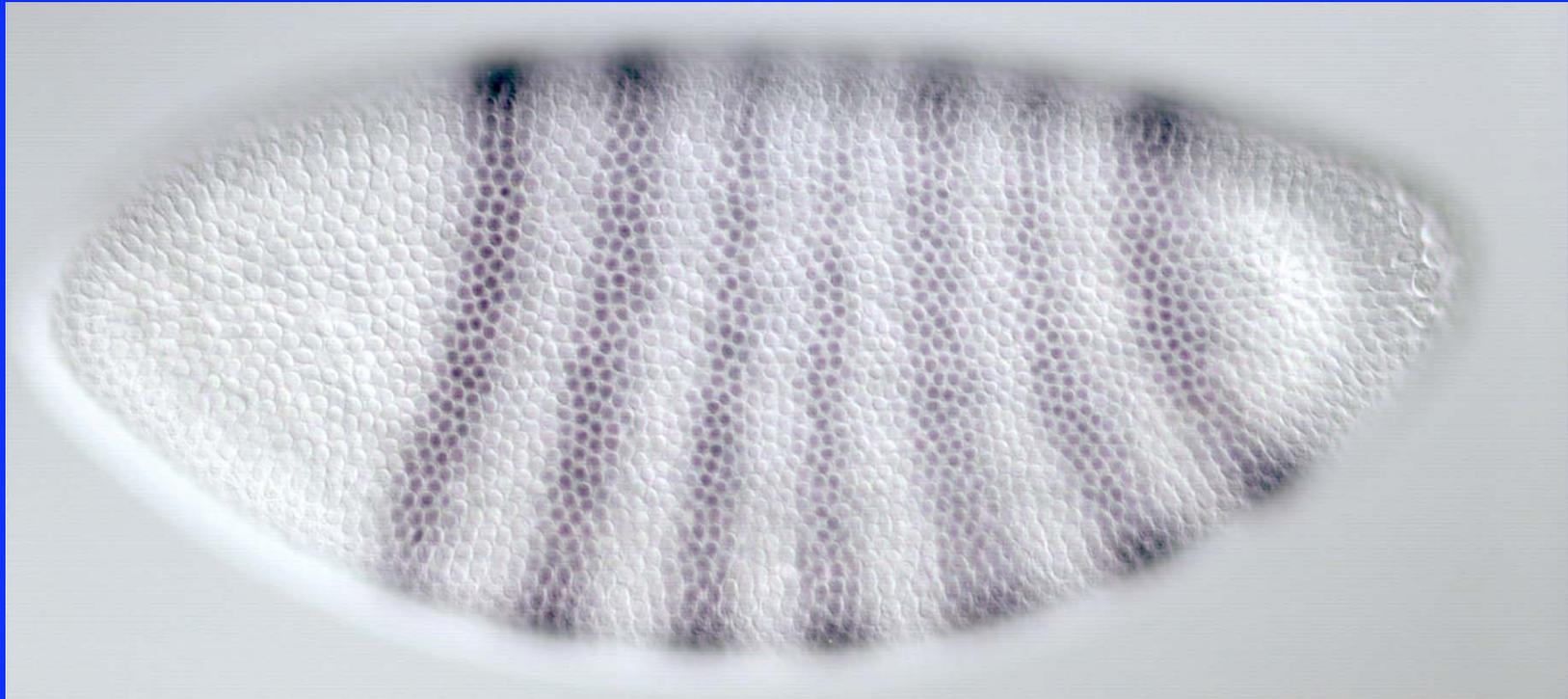
Organization of Talk

1. **Brief review of enhancer structure/function.**
2. **Introduction to embryogenesis and segmentation in the fruitfly.**
3. **Experiments on structure/function and evolution of the *even-skipped* stripe 2 enhancer (*eveS2E*).**
 - **Reporter transgene experiments with native and chimeric *eveS2E*'s.**
 - **Functional replacement of the native *eveS2E* with the enhancer from other species.**
4. **Canalization of *eveS2E* gene expression within and between species.**

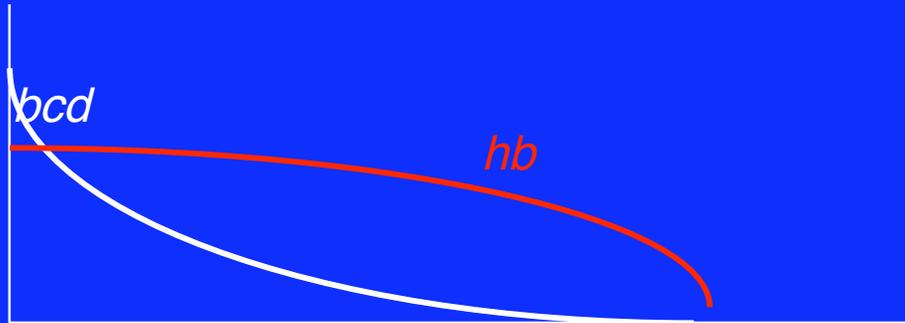
Enhancer types in different organisms



even-skipped in *D. melanogaster*
blastoderm stage

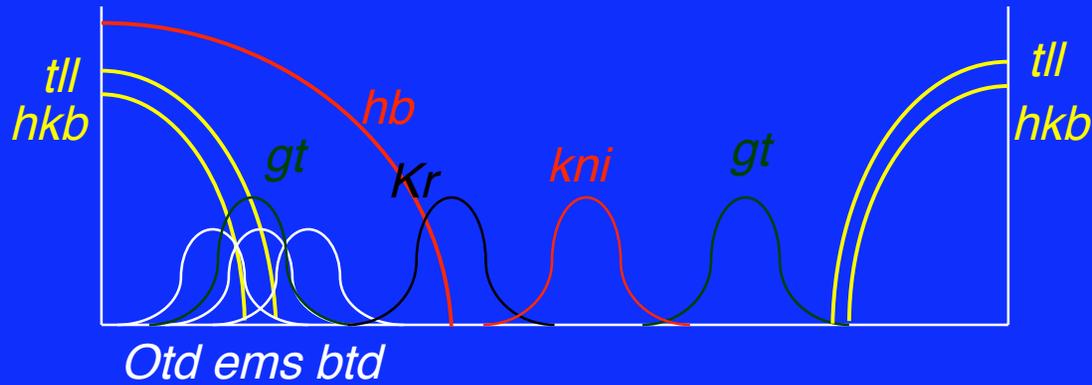


A



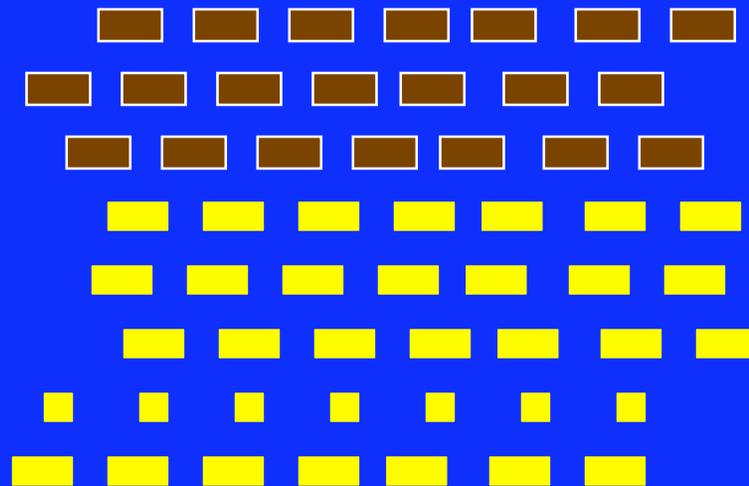
P

maternal



gap

run
h
eve
ftz
opa
odd
slp
prd

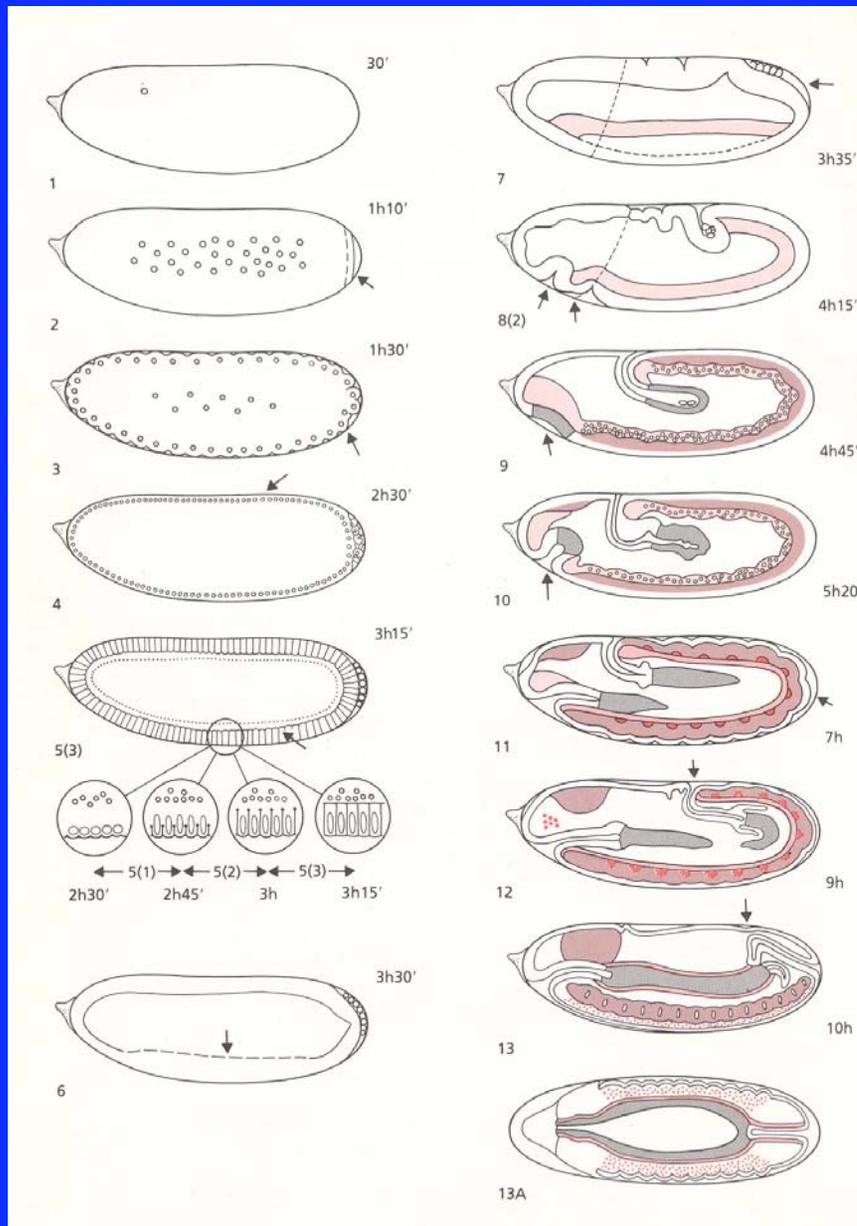


Primary pair-rule

Secondary pair-rule

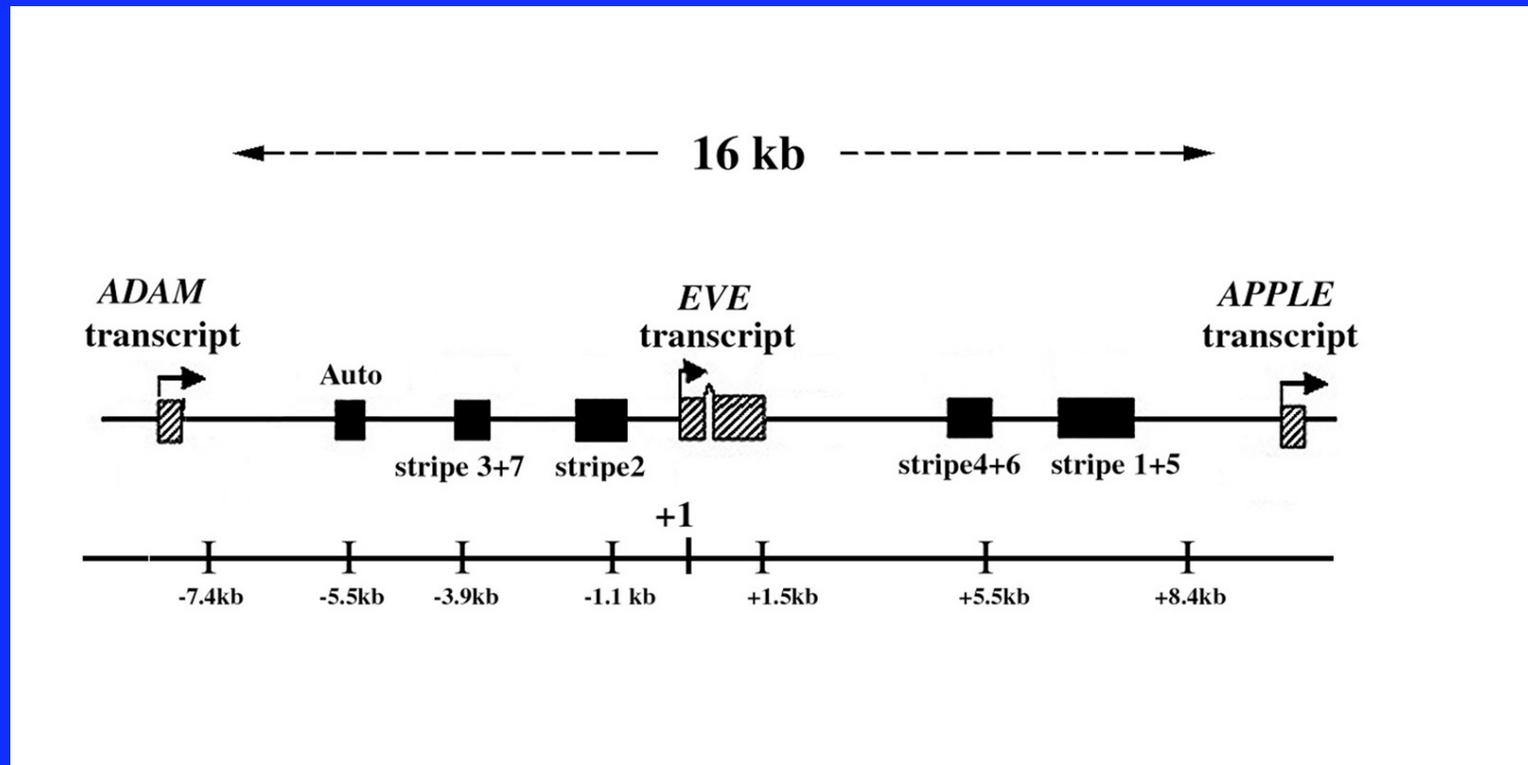
From: Pankratz & Jackle (1993)

Embryogenesis in the fruitfly



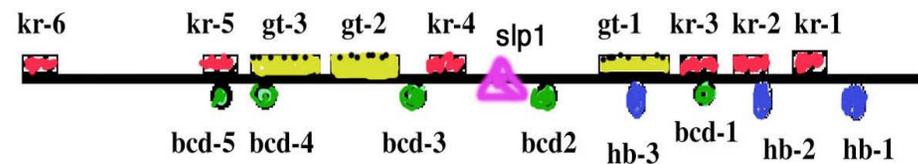
From: P. Lawrence,
The Making of a Fly

cis-elements (enhancers) regulate
even-skipped stripe expression
(after Sackerson, et al., 1999)



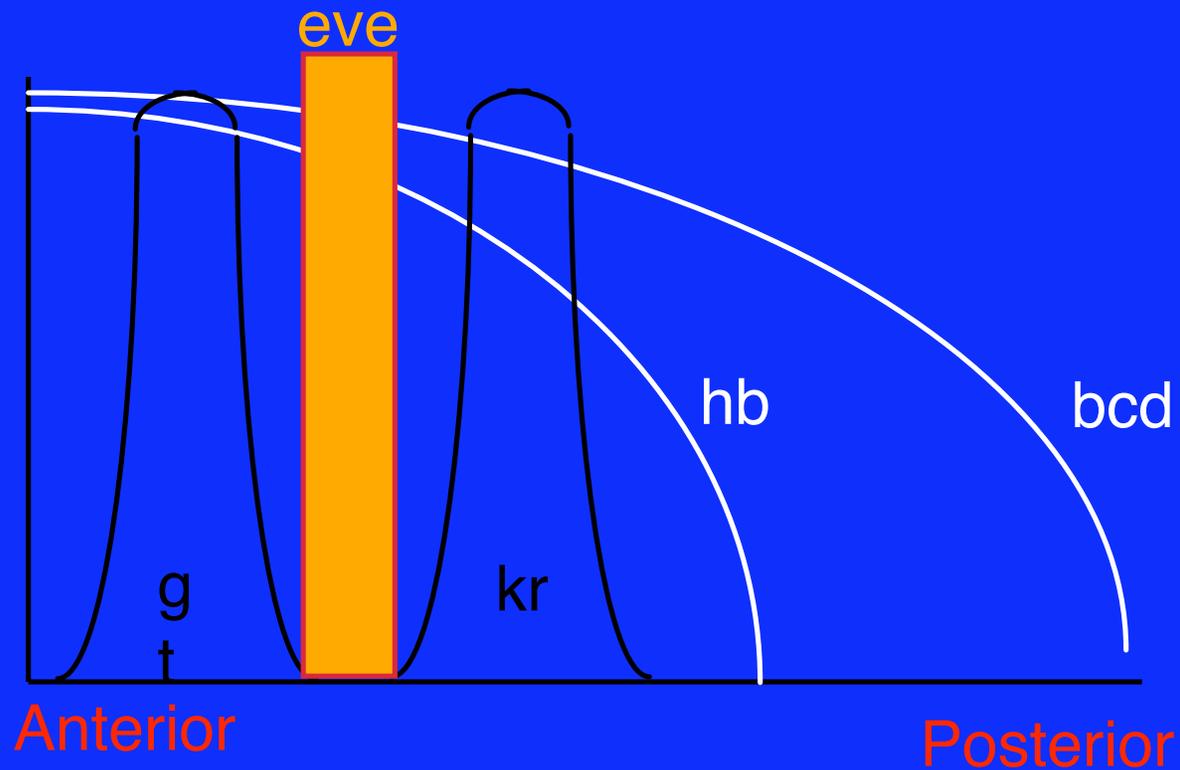
D. melanogaster even-skipped

stripe-2 enhancer region (798 bp)

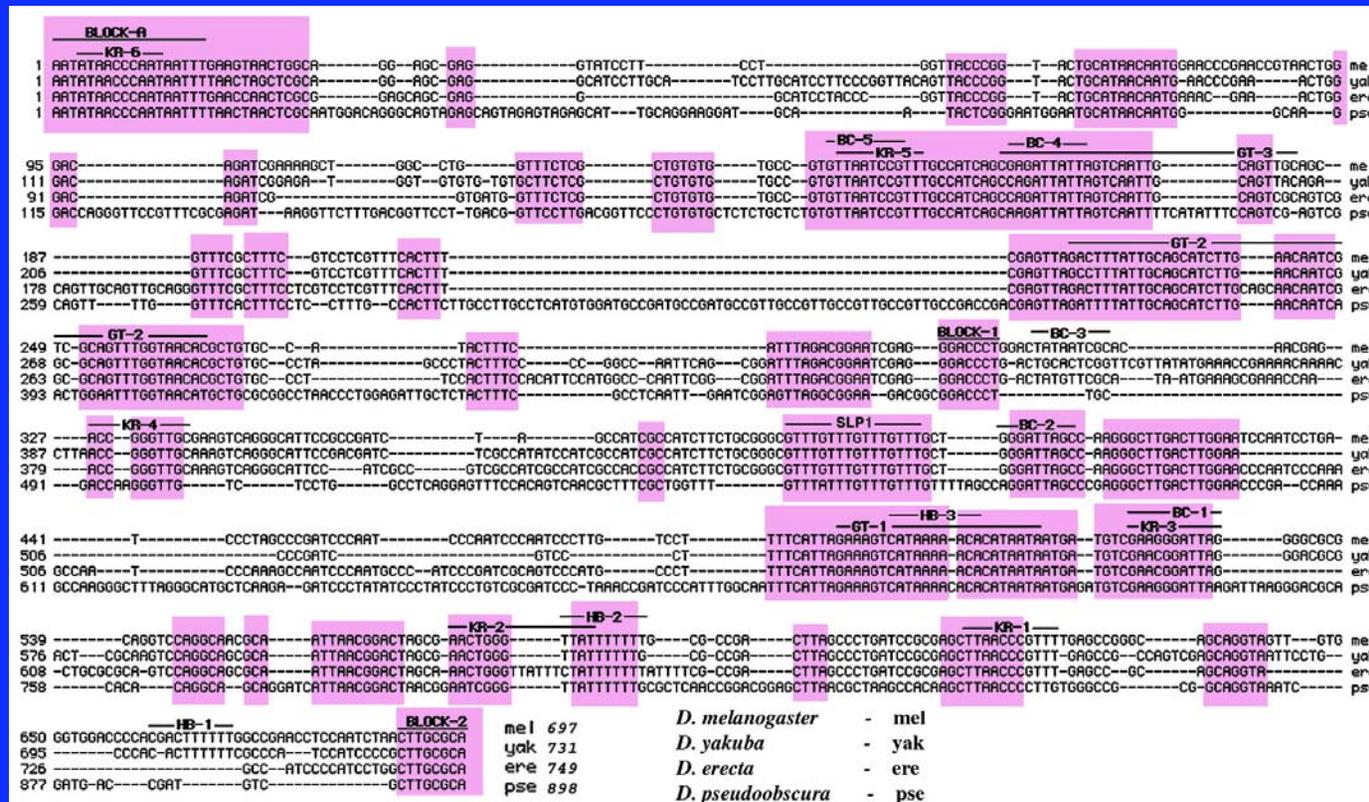


after M. Levine, S. Small, and D. Arnosti

Model of *eve* stripe 2 regulation

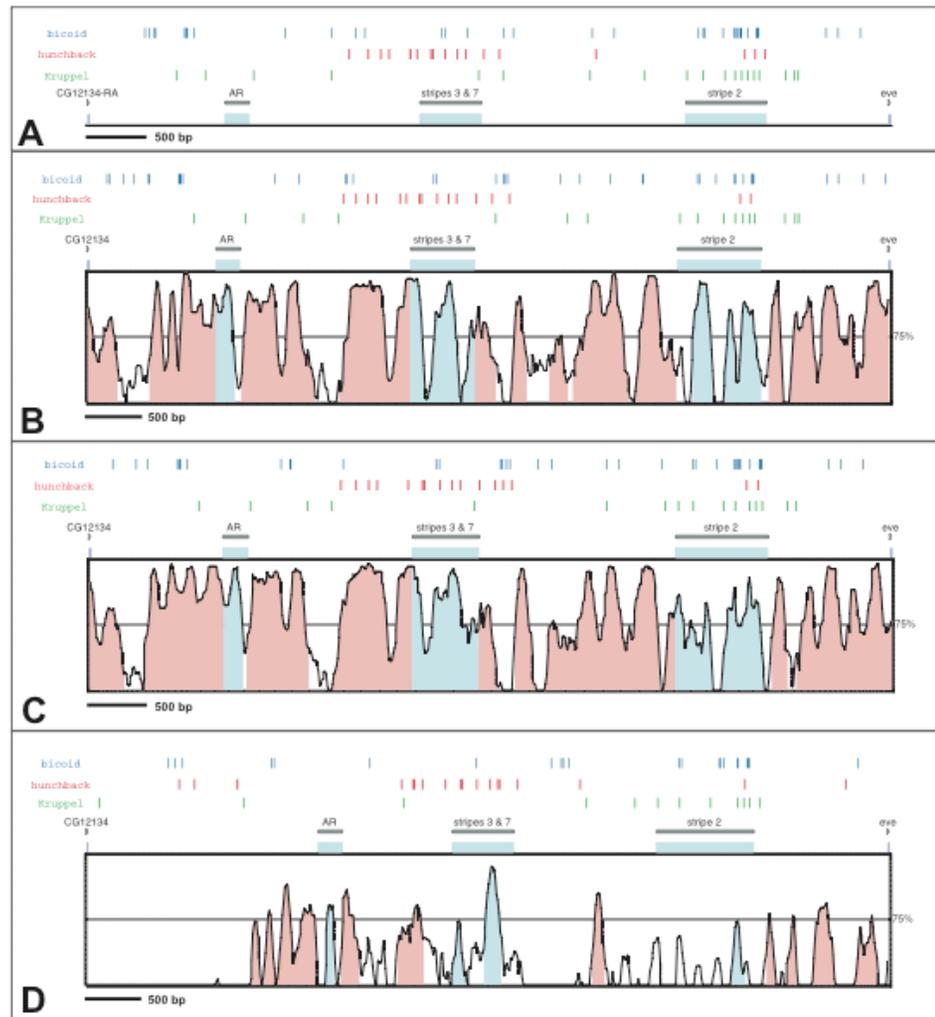


Alignment of *eve* stripe-2 enhancer regions in four *Drosophila* species



(pink - conserved sequences)

Divergence of the *eve* 5' regulatory region



D. melanogaster

vs.

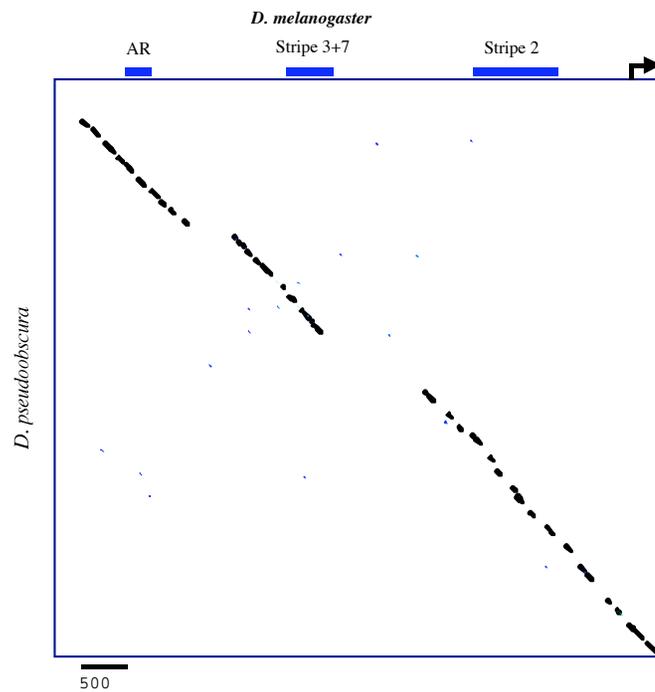
D. yakuba

D. erecta

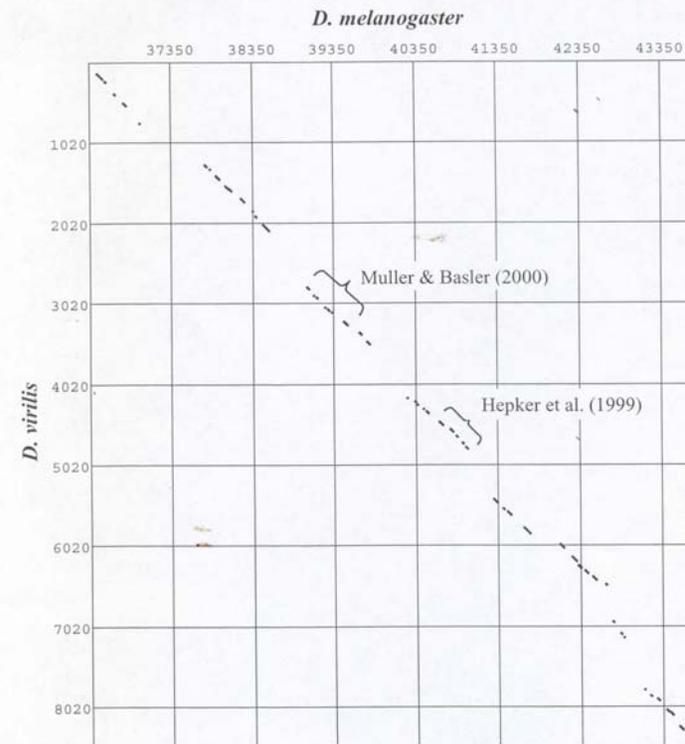
D. pseudoobscura

Enhancers are weakly conserved between fly species

Eve pair-rule enhancers



Dpp downstream enhancers



Putative binding sites in *eve* stripe 2 enhancer

	kr-6	kr-5	kr-4	kr-3	kr-2	kr-1
<i>mel</i>	ATAACCCAT	TTAATCCGT	ACC--GGTGC	GAGGGATTAG	ACTGGGTAT	TTAACCCGTT
<i>yak</i>-.....	...C.....
<i>ere</i>-.....	...C.....
<i>pse</i>AA.....-A	.TC.....C..G

	bcd-5	bcd-4	bcd-3	bcd-2	bcd-1
<i>mel</i>	GTTAATCCG	GAGATTATT	TATAATCCG	GGATTAGC	GAAAGGATTAG
<i>yak</i>	C.....	N/AC.....
<i>ere</i>	C.....	N/AC.....
<i>pse</i>	A.....	N/A	A.....A

	hb-3	hb-2	hb-1	gt-3
<i>mel</i>	CATAAAA-ACA	TTATTTTTT	CGATTTTTT	CGAGATTATTAGTCAATTG-----CAGTGC
<i>yak</i>G	..-C.....	.C.....A.
<i>ere</i>	C.....	N/A	.C.....C..
<i>pse</i>C..	N/A	.C.....TTCATATTC....C.-

	gt-2	gt-1
<i>mel</i>	GACTTATTTCAGCATCTTG----AACAACTGTC-GCACTTGGTAACAC	GAAAGTCATAAAA-ACACATAATA
<i>yak</i>	.C.....G.....
<i>ere</i>CAGC.....G.....
<i>pse</i>	.T.....AA.T.G.A.....TC.....

D. melanogaster (*mel*), *D. yakuba* (*yak*), *D. erecta* (*ere*),
D. pseudoobscura (*pse*)

bicoid (*bcd*), Kruppel (*kr*), giant (*gt*), hunchback (*hb*)

Putative binding sites for transcription factors Kruppel and bicoid in *eve s2e* of 13 *Drosophila* species.

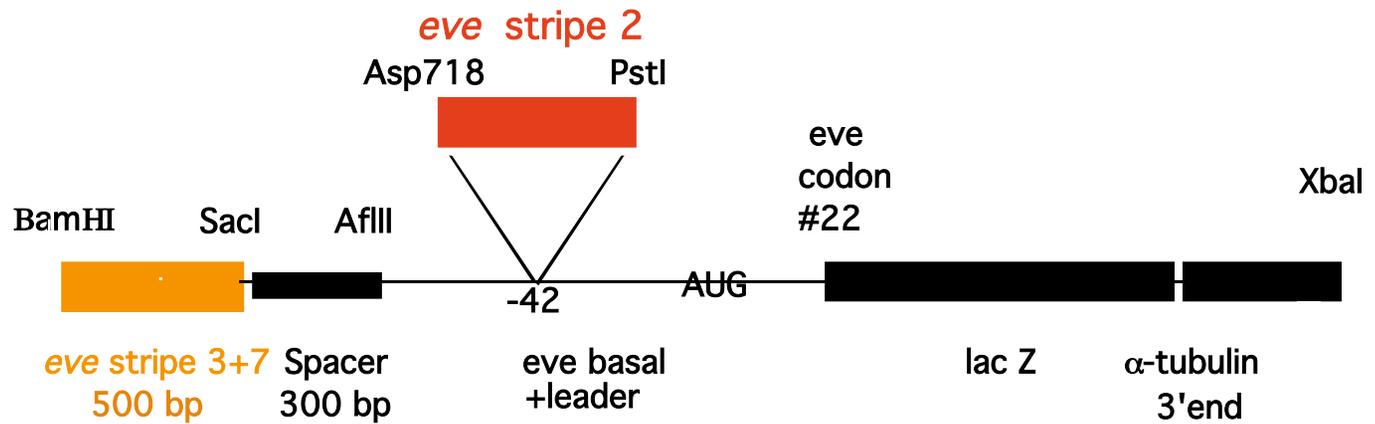
	kr-5	kr-4	kr-3	kr-2	kr-1
<i>mel</i>	TTAATCCGTT	ACC--GGGTTGC	GAAGGGATTAG	ACTGGGTAT	TTAACCCGTTT
<i>sim</i>--.....
<i>mau</i>--.....C.
<i>sec</i>--..T....
<i>yak</i>--.....	...C.....
<i>tei</i>--.....	...C.....
<i>ere</i>--.....	...C.....
<i>ore</i>--.....	...C.....
<i>tak</i>G--...TAG.....
<i>ana</i>C..	..G--.....	...-...C	..C.....	..C.G...T...
<i>pse</i>AA.....TA	..TC.....C..G
<i>vir</i>--...A.	AC.....	..T.....	N/A
<i>pic</i>--...A.	AGG.....	..T...C....	N/A
	bcd-5	bcd-4	bcd-3	bcd-2	bcd-1
<i>mel</i>	GTTAATCCG	GAGATTATT	TATAATCGC	GGGATTAGC	GAAGGGATTAG
<i>sim</i>	C.....	.C.....
<i>mau</i>	C.....	.C.....
<i>sec</i>C.....
<i>yak</i>	C.....	N/AC.....
<i>tei</i>	C.....	N/AC.....
<i>ere</i>	C.....	N/AC.....
<i>ore</i>	C.....	N/AC.....
<i>tak</i>	C.....	N/A
<i>ana</i>C	C.....	N/A	C.....	...-...C
<i>pse</i>	A.....	N/A	A.....A
<i>vir</i>C.....	N/A	T.....TA	AC.....
<i>pic</i>C.....	N/A	..A.....G	AGG.....

eve s2e binding sites in *D. melanogaster* [30] and homologous sequences from 12 other *Drosophila* species [7,8]. Residues homologous to *D. melanogaster* are indicated as dots. Gaps in aligned sequences are indicated by dashes. bcd and kr are the binding sites in *D. melanogaster* for the transcription factors bicoid and Kruppel. N/A, not available (no homologous sequence identified). *Drosophila* species: *mel*, *D. melanogaster*; *sim*, *D. simulans*; *mau*, *D. mauritiana*; *sec*, *D. sechellia*; *ere*, *D. erecta*; *ore*, *D. orena*; *yak*, *D. yakuba*; *tei*, *D. teissieri*; *tak*, *D. takahashii*; *ana*, *D. ananassae*; *pse*, *D. pseudoobscura*; *vir*, *D. virilis*; *pic*, *D. picticornis*.

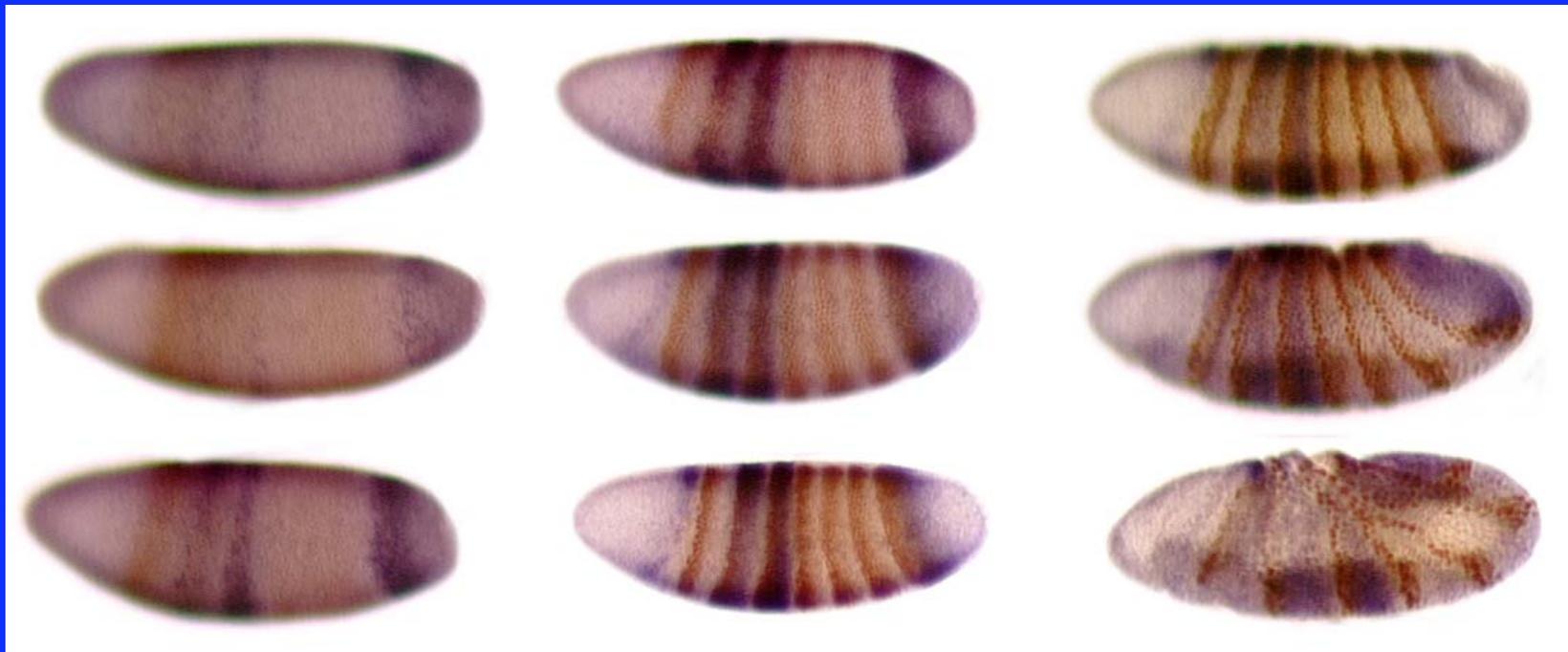
Transcription factor binding sites are not strictly conserved

- 1. Nucleotide substitutions are present in almost all regulatory protein binding sites**
- 2. Some binding sites are either absent or highly modified in some species**
- 3. Spacing between binding sites evolves quickly**

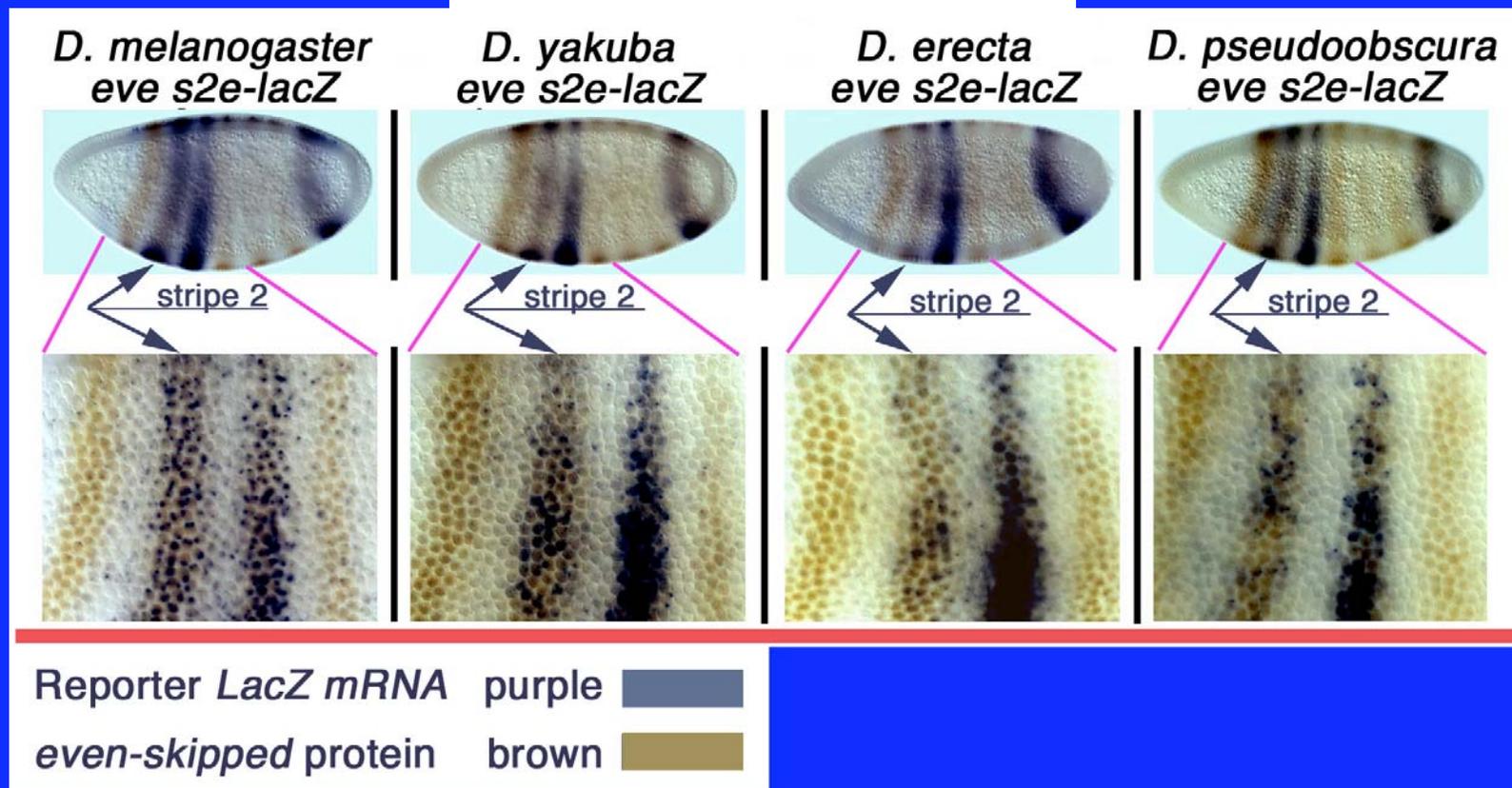
CaSpeR [*eve* (s2e,s3+7e), *lacZ*]



Eve expression in early development

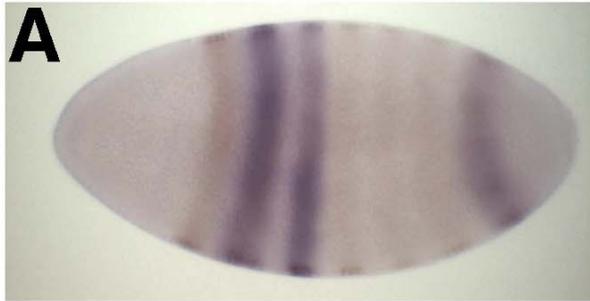


Functional conservation of *eve* s2e-lacZ transgene expression



(after Ludwig et al., 1998)

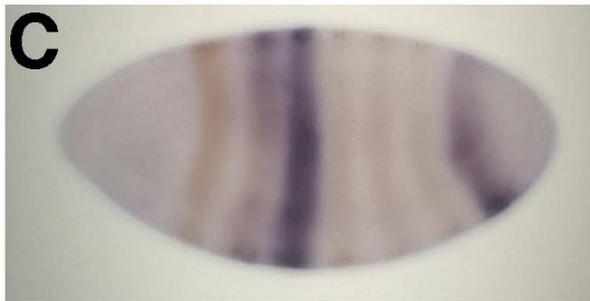
D. melanogaster embryos, piggyBac vector



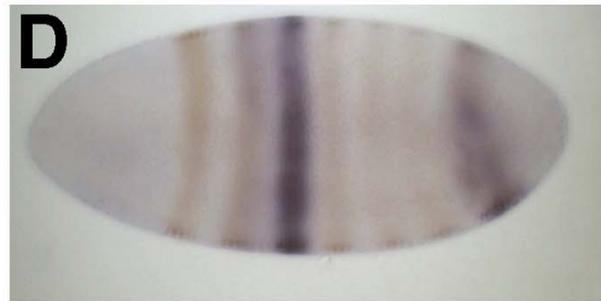
M s(3+7)e; M s2e; M core promoter



M s(3+7)e; M s2e; Vir core promoter

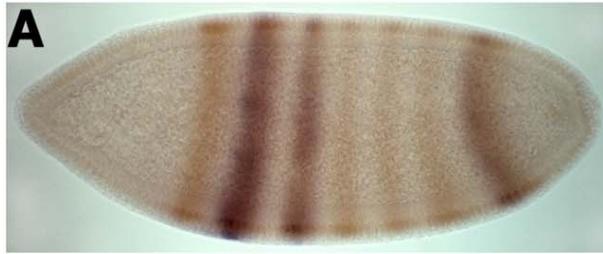


M s(3+7)e; Vir s2e; M core promoter

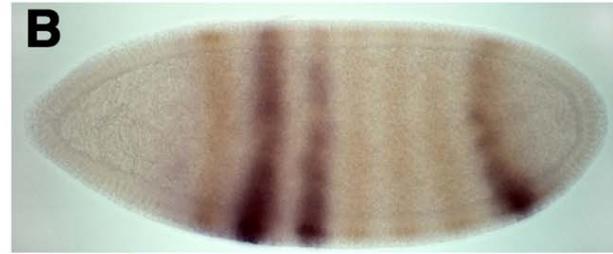


M s(3+7)e; Vir s2e; Vir core promoter

D. virilis embryos, piggyBac vector



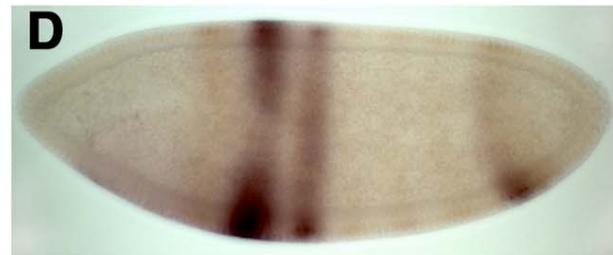
M s(3+7)e; M s2e; M core promoter



M s(3+7)e; M s2e; Vir core promoter



M s(3+7)e; Vir s2e; M core promoter

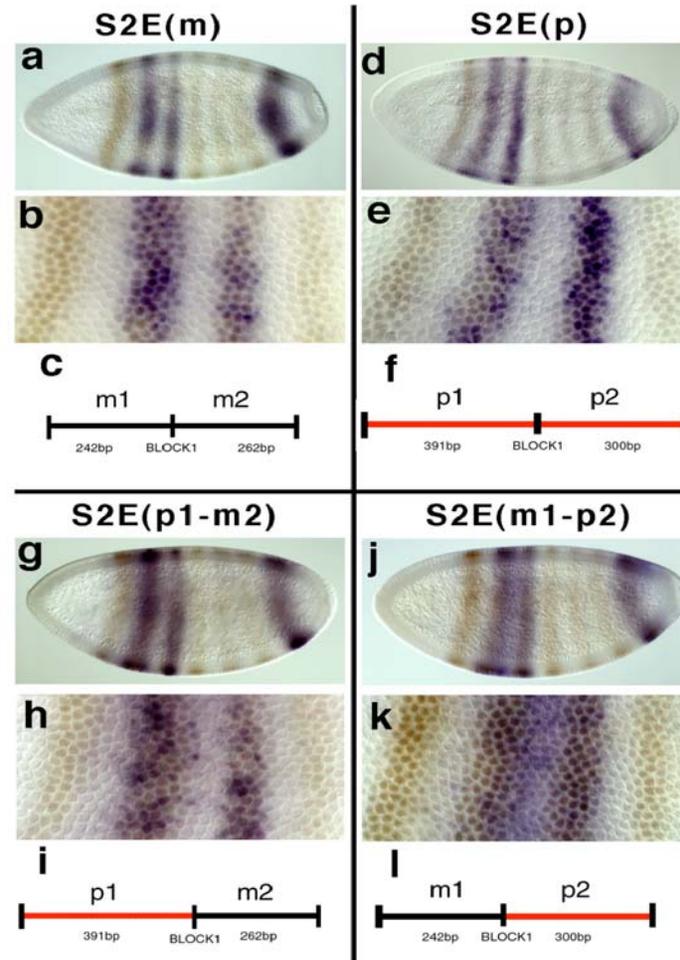


M s(3+7)e; Vir s2e; Vir core promoter

Interpretation of transgene experiments

- Strong functional conservation of spatio-temporal expression of stripe 2 Eve by the S2E in *Drosophila*
- No evidence for enhancer - proximal promotor coevolution between two distantly related species
- Expression levels of stripe 2 Eve driven by the *D. erecta* S2E is sensitive to species genetic background
 - Perhaps the trans-acting “activation” environment is stronger in *D. virilis* than in *D. melanogaster*. S2E's have evolved to compensate.

Misexpression of a S2E chimera between *D. pseudoobscura* and *D. melanogaster*



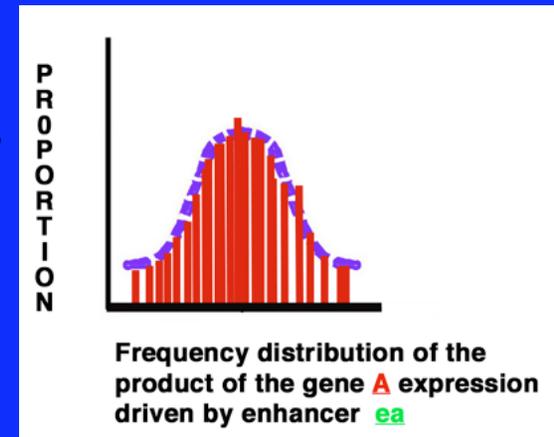
(After Ludwig
et al. 2000)

Interpretation of chimera misexpression

- Coevolved differences in 5' and 3' halves of *s2e* are required for functional conservation
 - » Implies that individual changes were themselves not functionally “neutral”
 - » Suggests a model of stabilizing selection on enhancer function with evolutionary accumulation of slightly deleterious and slightly advantageous mutations

Enhancer activity is a quantitative character

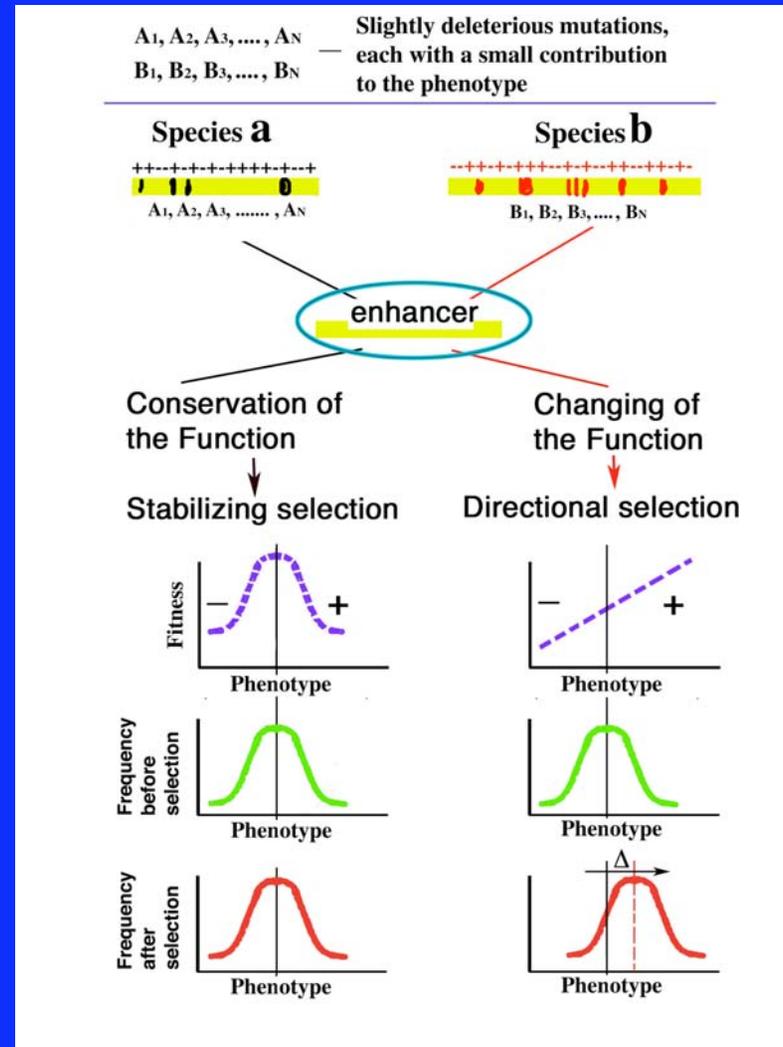
1. Enhancer architecture suggest that many independent mutations with subtle changes in both binding sites and spacing between them and neighbors sequences contribute to continuous variation in gene expression



Predictions:

- Polymorphism within species
- Structural divergence between species

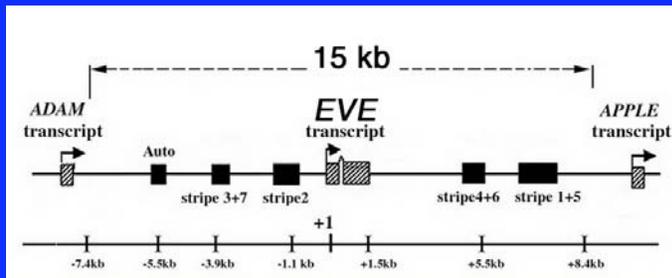
Stabilizing selection on spatio-temporal expression of the *eve* S2E



Is the reporter transgene assay a good proxy for enhancer functional readout?

Rescue of *eve s2e* deficiency mutant to adulthood
using the *eve s2e* from *D. melanogaster*, *D.*
yakuba, *D. erecta* and *D. pseudoobscura*

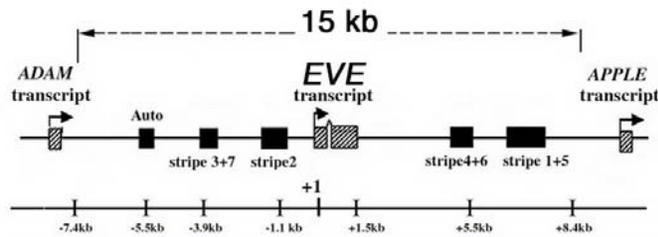
Construction of the *eve* stripe 2 enhancer deficiency mutant – *Df(eve s2e)*



As a transgene, the ~15kb region is capable of rescuing the *eve* null mutant flies to fertile adulthood

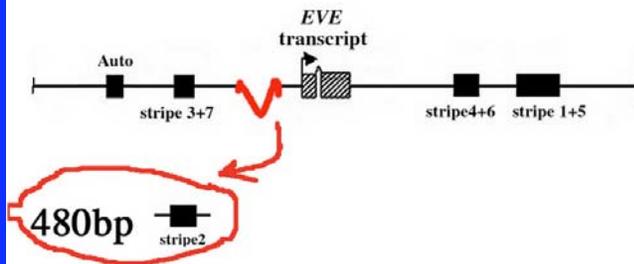
Miki Fujioka and Associates, 1999

Construction of the *eve* stripe 2 enhancer deficiency mutant – *Df (eve s2e)*



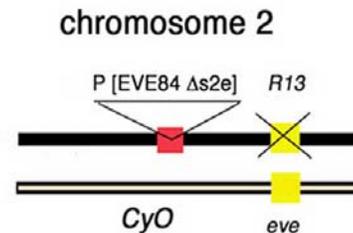
As a transgene, the ~15kb region is capable of rescuing the *eve* null mutant flies to fertile adulthood

Miki Fujioka and Associates, 1999



The transgene was recombined onto the *eve* null mutant chromosome

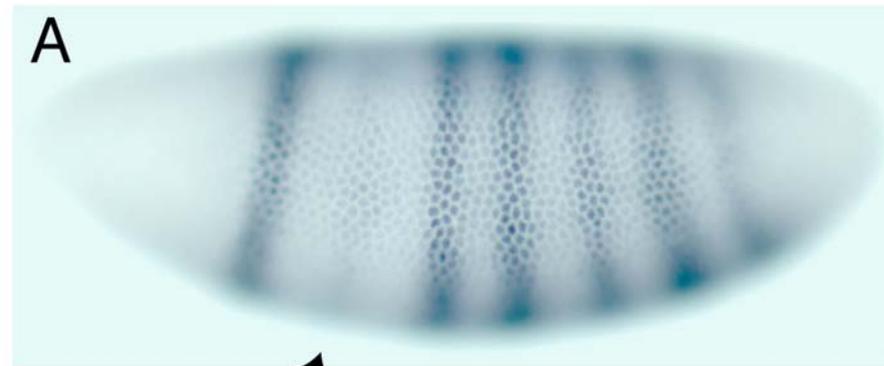
balanced stock
Df (eve s2e)



R13 = *eve*^{R13} (Gln 106 Ter)

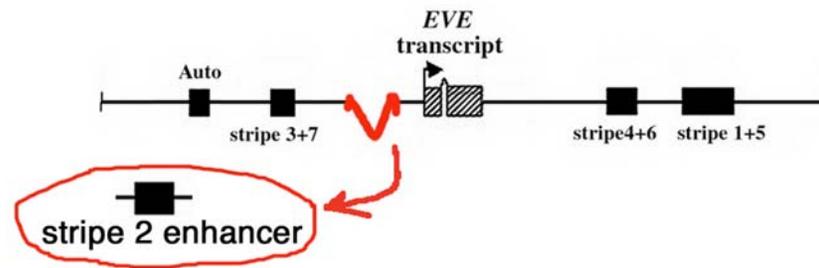
P [EVE84 Δs2e] =
eve -6.4 to +8.4 kb transgene
without stripe 2 enhancer

***Df* (*eve* stripe 2 enhancer) mutant:
A. *EVE* protein; B. Map of the *eve* locus**

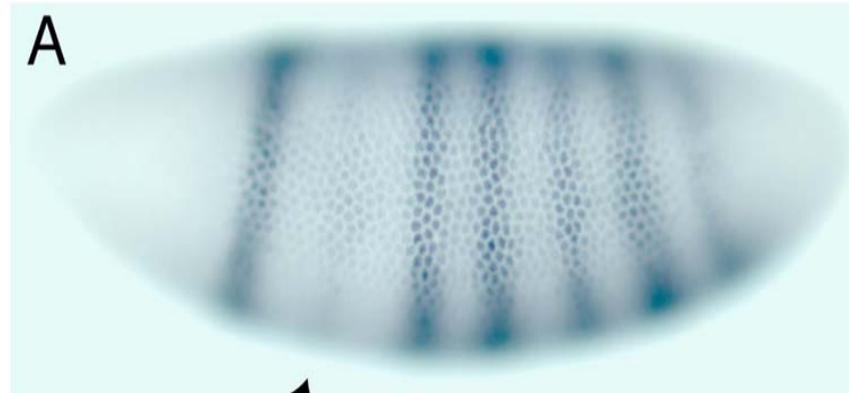


↑
even-skipped stripe 2

B



even-skipped (A) and *engrailed* (B)
in *Df* (*eve* stripe2 enhancer) mutant

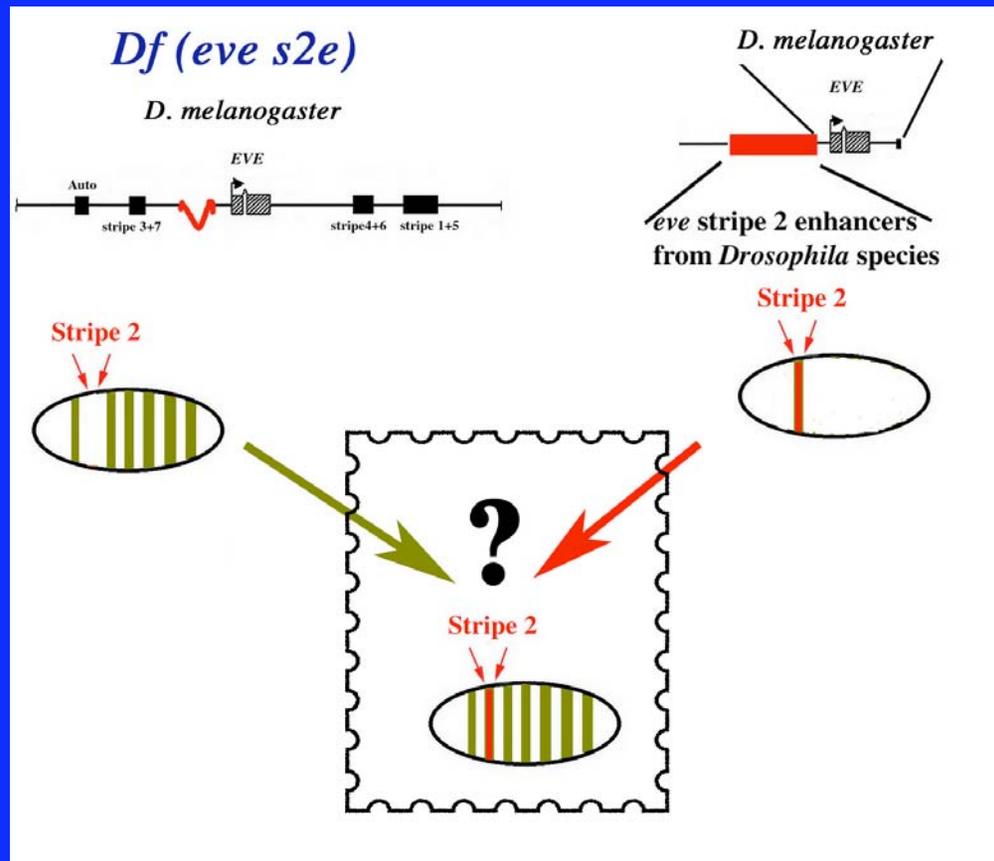


↑
even-skipped stripe 2



↑
engrailed stripe 4

Experiment to investigate *cis*-regulatory structure-function and sequence evolution



Questions

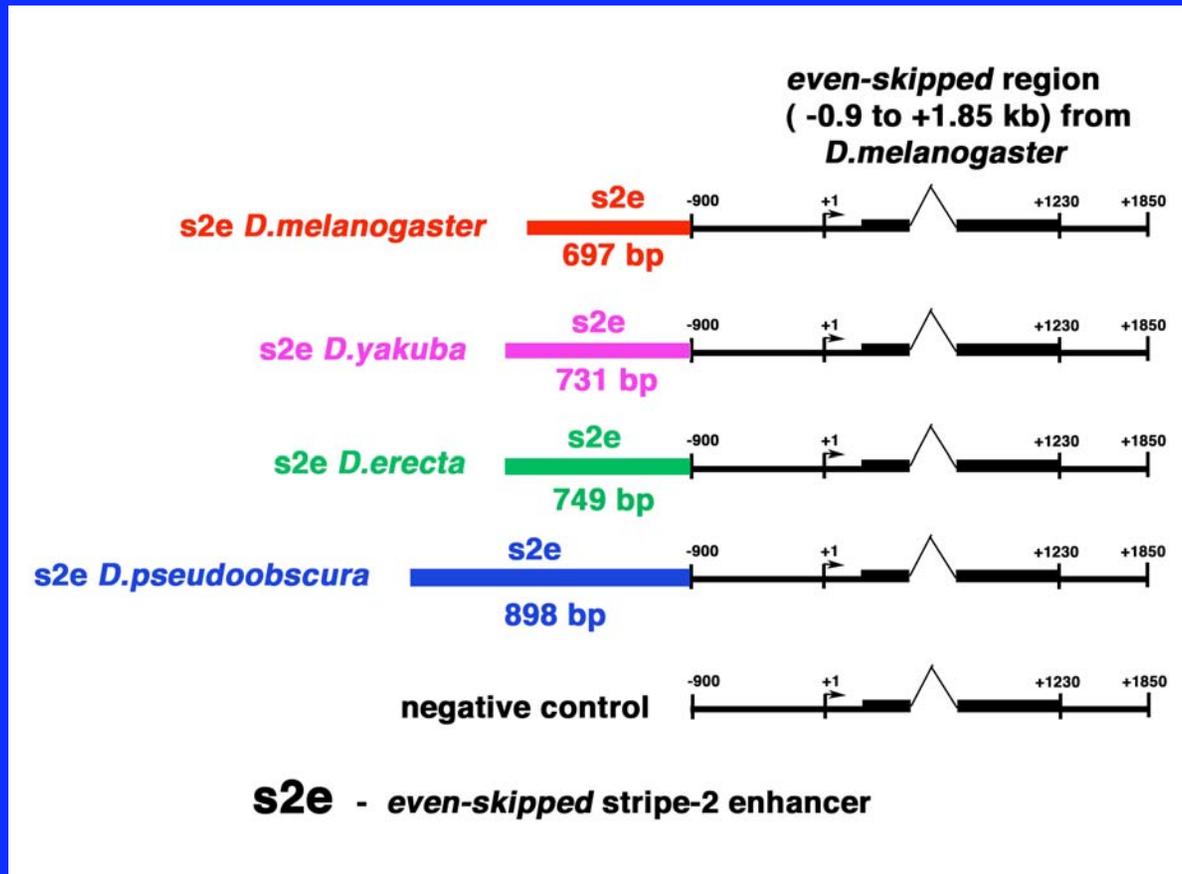
Can *eve* stripe 2 enhancers from different species rescue the phenotype of *Df(eve stripe2enhancer)* mutants?

Does rescue ability differ between species?

If there is functional evolution of s2e's, over what time-scale does it occur?

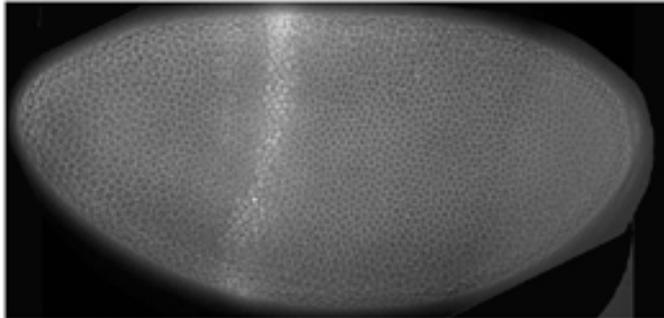
Rescue constructs

eve stripe-2 enhancers from *Drosophila* species drive expression of *D. melanogaster eve*

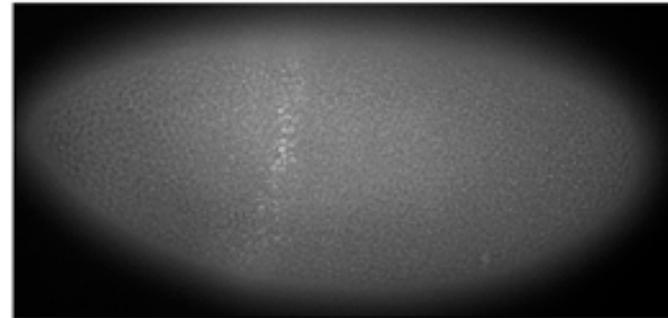


Expression of the transgenes containing *eve* stripe-2 enhancers from four species fused to *melanogaster eve* coding region (-0.9 to +1.85 kb) blastoderm stage; *eve* protein

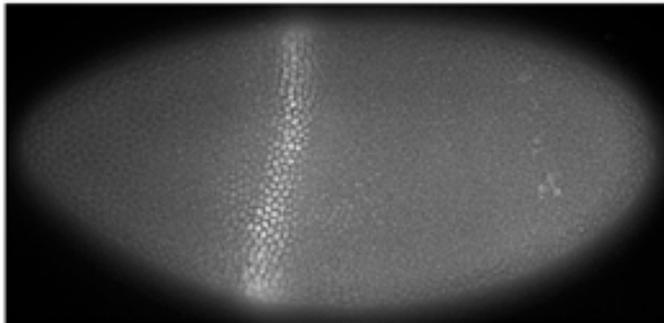
melanogaster s2e EVE



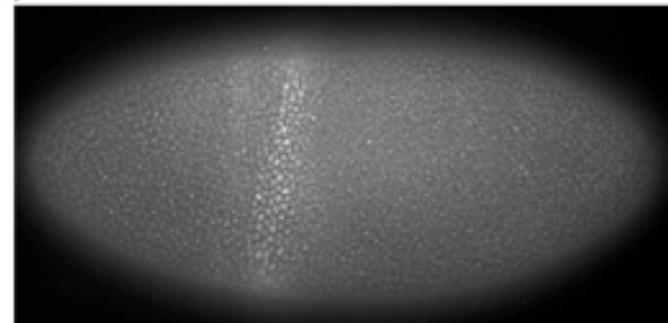
erecta s2e EVE



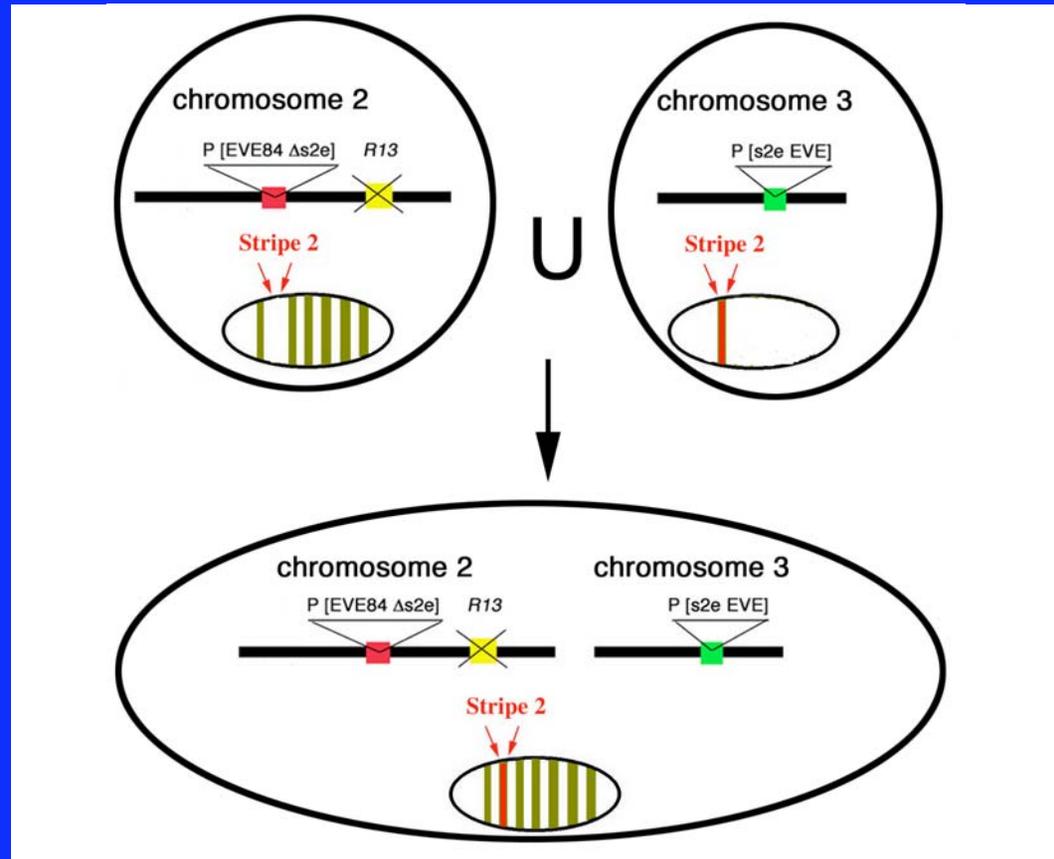
yakuba s2e EVE



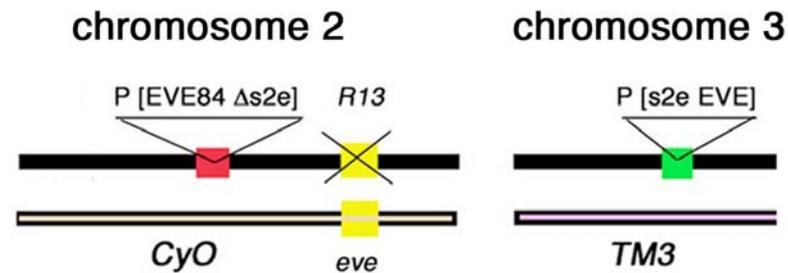
pseudoobscura s2e EVE



Genetic design



Genetic design

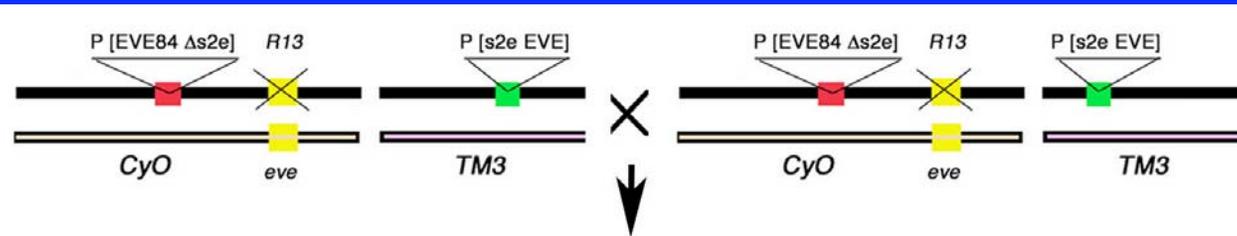


R13 = *eve*^{*R13*} (Gln 106 Ter)

P [EVE84 Δ s2e] = *eve* -6.4 to + 8.4 kb transgene
without stripe 2 enhancer

P [s2e EVE] = transgene containing stripe 2 enhancer
from different species fused to *eve*
coding region (- 0.9 to +1.85)

The cross to rescue of *Df(s2e)* mutant

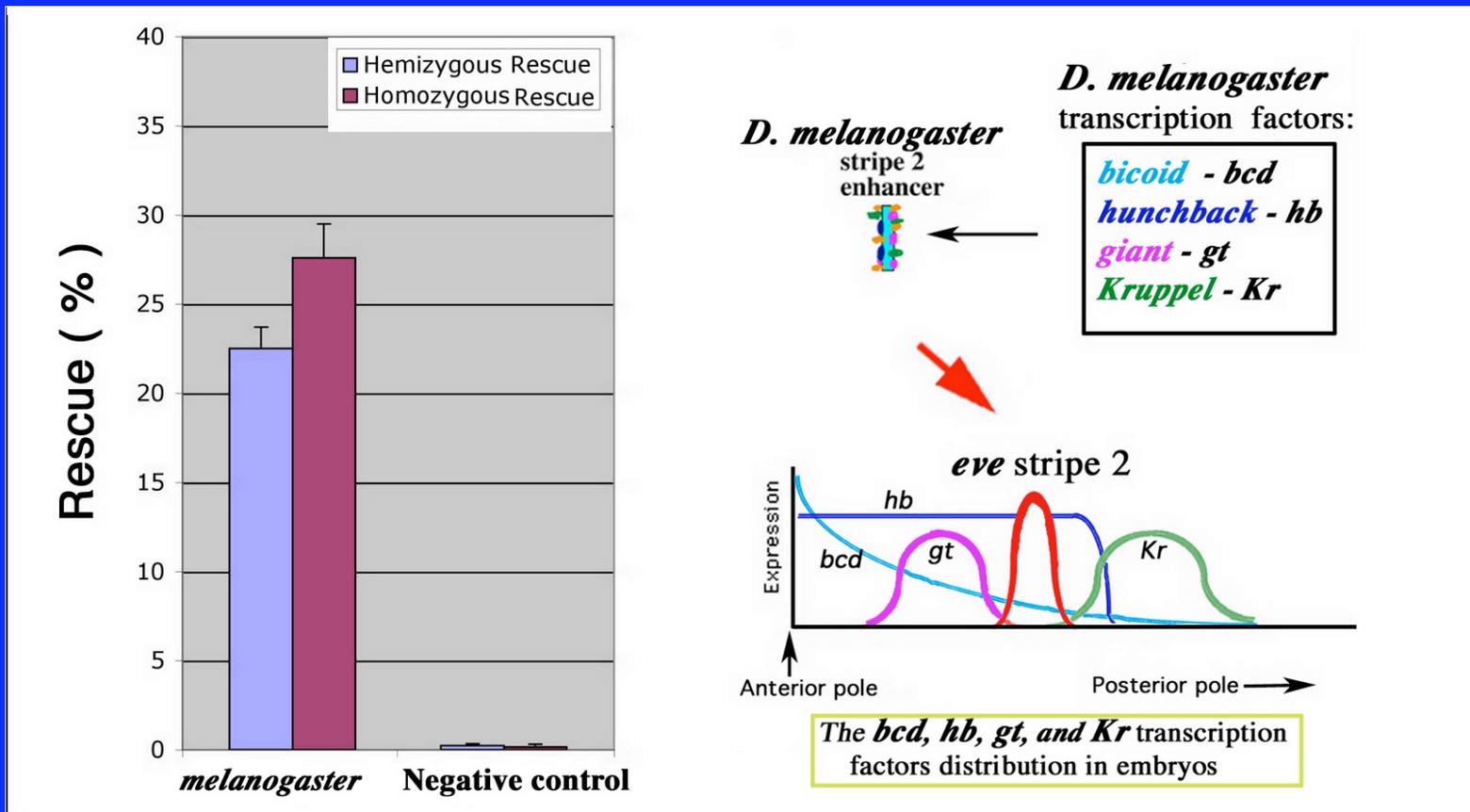


Offspring	Expected frequency	
	4 / 9	
	2 / 9	
	2 / 9	1 copy Rescue Hemizygous
	1 / 9	2 copies Rescue Homozygous

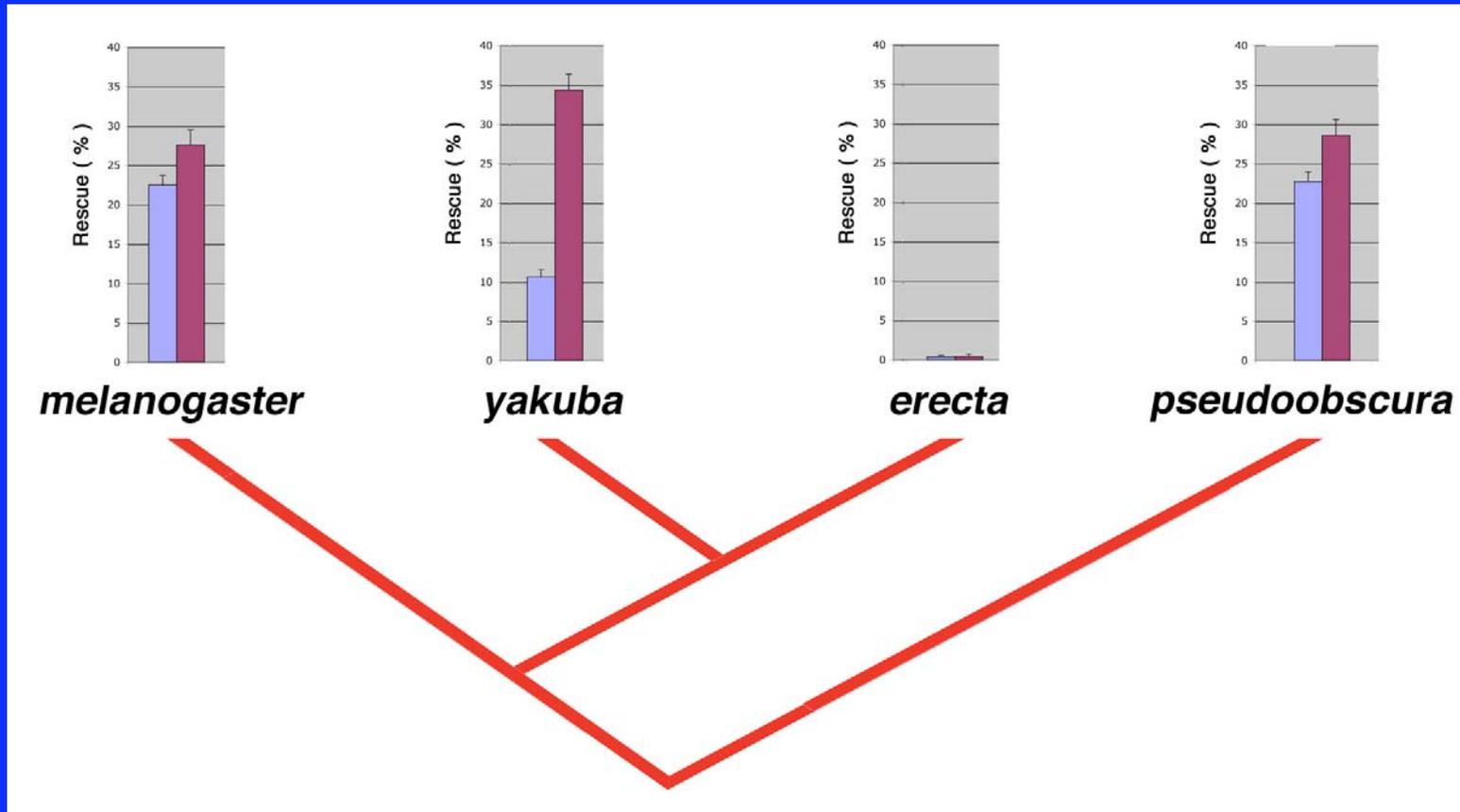
Number of Adults

Sex	Genotype	51 yak	51 x 74 yak	74 x 51 yak	74 yak	YAK sum
M	Cy; TM3	1711	1131	1065	1079	4986
F	Cy; TM3	1751	1098	1168	1106	5123
M	Cy; N	592	571	502	517	2182
F	Cy; N	586	589	537	526	2238
M	N; TM3	25	46	55	74	200
F	N; TM3	30	68	68	86	252
M	N; N	37	59	90	86	272
F	N; N	79	103	114	91	387

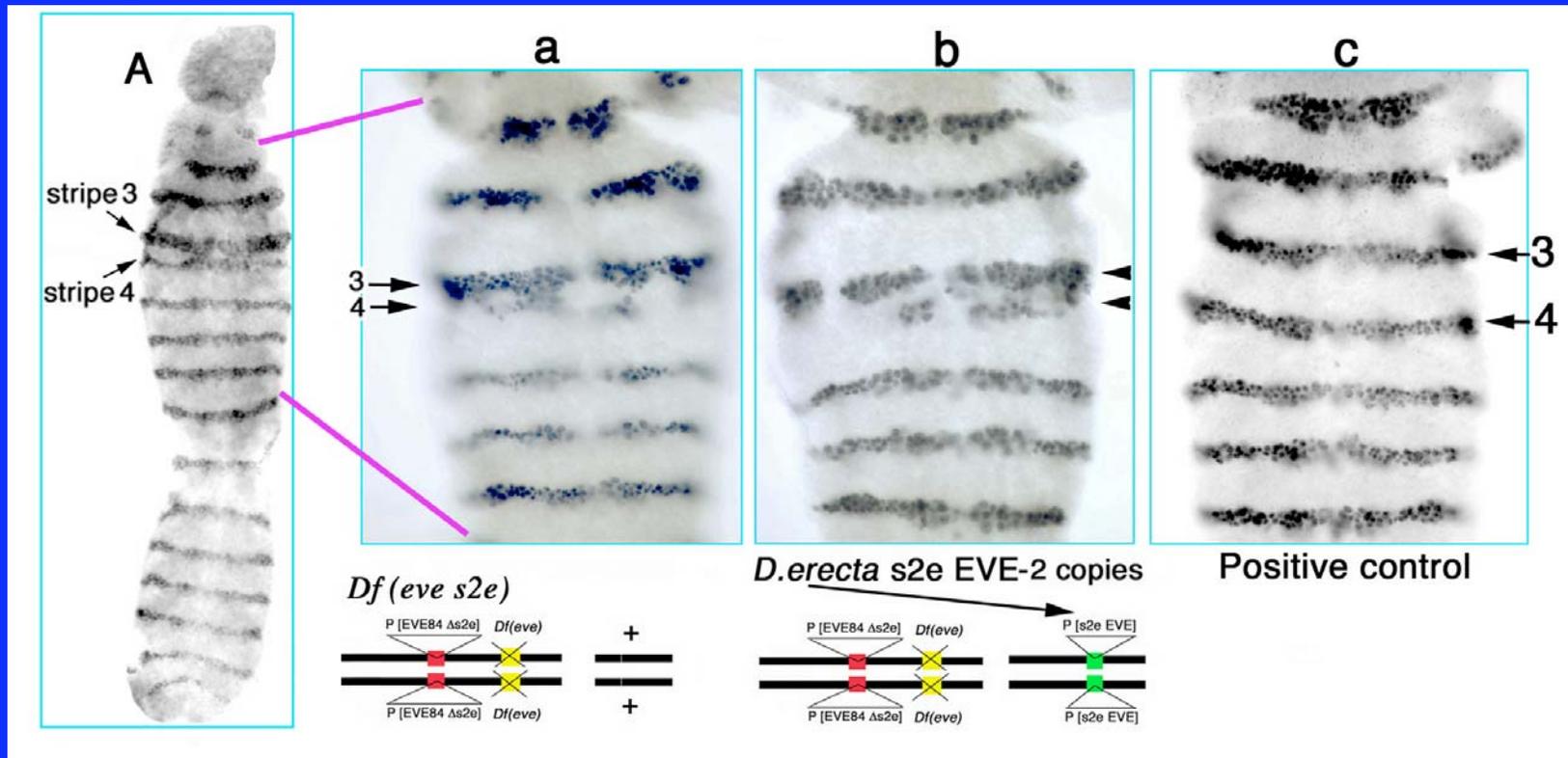
eve s2e from *D. melanogaster* Rescue Ability



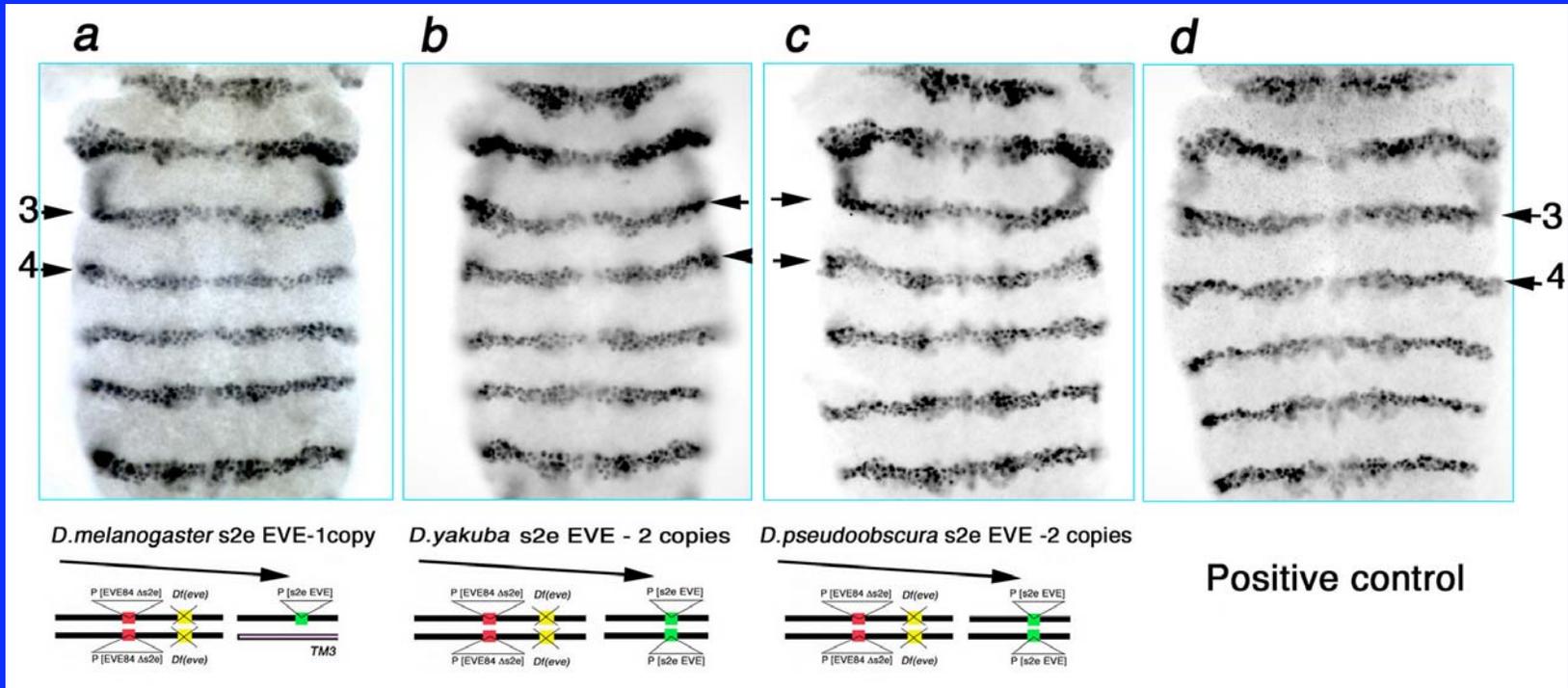
eve s2e from four *Drosophila* species Rescue Ability



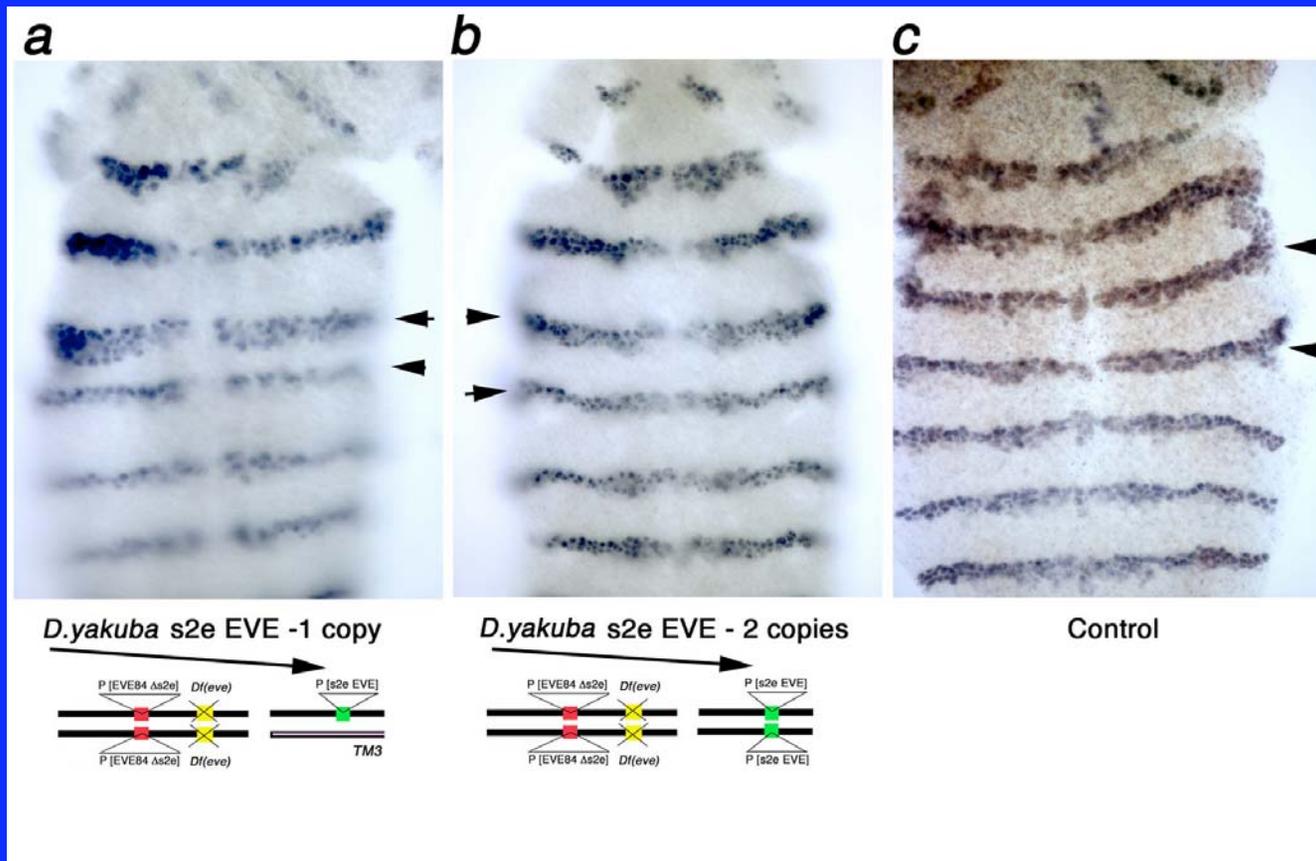
engrailed in *Df(s2e)* mutant (A; a) +
P [erecta s2e EVE] (b)



**engrailed in *Df(s2e)* mutant +
**P [*melanogaster s2e* EVE](a), P [*yakuba s2e*
EVE](b), and P [*pseudoobscura s2e* EVE](c)****



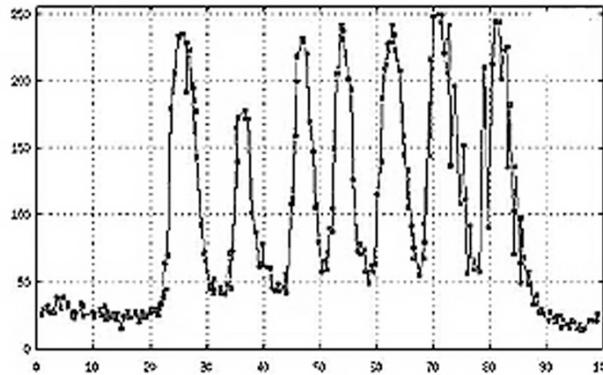
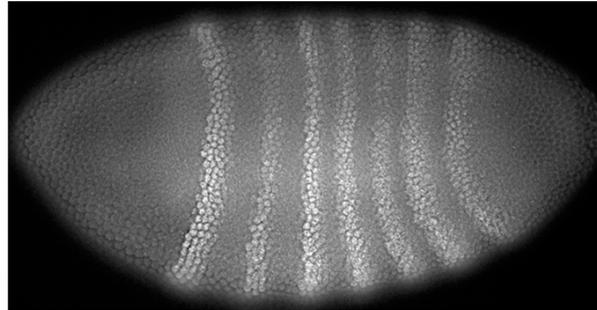
engrailed in *Df(s2e)* mutant +
P [yakuba s2e EVE]

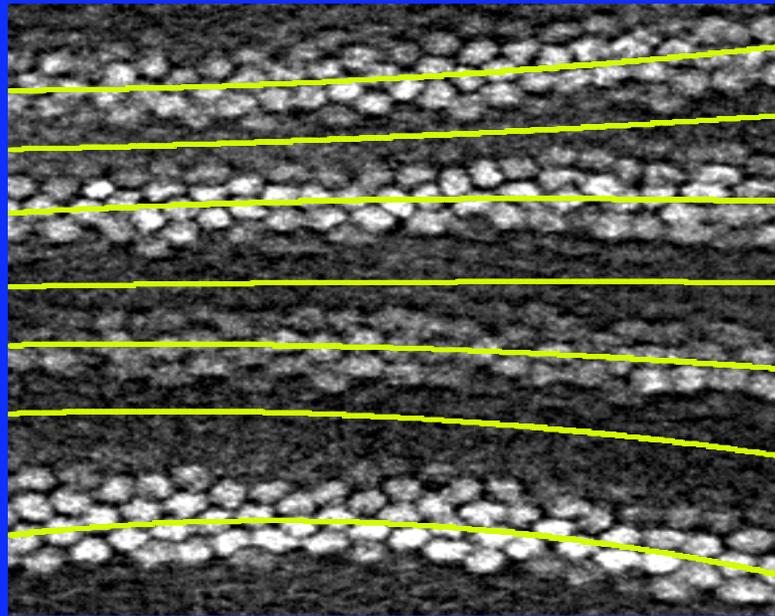
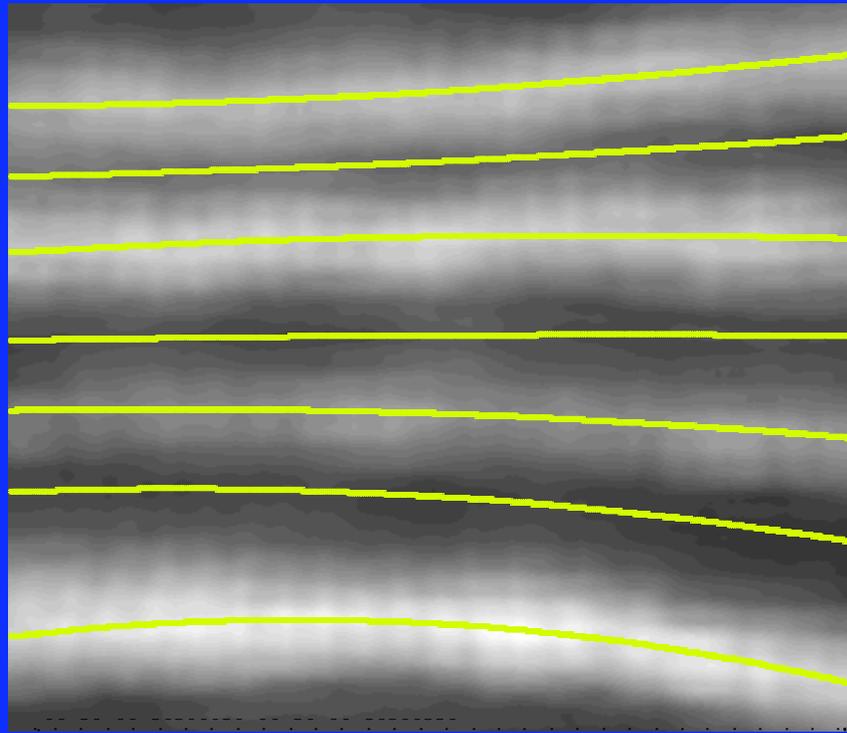


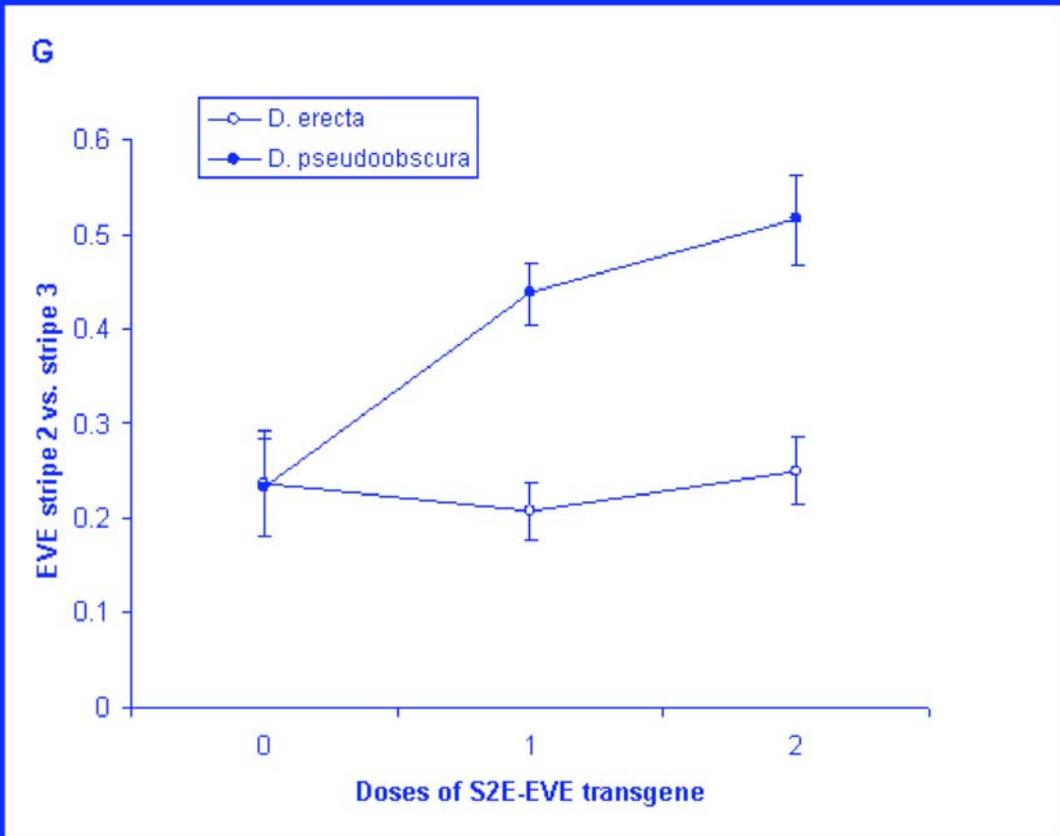
Conclusions

1. Engrailed expression in 5-hour old embryos correlates with adult viability.
2. The lethality of the *D. erecta s2e eve* rescue transgene is almost certainly caused by the inability of the weak stripe 2 it produces to correctly regulate downstream developmental processes.
3. The mild viability and morphological effects seen in the *D. yakubai s2e eve* 1-copy rescue genotype suggests that modulation of these very early developmental landmarks CAN influence traits later in development. Do they contribute to quantitative trait variation?

Eye Stripe Quantification







Conclusions

1. Stripe expression is additive (with gene dose), as expected.
2. The lethality of the *D. ere s2e eve* rescue transgene is almost certainly its inability to turn on *eve* expression in *D. melanogaster*.

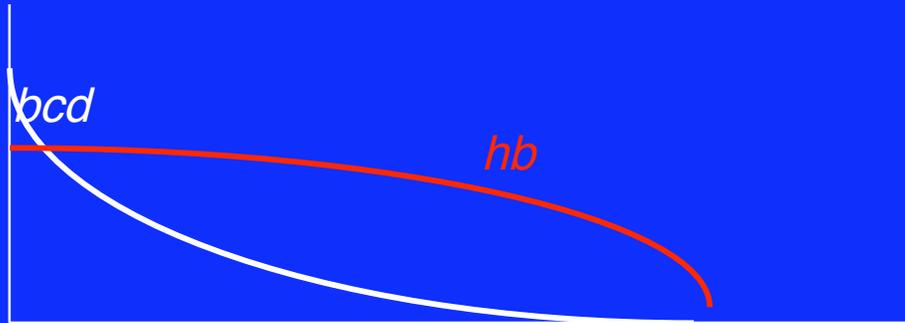
Question:

Do *D. erecta* and *D. melanogaster* have different morphogen gradients?

Organization of Talk

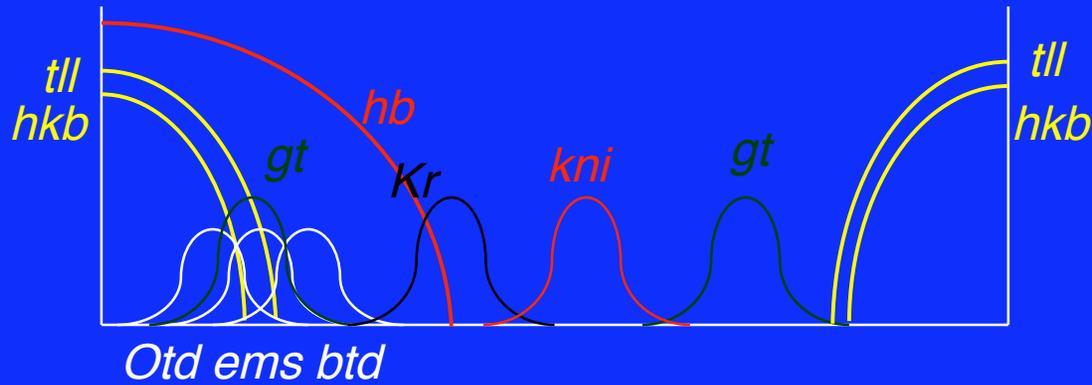
1. Brief review of enhancer structure/function.
2. Introduction to embryogenesis and segmentation in the fruitfly.
3. Experiments on structure/function and evolution of the *even-skipped* stripe 2 enhancer (*eveS2E*).
 - Reporter transgene experiments with native and chimeric *eveS2E*'s.
 - Functional replacement of the native *eveS2E* with the enhancer from other species.
4. Canalization of *eveS2E* gene expression within and between species.

A



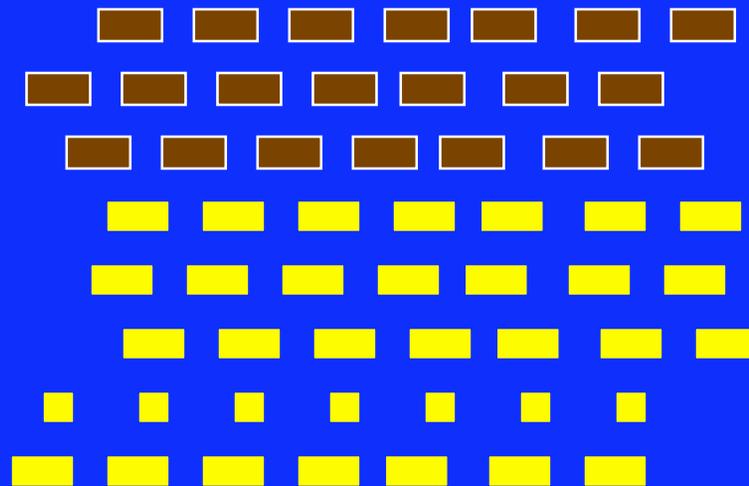
P

maternal



gap

run
h
eve
ftz
opa
odd
slp
prd

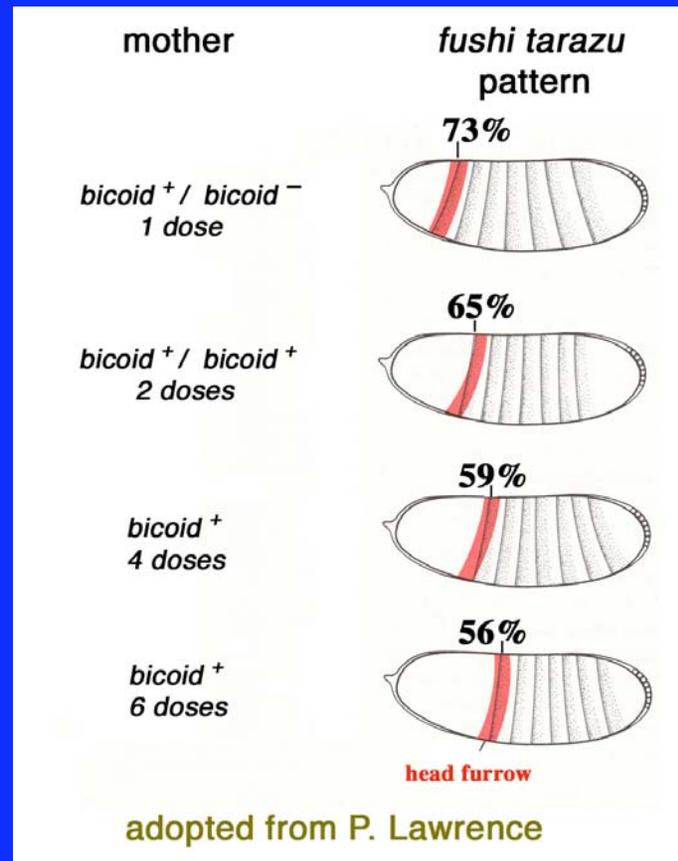


Primary pair-rule

Secondary pair-rule

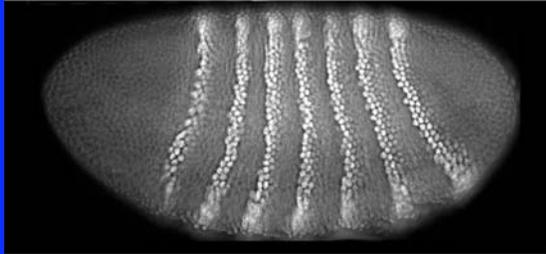
From: Pankratz & Jackle (1993)

Quantitative relationship between the number of *bicoid*⁺ genes in the mother and the pattern of the embryo

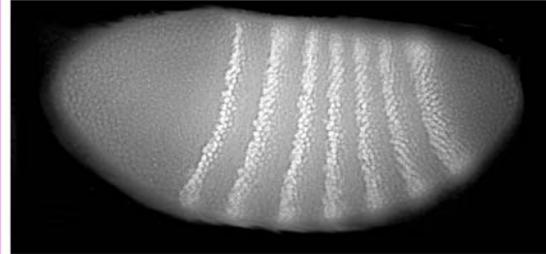


even-skipped in *D.erecta* and *D.melanogaster*
stage: late blastoderm

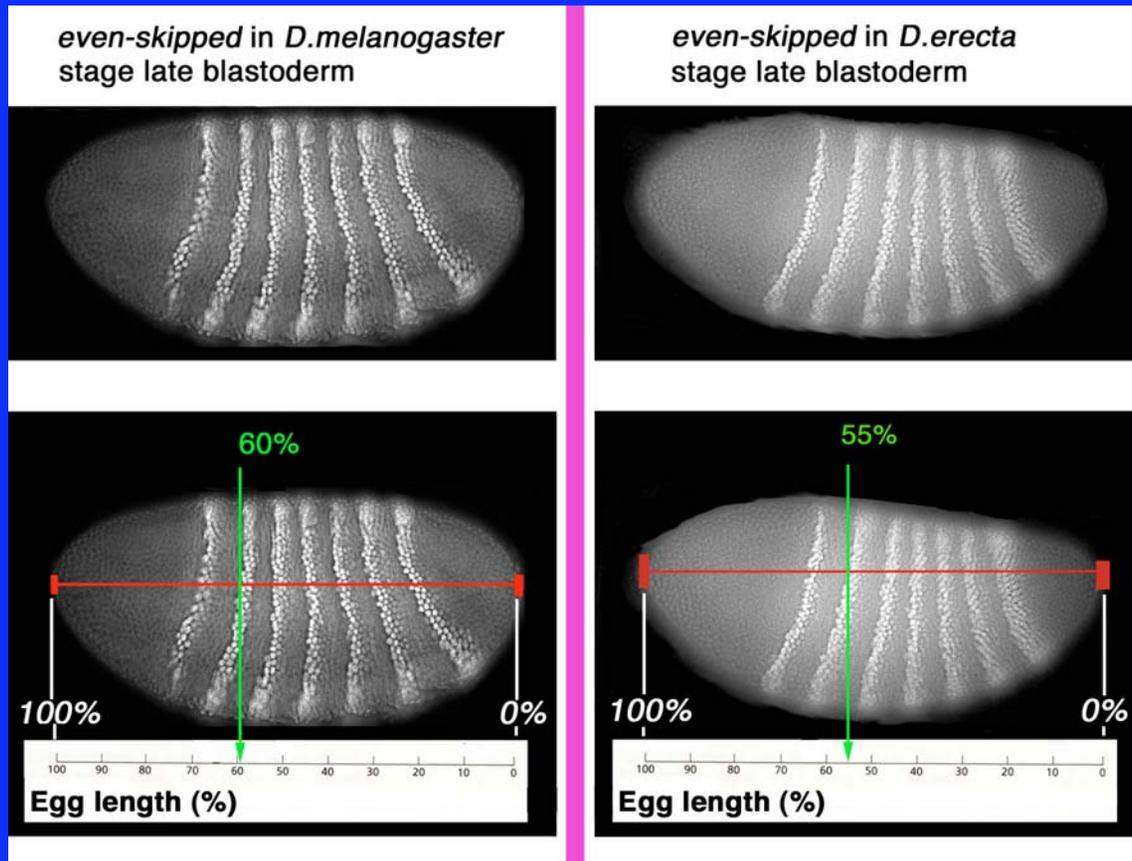
even-skipped in *D.melanogaster*
stage late blastoderm



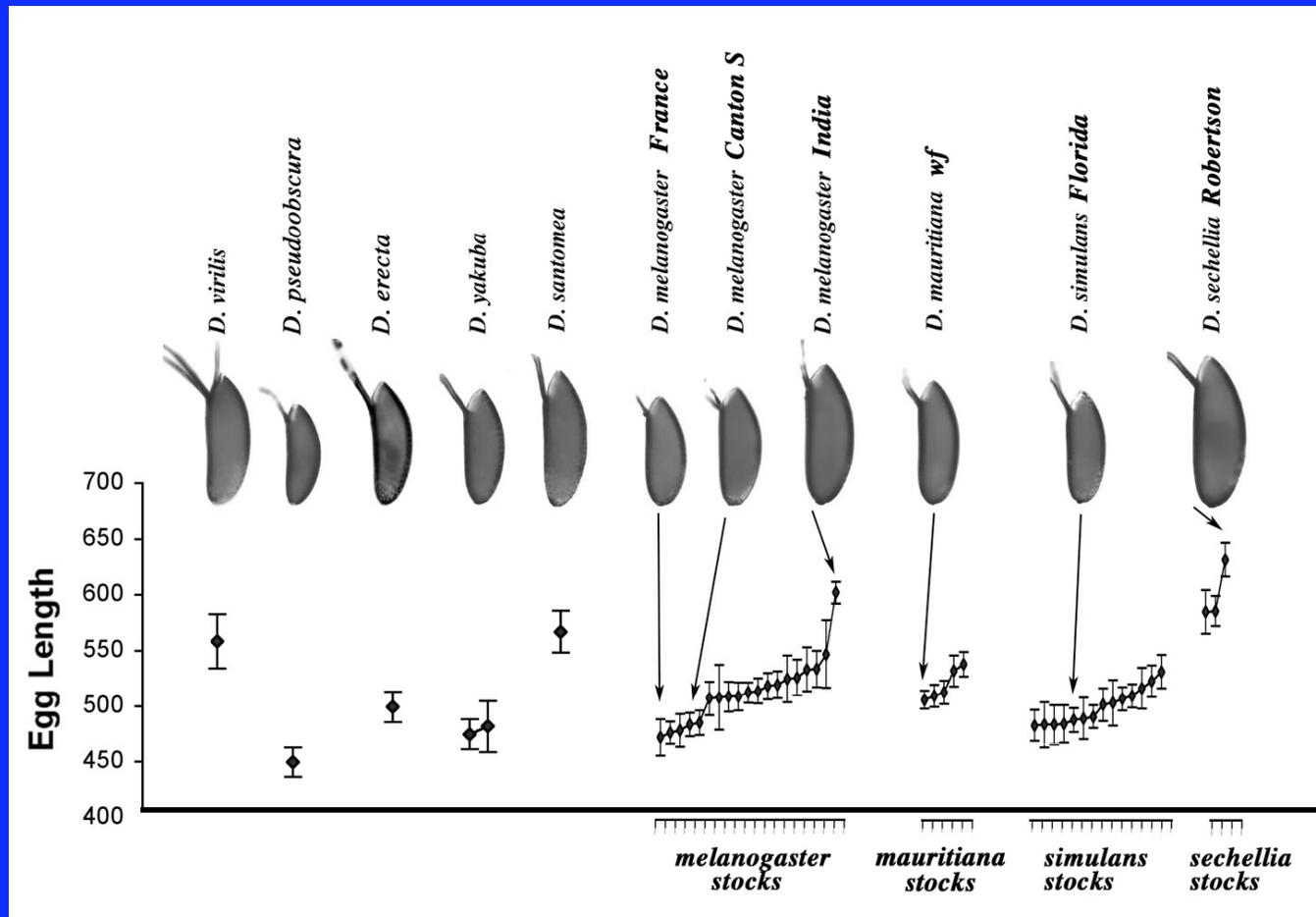
even-skipped in *D.erecta*
stage late blastoderm



even-skipped in *D.erecta* and *D.melanogaster*
stage: late blastoderm



Egg size variability within and between *Drosophila* species

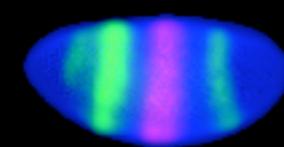
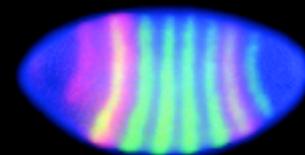


eve kr

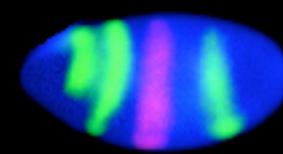
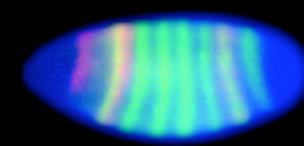
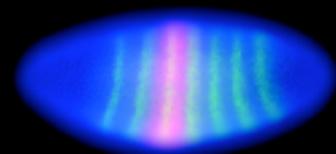
eve gt

gt kr

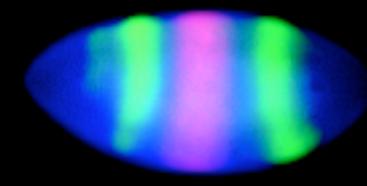
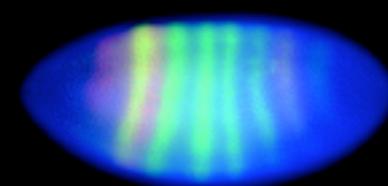
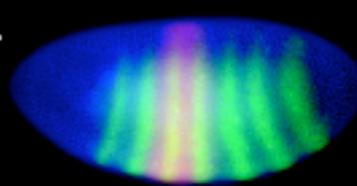
D.melanogaster
Canton-S



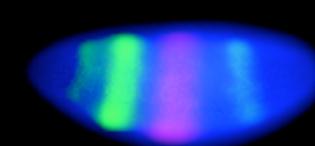
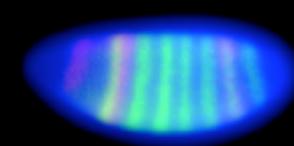
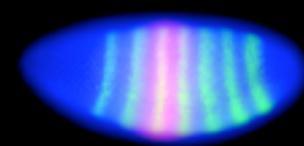
D. melanogaster
France



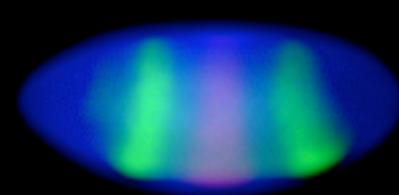
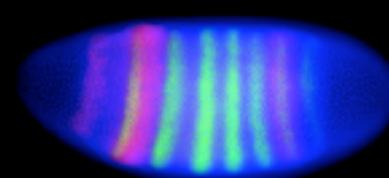
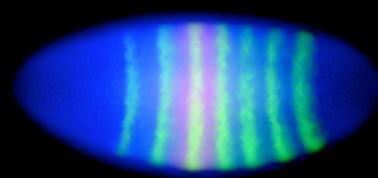
D. melanogaster
India



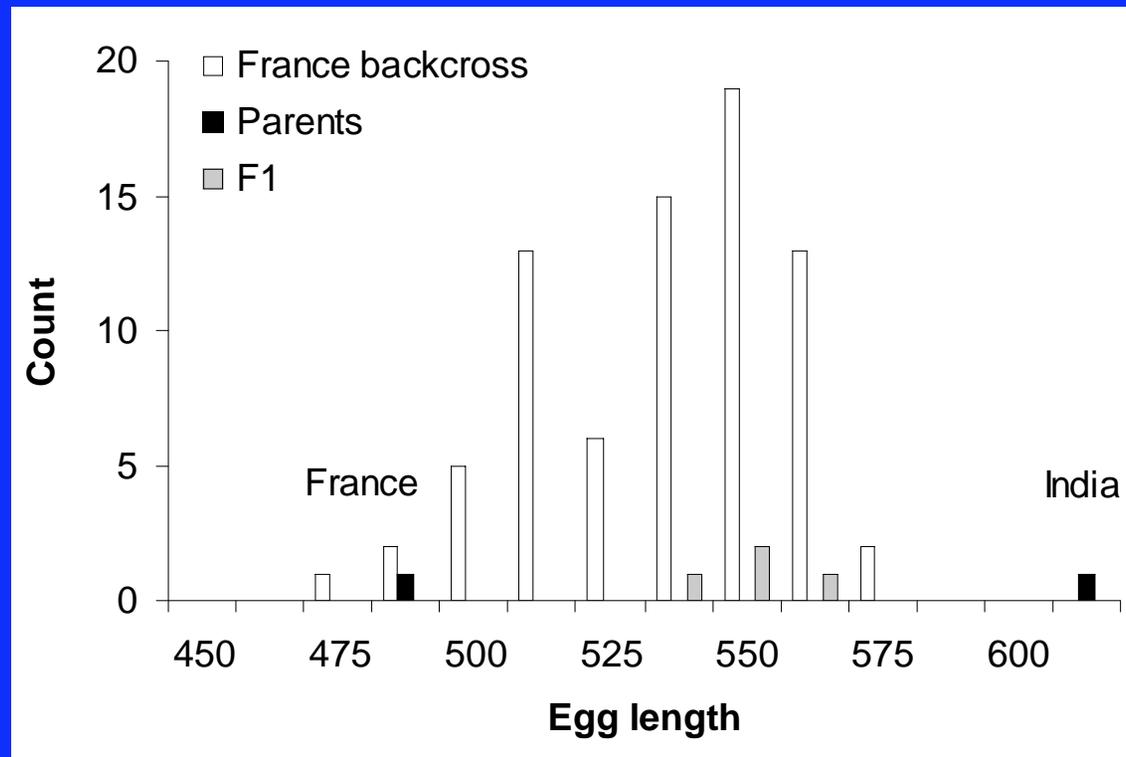
D. simulans



D. sechellia

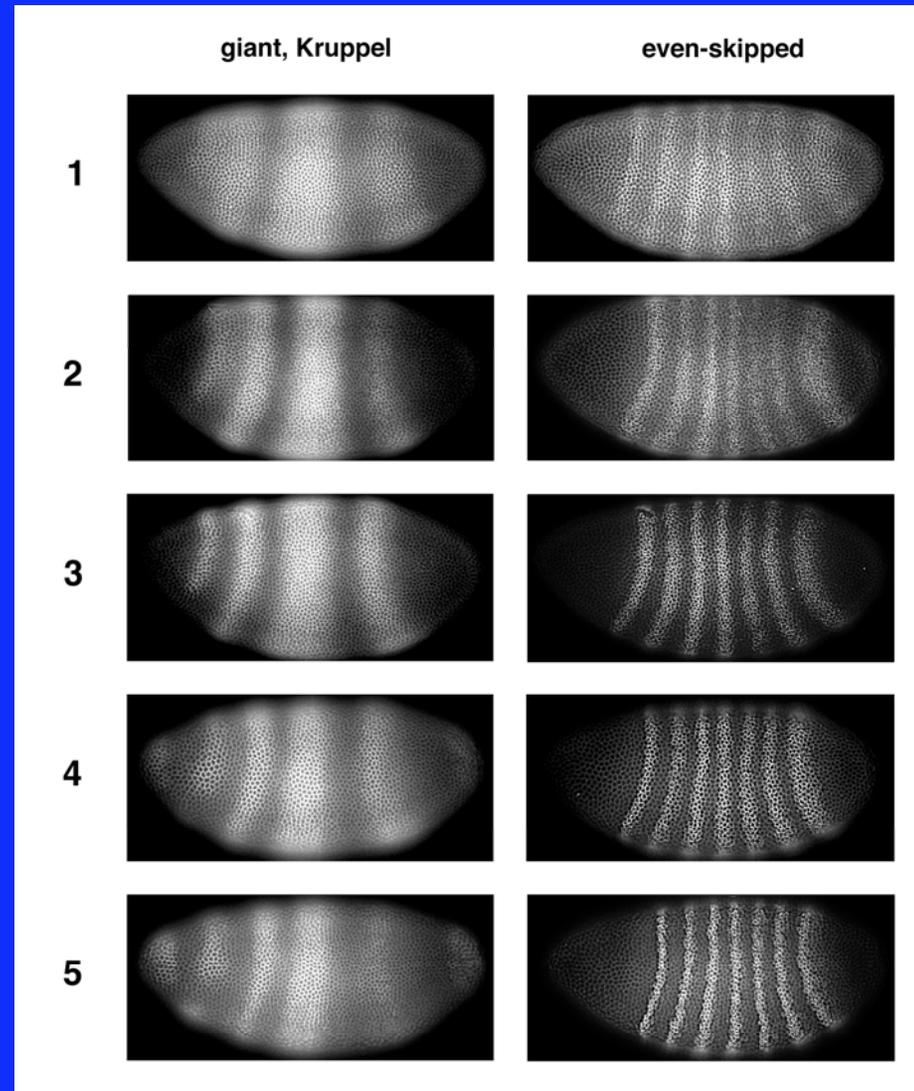


Genetic analysis of egg size between France and India lines

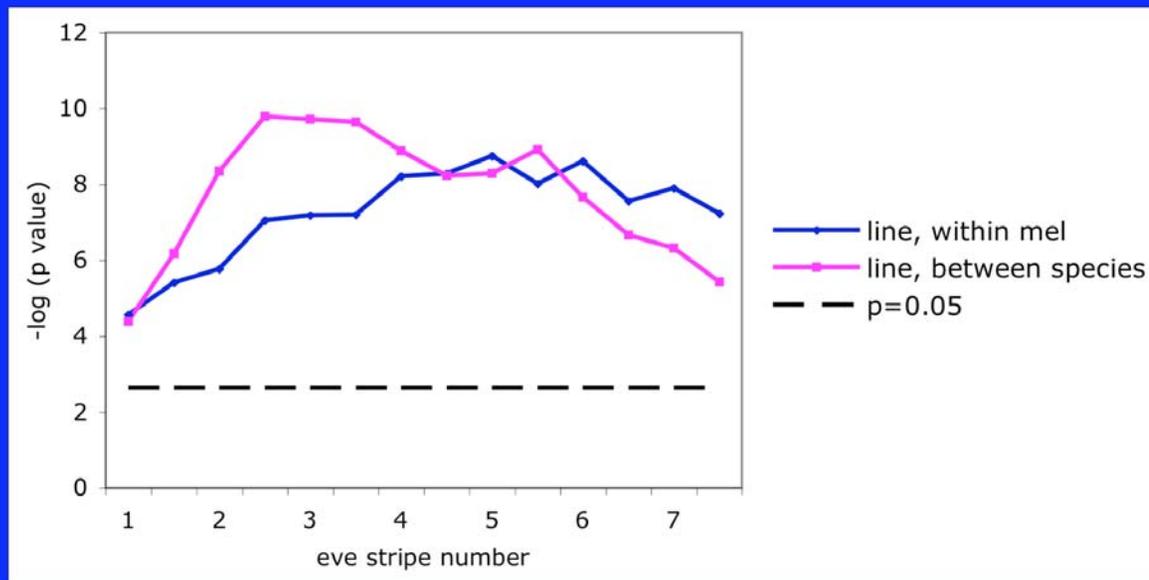
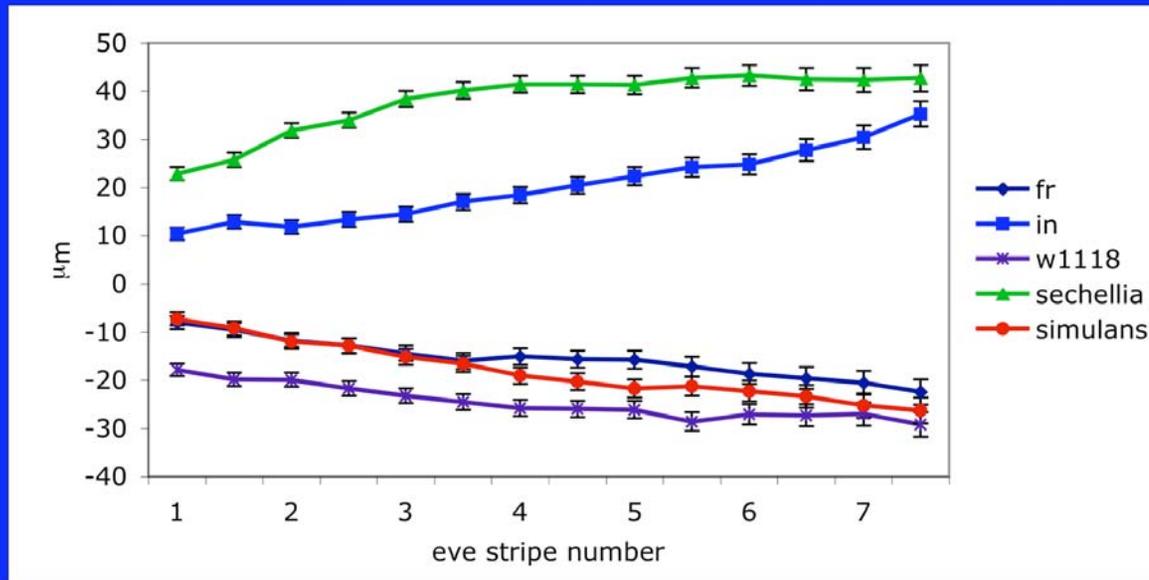


Egg size is a maternally inherited quantitative trait with additive effects

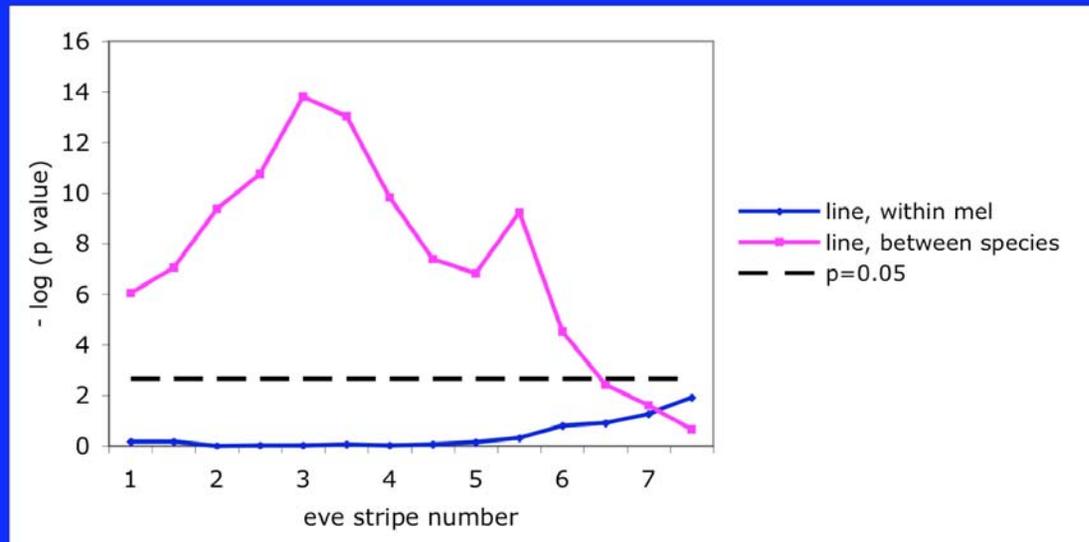
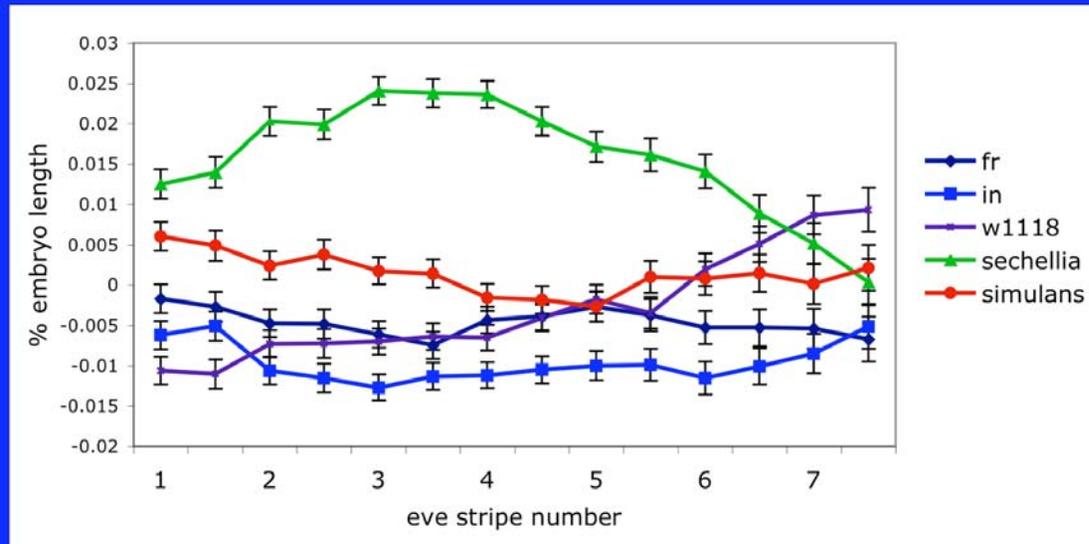
Stage classification



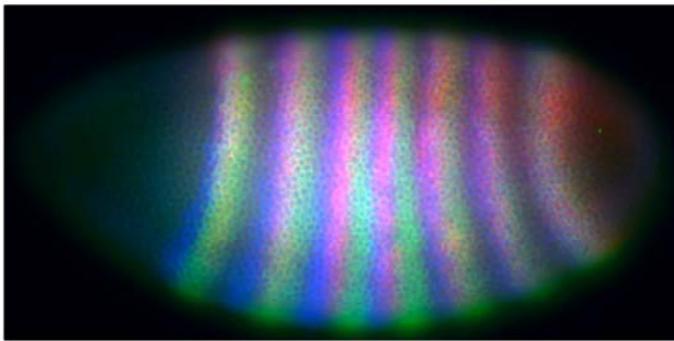
Eve stripe boundaries, absolute distances



Eve stripe boundaries, scaled to embryo length



Overlay of embryos, scaled to same size



D. melanogaster



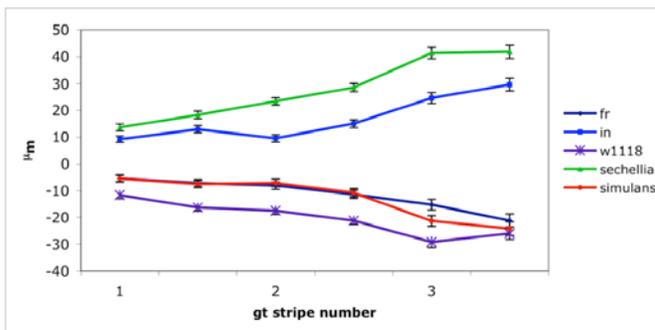
D. simulans



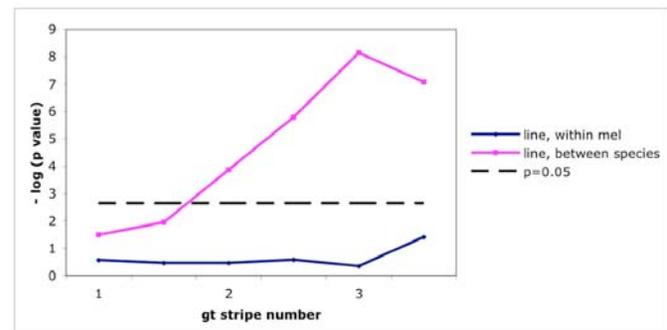
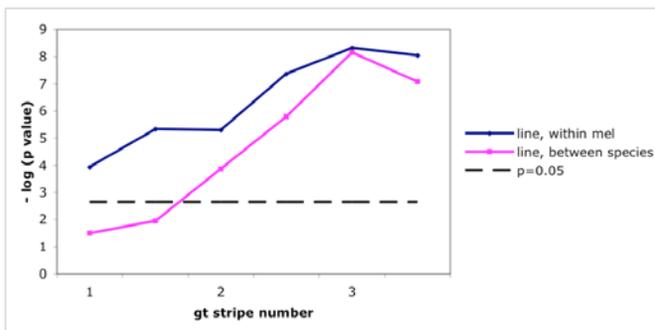
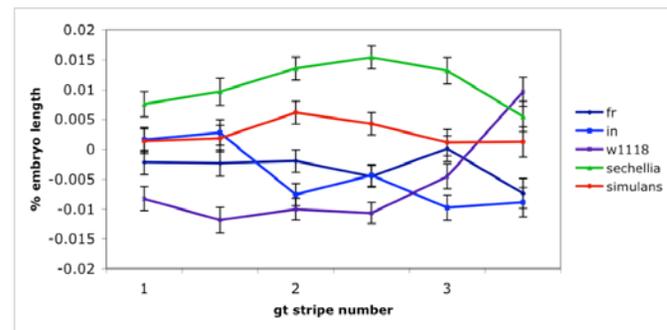
D. sechellia

Eve stripes 1 and 7 are anchored at nearly same location
Note the sizable posterior shift of middle stripes in *D. sechellia*

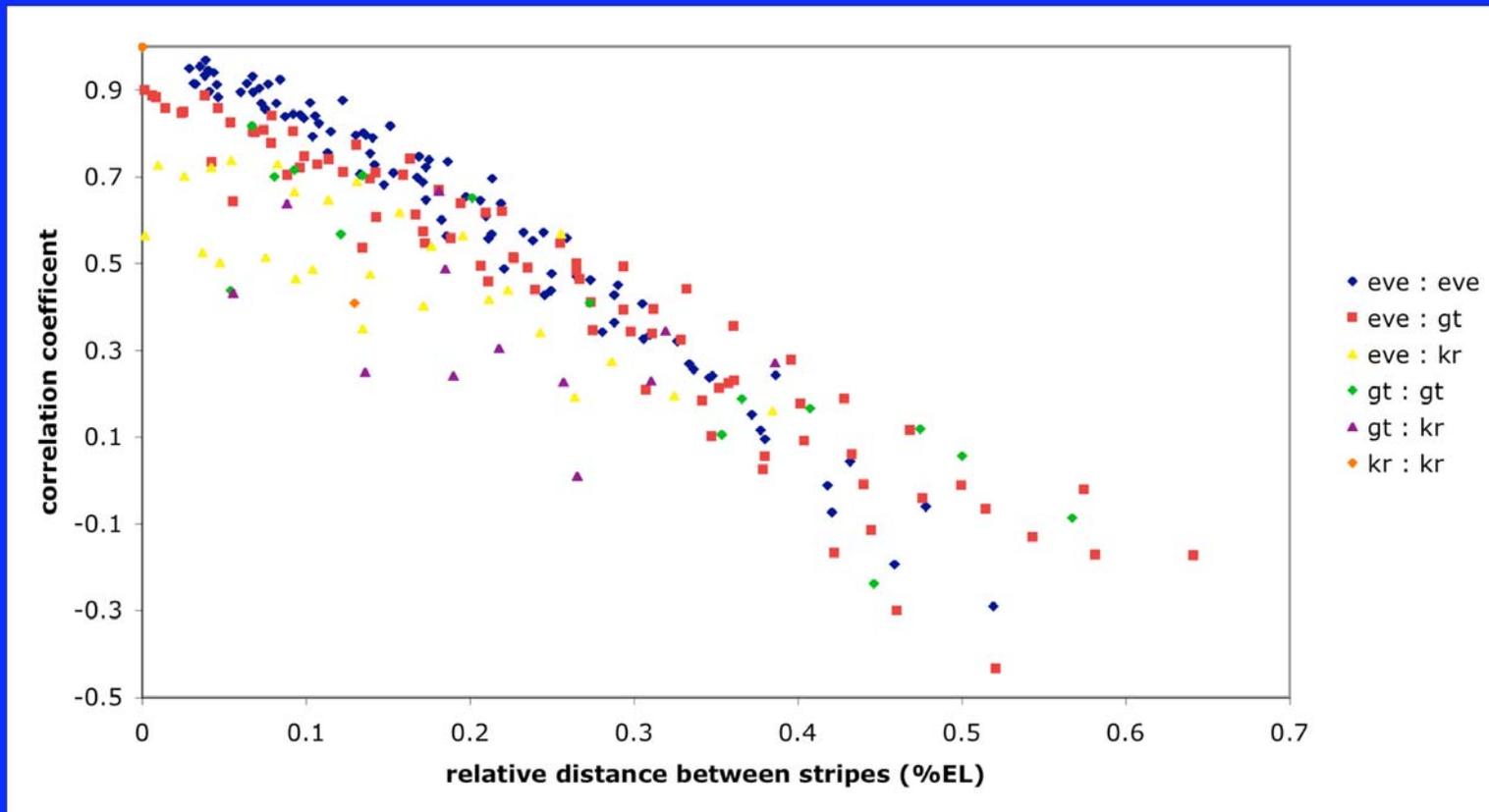
Absolute distance from mean stripe position



Relative distance from mean stripe position



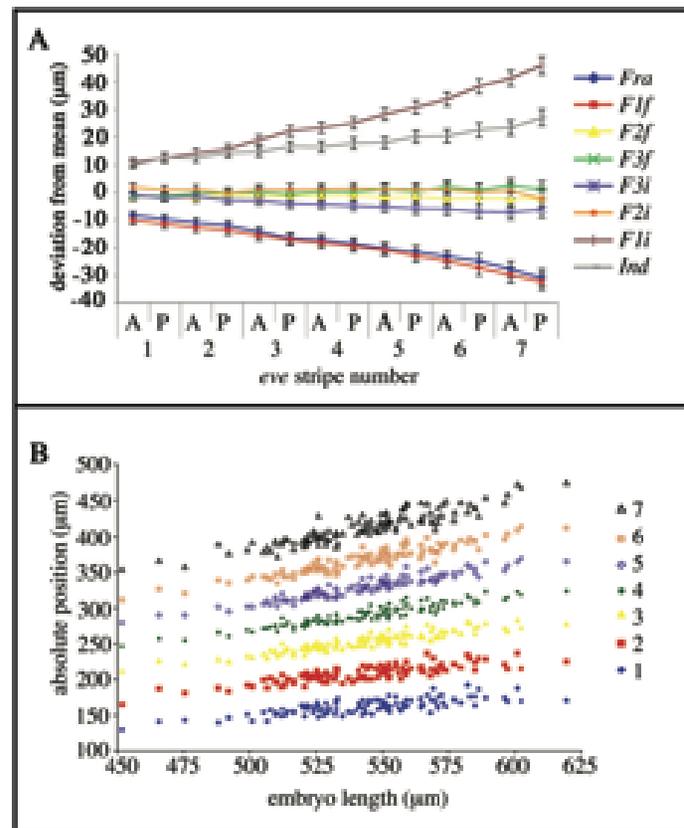
Spatial autocorrelation of stripe locations within embryos



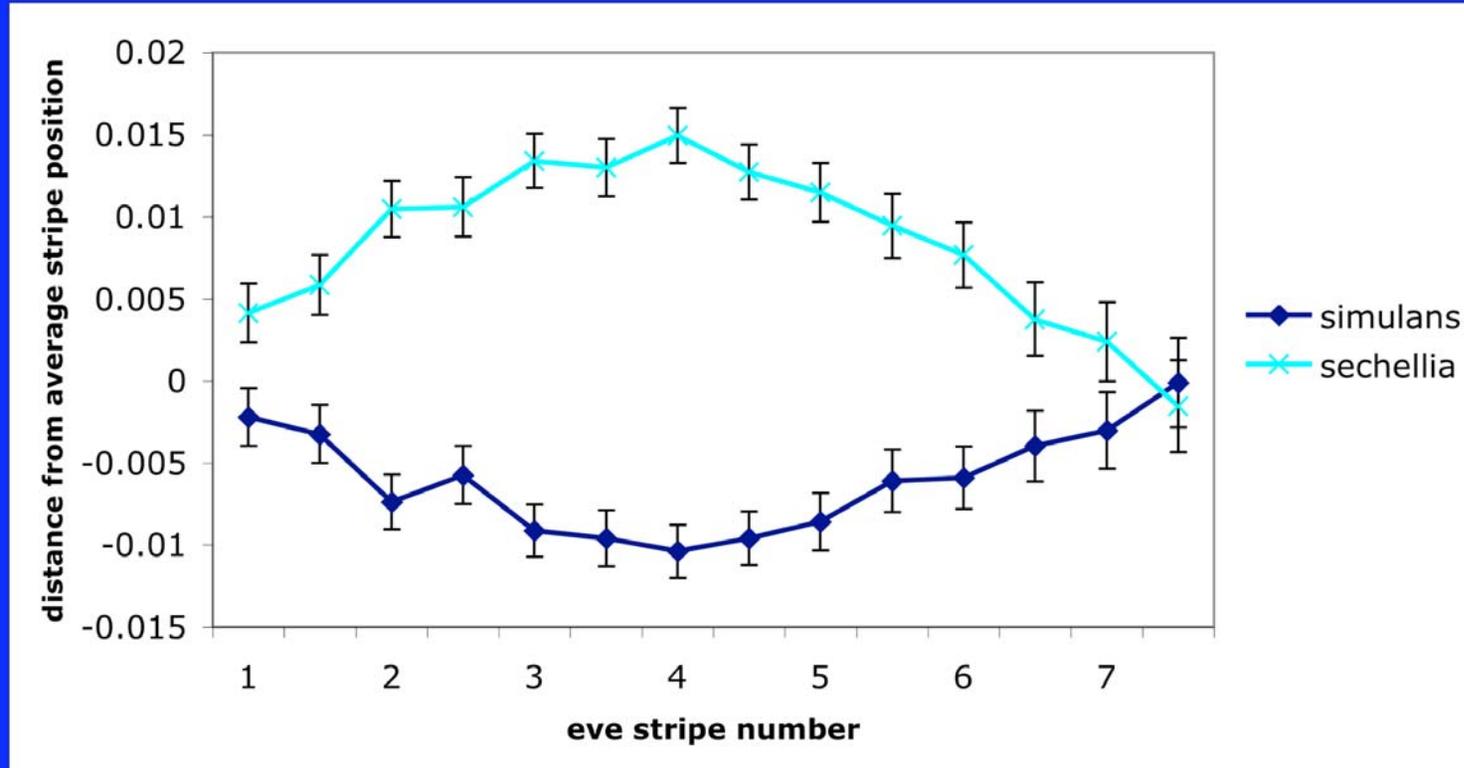
Adjacent stripe locations are highly correlated

Stripes at some distance from one another are not correlated

Genetic analysis, absolute stripe boundary locations

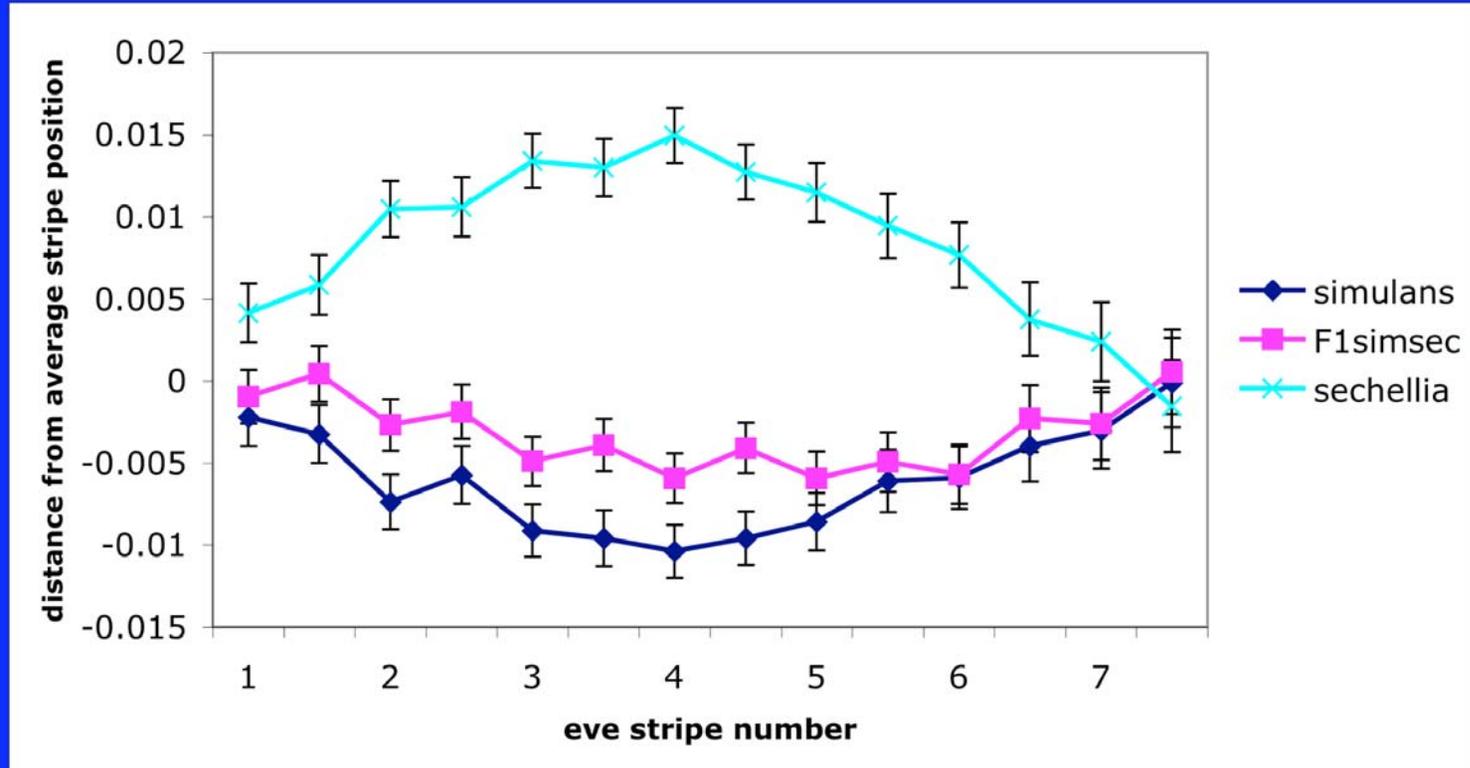


Genetic analysis of *D. simulans* - *D. sechellia* stripe Location difference



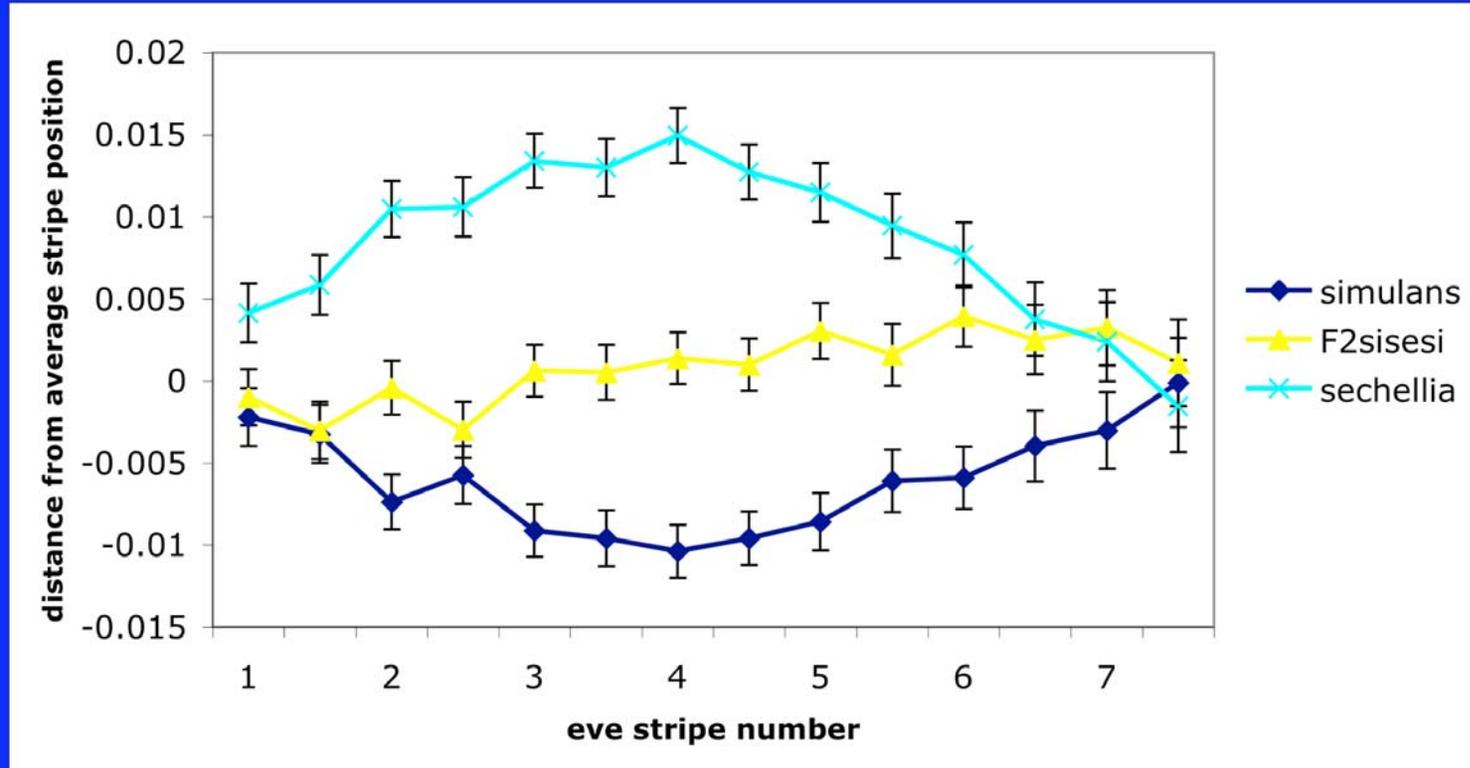
Stripe location is maternally inherited with additive effects

Genetic analysis of *D. simulans* - *D. sechellia* stripe Location difference



Stripe location is maternally inherited with additive effects

Genetic analysis of *D. simulans* - *D. sechellia* stripe Location difference



Stripe location is maternally inherited with additive effects

Summary

- *Cis*-regulatory modules, as exemplified by the *eS2E*, evolve relatively quickly in sequence and function.
 - *Chimera mis-expression*
 - Replacements between closely related species don't complement
- Eve stripe positions are buffered against genetic variation for embryo size within species
- Setpoints for stripe positioning changes rapidly, even between very closely related species

Current and Future directions



- Investigate s2e expression differences in reciprocal species transformations
- Implement site-specific replacement technology for the s2e
- Investigate the genetic basis for evolution of segmentation setpoints
- Are enhancers themselves responsible for establishing robustness in segmentation?

Acknowledgements

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NATALIA TAMARINA

JEFFERSON UNIVERSITY:

MIKI FUJIOKA

SUNY STONY BROOK :

JOHN REINITZ AND MEMBERS OF HIS LAB

eve protein in stripe two of embryos:

a. *Df(eve s2e)*

b. *Df(eve s2e)* + 2 copies of P [erecta s2e EVE] embryo

c. *Df(eve s2e)* + 1 copy of P [yakuba s2e EVE] embryo

