Hybrid sterility and hybrid inviability: "postzygotic isolation"

Permanent reproductive isolation?

Post-mating barriers

Darwin argued that hybrid sterility was not an adaptation (nor a God-given "isolating mechanism"), in his chapter 8 Hybridism:

"Now do these complex and singular rules indicate that species have been endowed with sterility simply to prevent their becoming confounded in nature? I think not. … "

"The foregoing rules and facts, on the other hand, appear to me clearly to indicate that the sterility, both of the first crosses and of hybrids is simply incidental or dependent on unknown differences, chiefly in the reproductive systems, of the species which are crossed."

Today, we'd tend to agree with this, and *we are now beginning to understand how hybrid incompatibilities might evolve.*

Post-mating barriers
(hybrid sterility & inviability)

Coyne & Orr 2004 (and others) argued that once hybrid sterility and inviability evolve, it is like “closing the barn door” on speciation. There’s no return.

But what sorts of genetic effects lead to hybrid sterility and inviability?

And are these barriers really so stable to gene flow?

Types of post-mating barriers (hybrid sterility & inviability)

Genic:

Dobzhansky-Muller incompatibilities (DMIs)

Duplications/translocations of genes

Heterozygote disadvantage (“underdominance”), peak epistasis

Chromosomal:

Underdominance of chromosomal rearrangements

Accumulation of DMIs in low recombination regions

Genomic effects (e.g. meiotic pairing)

Epigenetic dysregulation (e.g. transposable elements)

Reifová et al. 2023 Cold Spring Harb Perspect Biol

Possible types of 1- and 2-locus post-mating barriers

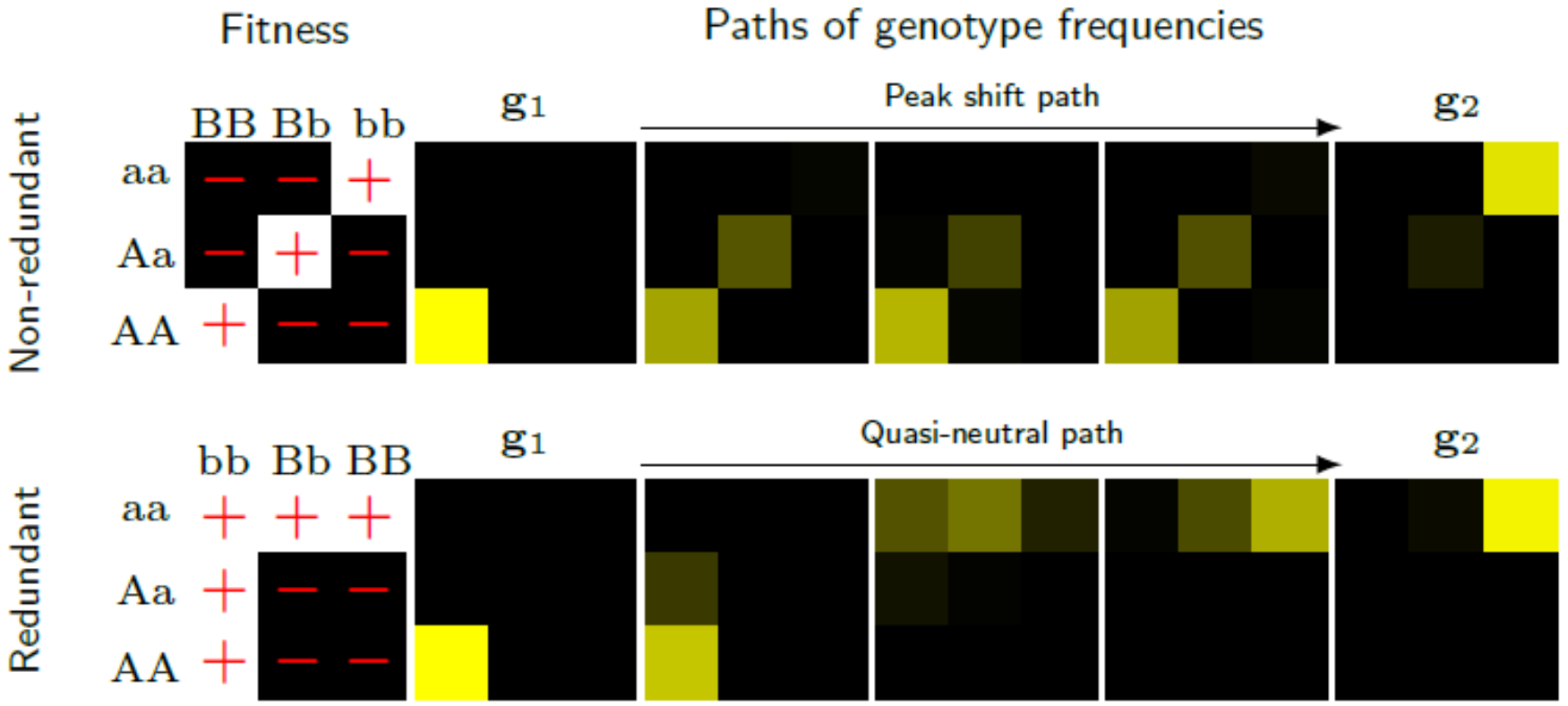
One locus, Heterozygote disadvantage: AA Aa aa

Fitnesses + – +

Two-locus examples, with epistasis, showing routes of evolution

Incompatibilities due to two adaptive peaks **AABB** and **aabb**. Evolution requires stochastic process. Stable to gene flow

Classical "Dobzhansky-Muller Incompatibilities" **AAbb**, **aaBB** and **aabb** most fit. No stochasticity required. But not stable to gene flow.



… and then there's many loci, more complex!!

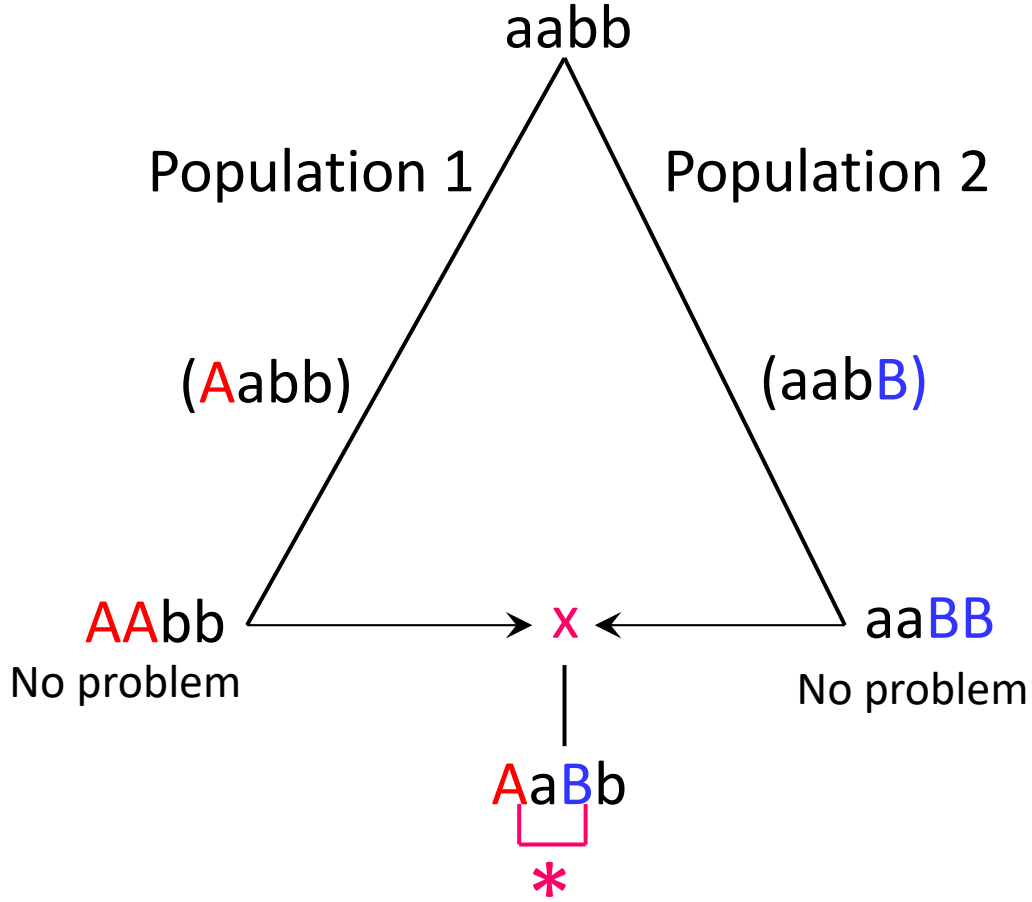
Xiong & Mallet 2022

“Dobzhansky-Muller” incompatibilities (DMIs)

How hybrid sterility can evolve (unopposed by natural selection)!

*Negative **epistasis***:

Population 2 \ Population 1	a	A
b	OK	OK
B	OK	dies

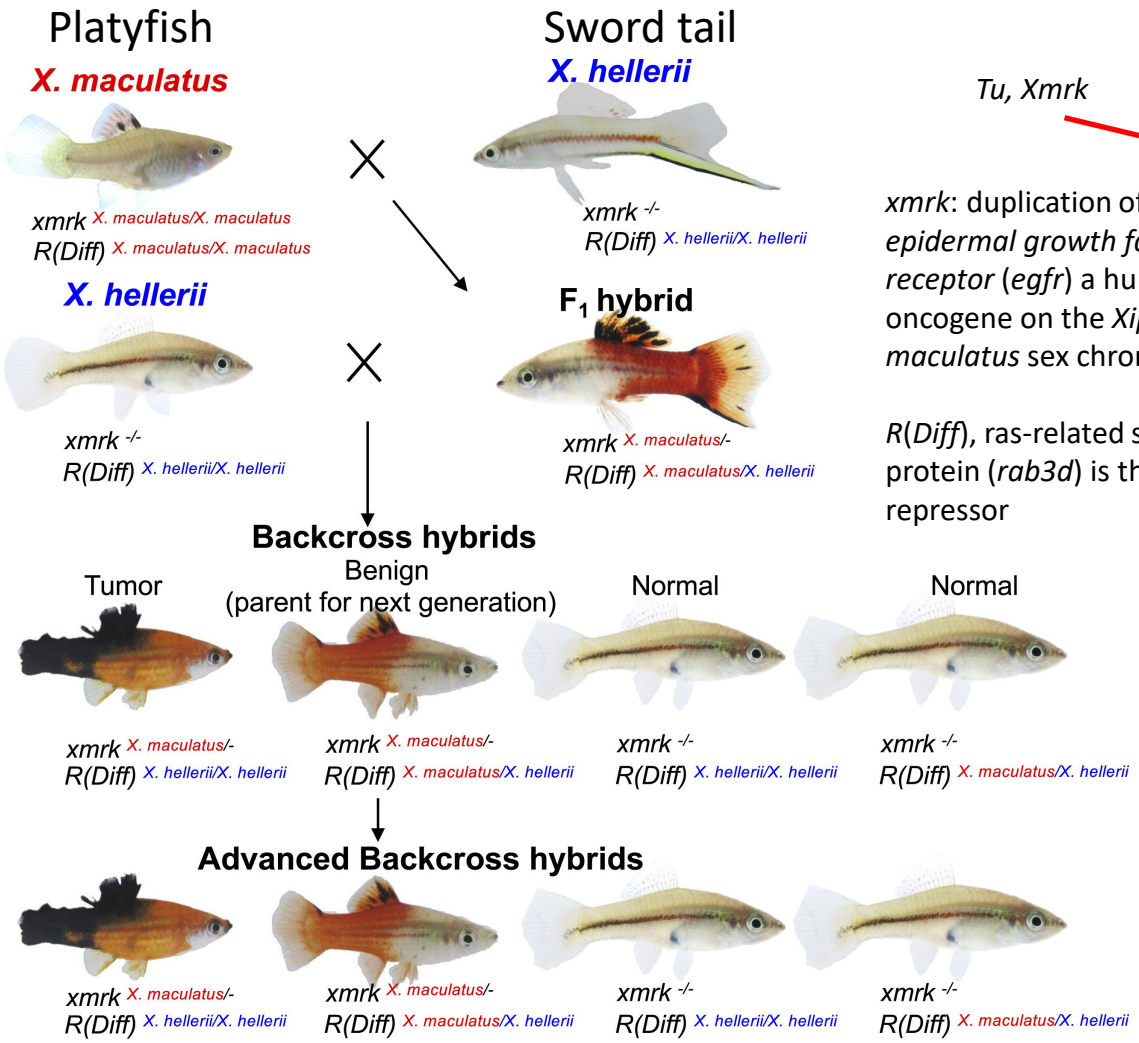


Hybrids sterile or inviable

“A” and “B” have never “seen” each other before, and have a negative interaction

Platyfish x Sword tail hybrids – melanoma, genus *Xiphophorus*

xmrk: Xiphophorus melanoma receptor kinase, *Tu*: tumorigenesis
R: repressor, *Diff*: differentiation

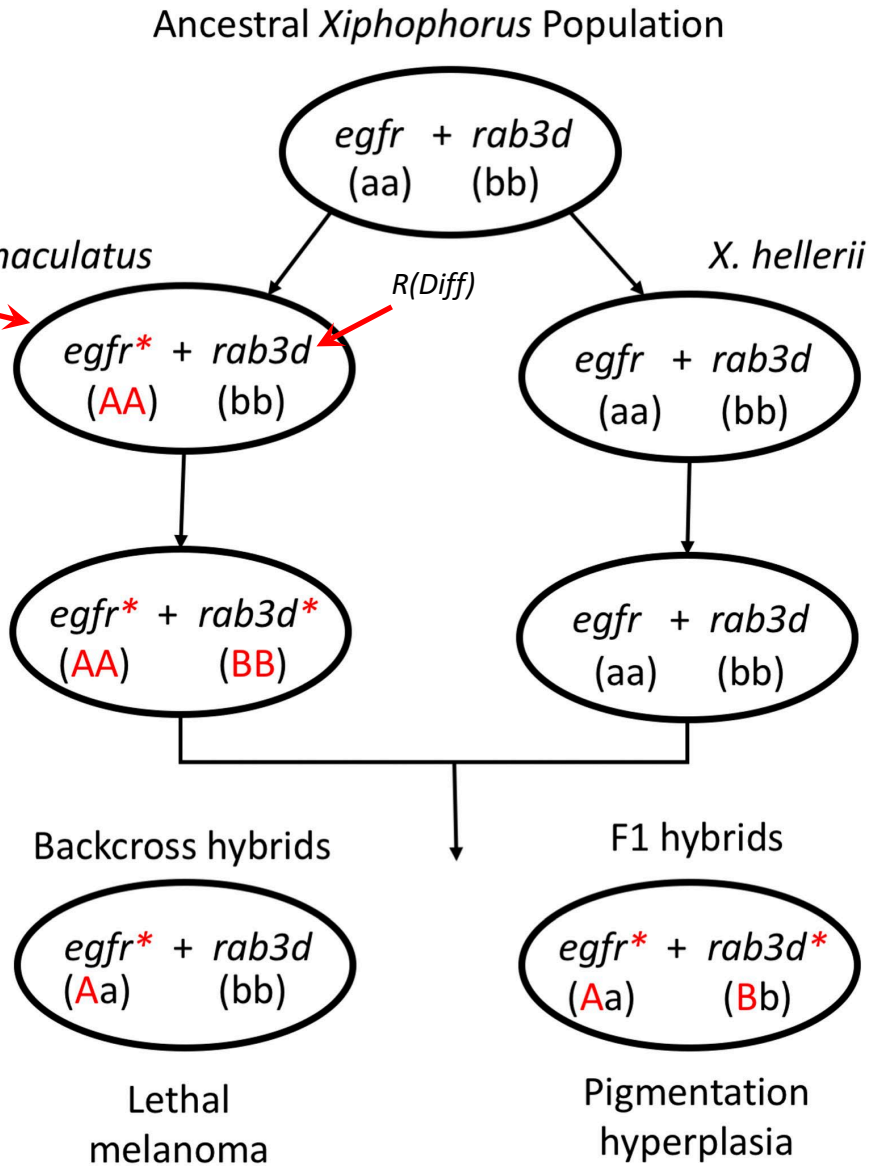


Lu et al. 2020 PNAS

Tu, Xmrk

xmrk: duplication of *epidermal growth factor receptor* (*egfr*) a human oncogene on the *Xiphophorus maculatus* sex chromosome †

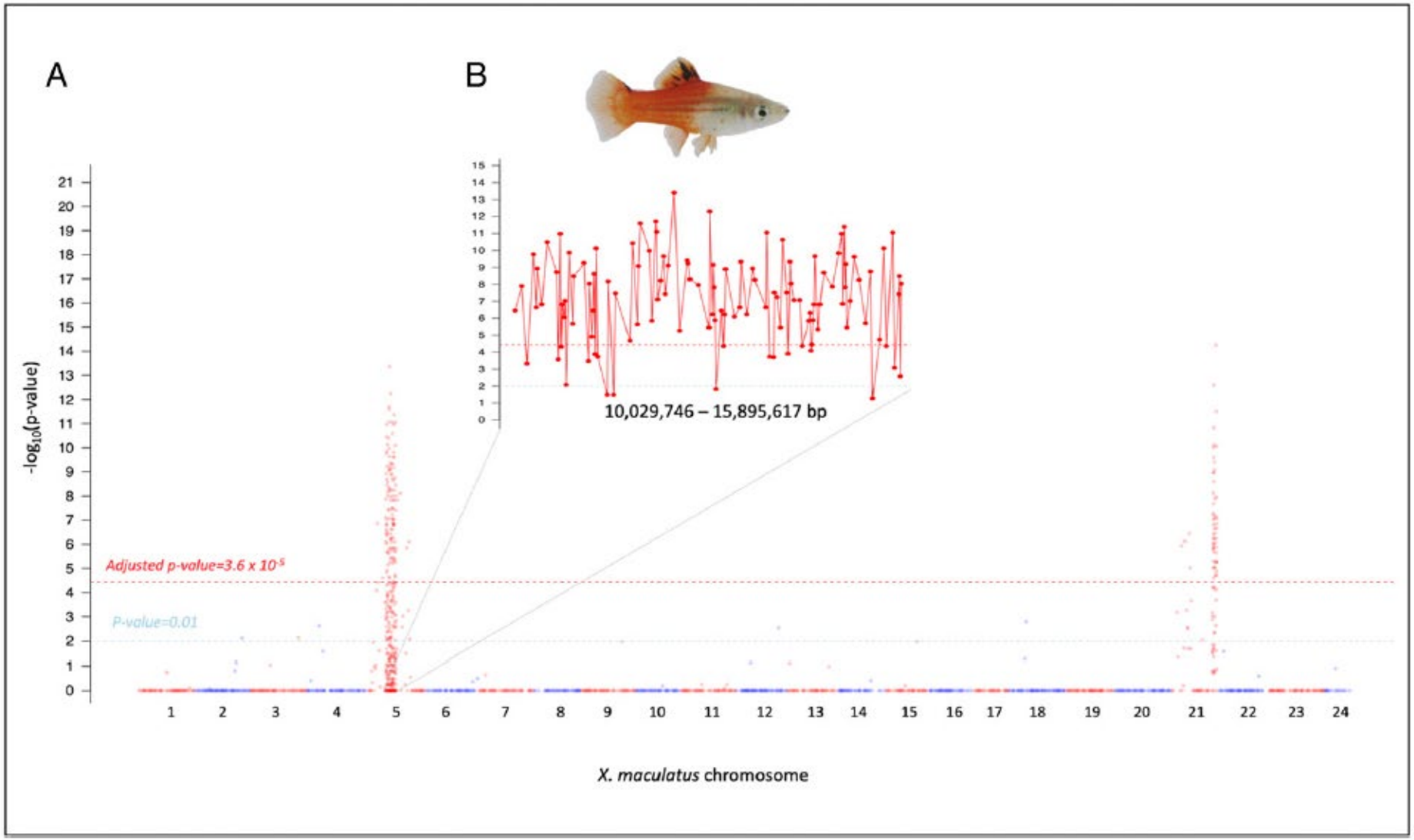
R(*Diff*), ras-related small protein (*rab3d*) is the repressor



Johnson & Phadnis 2020 PNAS

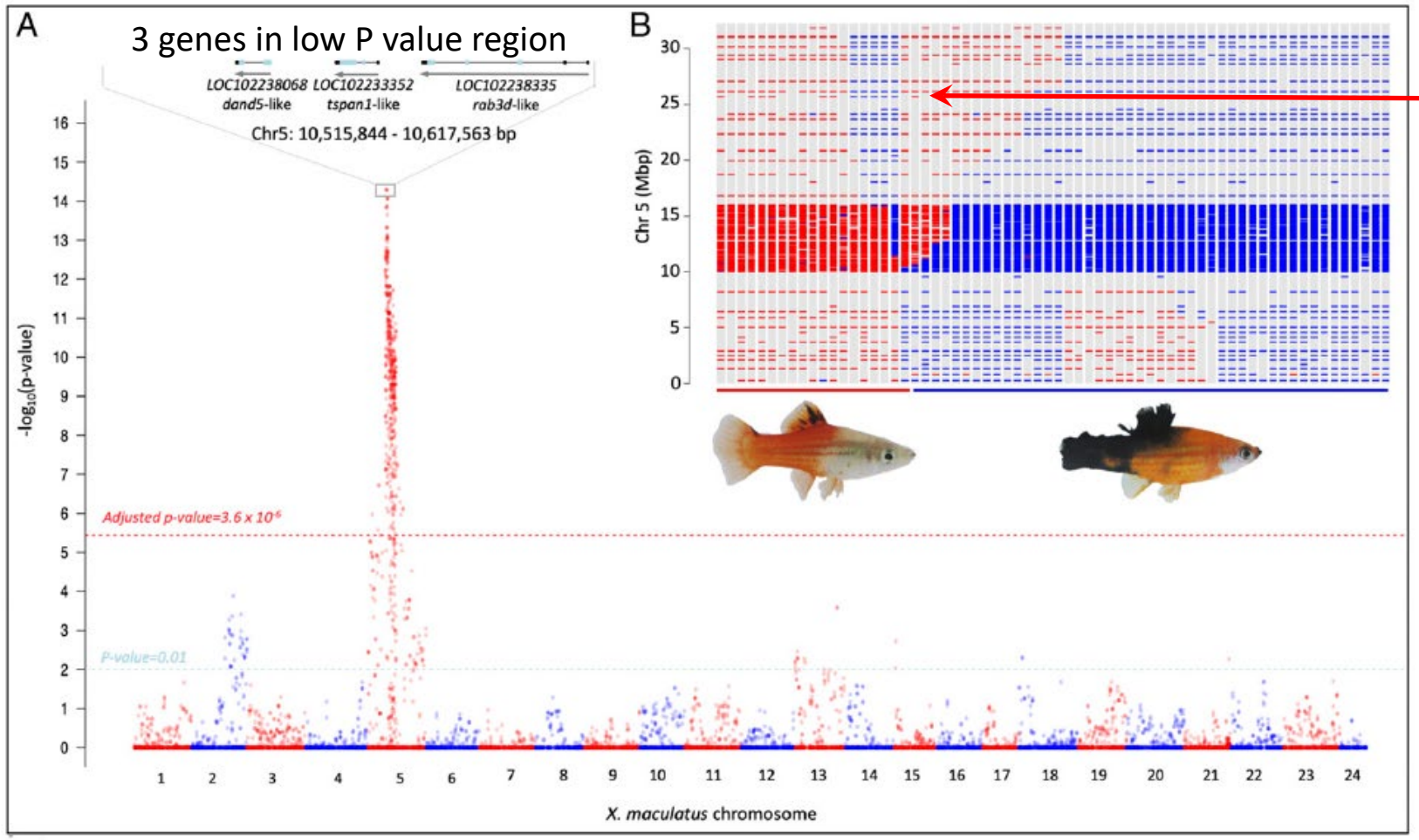
† Sex determination is complex. Males can be XY or YY, and females can be XX, XW and YW

Genome mapping in Platyfish x Sword tail hybrids. Chr 21 (sex chromosome) peak is *xmrk* (previously mapped), *R(Diff)* is on chr 5

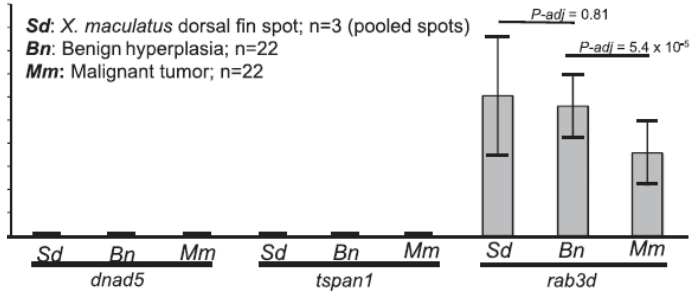


Lu et al. 2020 PNAS

Mapping *R(Diff).* All hybrids are *Xmrk/-*; benign hyperplasia (red): tumor (blue)



Chr 5 genes: Red is heterozygous, blue is homozygous



Differential gene expression inside melanic spots, benign, & melanoma for the 3 genes in the 100 kb region

Lu et al. 2020 PNAS

Hybrids between *Xiphophorus birchmanni* and *X. malinche* also show involvement of *xmrk*, but the tumor suppressor gene is apparently different – *myrip* not *rab3d*

Post-mating barriers: Haldane's Rule

Special case of hybrid inviability and epistasis: Haldane's Rule:

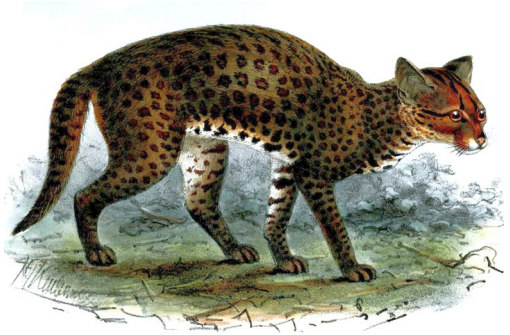
"When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex"

J.B.S. Haldane (1922) J. Genetics 12, 101-109

One of the few good rules or laws in evolutionary biology!

Sex chromosomes vs. autosomes (the non-XY chromosomes)

Mammals, Drosophila
(XY males, XX females)



Birds, butterflies
(ZZ males, WZ females)

Haldane's rule

Table 15.2 Support for Haldane's rule. Asymmetry in the column "hybridizations with asymmetry" means that one sex is affected more than the other with respect to the trait, such as fertility. Many species of butterflies, moths, and mosquitoes are also known to follow the same rule. From Coyne and Orr (1989b) , Presgraves (2002)

Group	Trait	Hybridizations with asymmetry	Number obeying Haldane's rule
Mammals	Fertility	20	19
Birds	Fertility	43	40
	Viability	18	18
Drosophila	Fertility and viability	145	141
Lepidoptera	Fertility	30	29
	Viability	84	81

Agreement 340 Disagreement 12

Post-mating barrier

e.g.
Haldane's Rule in
Drosophila

The "large-X effect"

D. pseudoobscura

D. persimilis

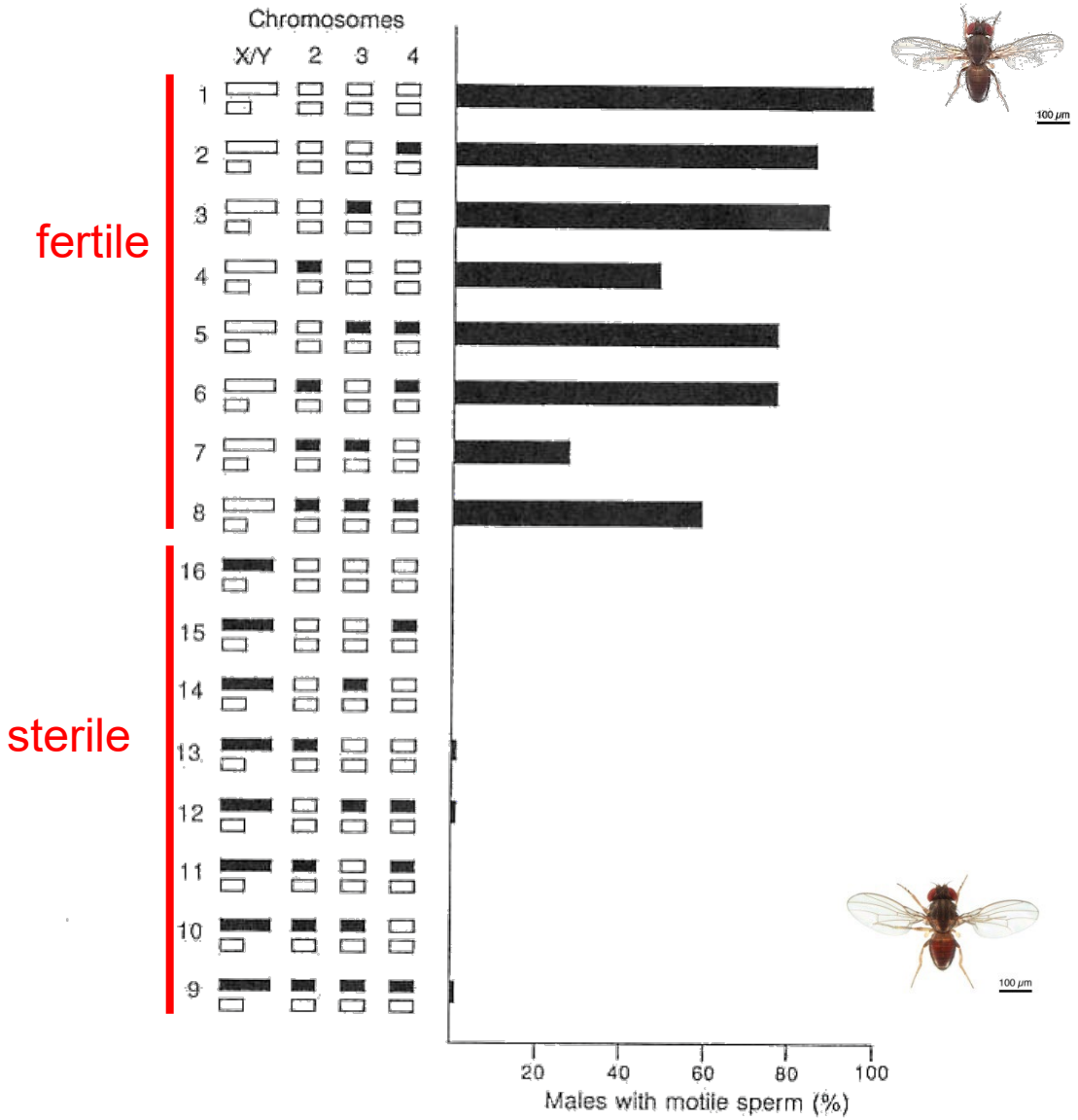


FIGURE 2. Results from a typical backcross analysis of hybrid sterility. Backcross males were produced from the backcross of *D. pseudoobscura–D. persimilis* hybrid F_1 females to *D. pseudoobscura* males (from Orr 1987). *D. pseudoobscura* chromosomes are shown in white and *D. persimilis* in black. Substitution of a *D. persimilis* X chromosome causes almost complete sterility.

Genetics of speciation

"Two of the strongest patterns in evolutionary biology, Haldane's rule and the large effect of the sex chromosomes on postzygotic isolation, still lack wholly convincing explanations."

Jerry A. Coyne (1992) Genetics and speciation. *Nature* 355, 511-515.

But not any more! By the late 1990s we had some answers.

What explains Haldane's Rule and the large-X effect?

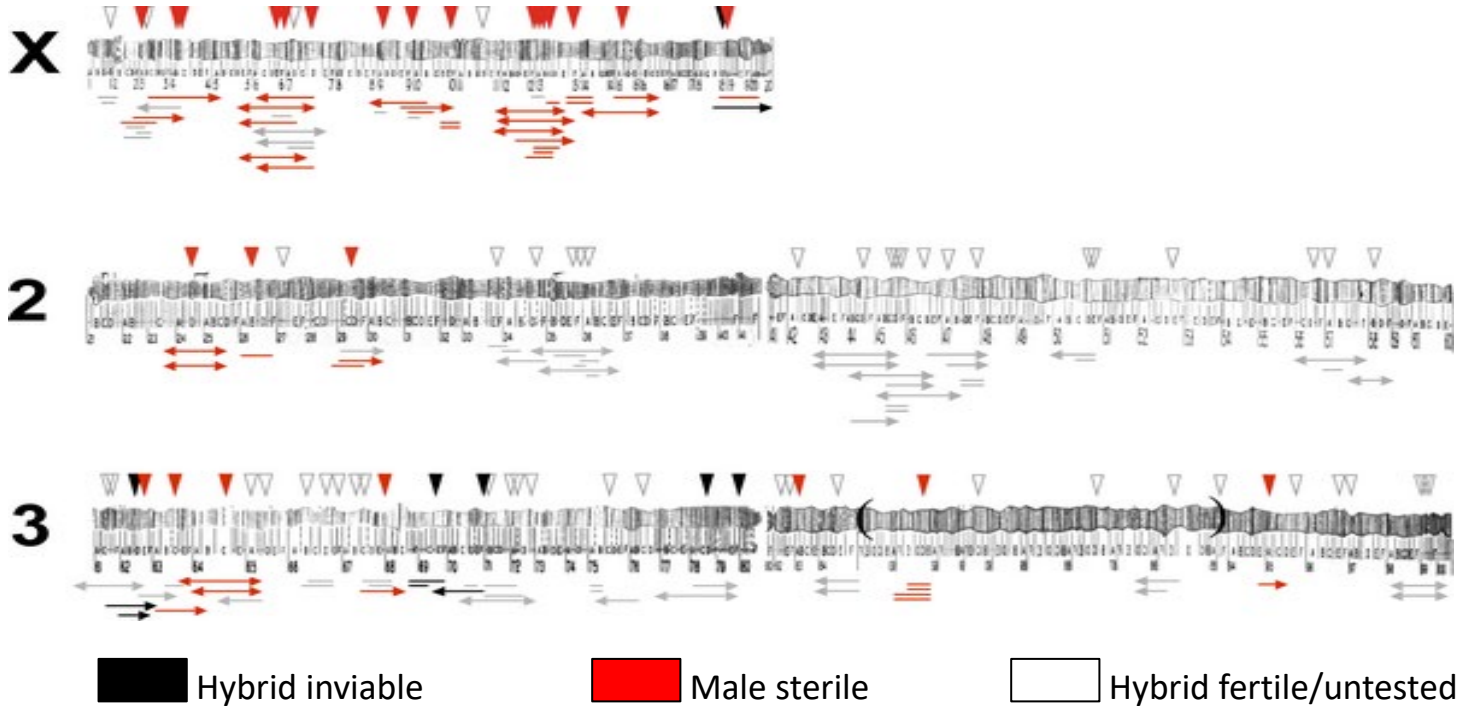
"Dominance theory": *recessive* alleles on X are always expressed in the heterogametic sex (XY)
Maybe genes contributing to postzygotic isolation are predominantly recessive (therefore are expressed only in the heterogametic sex of hybrids)

Predictions: (1) Most genes contributing to postzygotic isolation should be recessive.
(2) large X chromosomes should evolve Haldane's rule faster (more opportunity for incompatibilities to arise)

We can test these theories by making hybrids with autosomal regions hemizygous (or balanced by deletion) or homozygous

“Introgression” experiment: *D. mauritiana* into *D. sechellia*

Distribution of *D. mauritiana* introgressions in *the D. sechellia* genome

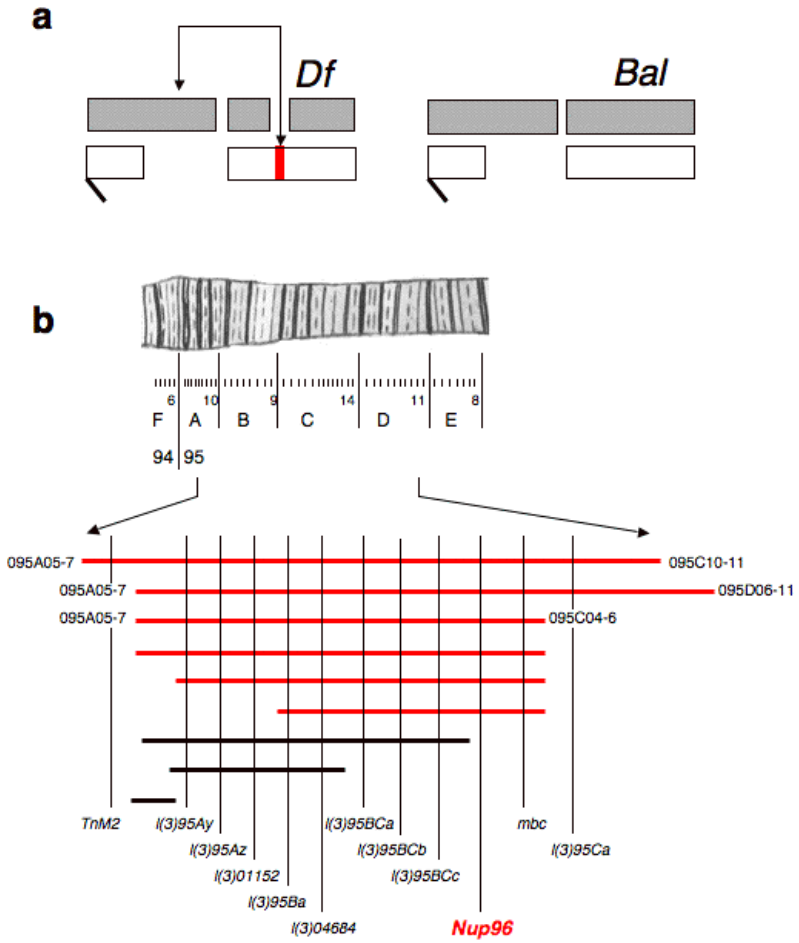


Dominance theory is (partly) correct: The vast majority of regions causing problems are recessive. Many of these are on autosomes, so do not express in F1 hybrids

Also: ‘Faster male:’ Male sterility much commoner than female sterility (in *Drosophila*)
‘Faster X:’ Only 18% of autosomal, but 60% of X chromosome introgressions cause recessive sterility problems

Masly & Presgraves 2007

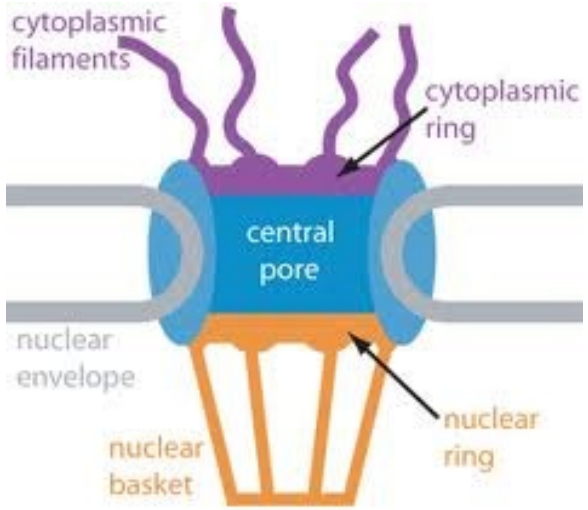
Deletions to uncover recessive autosomal genes



D. melanogaster x *D. simulans* → ***Nup96***

Nup160 is incompatible partner on X chromosome

Nup96 on an autosome interacts with X-linked ***Nup160***, two of the proteins that line the pore to a cell's nucleus, a favorite target for viruses and even malicious genes within the fly's own genome. That role could be key to their rapid evolution (and positive selection).



Good evidence for positive selection at these genes

Amino acid encoding sites in these genes evolve faster than putatively neutral sites nearby

Tang & Presgraves 2009

“Speciation genes”

“This perhaps unfortunate term, which is now entrenched in the literature, refers to any locus that causes reproductive isolation, whether in F1 or later generation hybrids, and whether the gene was amongst the first to cause isolation or not” (H. Allen Orr)

Nup96, Nup160	*Drosophila*	inviability
Odyh	*Drosophila*	sterility
Hmr	*Drosophila*	inviability
Lhr	*Drosophila*	inviability
JYalpha	*Drosophila*	sterility
Prdm9	mice	inviabiity
Xmrk2	*Xiphophorus* fish	inviability
NB-Lrr	*Arabidopsis*	inviability

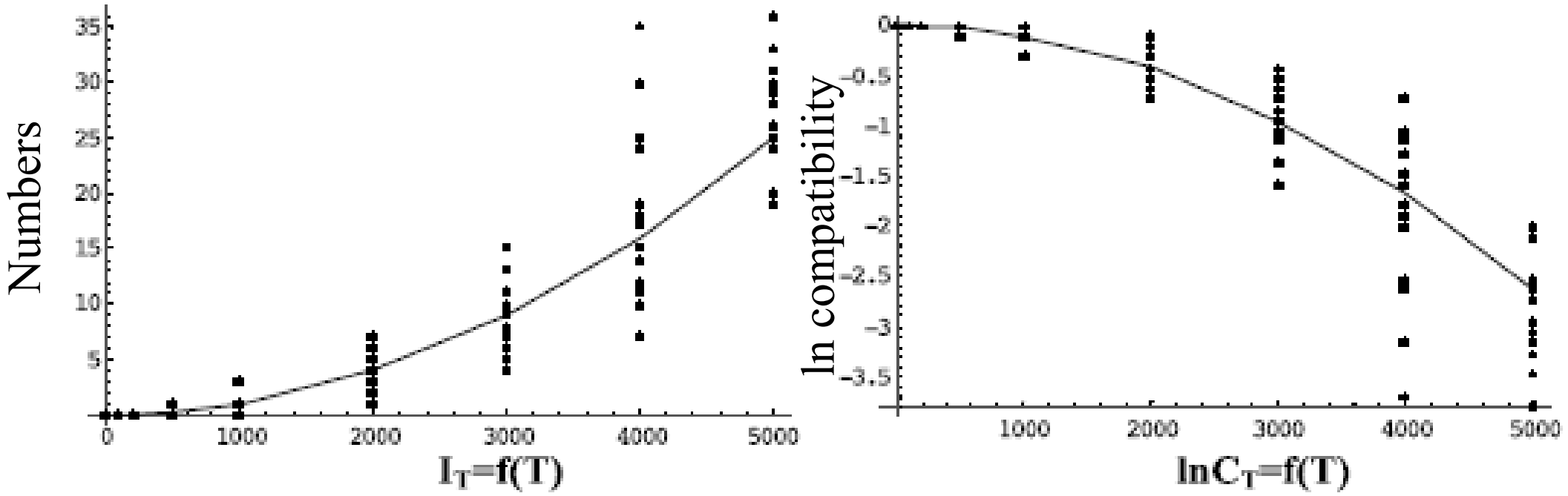
..and a handful more

- Most of these now thought to be Dobzhansky-Muller incompatibilities
- Often rapidly evolving for reasons unknown: local adaptation or genomic conflict?
- Not known to be “genes that caused speciation.” They are thought to have evolved as a by-product of divergence in general, rather than as an “isolating mechanism.”

Dobzhansky-Muller "snowball effect"

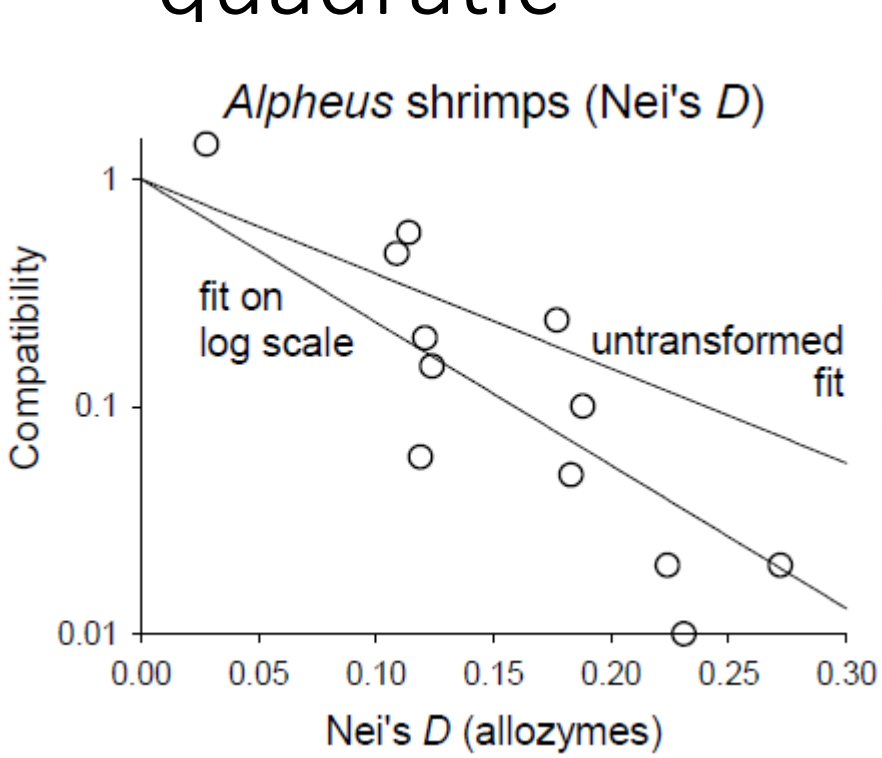
- Dobzhansky-Muller incompatibilities are expected to accumulate faster than linearly with time. The numbers of 2-locus DMIs are expected to accumulate with (divergence)^2

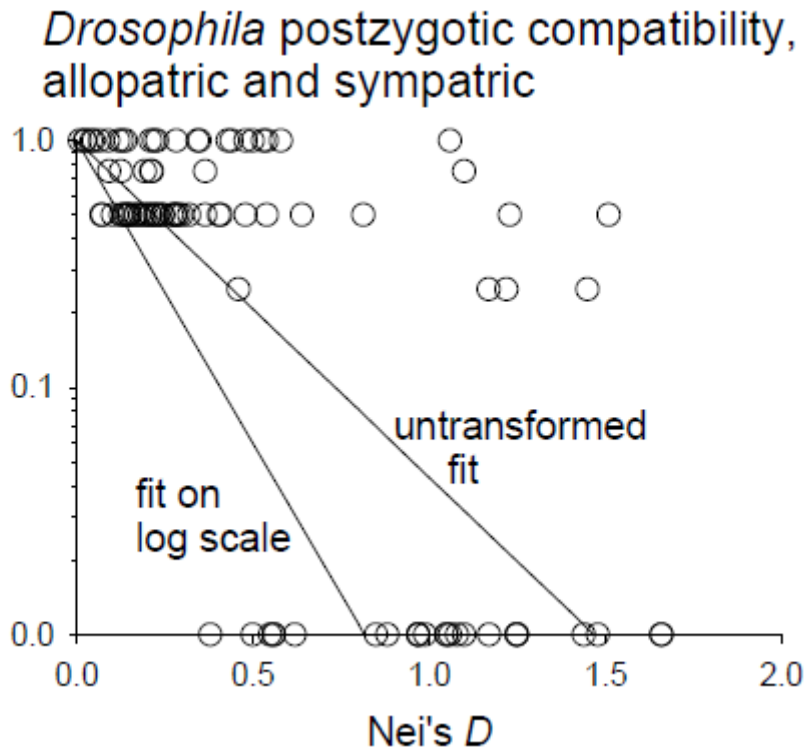
B Snowball. Constant substitution rate and Dobzhansky-Muller incompatibilities (p=0.005, s=0.1, and K= 0.01). Note especially the low initial variance.

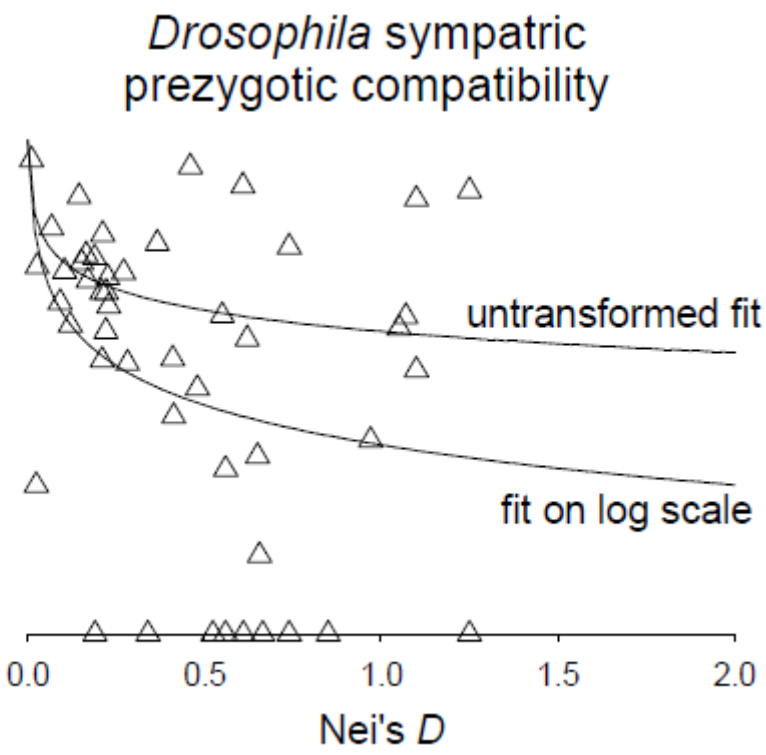


Gourbiere & Mallet 2010

Assuming each DMI has a small effect, we might expect "reproductive isolation" to accumulate as a quadratic





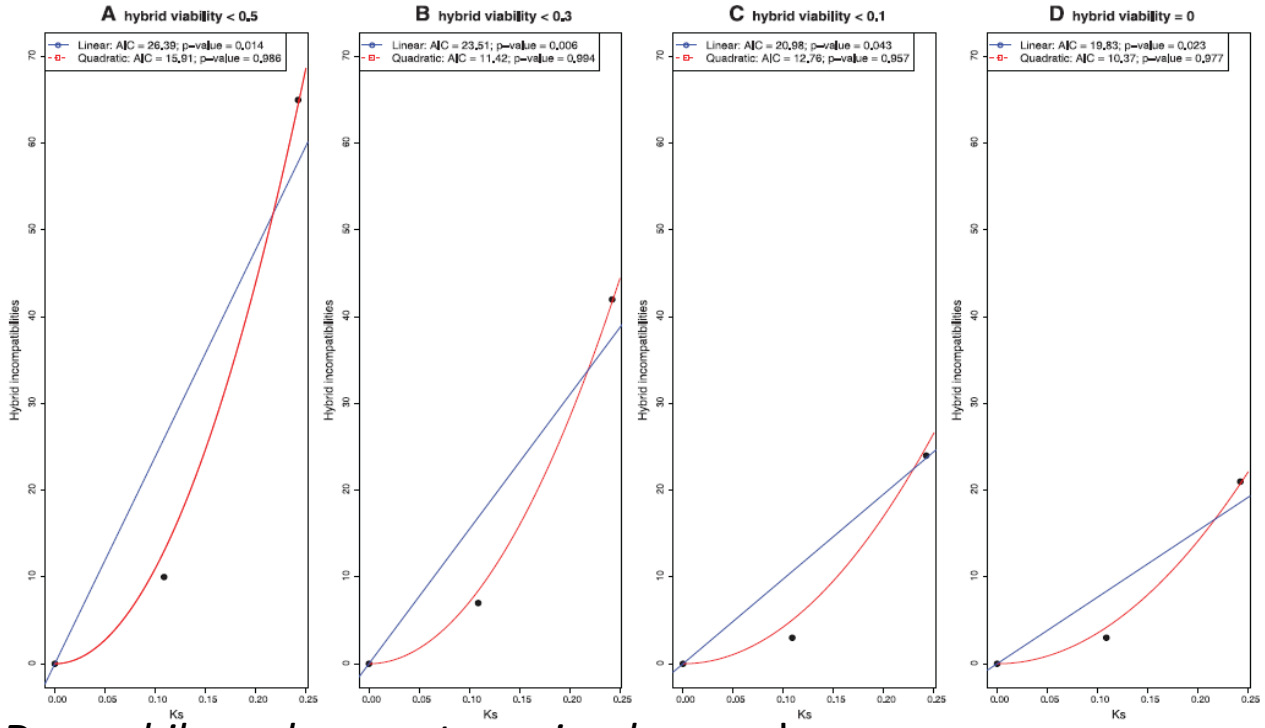


Result: not much evidence for snowball effects! Some evidence for a "slowdown" effect on Drosophila assortative mating, especially in sympatry."

Gourbiere & Mallet 2010

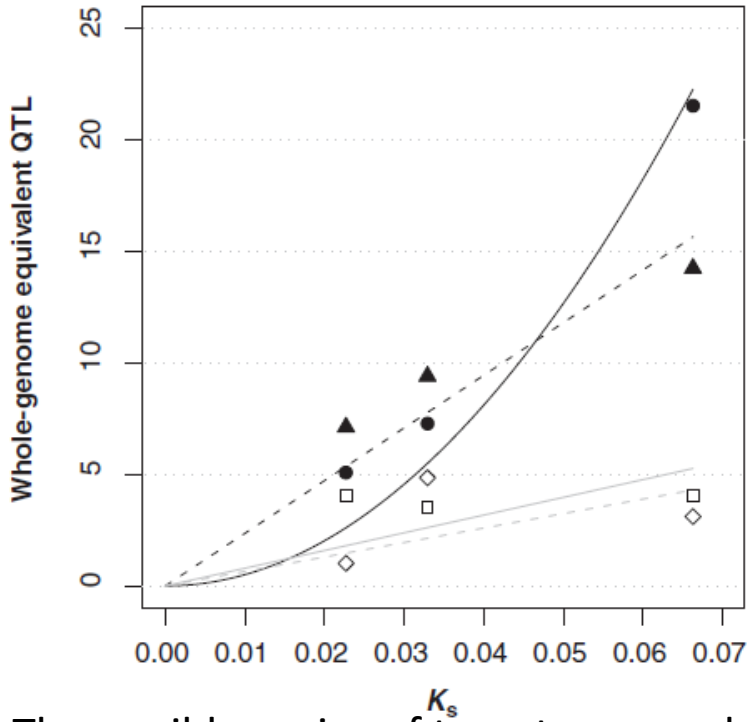
Dobzhansky-Muller "snowball effect"

Dobzhansky-Muller incompatibilities are expected to accumulate faster than linearly with time. For 2-locus DMIs, number of incompatibilities expected to accumulate with $(\text{divergence})^2$



Drosophila melanogaster x simulans and *D. melanogaster x santomea*

Matute et al. 2010



Three wild species of tomato crossed with *Solanum lycopersicum* ("the tomato")

Moyle & Nakazato 2010

Hybrid sterility or inviability between species is not necessarily permanent

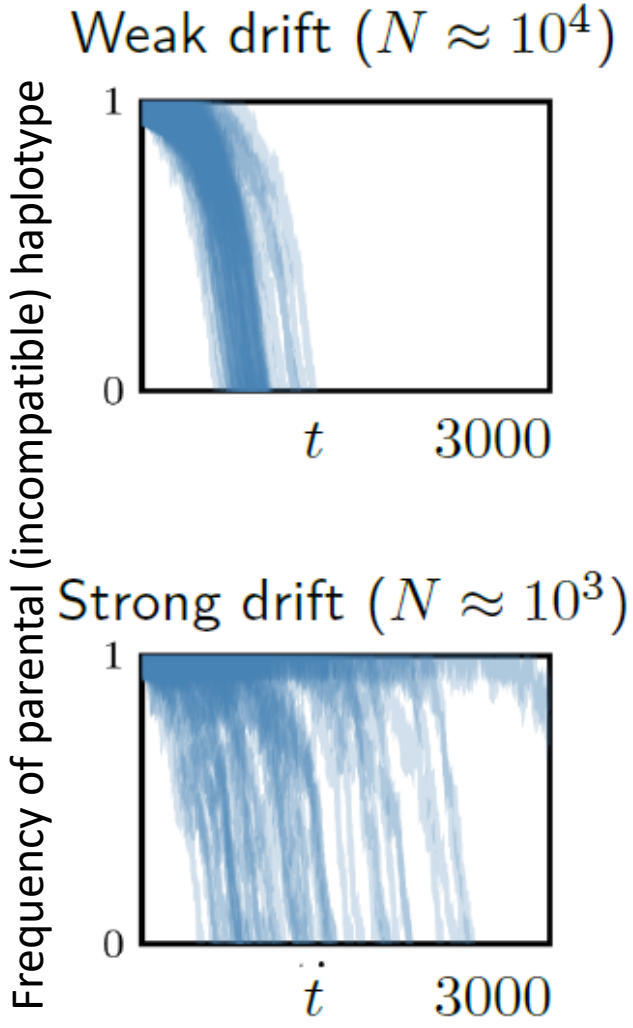
- If any fertile hybrids are produced, selection can drive the collapse of the incompatibility

Here we assume a simple 2 locus Dobzhansky-Muller incompatibility

Gene flow (*m*) = 1%

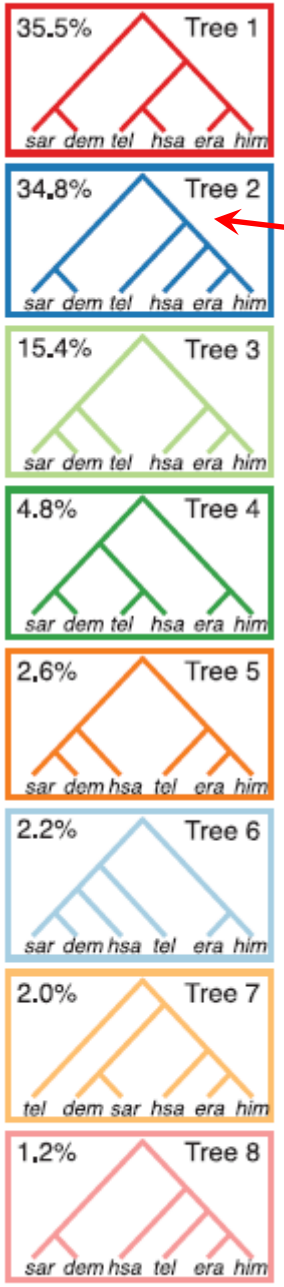
Survival of incompatible hybrids = 10%

Population sizes (*N*) as shown



Bank et al. 2012
Xiong & Mallet 2022

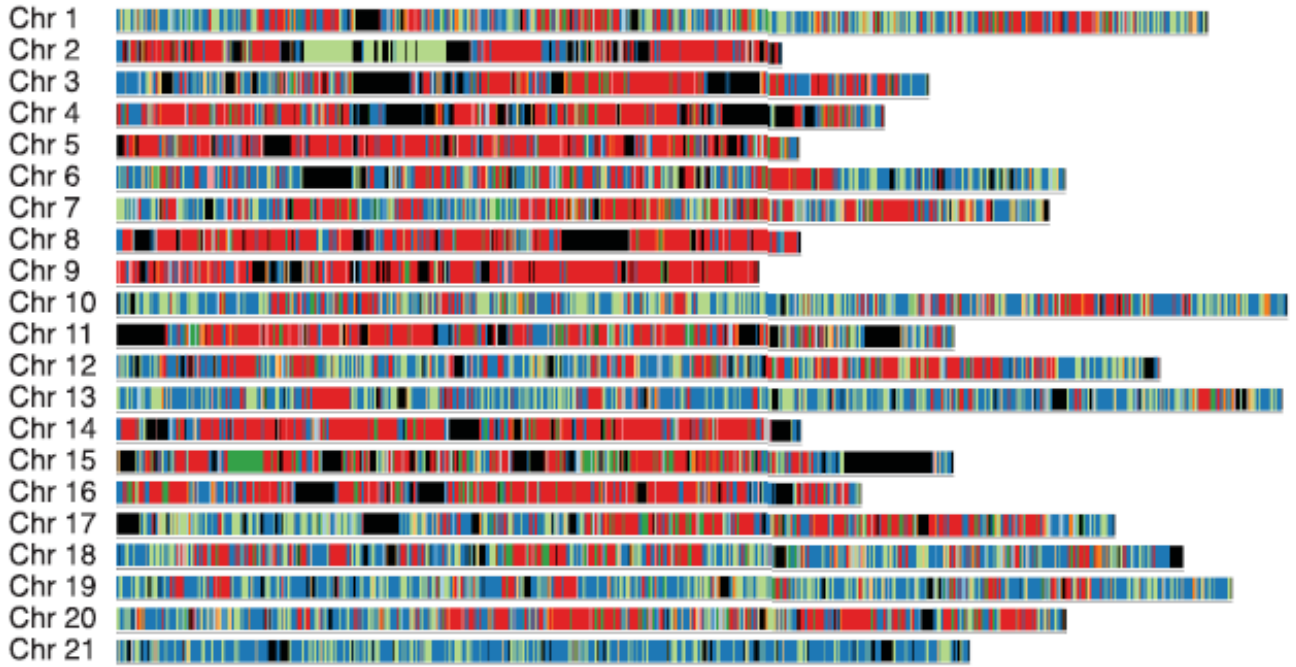
Genomic evidence: hybrid incompatibilities appear to be multilocus



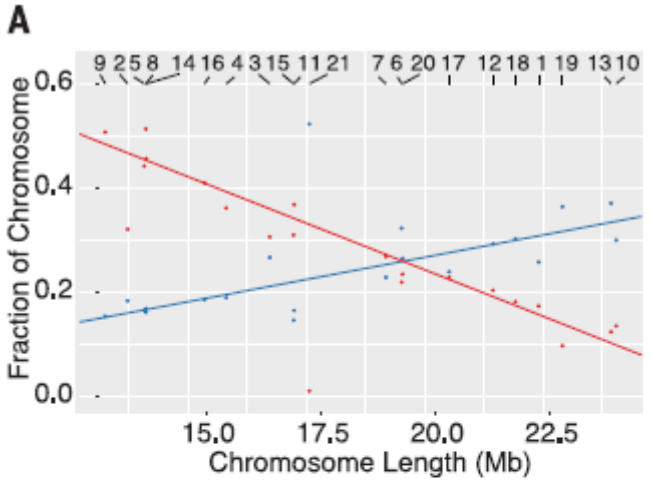
"true" species tree

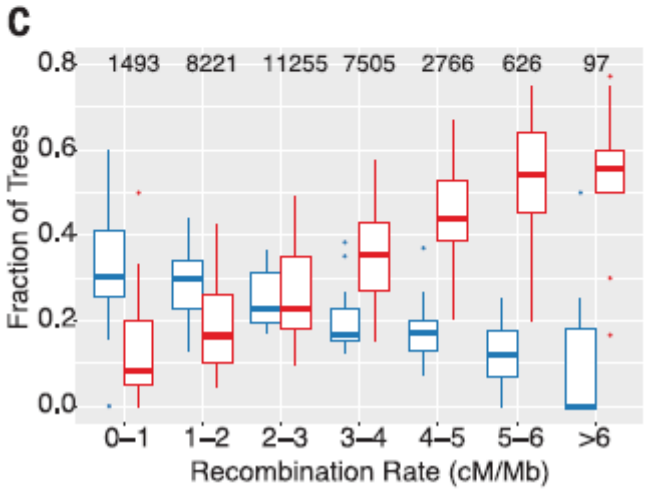
Often less gene flow on sex chromosome

More gene flow where recombination rate is high



Z chromosome





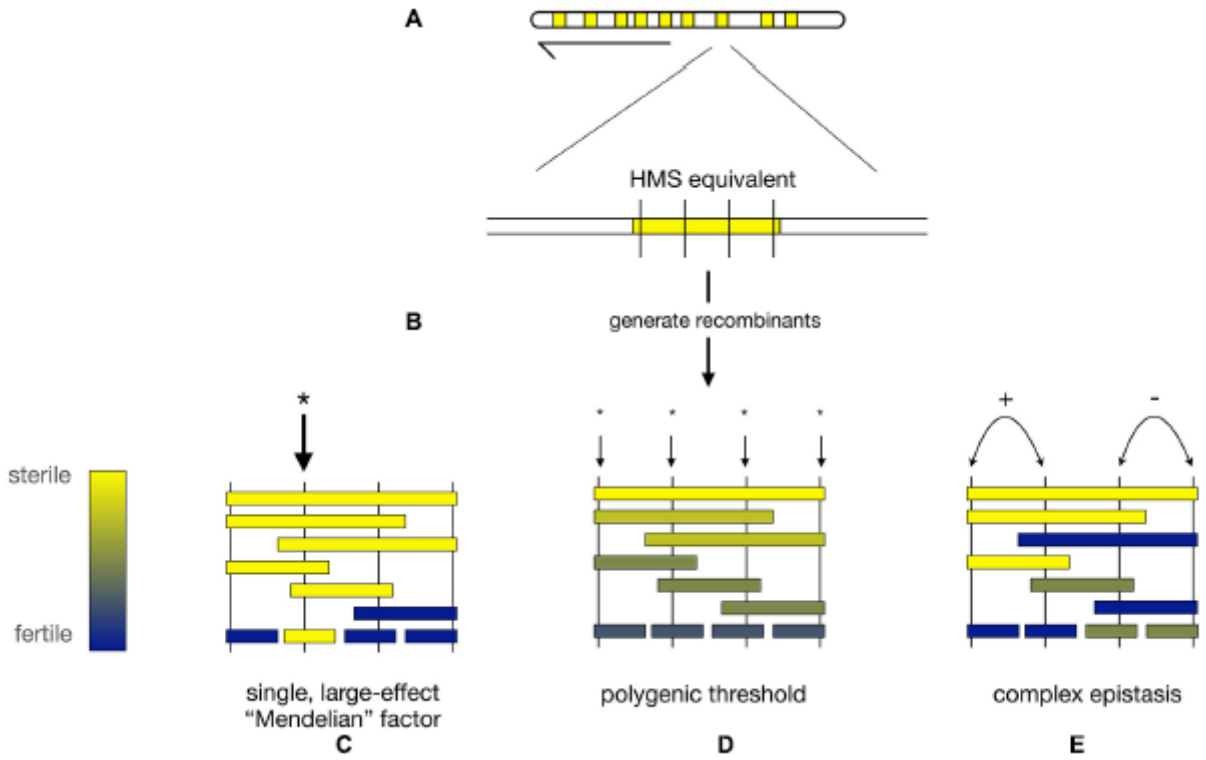
Similar results in *Xiphophorus* fish

And in Drosophila

Also Neanderthal/Denisovan -> modern human gene flow

Edelman et al. 2019
Schumer et al. 2018

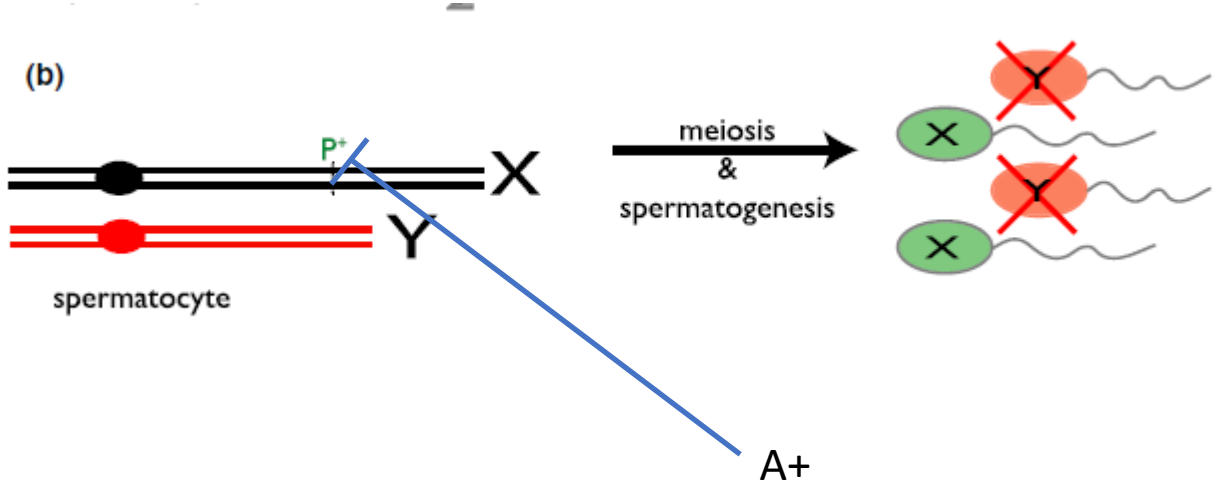
Male sterility (esp. *Drosophila*) often *many* loci



- Recombination mapping of introgressions causing sterility:
- Various other patterns – polygenic threshold & "complex epistasis"

Presgraves & Meiklejohn 2021

Meiotic driver loci on sex chromosome may explain some of this



- P+ = “poison” allele, kills off sensitive Y chromosome (red)
- Then A+ = “antidote” allele evolves on autosomes (which prefer 1:1 sex ratio), suppresses driver, restores sex ratio

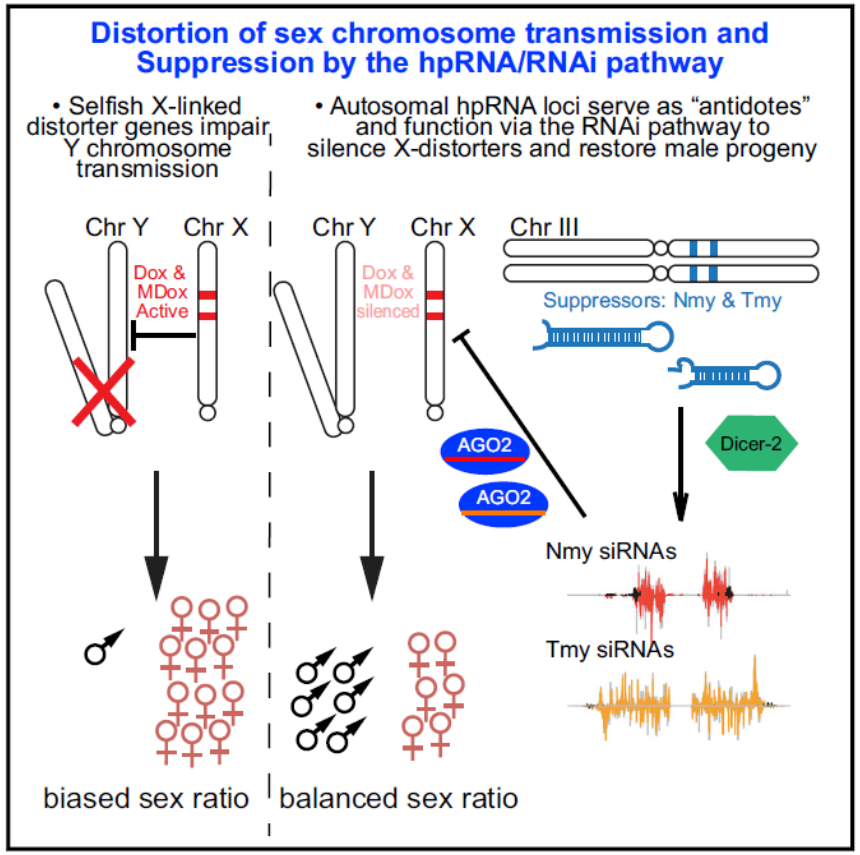
e.g. “Distorter on the X chromosome”, *Dox* loci

- Sex chromosomes often involved in selfish drive
- However, 1:1 sex ratio beneficial at individual level
- Suppressors evolve on autosomes to suppress drive
- *Dox /Nmy –Tmy* systems in *Drosophila simulans*

- Imbalance between species may cause hybrid male sterility

Intragenomic Conflict in the *Drosophila* Male Germline

Graphical Abstract



Authors

Ching-Jung Lin, Fuqu Hu, Raphaelle Dubruille, ..., Peter Smibert, Benjamin Loppin, Eric C. Lai

Correspondence

laie@mskcc.org

In Brief

Lin and Hu et al. reveal a critical biological usage of RNAi in *Drosophila simulans* to tame meiotic drive systems. Multiple autosomal hairpin-siRNA loci are deployed to suppress X-encoded distorter loci that bias progeny sex ratio. These loci are rapidly evolving and testis restricted, and such intragenomic conflicts may fuel speciation.

Dox and *Dox*-like genes in *D. simulans* and close relatives

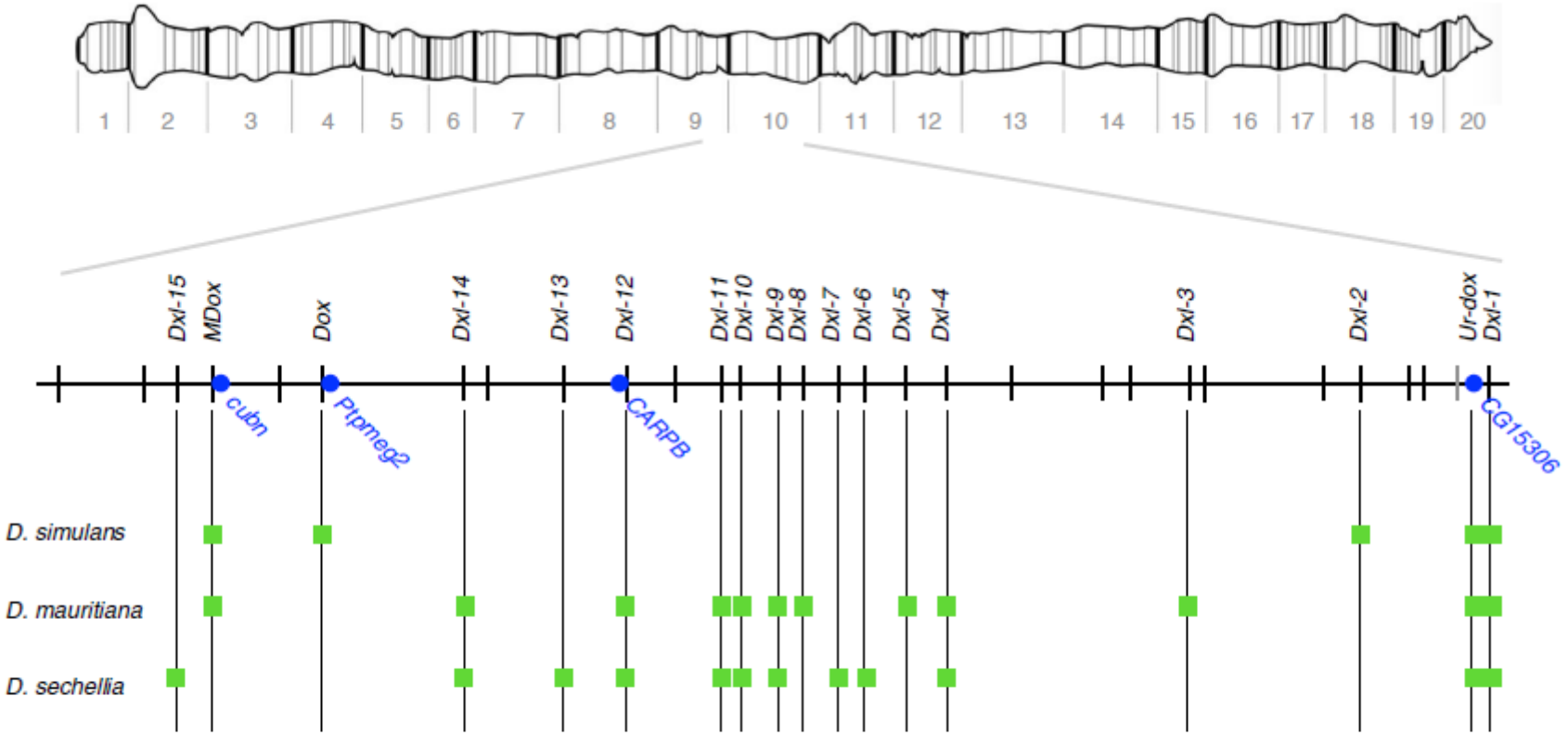


Fig. 1 | Physical distribution of known *Dxl* genes in *D. simulans*, *D. mauritiana* and *D. sechellia*. A schematic of the polytene X chromosome (top) shows location of the *Dxl*-containing region (*Dmel* r6 X:9400000-10400000). Tick marks show locations of sat359 islands conserved in all three *D. simulans* clade species and in the outgroup *D. melanogaster*; the single grey tick mark distal to *Ur-Dox* is a sat359 island found in the *D. simulans* clade species but not in *D. melanogaster*; the green squares show sat359 islands with a *Dxl* insertion; and the blue dots show protein-coding genes of interest. While *Dxl* insertions with the same name occupy orthologous sat359 islands in different species, the *Dxl* sequences are not necessarily orthologous due to the possibilities of independent, parallel insertion and ectopic exchange.

Muirhead & Presgraves 2021

How *Dox* and *Dxl* X-driving genes evolved

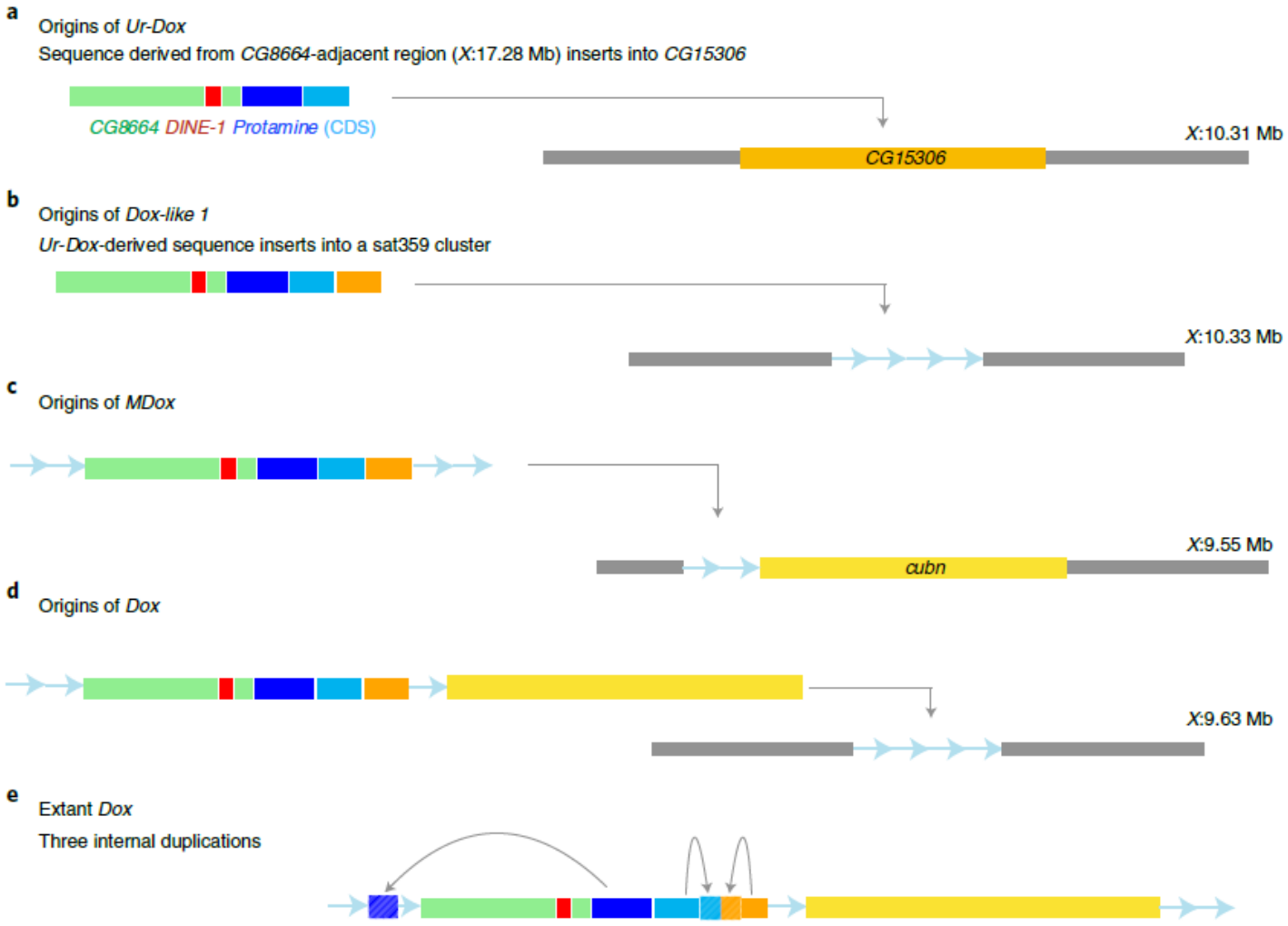
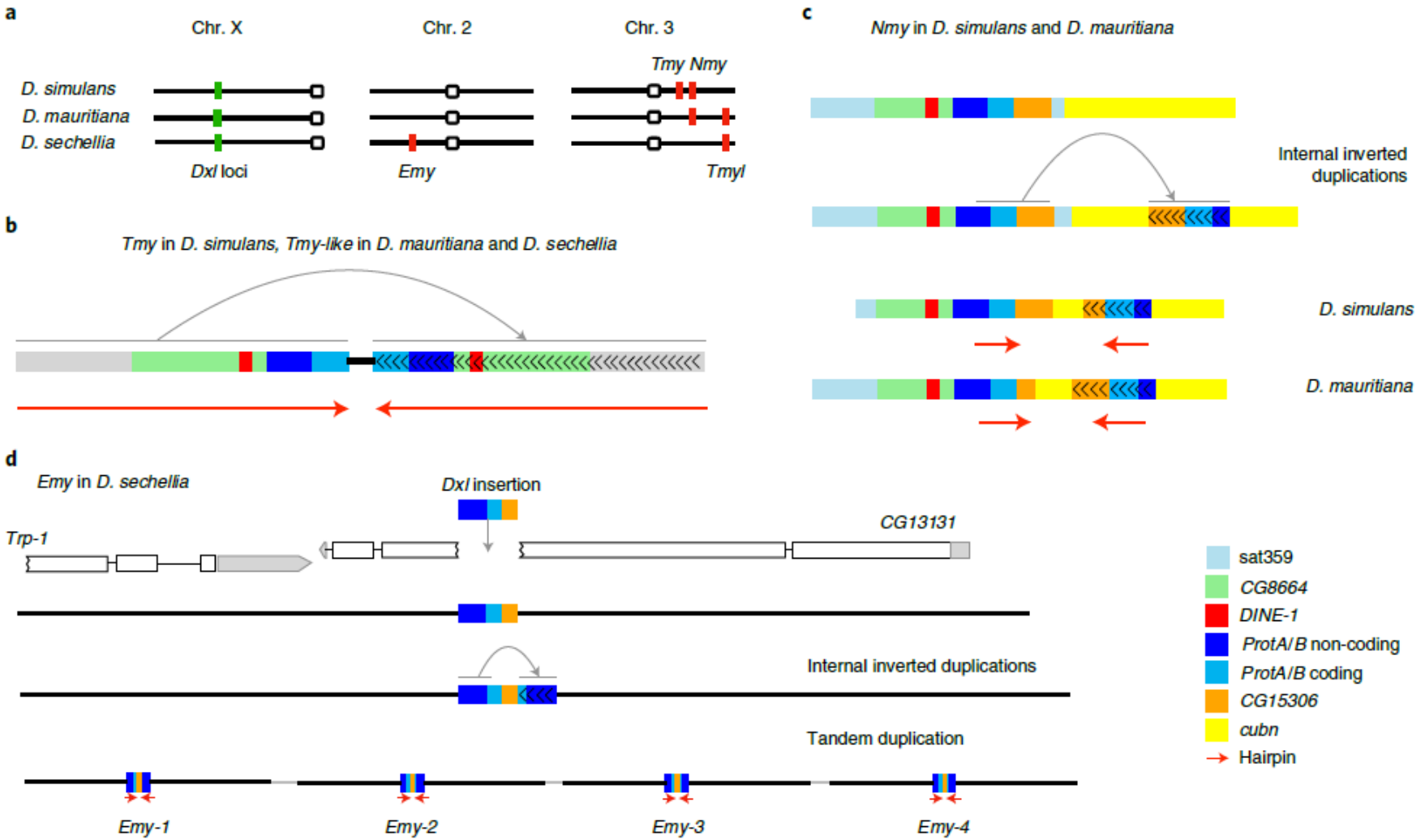


Fig. 2 | Inferred stepwise historical origins of *Dox*. Colour coding of sequence blocks indicates the putative sequence homology and light blue arrows represent sat359 repeats.

Muirhead & Presgraves 2021

How *Dox/Dxl* drive suppressors, *Tmy, Nmy, Emy* etc., evolved



Endogenous small interfering RNAs (esiRNA) – hairpin structures

Not much yang, a retroduplicate of Dox that suppresses via production of endogenous small interfering RNAs

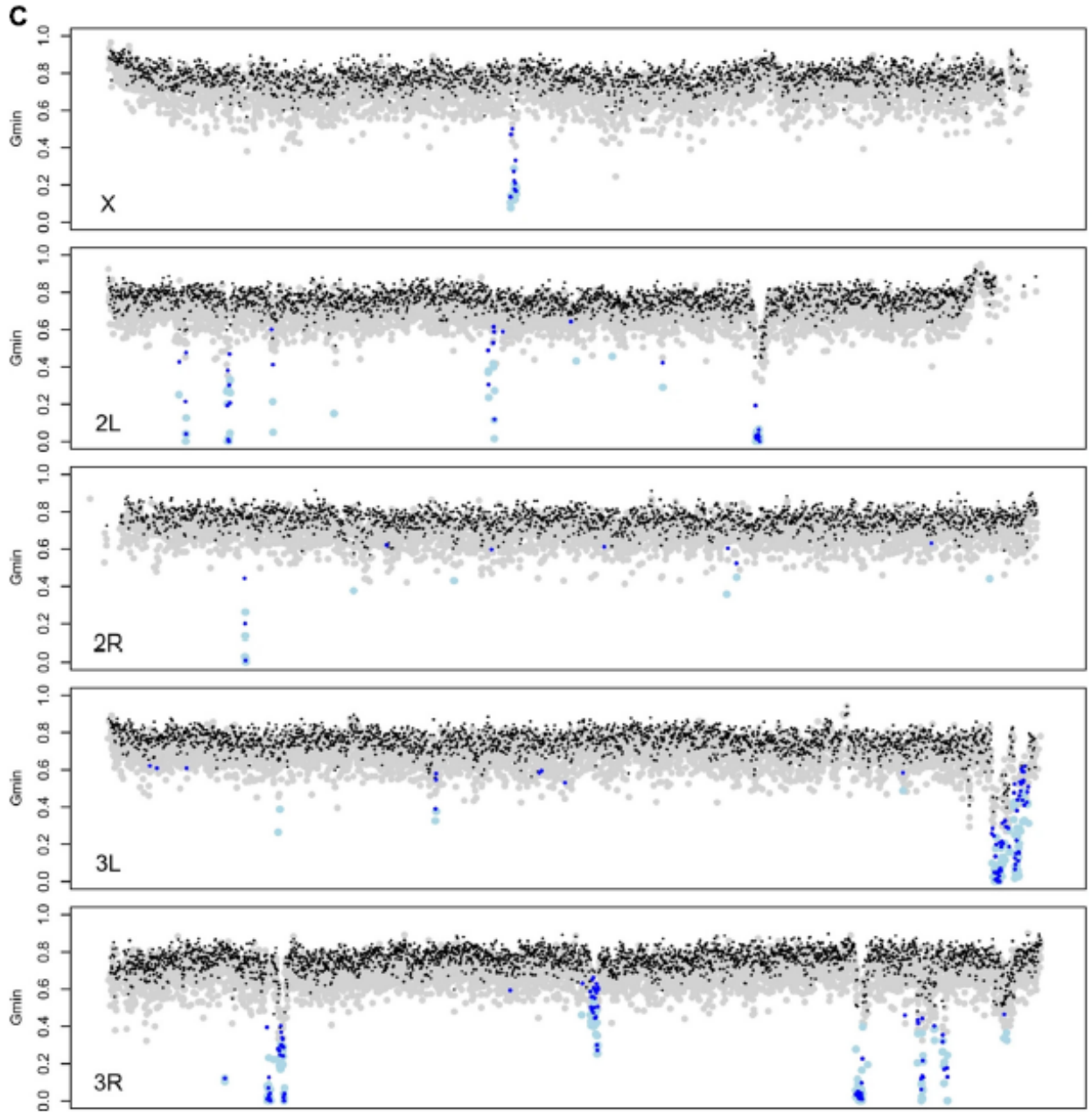
Interspecific divergence between *Drosophila simulans* and *D. mauritiana*

The two species have male sterile hybrids

Obviously there has been introgression between the species, in spite of occurring on different islands.

The X chromosome is underrepresented in terms of gene flow. Paradoxically, the *Dox/Mdox* region has introgressed! By NOT causing sterility!

The X chromosome also has many more sterility alleles that are involved in hybrid sterility. Likely due to unbalanced drive/suppressor systems.



"Gmin statistic identify haplotypes with interspecific distances too low to be consistent with simple allopatric speciation history." Blue = gene flow.

Presgraves & Meiklejohn 2021

Hybrid sterility and hybrid inviability

Logic of Dobzhansky-Muller is inescapable. It happens!

Sex-linkage of Haldane's Rule effects is expected under dominance theory – and also meiotic drive/suppressor evolution

But: hybrid problems are not just simple Dobzhansky-Muller 2-locus incompatibilities!

- Heterozygous disadvantage (e.g. chromosomal translocation differences – see later topic)
- Likely many more than 2 loci interacting in complex epistatic networks – such as Dox/dxl genetic conflict loci (though these are not known to cause hybrid sterility)
- Recombination difficulties when sequences are divergent (yeast, bacteria)
- Data on compatibility/postzygotic isolation are very noisy; much variation in the rate of incompatibility accumulation
- Models show that many systems of hybrid incompatibility can be lost in the face of gene flow; so hybrid sterility/inviability not necessarily permanent!

To read ...

- Barbash, D.A. 2012. Ninety years of *Drosophila melanogaster hybrids. Genetics 186:1-8. https://doi.org/10.1534/genetics.110.121459*
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