Sex and the Single Fly: A Perspective on the Career of Bruce S. Baker

陈洁 金思慧 邢丽敏

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Bruce S. Baker

Affiliations: 1976-1985 University of California, San Diego, La Jolla, CA
1986-2008 Stanford University, Palo Alto, CA
2008- HHMI Janelia Farm Research Campus, Ashburn, VA, United States

Area: Genetics, Neuroscience

Parents

Lawrence Sandler	grad student	1971	University of Washington
(Sex chromosome meiotic mutants in Drosophila melanogaster : detection and preliminary characterization)			
James F. Crow	post-doc	1972-1974	UW Madison

Bruce S. Baker

Affiliations: 1976-1985 University of California, San Diego, La Jolla, CA
1986-2008 Stanford University, Palo Alto, CA
2008- HHMI Janelia Farm Research Campus, Ashburn, VA, United States

Area: Genetics, Neuroscience

Children

Dori Allen	grad student		
Debbie Andrew	grad student		
Deborah J. Andrew	grad student		(E-Tree)
Monica Gorman	grad student		Stanford
Elizabeth H. Chen	grad student	1998	Stanford (Neurotree)
Carrie M. Garrett-Engele	grad student	2000	Stanford
Suzanne D. Plump	grad student	2000	Stanford
Eric L. Keisman	grad student	2001	Stanford
Shaad M. Ahmad	grad student	2002	Stanford
Devanand S. Manoli	grad student	2007	Stanford (Neurotree)
Joy Hatzidakis	grad student	2008	Stanford
David J. Mellert	grad student	2009	Stanford
Alexander G. Vaughan	grad student	2012	Stanford University, I
Ounissa Ait-Ahmed	post-doc		
Michelle N. Arbeitman	post-doc		Stanford
John Belote	post-doc		
Kenneth C. Burtis	post-doc		
Bruce Chase	post-doc		
Audrey Christiansen	post-doc		
Mitzi Kuroda	post-doc		
Bill Mattox	post-doc		
Michael McKeown	post-doc		
Rod Nagoshi	post-doc		
Brian Oliver	post-doc		
Lisa Ryner	post-doc		
Mariana Wolfner	post-doc		
Yufeng Pan	post-doc	2009-	Janelia Farm
Chuan Zhou	post-doc	2011-	Janelia Farm (Neurotree)
Ignacio Marin	post-doc	1993-1998	
Mark L. Siegal	post-doc	1998-2004	Stanford
Delphine Fagegaltier	post-doc	2001-2004	Stanford

Sex and the Single Fly: A Perspective on the Career of Bruce S. Baker

- Sex Determination and Dosage Compensation in *Drosophila* (CJ)
- The Development of Sexually Dimorphic Structures and the Evolution of Sex (JSH)
- Sex Behavior Meets the Sex Determination Regulatory Hierarchy: The Genetic Control of Sexual Behavior (XLM)

Sex Determination and Dosage Compensation in *Drosophila*

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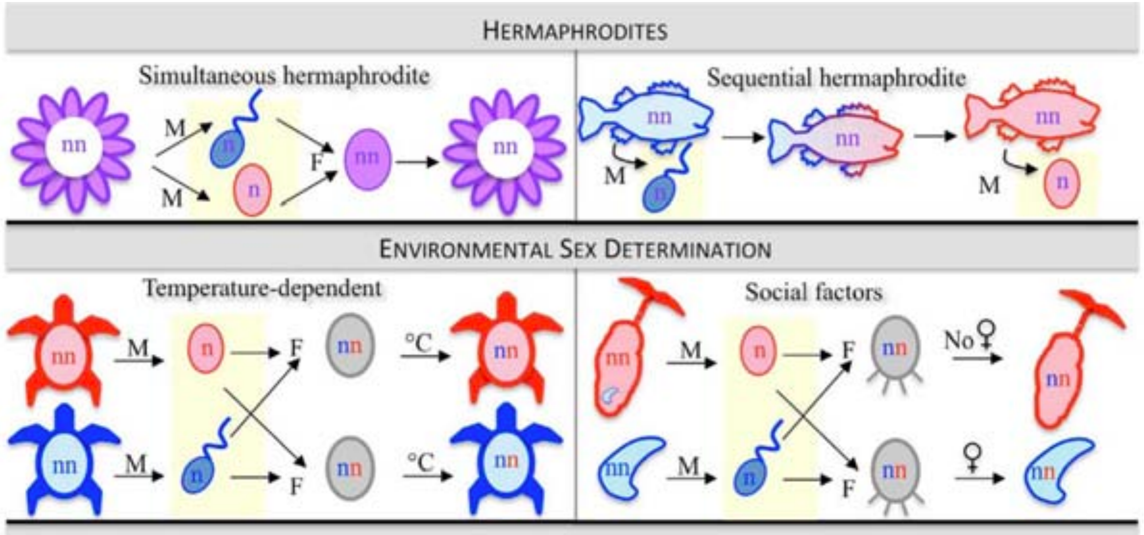
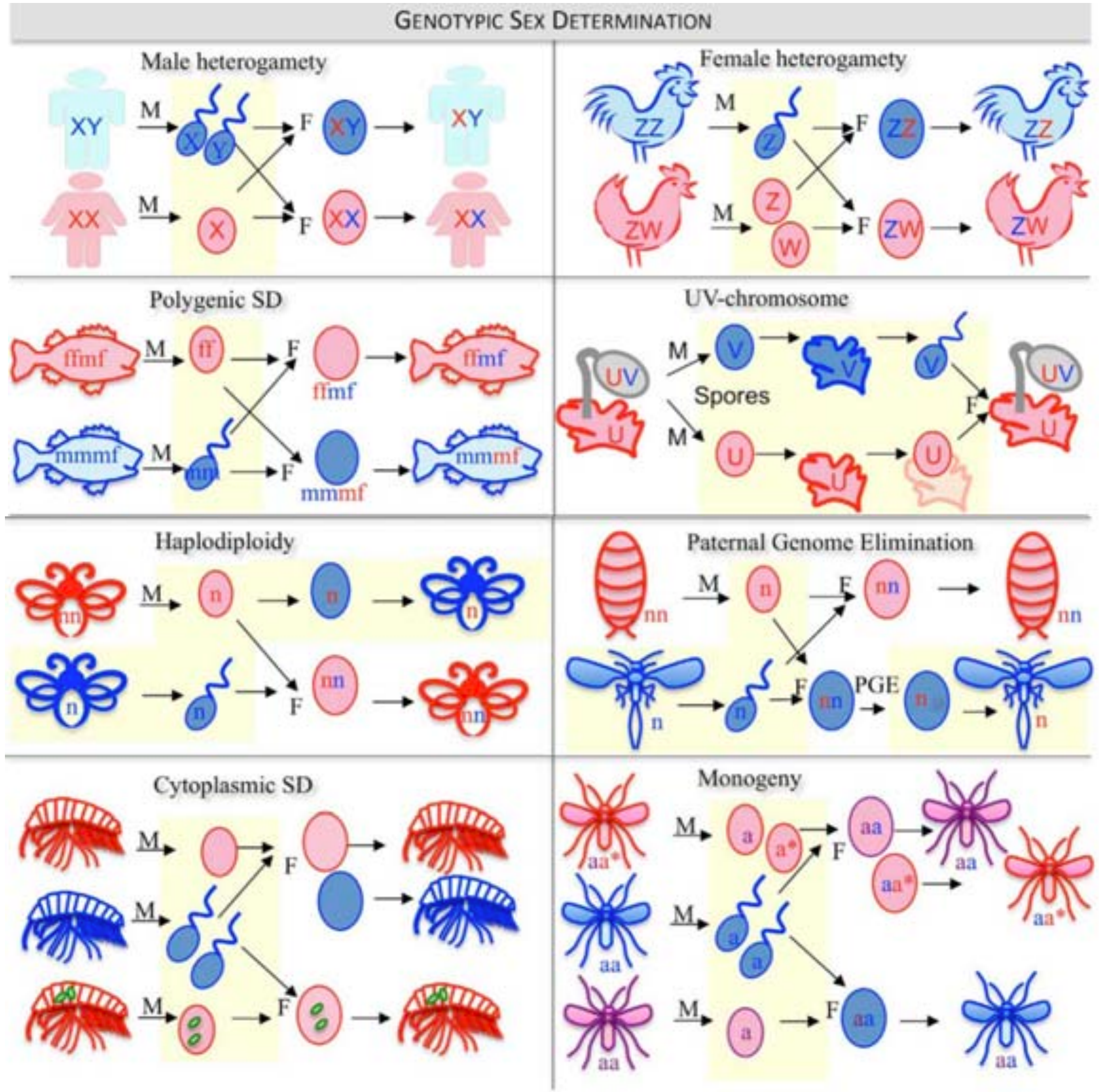
Sex Determination





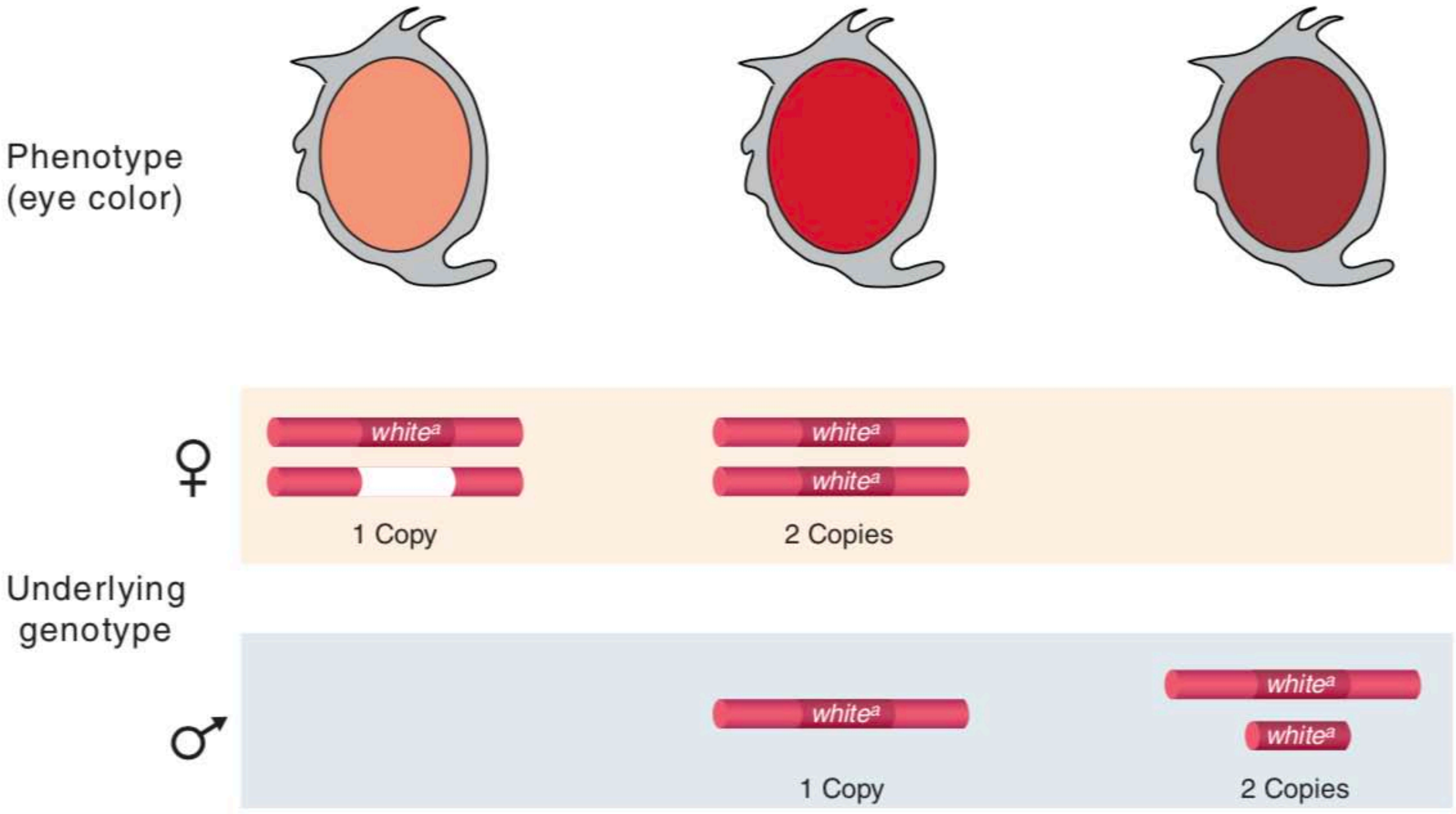
Doris Bachtrog et al. PLOS Biology .2014

Sex Determination and sex chromosomes

	Sex Determination
Drosophila	X chromosome: autosome ratio (X:A ratio) In Drosophila, the Y chromosome has no role in sex determination
Human	The presence or absence of a Y chromosome

	XY（2A）	XX（2A）	XXY（2A）	XYY（2A）
Drosophila	male	female	female	male
Human	male	female	metafemale	metamale

The discovery of dosage compensation in drosophila



John C. Lucchesi and Mitzi I. Kuroda . Cold Spring Harb Perspect Biol. 2015

Dosage compensation

Across species, different sexes are often characterized by different types and numbers of sex chromosomes. In order to account for varying numbers of sex chromosomes, different organisms have acquired unique methods to equalize gene expression amongst the sexes.

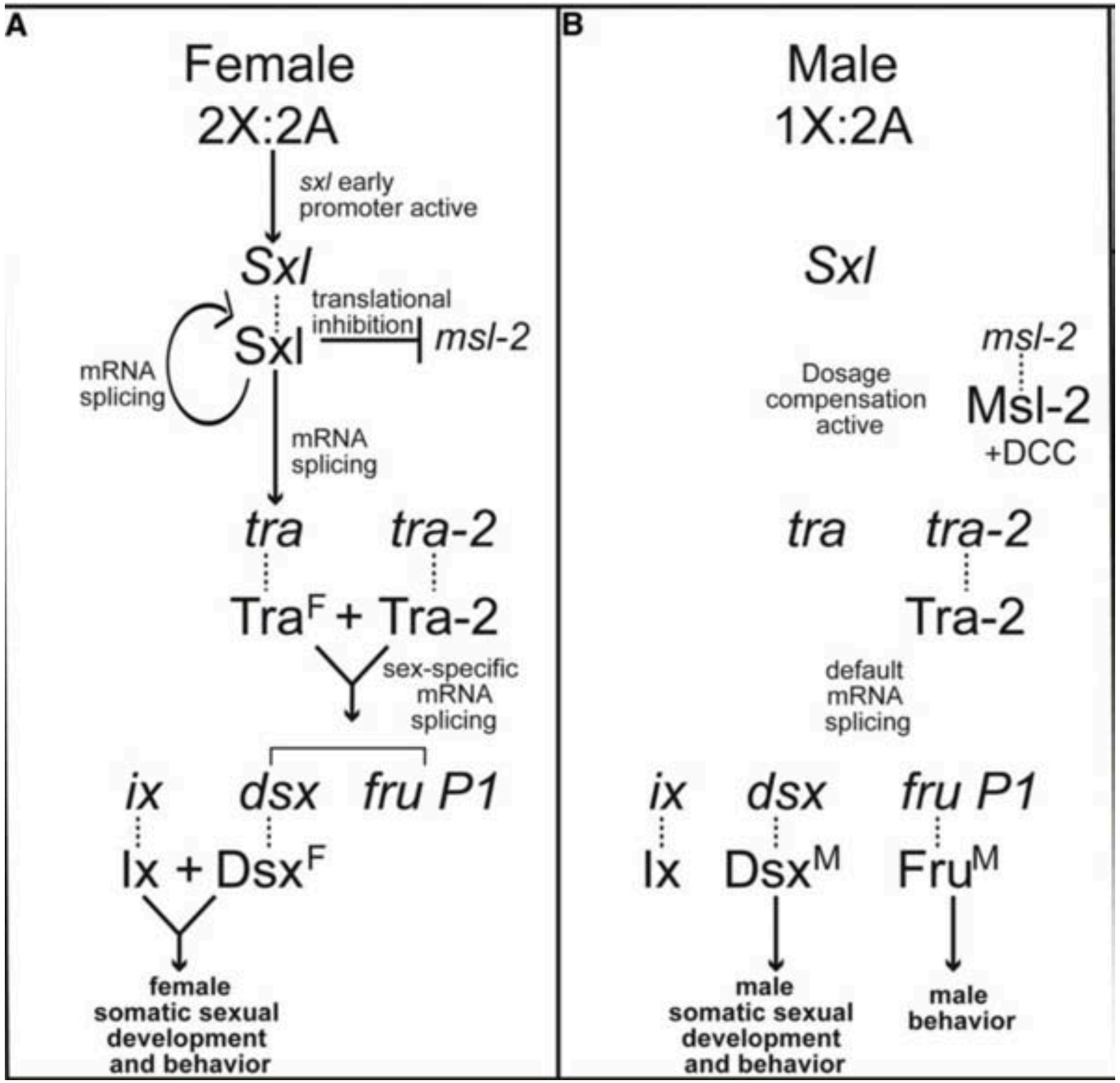
There are three main mechanisms of achieving dosage compensation which are widely documented in the literature and which are common to most species.

1. random inactivation of one female X chromosome (as observed in Mus musculus)

2. a two-fold increase in the transcription of a single male X chromosome (as observed in Drosophila melanogaster)

3. decreased transcription by half in both of the X chromosomes of a hermaphroditic organism (as observed in Caenorhabditis elegans).

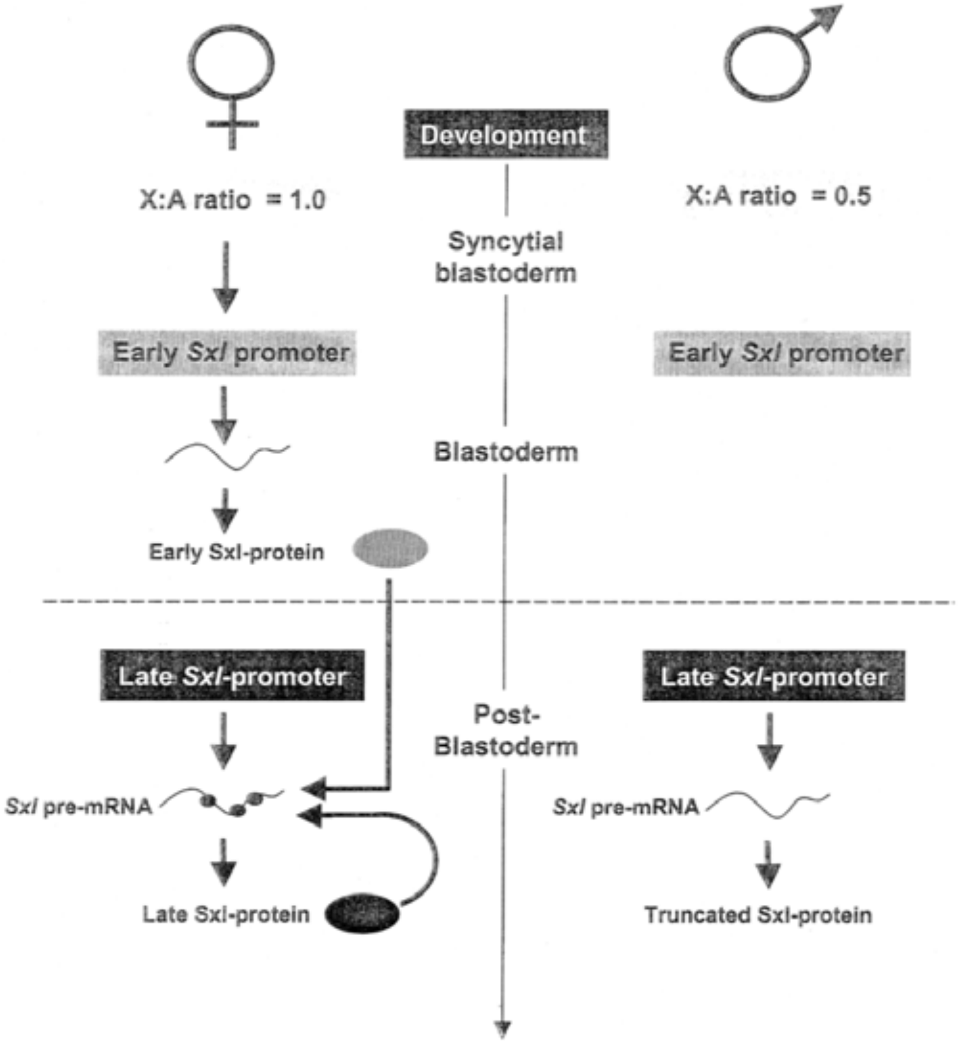
The somatic sex determination hierarchy in *Drosophila*



Deborah J. Andrew et al. Genetics.2019

Thomas W. Cline and *Sex lethal* (*Sxl*)

Regulation of *Sxl* expression



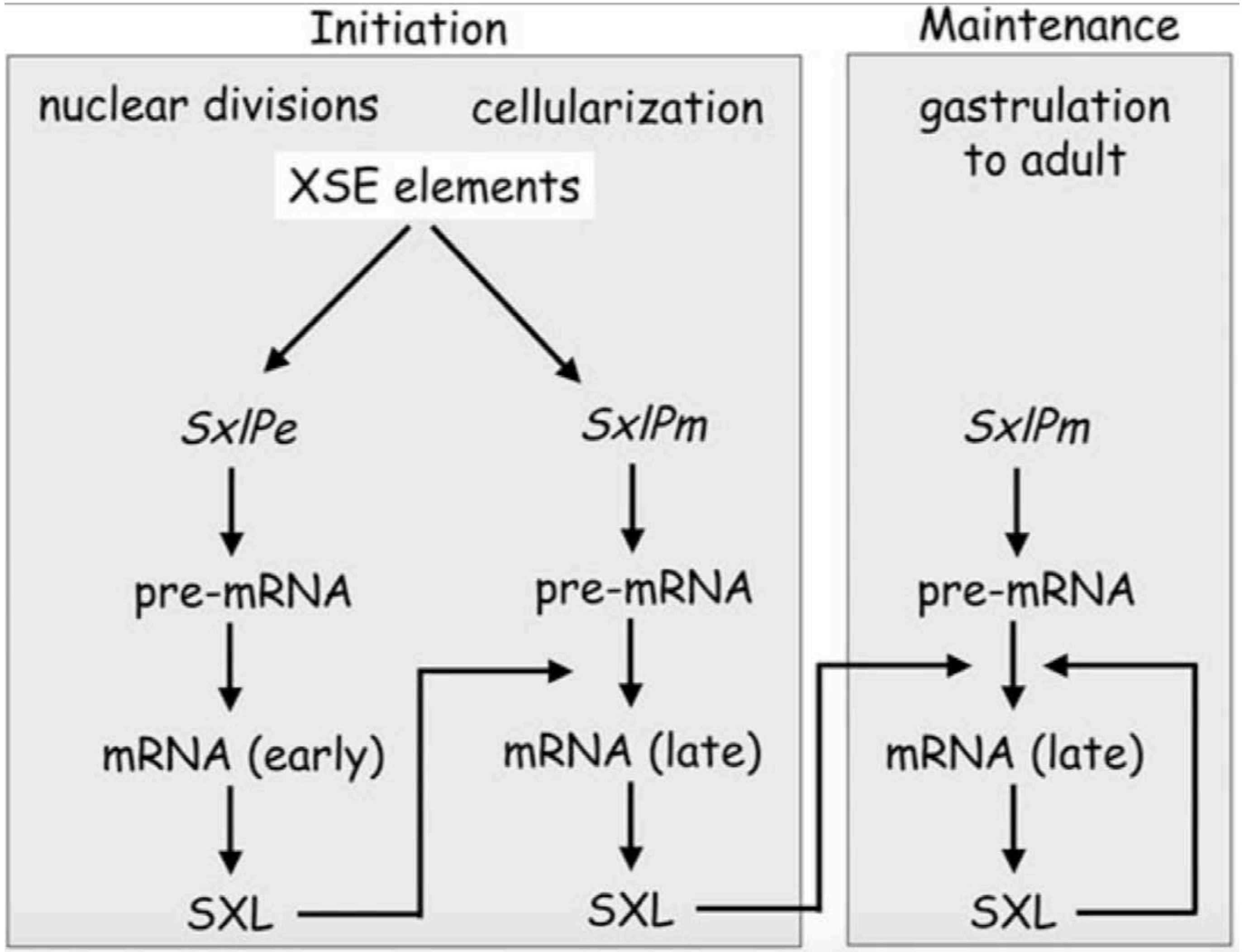
Luiz O. F. Penalva et al. Microbiol Mol Biol Rev. 2003

How does *SxlPe* reliably distinguish between the ratio of X chromosomes and autosomes ?

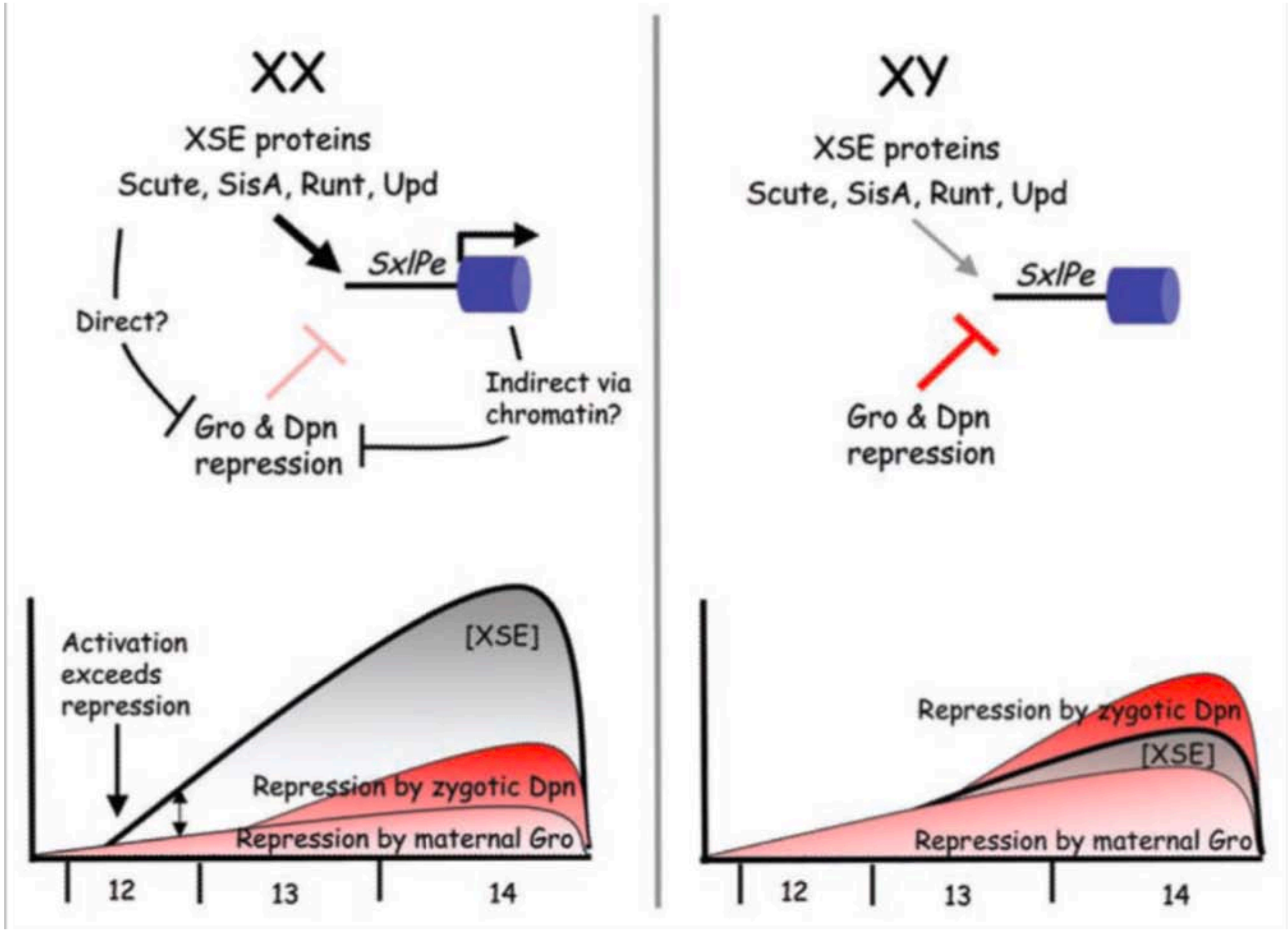
XSE :X-linked signal elements

four X- encoded proteins, encoded by the *scute*, *sisA*, *runt and unpaired*

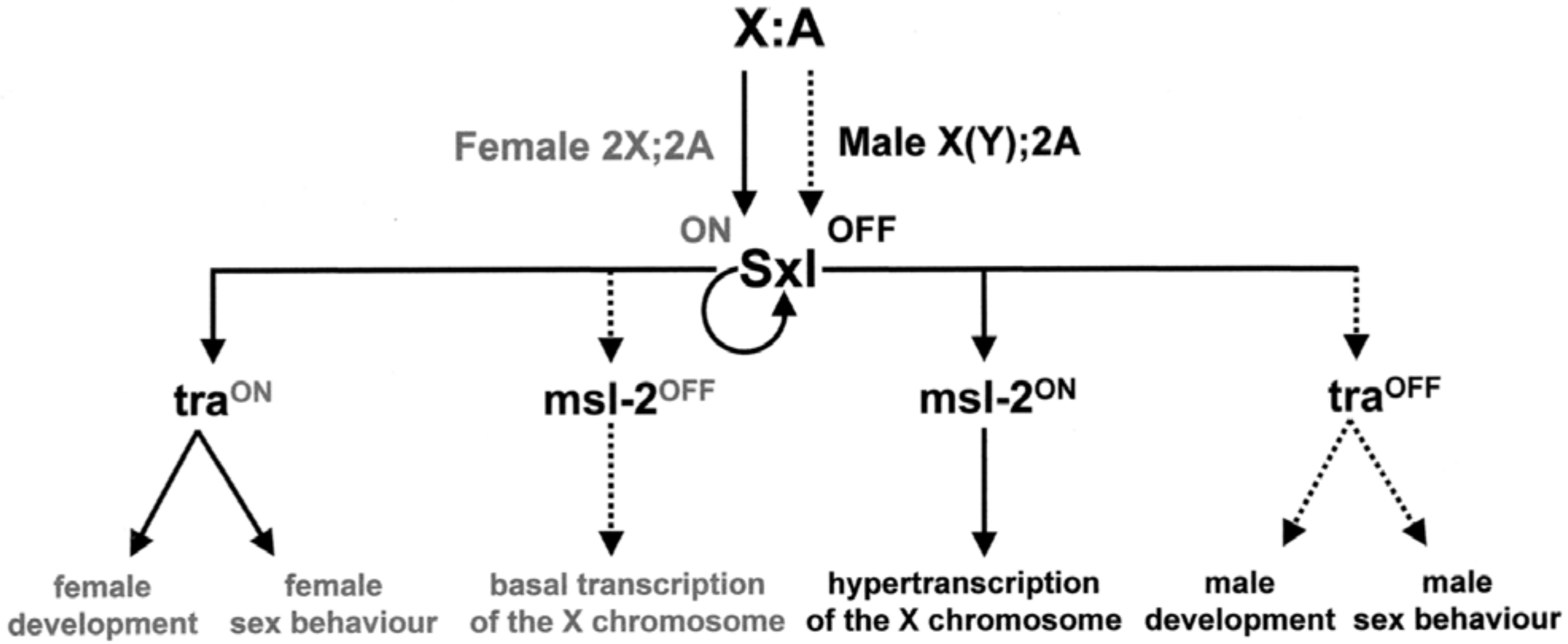
Overview of the regulatory logic that guarantees *Sxl* protein expression in XX animals.



Threshold response model

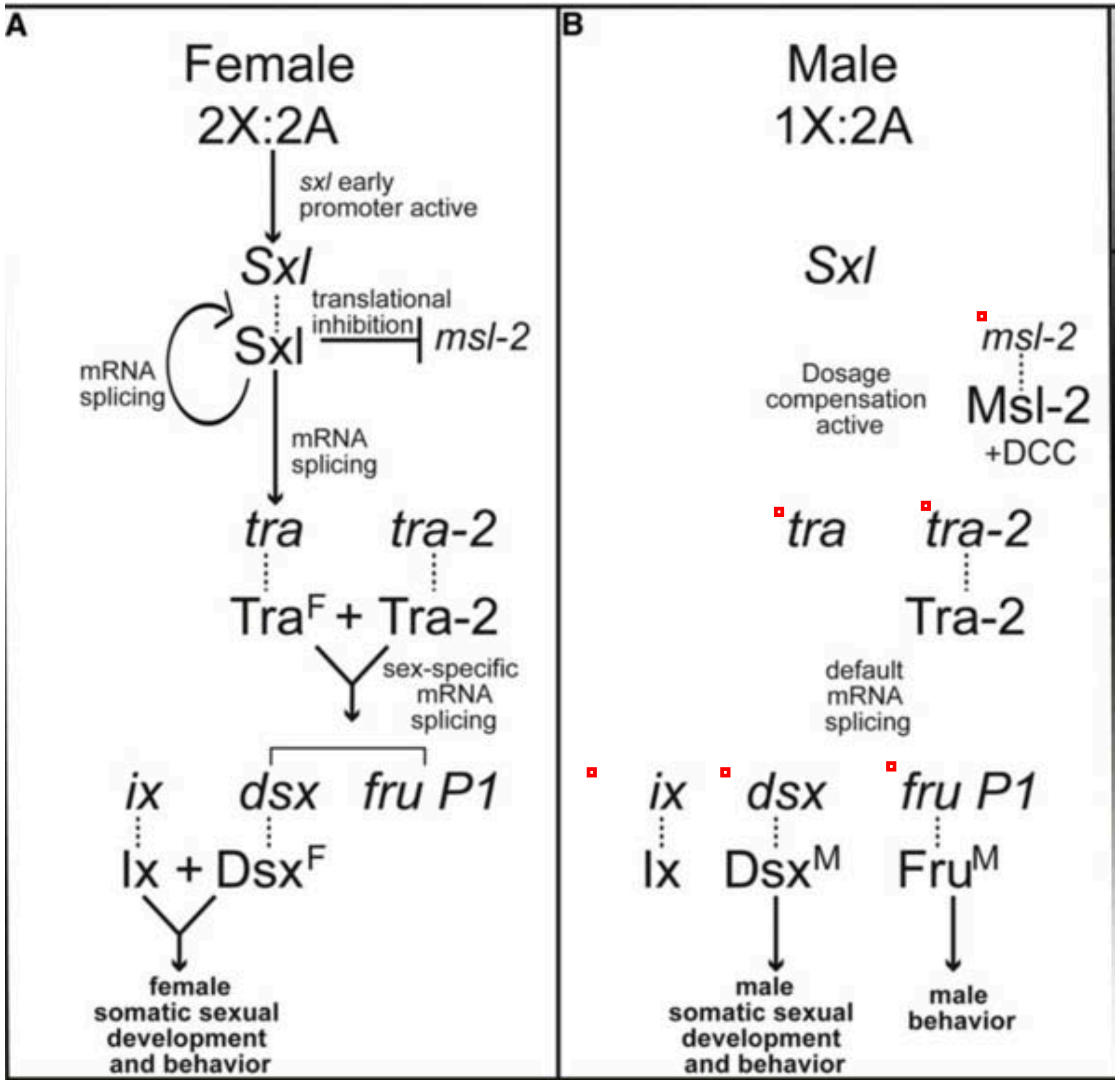


Sxl , sex determination and dosage compensation



Deborah J. Andrew et al. Genetics.2019

The somatic sex determination hierarchy in *Drosophila*



Deborah J. Andrew et al. Genetics.2019

Sex and single cell

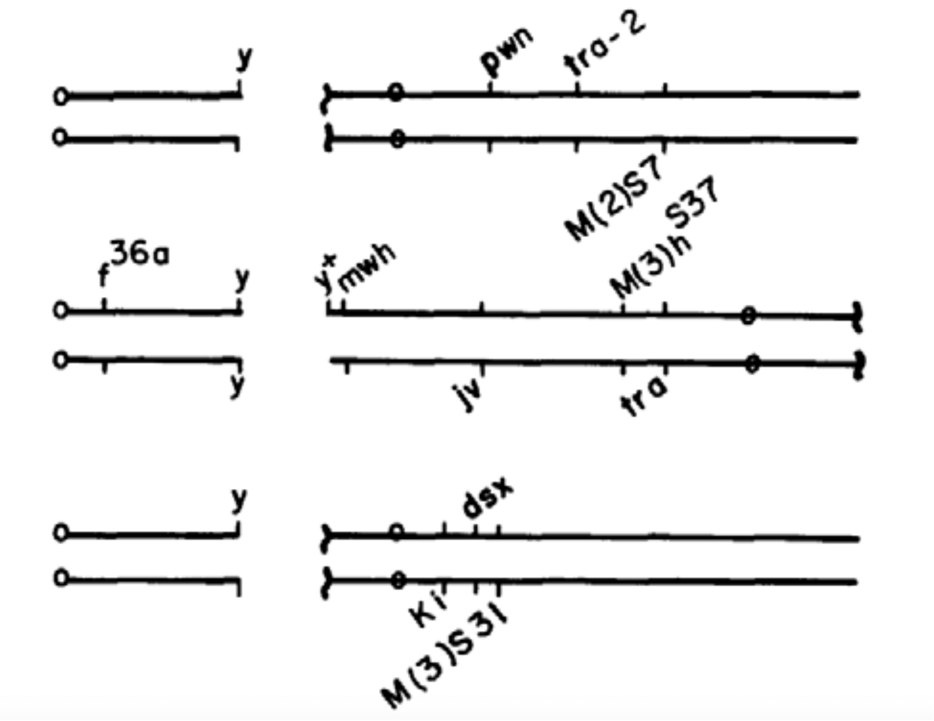
SEX AND THE SINGLE CELL. I. ON THE ACTION OF MAJOR LOCI AFFECTING SEX DETERMINATION IN *DROSOPHILA MELANOGASTER*[1]

BRUCE S. BAKER AND KIMBERLY A. RIDGE

- That the four genes known at the time to cause dramatic sex-transformation phenotypes in Drosophila— *transformer (tra), transformer-2 (tra-2), doublesex (dsx)*, and *intersex (ix)*—function in a cell-autonomous manner
- That these genes function in a shared genetic pathway

Locus (symbol)		Chromosome-map position	Relevant properties	Major references†
I. Sex determination mutants:				
transformer-2	(*tra-2*)	2–70	Transforms females into males; males sterile	WATANABE 1975
	($tra\text{-}2^{OTF}$)		Incomplete transformation of females into males; males fertile	FUJIHARA, KAWABE and OISHI 1978
intersex	(*ix*)	2–60.5	Transforms females into intersexes; males normal	KROEGER 1959
	(ix^2)		Like *ix*	
transformer	(*tra*)	3–45	Transforms females into males; males normal	STURTEVANT 1945; SEIDEL 1963
	(tra^{AC})		Like *tra*	This report
doublesex	(*dsx*)	3–48.1	Transforms both males and females into intersexes	HILDRETH 1965
doublesex-dominant	(dsx^D)		Dominant, $dsx^D/+$ transforms females into intersexes; male unaffected	FUNG and GOWEN 1957; DUNCAN and KAUFMAN 1975
Masculanizer	($Mas = dsx^{Mas}$)		Like dsx^D	

That the four genes known at the time to cause dramatic sex-transformation phenotypes in Drosophila— *transformer (tra)*, *transformer-2 (tra-2)*, *doublesex (dsx)*, and *intersex (ix)*—function in a cell-autonomous manner



Analysis of autonomy of the tra-2 *locus and time of* tra-2+ *expression in the abdomen*

Time Irradiated	Clones: Tergites 5 and 6: Male *pwn*	Male no *pwn*	Indeterminate *pwn*	Female *pwn*	Tergites 2,3,4 *pwn*	Number of abdomens	Frequency male clones	Frequency *pwn* clones
No irradiation	2	2	1	0	4	295	0.014	0.024
Pre-pupariation, hrs:								
120–19	59	17	2	2	111	393	0.193	0.443
19–12	14	9	1	0	24	106	0.217	0.368
12–4	22	9	2	1	28	142	0.218	0.373
4–0	5	1	1	1	13	41	0.146	0.488
Post-pupariation, hrs:								
0–5.5	8	4	0	1	17	44	0.273±0.067	0.591
5.5–13.5	14	16	1	10	70	107	0.280±0.043	0.888
13.5–21.5	25	34	8	57	174	193	0.306±0.033	1.368
21.5–29.5	6	0	1	96	160	113	0.053±0.019	2.33

y/+; pwn tra-2/+ female progeny

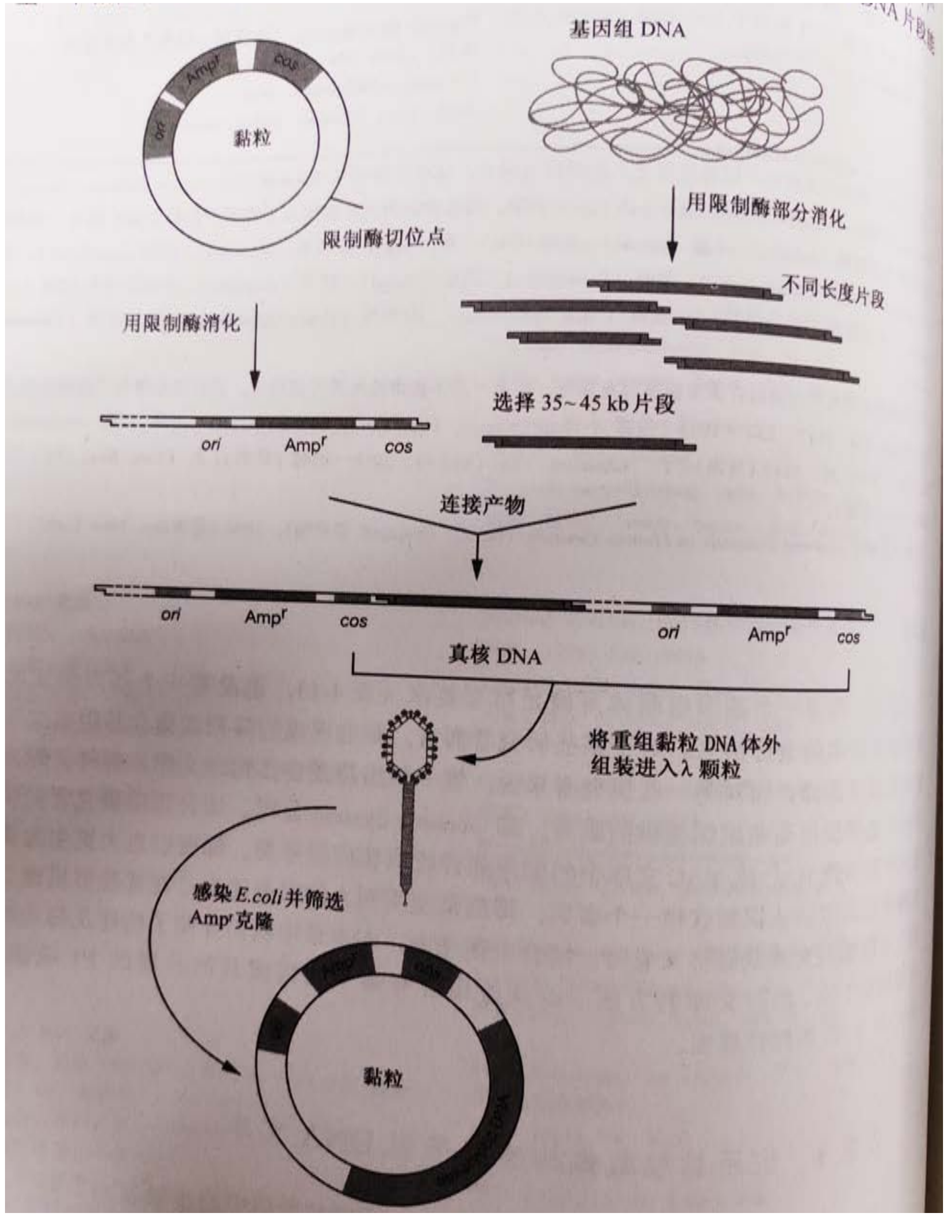
That these genes (*tra, tra-2, dsx*, and *ix*) function in a shared genetic pathway

Method: Double-mutant combinations

In a pathway, then the double-mutant combination should exhibit the same phenotype as one of the component single mutants.
In parallel pathways, the double-mutant combination should exhibit a phenotype that is a composite of the phenotypes produced by the two component single mutants.

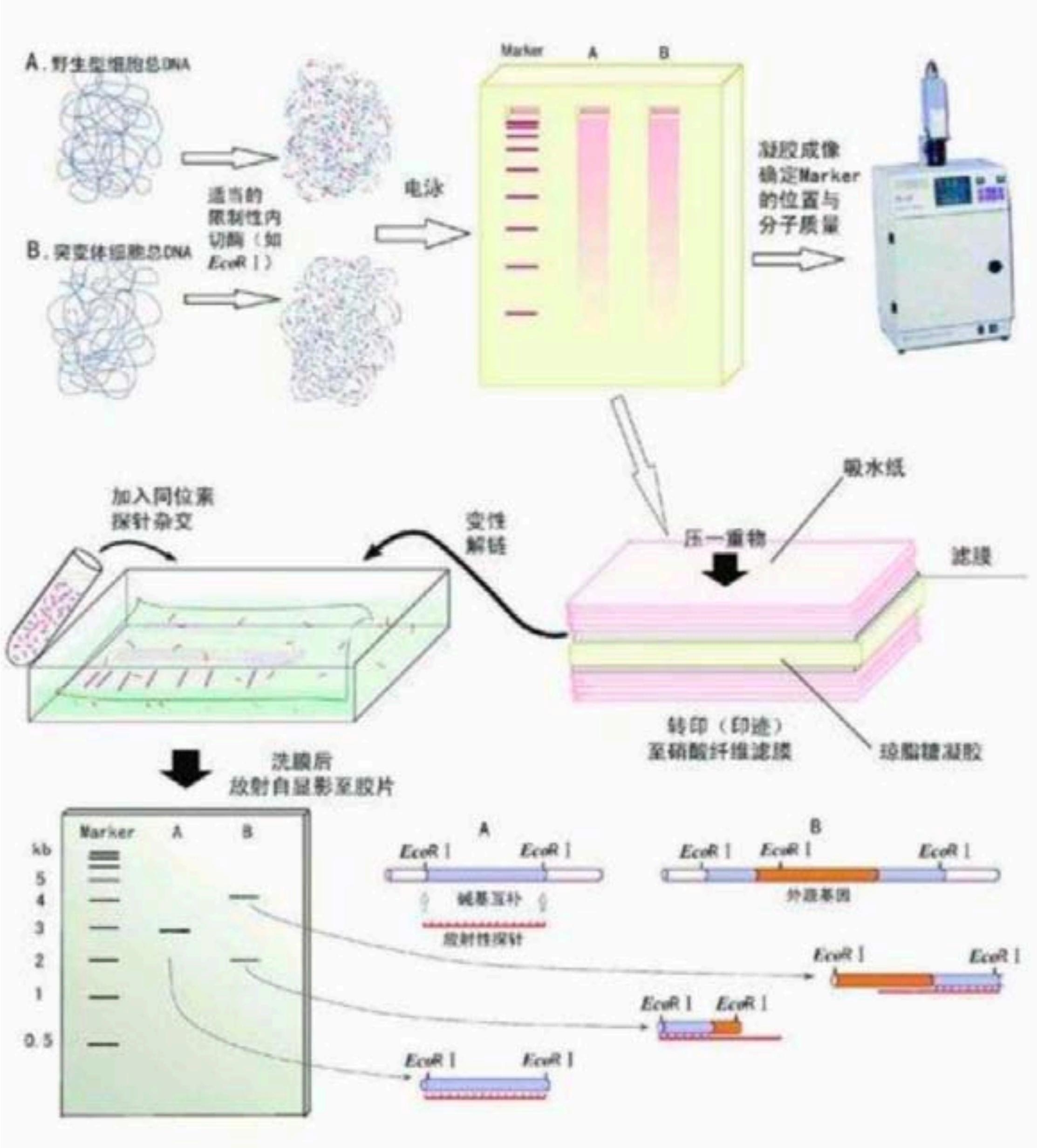
mutant	phenotype	Conclusion
dsx mutant	Intersexes	*dsx* is epistatic to *tra*
tra mutant	Transforms female into male	
dsx + *tra* mutant	Intersexes	

Cosmid clones for the chromosome walk



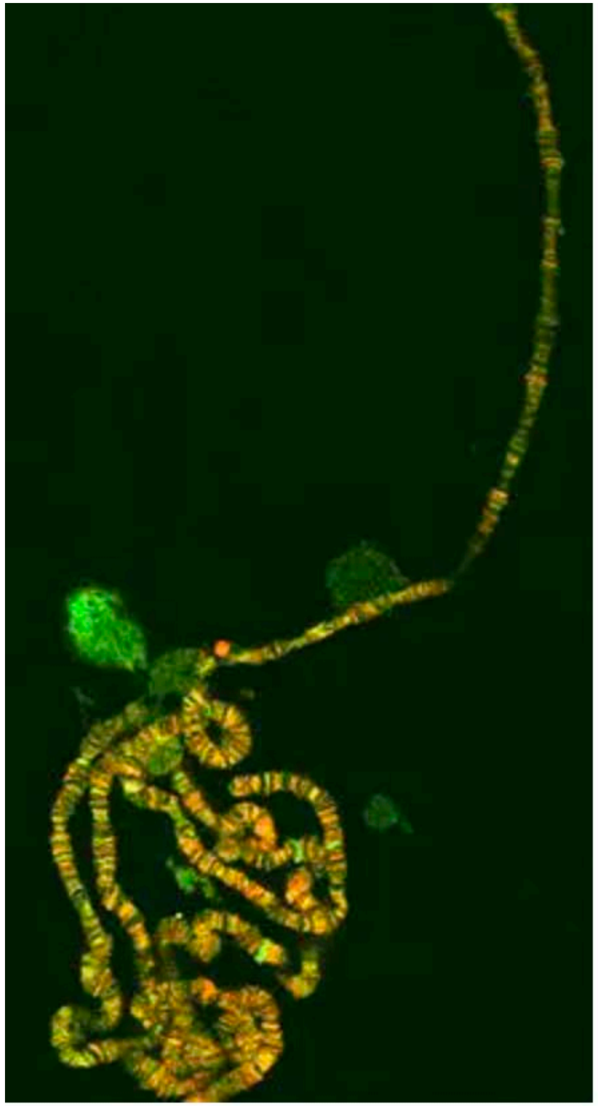
Southern blot

A Southern blot is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.



Polytene chromosome

Polytene chromosomes are oversized chromosomes which have developed from standard chromosomes and are commonly found in the salivary glands of Drosophila melanogaster. Specialized cells undergo repeated rounds of DNA replication without cell division (endomitosis), to increase cell volume, forming a giant polytene chromosome. Polytene chromosomes form when multiple rounds of replication produce many sister chromatids that remain fused together.

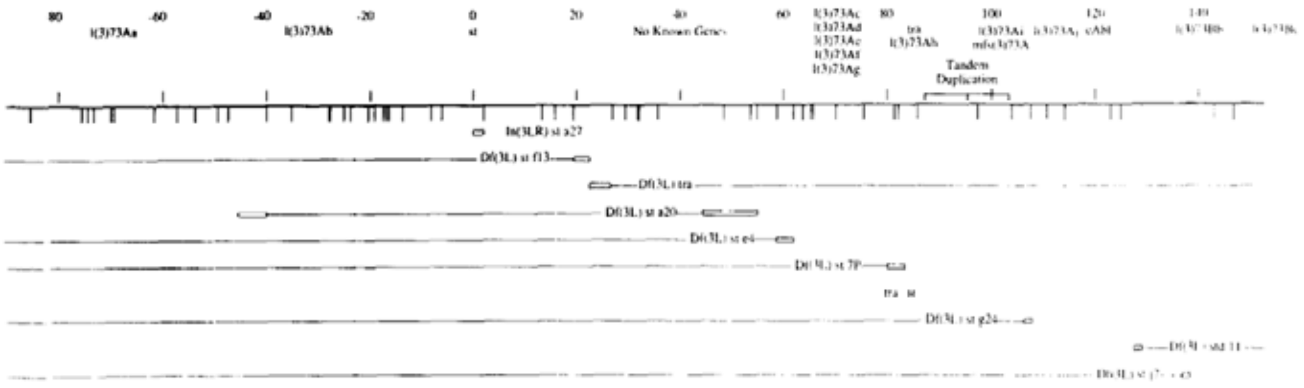


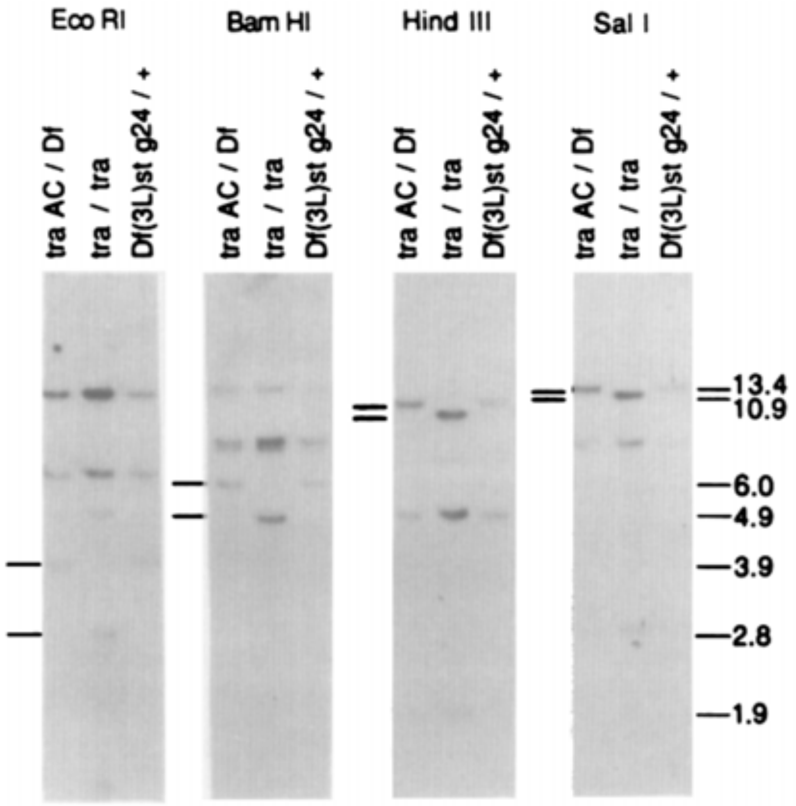
Polytene chromosome

Cell, Vol. 48, 489–499, February 13, 1987, Copyright © 1987 by Cell Press

A Molecular Analysis of *transformer*, a Gene in Drosophila melanogaster That Controls Female Sexual Differentiation

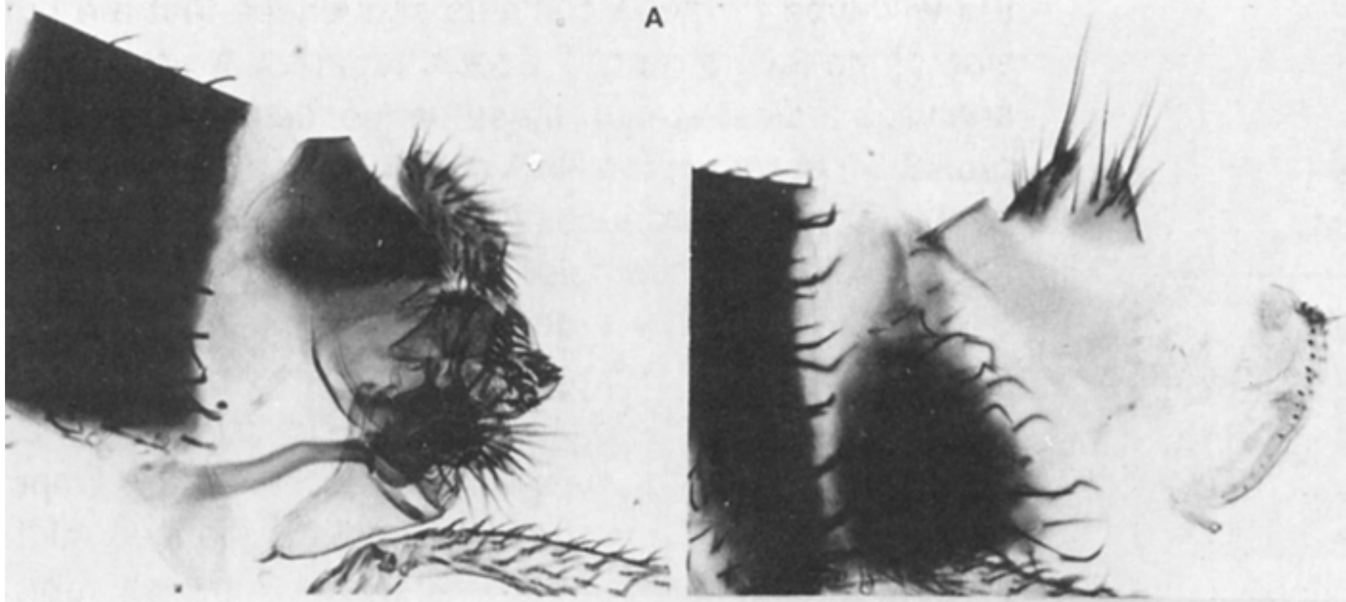
Michael McKeown,* † John M. Belote,* ‡
and Bruce S. Baker* §

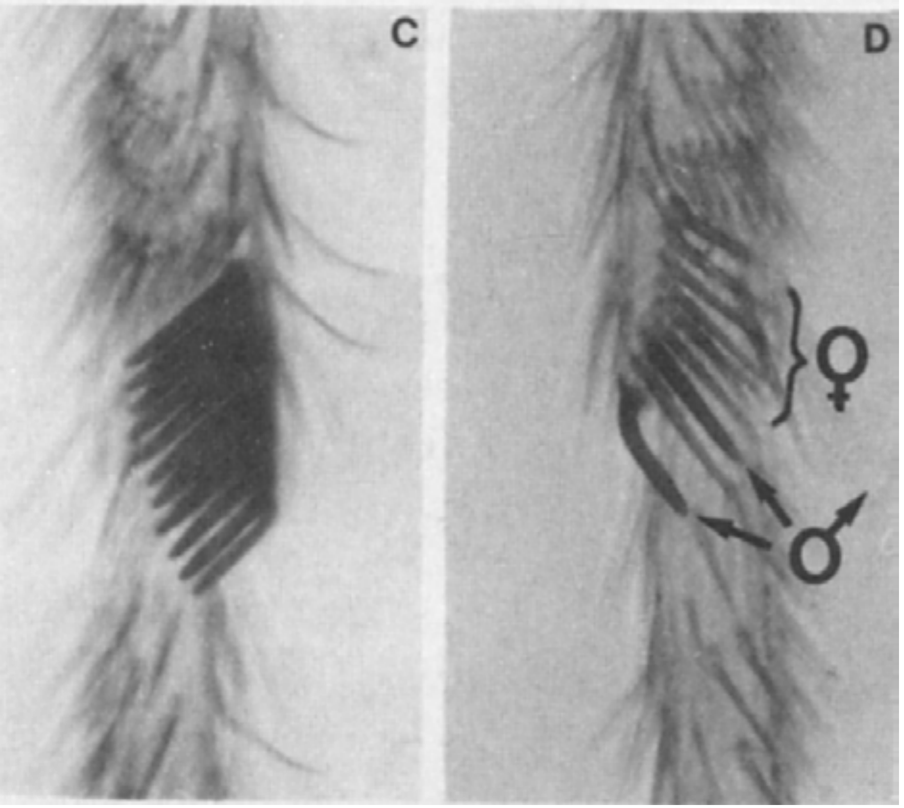




Southern blot

Identification of the tra Gene by Germ-Line Transformation



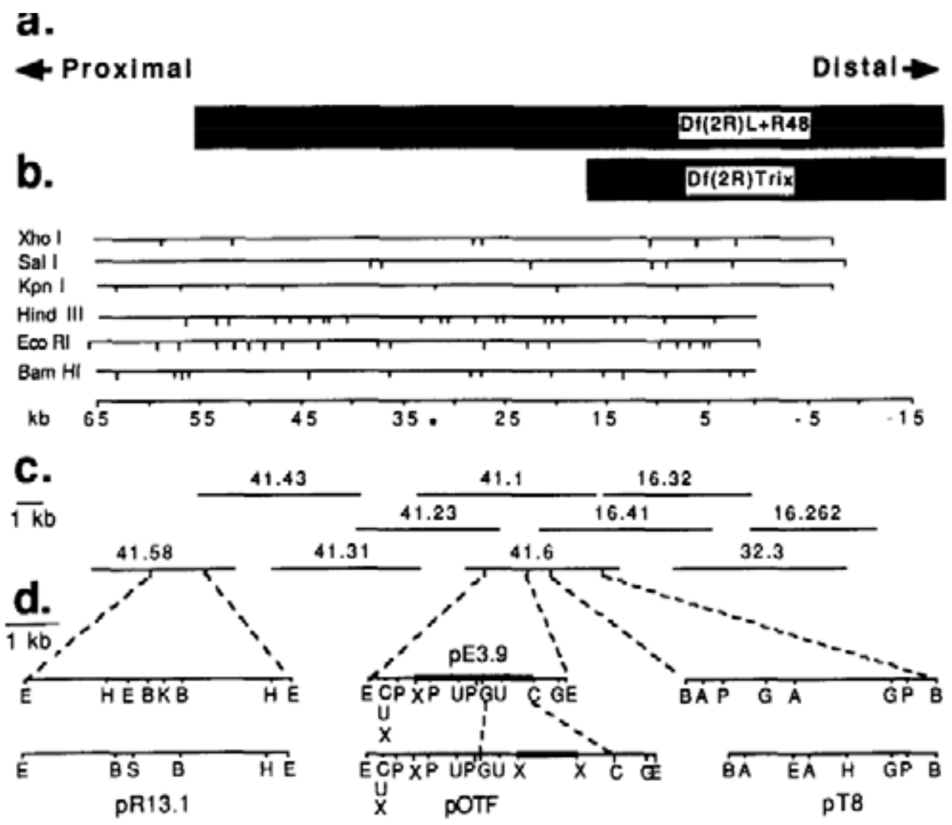


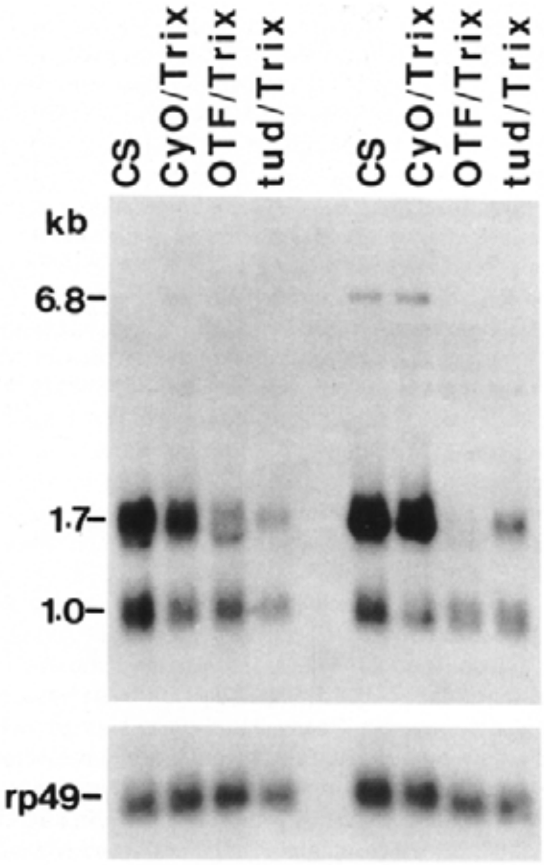
A) Male genitalia of XX; *tra*/Df(3L)st j7, Ki roe p^p transformed female. (B) Female genitalia of an XX; ; *tra*/Df(3L)st j7, Ki roe p^p wild-type female. (C) Sex comb of XX ; *tra*/Df(3L)st j7, Ki roe p^p transformed female. (D) Intersexual sex comb of an X, RB161X; ; *tra*/Df(3L)st j7, Ki roe p^p transformant.

Cell, Vol. 56, 1011–1018, March 24, 1989, Copyright © 1989 by Cell Press

Discovery of Tra-2

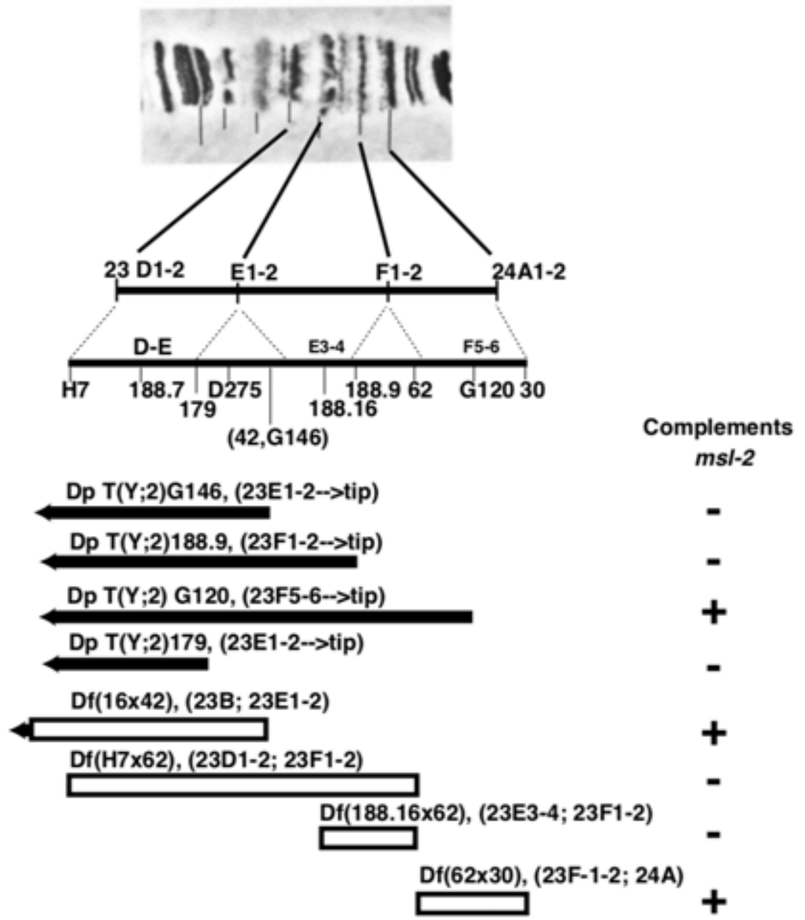
The Sex Determination Locus *transformer-2* of Drosophila Encodes a Polypeptide with Similarity to RNA Binding Proteins

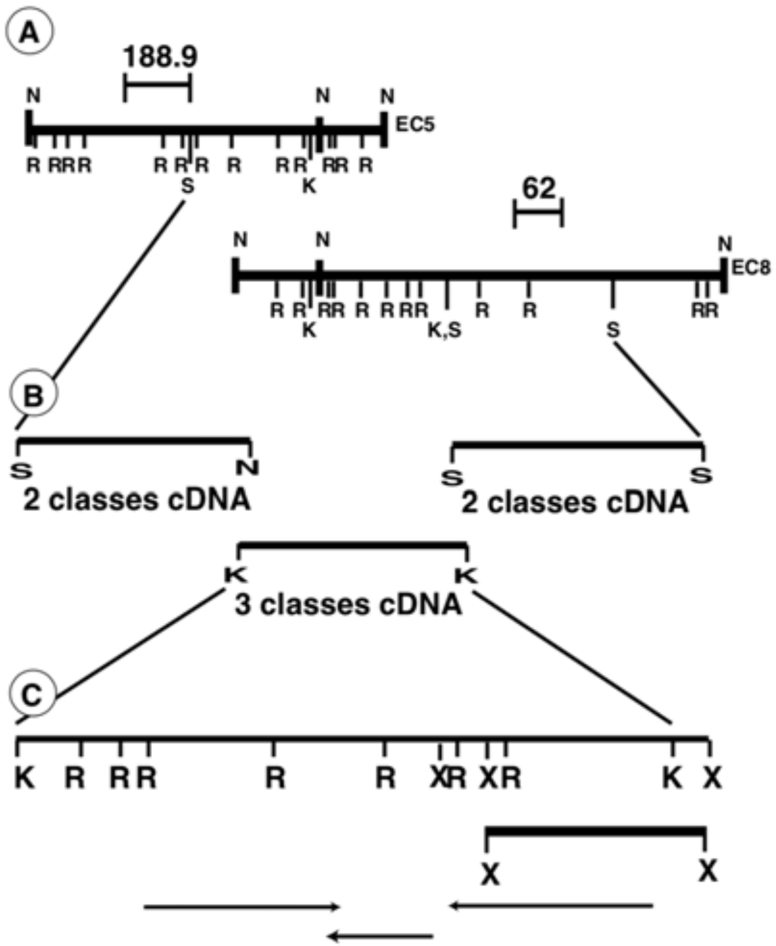




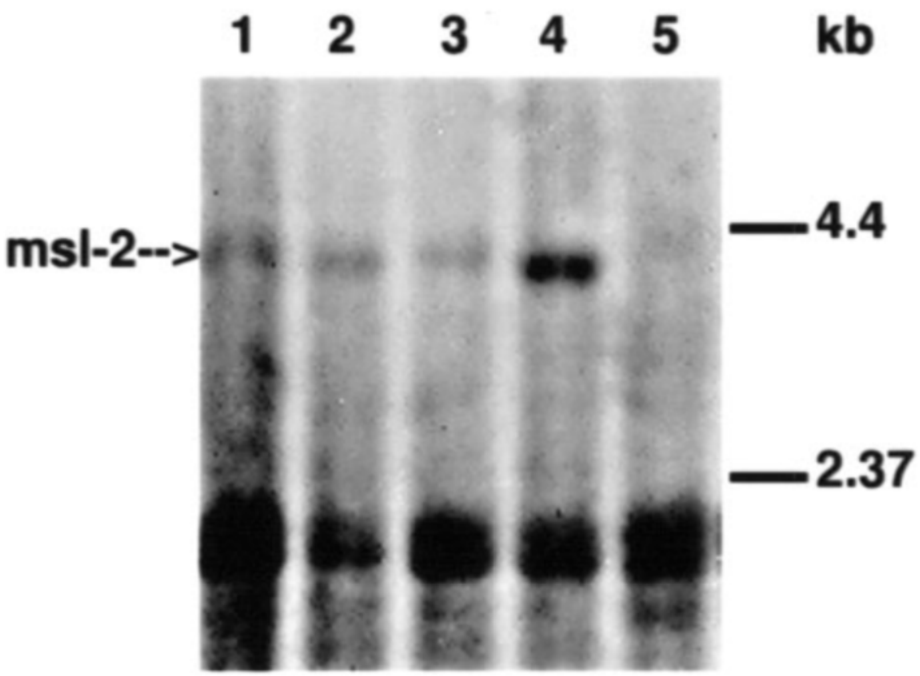
Northern blot

The *msl-2* dosage compensation gene of *Drosophila* encodes a putative DNA-binding protein whose expression is sex specifically regulated by *Sex-lethal*

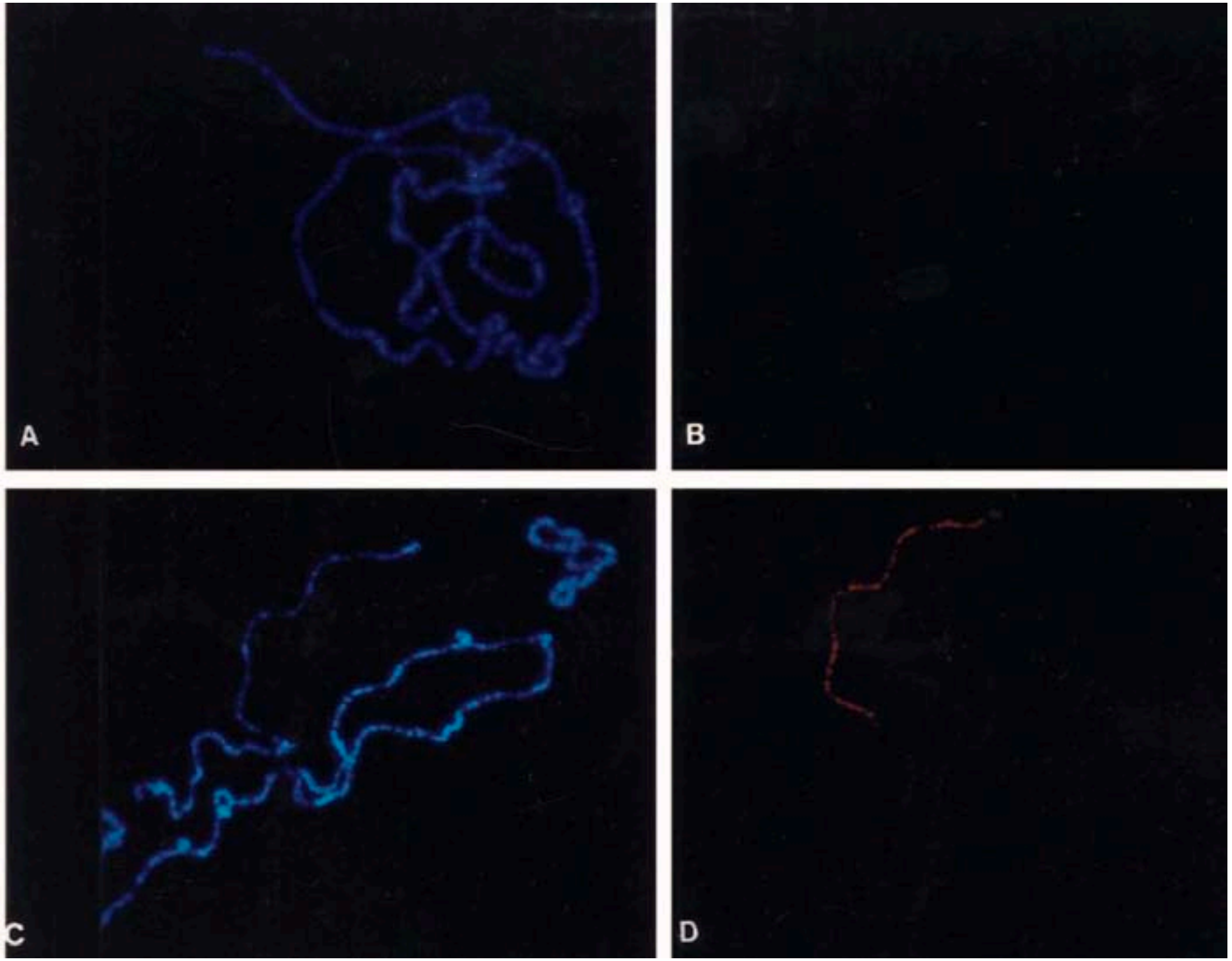




msl-2 and dosage compensation

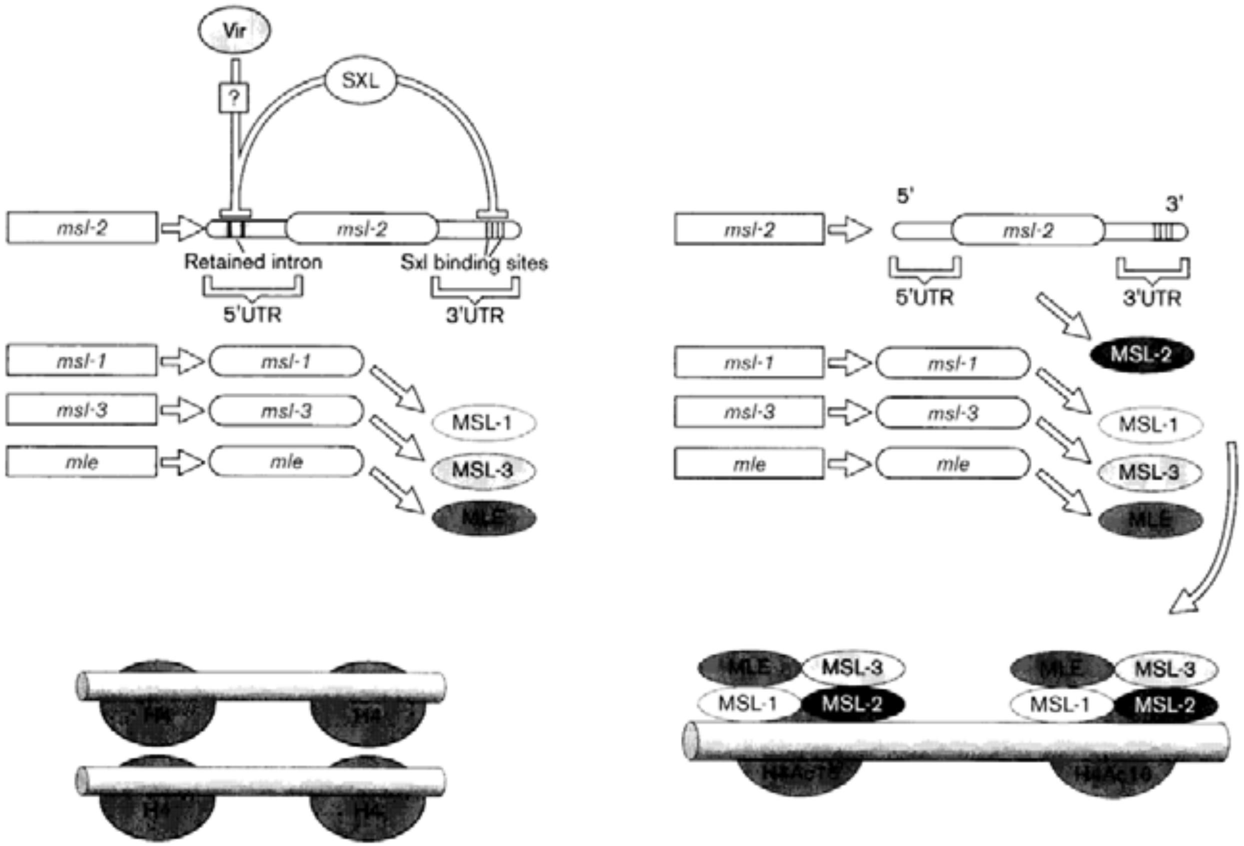


Northern analysis of 4-6 µg of poly(A)+ RNA prepared from: lane 1, Canton S females; lane 2, Canton S males; lane 3, *tudor* females; lane 4, *tudor* males; lane 5, *msl-2* females

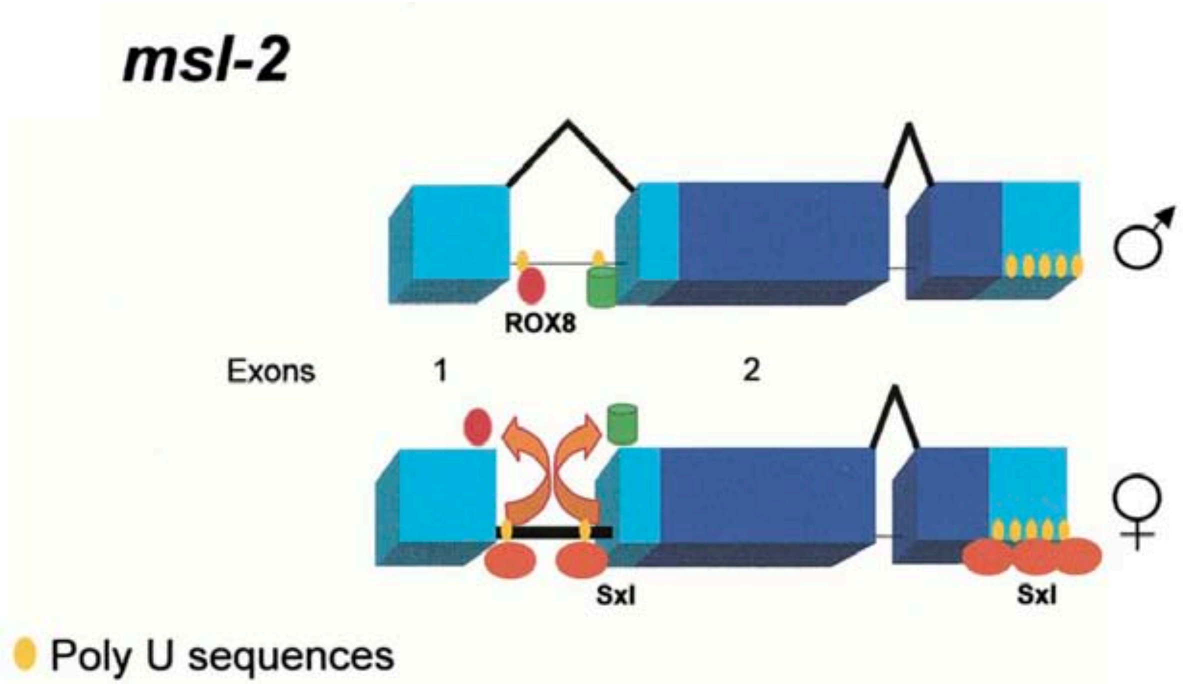


Anti-MSL-2 and anti-MSL-1 staining of wild-type male and female chromosomes. (A) DNA stain of wild-type female. (B) Anti-MSL-2 stain of wild-type female.

Dosage Compensation in *Drosophila*

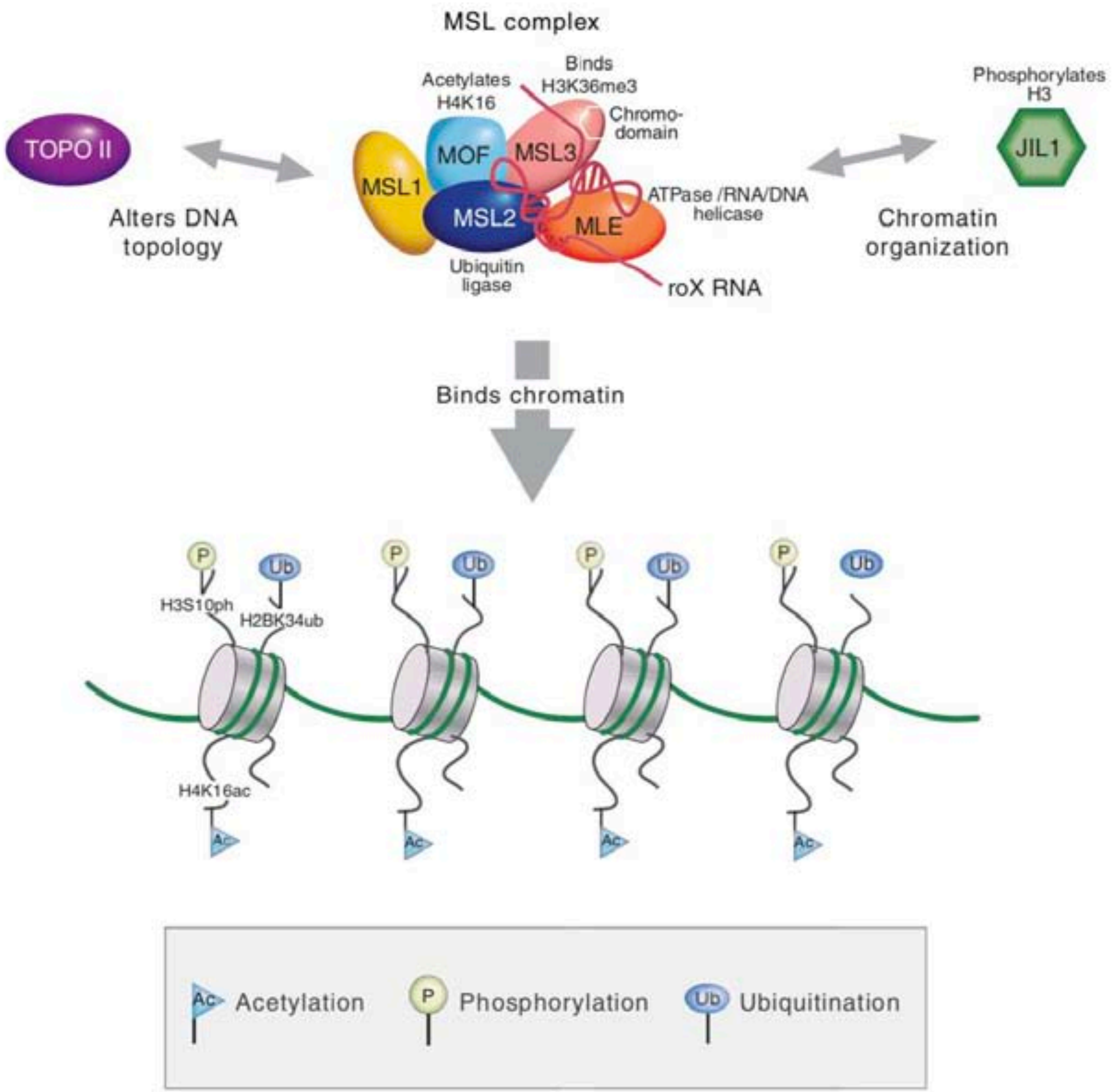


Greg J Bashaw and Bruce S Baker. Curr Opin Genet Dev. 1996



John C. Lucchesi and Mitzi I. Kuroda . Cold Spring Harb Perspect Biol. 2015

Assembly of the chromatin-remodeling complex responsible for dosage compensation

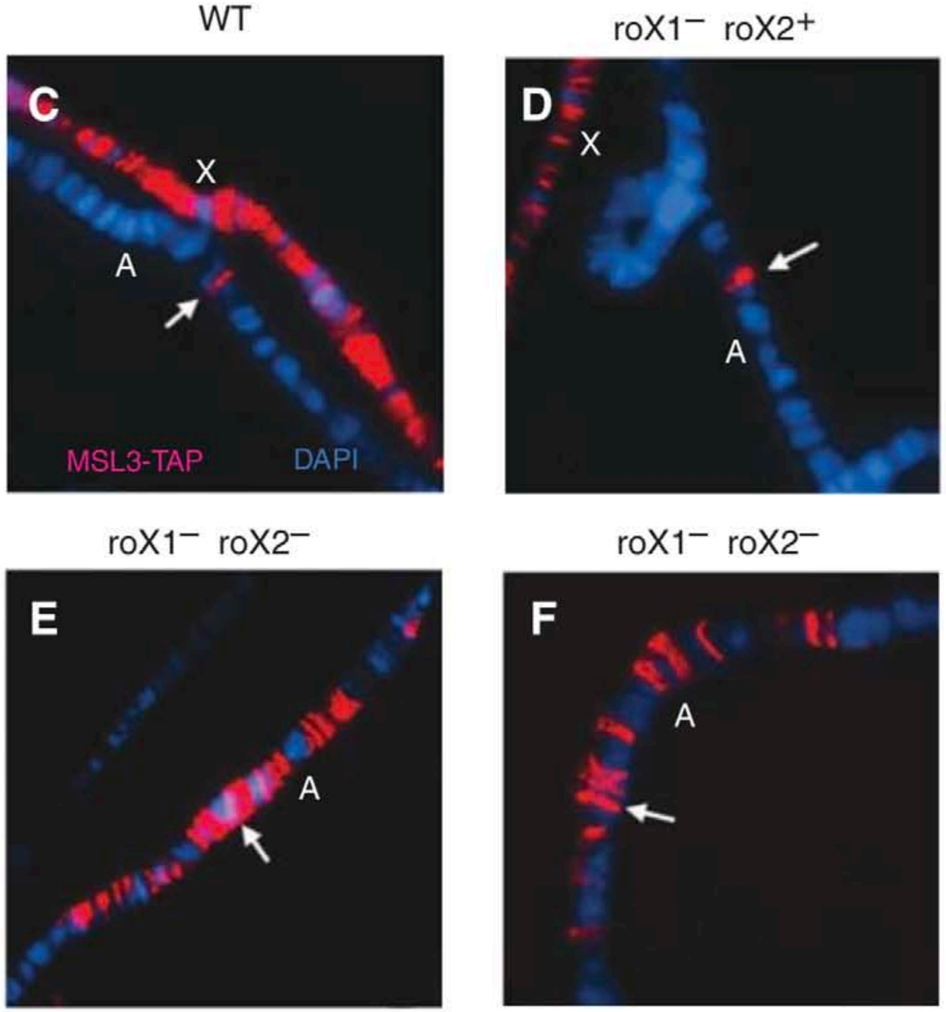


roX: X-specific noncoding RNAs (called 1 and roX2)

John C. Lucchesi and Mitzi I. Kuroda. Cold Spring Harb Perspect Biol. 2015

Why MSL complex can specific recognize the X chromosome?

1.The roX genes may reside on the X to target MSL complex assembly to this chromosome.



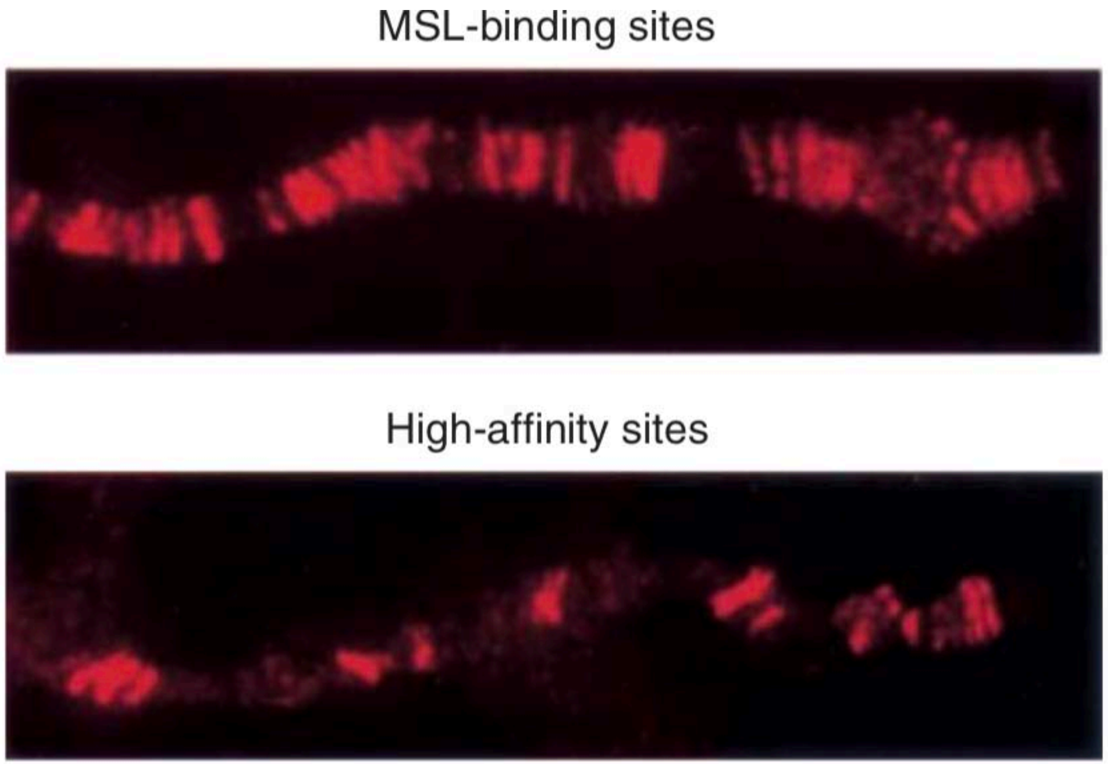
in all cases in which *roX* genes direct spreading in *cis* on autosomes, they also provide roX RNA in *trans* to cover the X chromosome (Meller and Rattner 2002). Therefore, it is clear the X chromosome has additional targeting signals beyond the two known *roX* genes.

John C. Lucchesi and Mitzi I. Kuroda. Cold Spring Harb Perspect Biol. 2015

Why MSL complex can specific recognize the X chromosome?

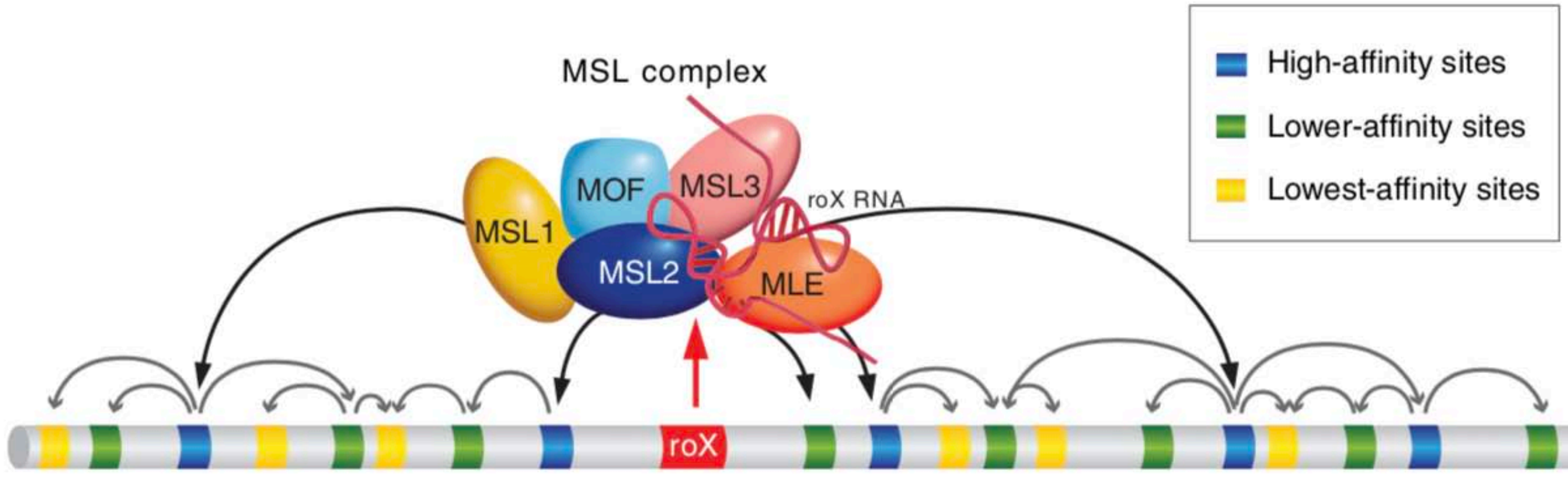
2. MSL1 and MSL2 are critical for the specific recognition of the X chromosome

In the absence of either MSL1 or MSL2, none of the remaining MSL proteins or roX RNAs appears to retain specific recognition for the X chromosome.
However, in the absence of MLE, MSL3, or MOF, partial MSL complexes bind a subset of approximately 35 – 70 sites by cytological mapping, including the two roX genes



John C. Lucchesi and Mitzi I. Kuroda. Cold Spring Harb Perspect Biol. 2015

Model for the targeting of the MSL complex to the X chromosome



John C. Lucchesi and Mitzi I. Kuroda. Cold Spring Harb Perspect Biol. 2015

References

- Amrein H., Gorman M., Nothiger R., 1988 The sex-determining gene tra-2 of Drosophila encodes a putative RNA binding protein. Cell **55**: 1025–1035. doi:10.1016/0092-8674(88)90247-4
- Andrew, D.J., et al., *Sex and the Single Fly: A Perspective on the Career of Bruce S. Baker.* Genetics, 2019. **212**(2): p. 365-376.
- Baker B. S., 1989 Sex in flies: the splice of life. Nature **340**: 521–524.doi:10.1038/340521a0
- Baker, B. S., and J. M. Belote, 1983 Sex determination and dosage compensation in Drosophila melanogaster. Annu. Rev. Genet. 17: 345–393.
- Baker, B. S., and K. A. Ridge, 1980 Sex and the single cell. I. On the action of major loci affecting sex determination in Drosophila melanogaster. Genetics 94: 383–423.
- Baker, B. S., M. Gorman, and I. Marin, 1994 Dosage compensation in Drosophila. Annu. Rev. Genet. 28: 491–521.
- Bashaw, G. J., and B. S. Baker, 1995 The msl-2 dosage compensation gene of Drosophila encodes a putative DNA-binding Sex-specific deployment of Perspectives 373 protein whose expression is sex specifically regulated by sex-lethal. Development 121: 3245–3258.
- Bashaw, G. J., and B. S. Baker, 1996 Dosage compensation and chromatin structure in Drosophila. Curr. Opin. Genet. Dev. 6: 496–501.
- Lucchesi, J.C. and M.I. Kuroda, *Dosage Compensation inDrosophila.* Cold Spring Harbor Perspectives in Biology, 2015. **7**(5).
- Penalva, L.O. and L. Sanchez, *RNA binding protein sex-lethal (Sxl) and control of Drosophila sex determination and dosage compensation.* Microbiol Mol Biol Rev, 2003. **67**(3): p. 343-59, table of contents.
- Miko, I., 2008 Sex chromosomes and sex determination. Nature Educ. 1: 108.
- Salz, H.K. and J.W. Erickson, *Sex determination in Drosophila: The view from the top.* Fly (Austin), 2010. **4**(1): p. 60-70.

The Development of Sexually Dimorphic Structures and the Evolution of Sex

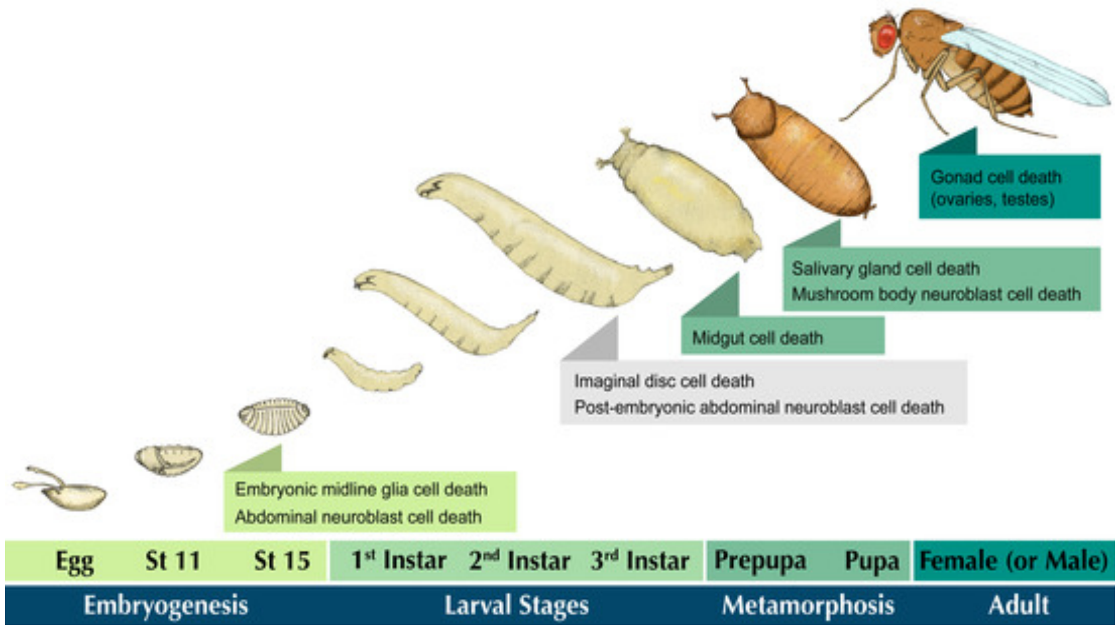
JSH

How does the sex determination pathway direct the development of sexually dimorphic morphologies?

How did sex differences evolve?

384 B. S. BAKER AND K. A. RIDGE

SEX determination in eukaryotes provides a striking example of the differential control of gene expression during development. Since sex determination affects the developmental fate of numerous organ primordia, information as to the nature of the genetic events involved in sex determination should contribute not only to our understanding of sex determination, but also to the elucidation of the mechanisms by which eukaryotes effect the expression of alternative developmental pathways.



- 1.a molecular–genetic investigation of genital imaginal disc development
- 2.genome-wide approaches to find downstream targets of the transcription factors found at the end of the sex determination pathway, Dsx and Fru M
- 3.an examination of the evolutionary conservation of genes in the sex hierarchy

PART1： sexually dimorphic structures

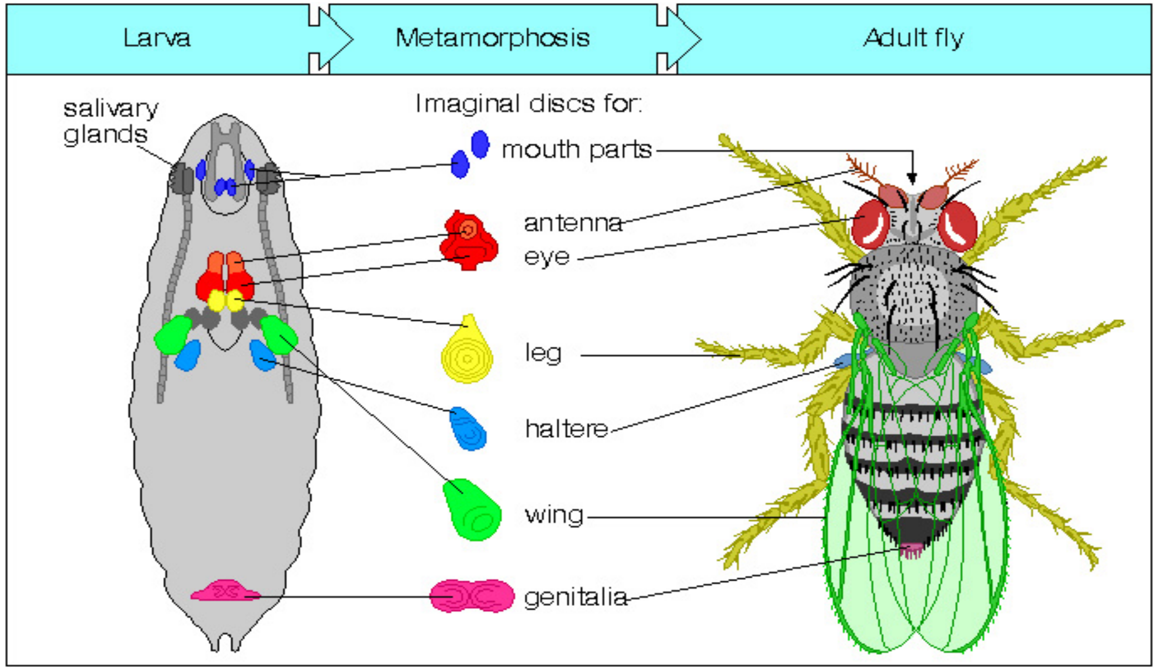
(genital imaginal disc development)

sex determintion hierarchy → ? → **sexual dimorphism**

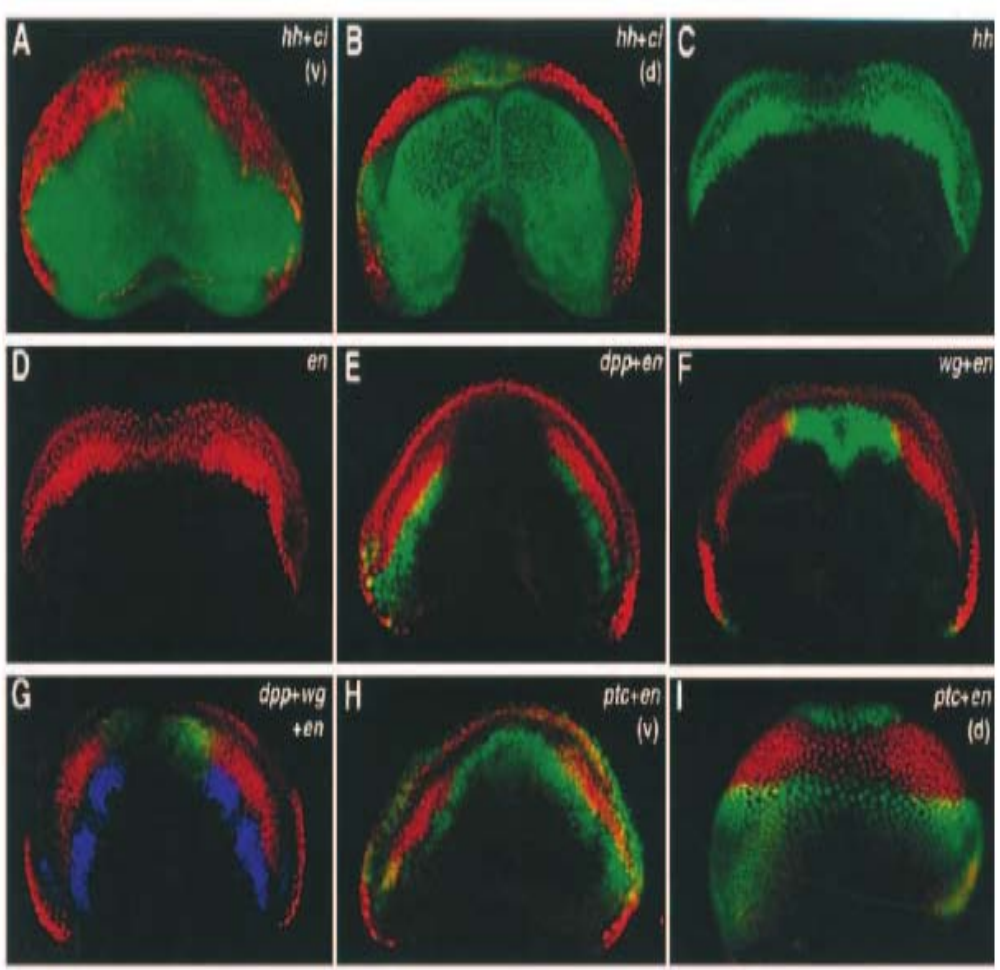
Elizabeth Chen

Shaad Ahmad

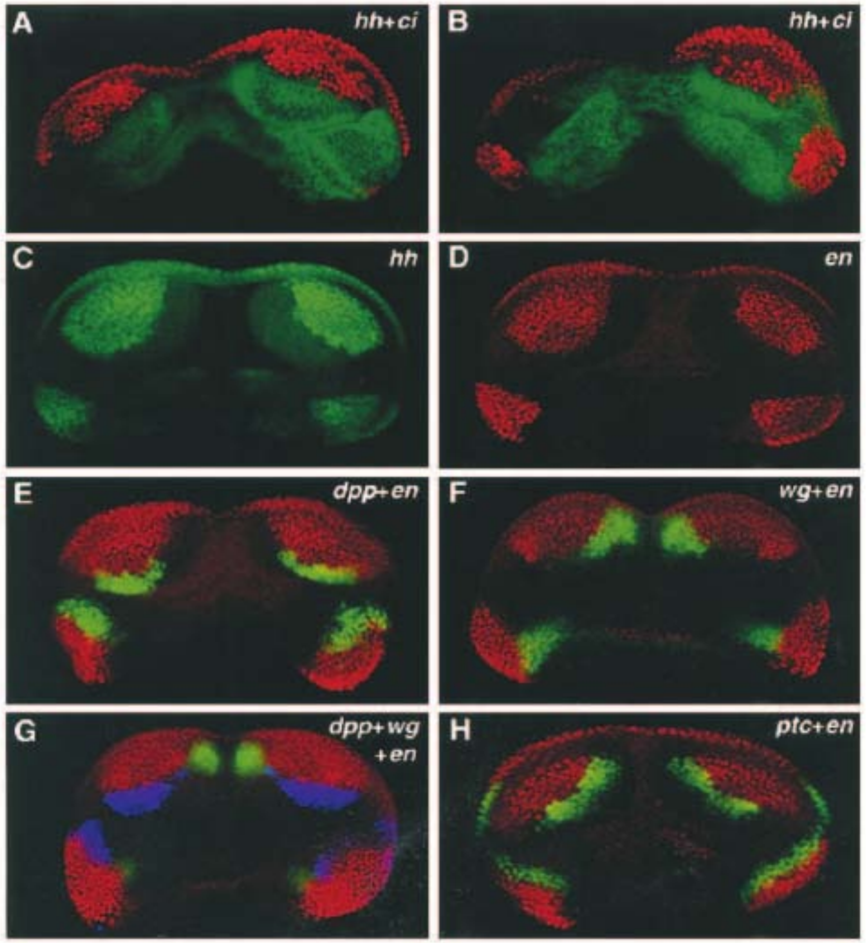
Audrey Christiansen



A new model for the A/P compartmental organization of the genital disc



female



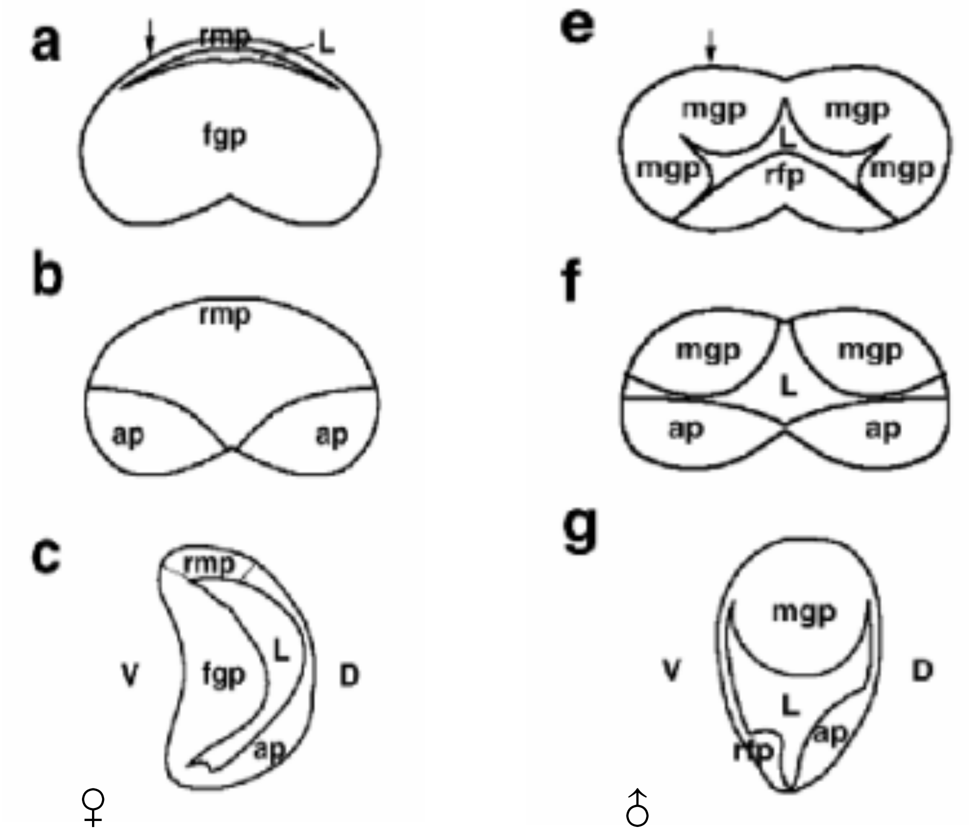
male

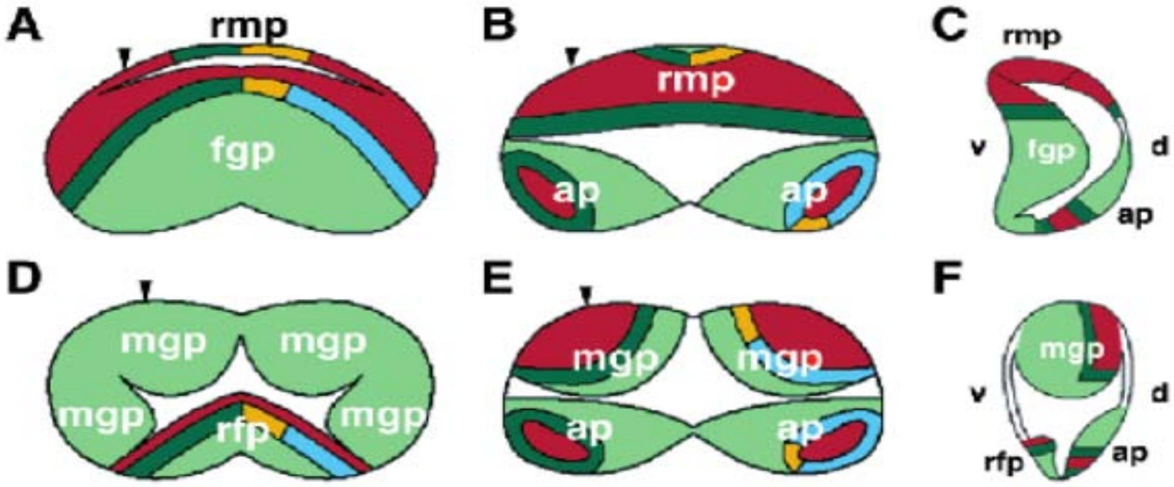
A/P patterning genes：*hh,en,ci,dpp,wg,ptc*

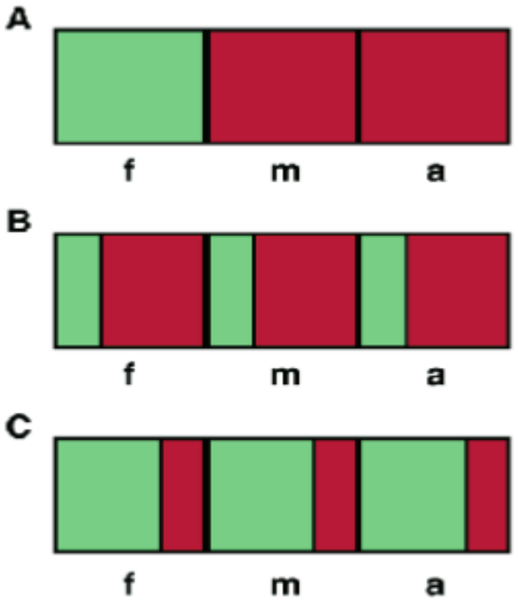
hh(en)— the posterior compartment
ci— the anterior compartment
dpp,wg,ptc—the border area

Elizabeth H. Chen and Bruce S. Baker.Development.1997

A new model for the A/P compartmental organization of the genital disc







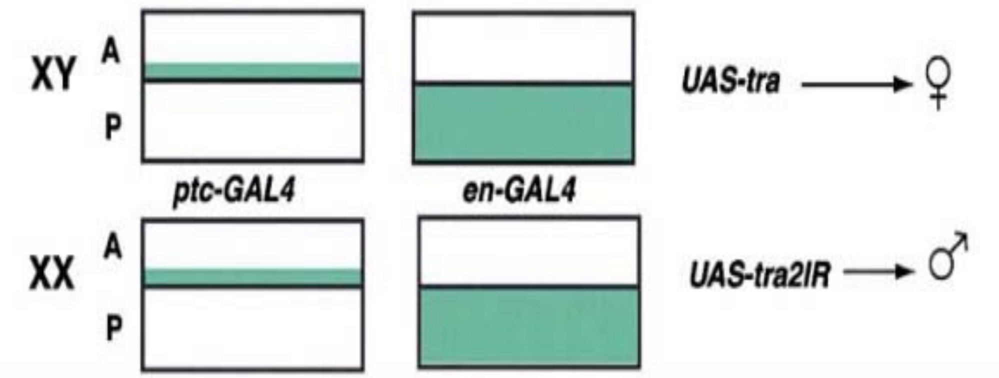
Lawrence and Struhl, 1982

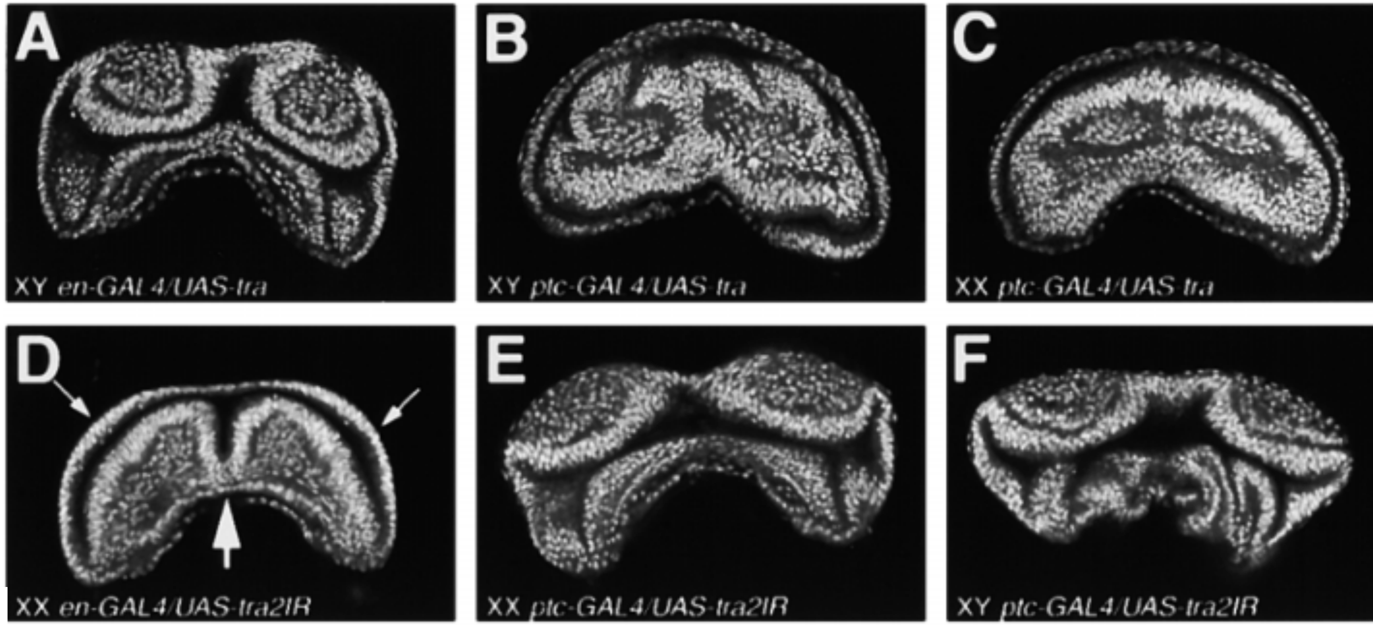
Epper and Sánchez, 1983

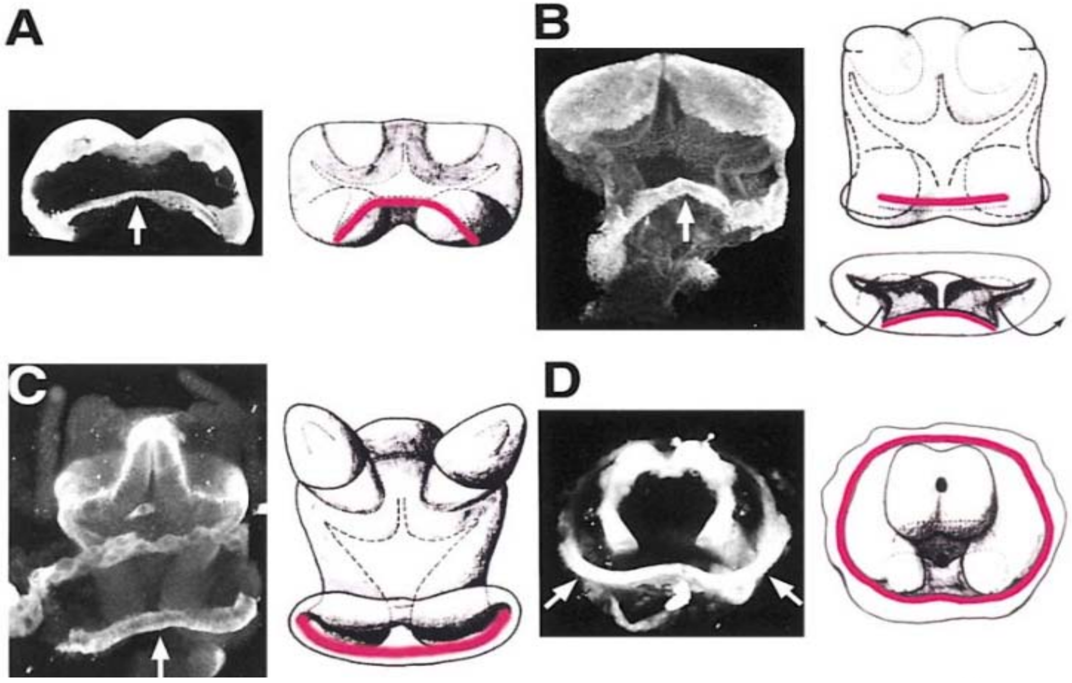
The genital disc：
female genital, male genital, and anal primordia.

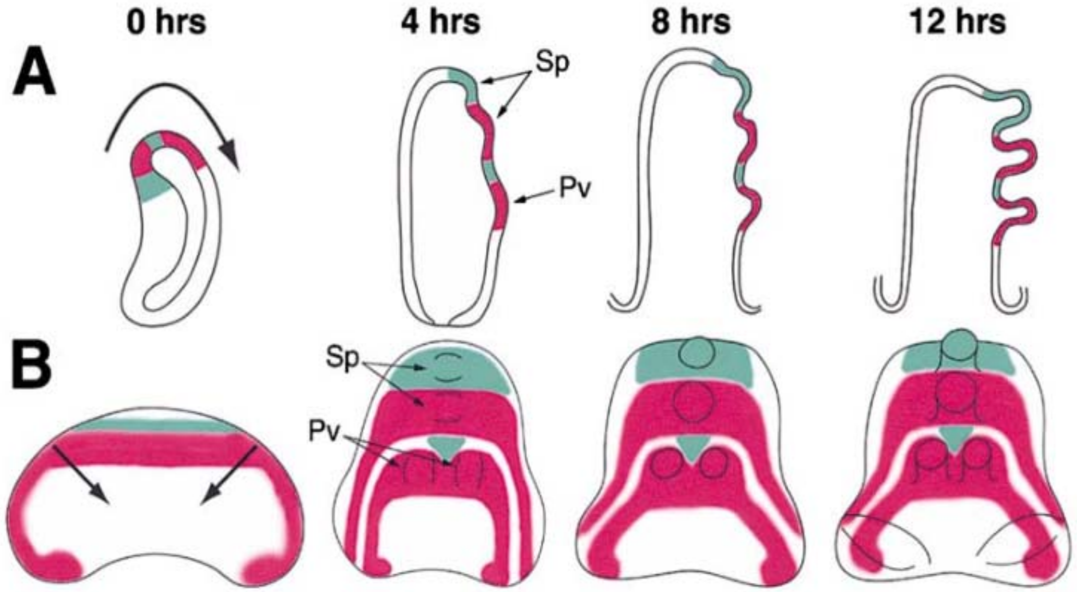
Elizabeth H. Chen and Bruce S. Baker.Development.1997

Sexual dimorphism in the genital disc is controlled from the A/P organizer



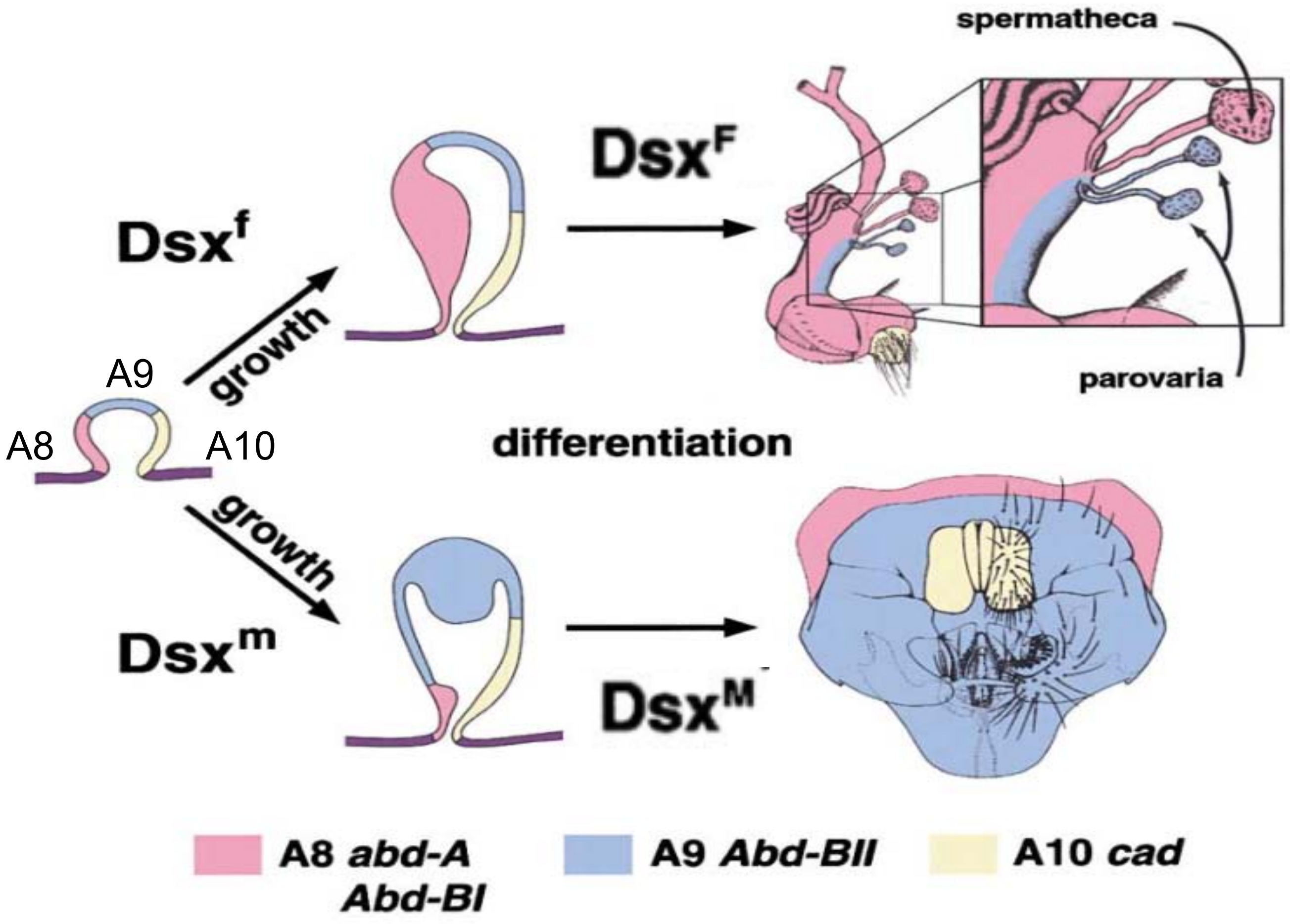






Eric L. Keisman, et al. Developmental Cell.2001

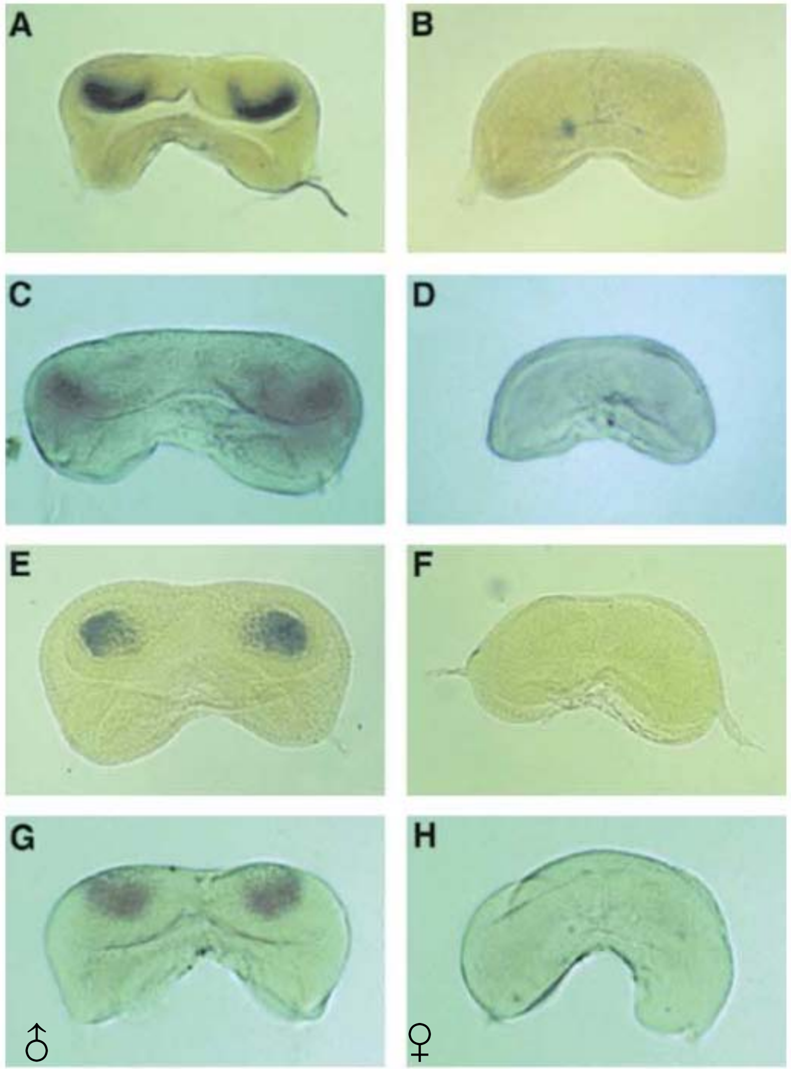
Dsx acts at multiple levels to instruct the sex-specific differentiation of the genital primordia



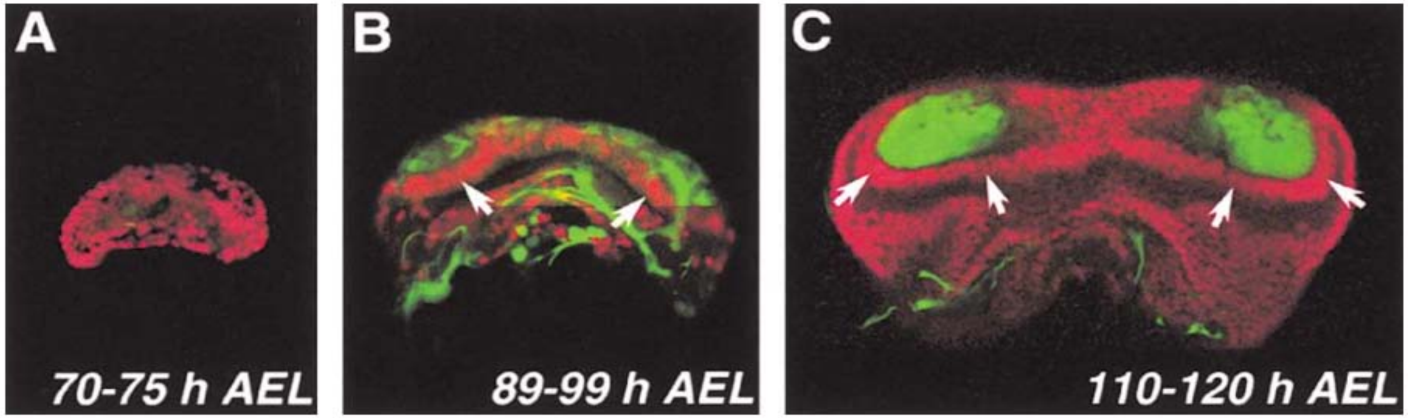
- Dsx acts in concert with the homeotic genes to direct the sex-specific fate of each primordium.
- Dsx controls growth by regulating the activity of the A/P organizer.
- Differentiation is controlled by dsx cell autonomously.

Eric L. Keisman, et al. Developmental Cell.2001

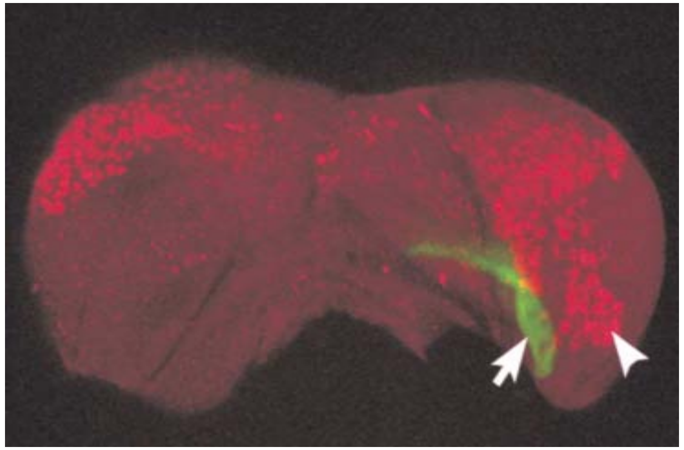
FGF is used to recruit mesodermal cells to the genital imaginal disc controlled by dsx



Bnl and *btl* are only expressed in the A9-derived developing “male” primordium. *bnl*(FGF);*btl*(FGFR)



recruitment of btl-expressing cells

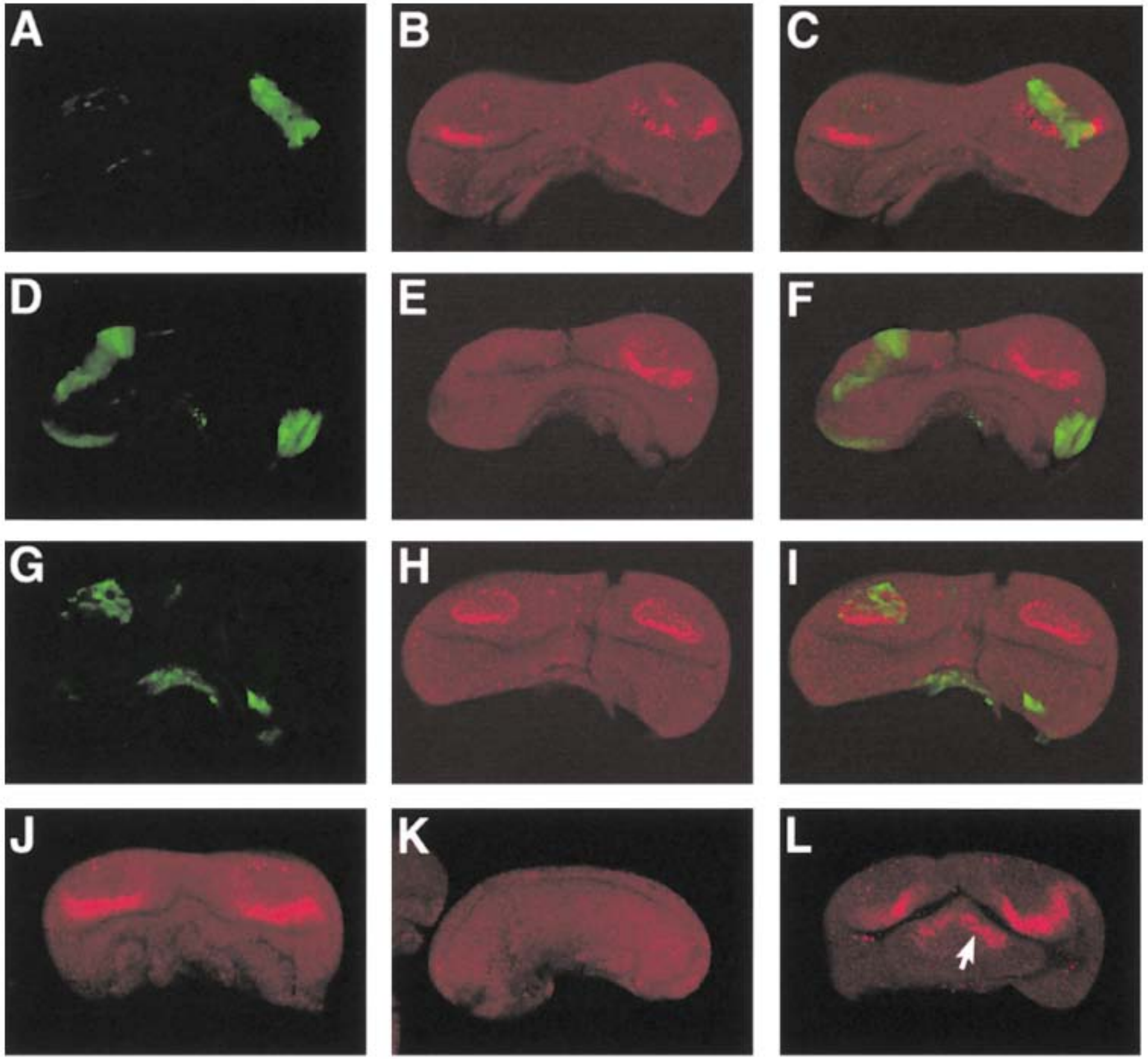


bnl was sufficient to recruit btl-expressing cells into locations

act5C>CD2>GAL4/Y; UAS-bnl/UAS-GFP; btl-lacZ/hs-FLP

Shaad M. Ahmad and Bruce S. Baker.Cell.2002

bnl is a target of sex determination hierarchy, being repressed byDsx^F in females



Bnl is a target of the sex determination hierarchy, downstream of tra.

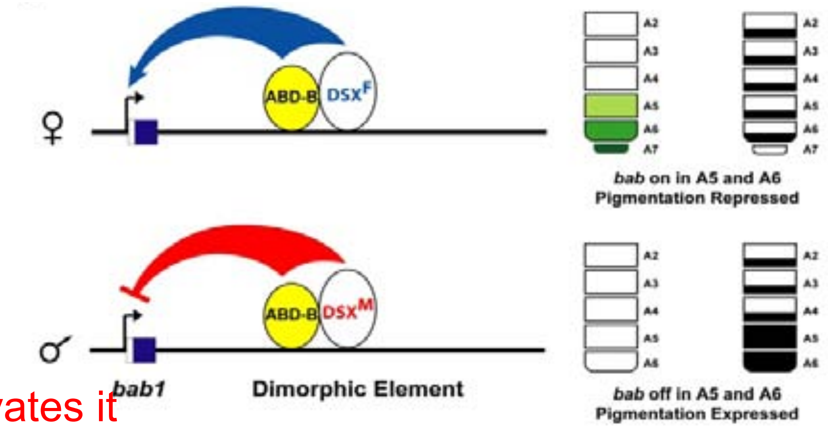
“lobe” “flatten”

Btl is not a direct target of the sex determination hierarchy

Shaad M. Ahmad and Bruce S. Baker.Cell.2002

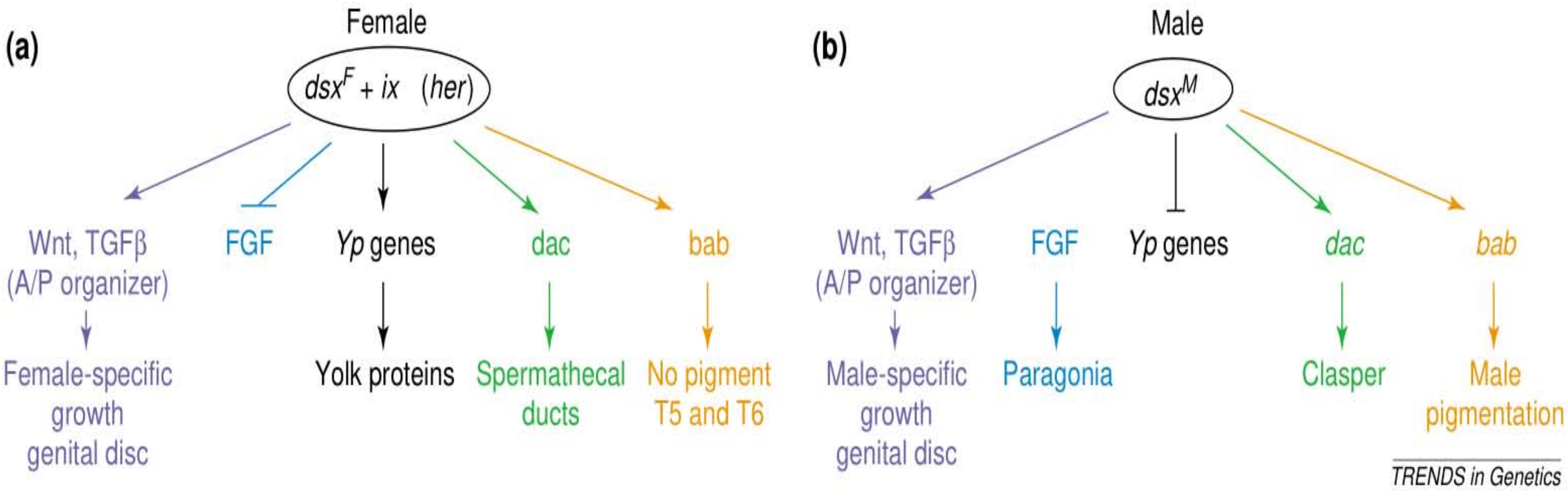
Regulation of sexual dimorphism

- early studies
  - dsx can act both negatively and positively to regulate various target genes (Yp1)
- the genital disc
  - 1.“repressed”genital primordia
  - 2. instructive function
- recent studies (DSX and the Hox genes)
  - Dsx&*wg dpp*:the A/P organizers
  - Dsx&FGF
  - Dsx&*bab*
  - Dsx&*dac*



XY:*wg* represses *dac* and *dpp* activates it
XX:*wg* activates *dac* and *dpp* represses it

The sex hierarchy modulates the activities of signaling molecules and transcription factors to direct various sex-specific aspects of growth and differentiation.

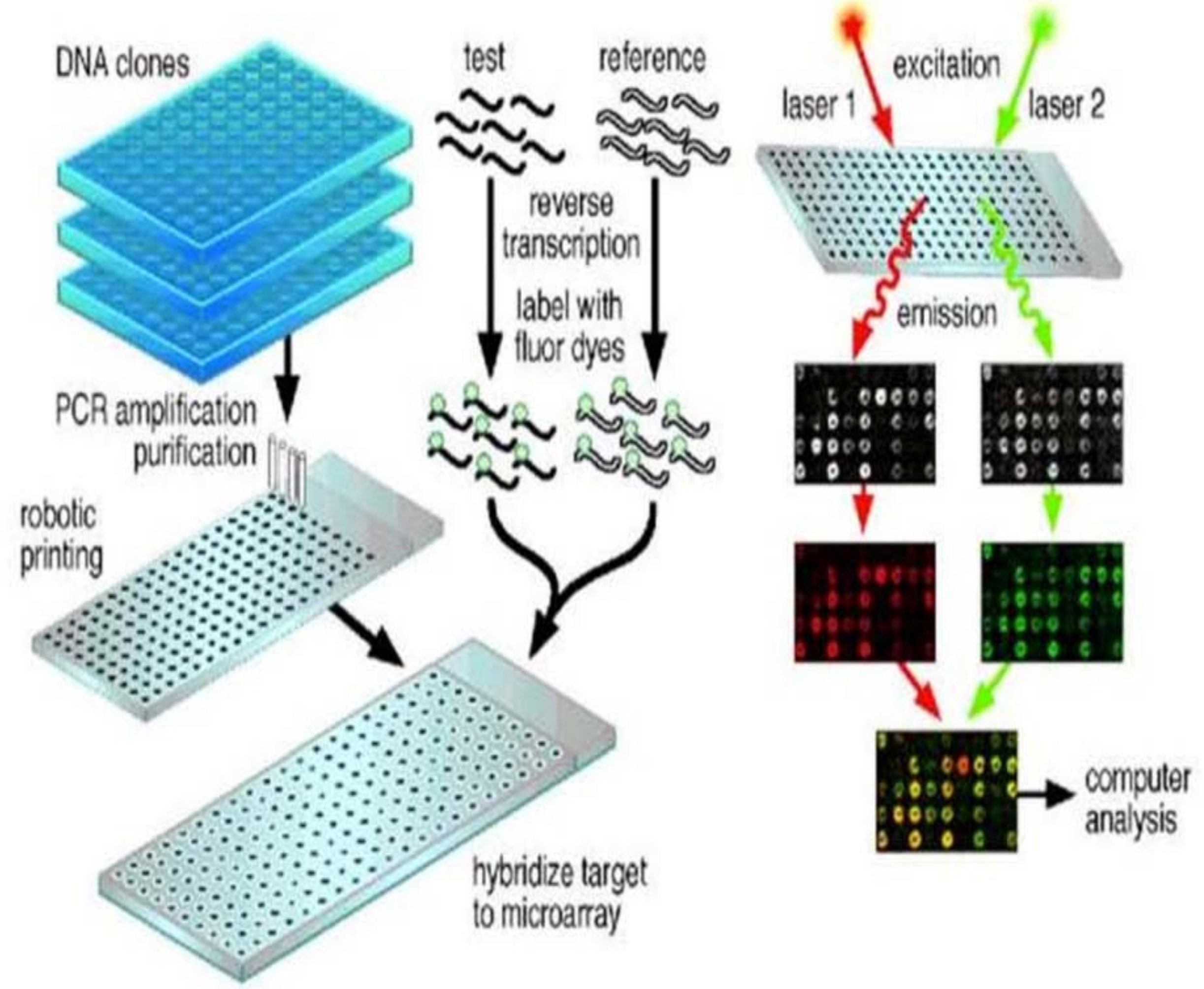


Audrey E.Christiansen, et al. Trends in genetics.2002

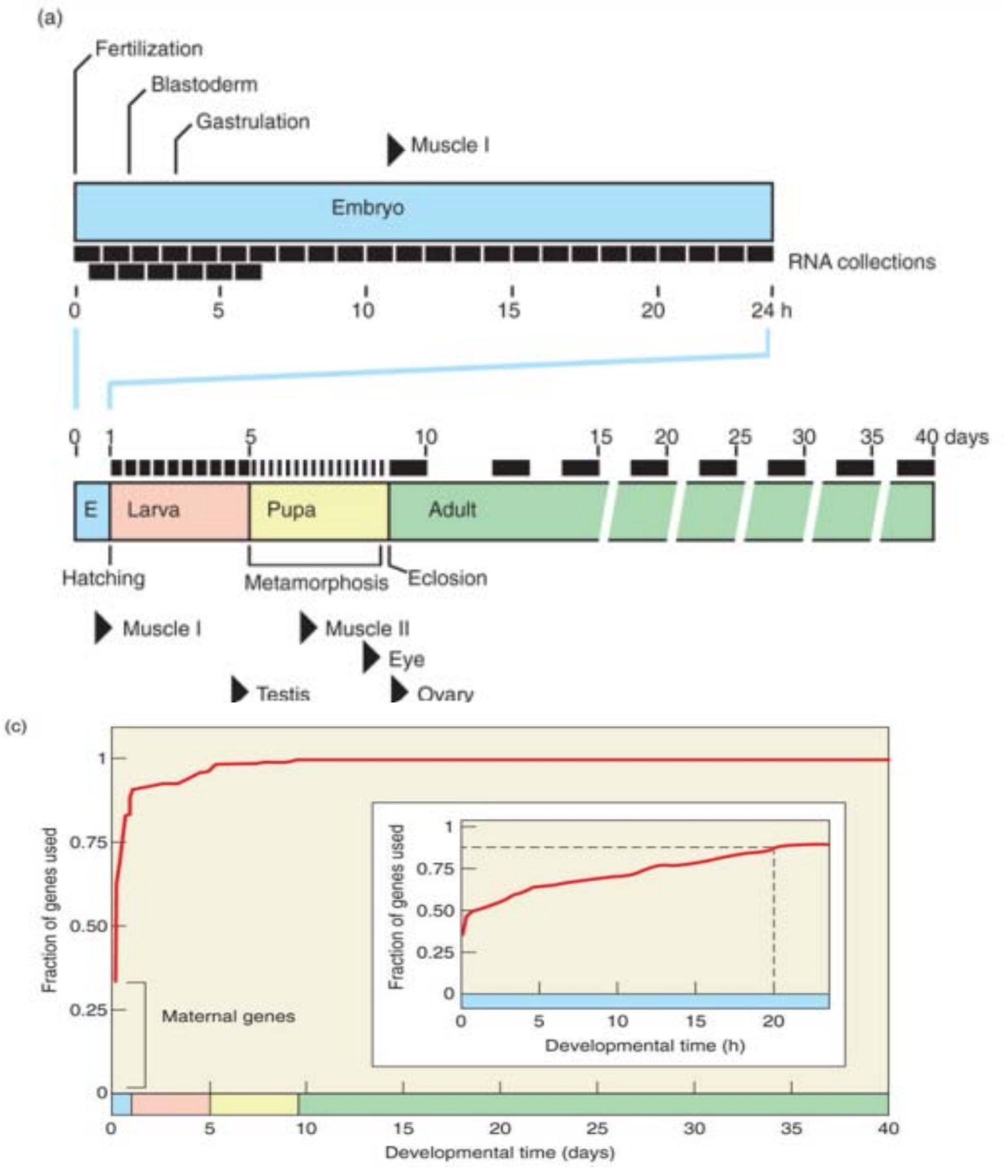
PART2：genome-wide approaches to find downstream targets

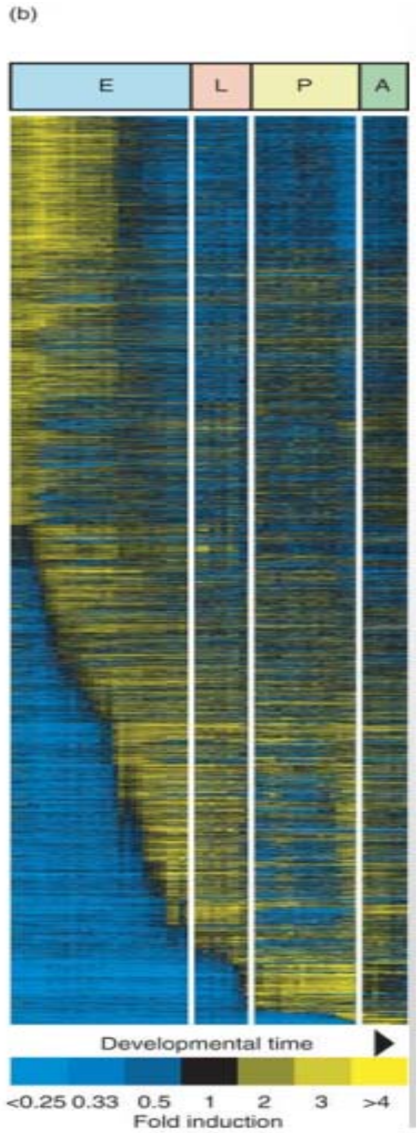
DNA Microarray

•"Quantitative Monitoring of Gene Expression Patterns with a complementary DNA microarray" reported by **Patrick Brown**, Mark Schena and colleagues in *Science (1995).*



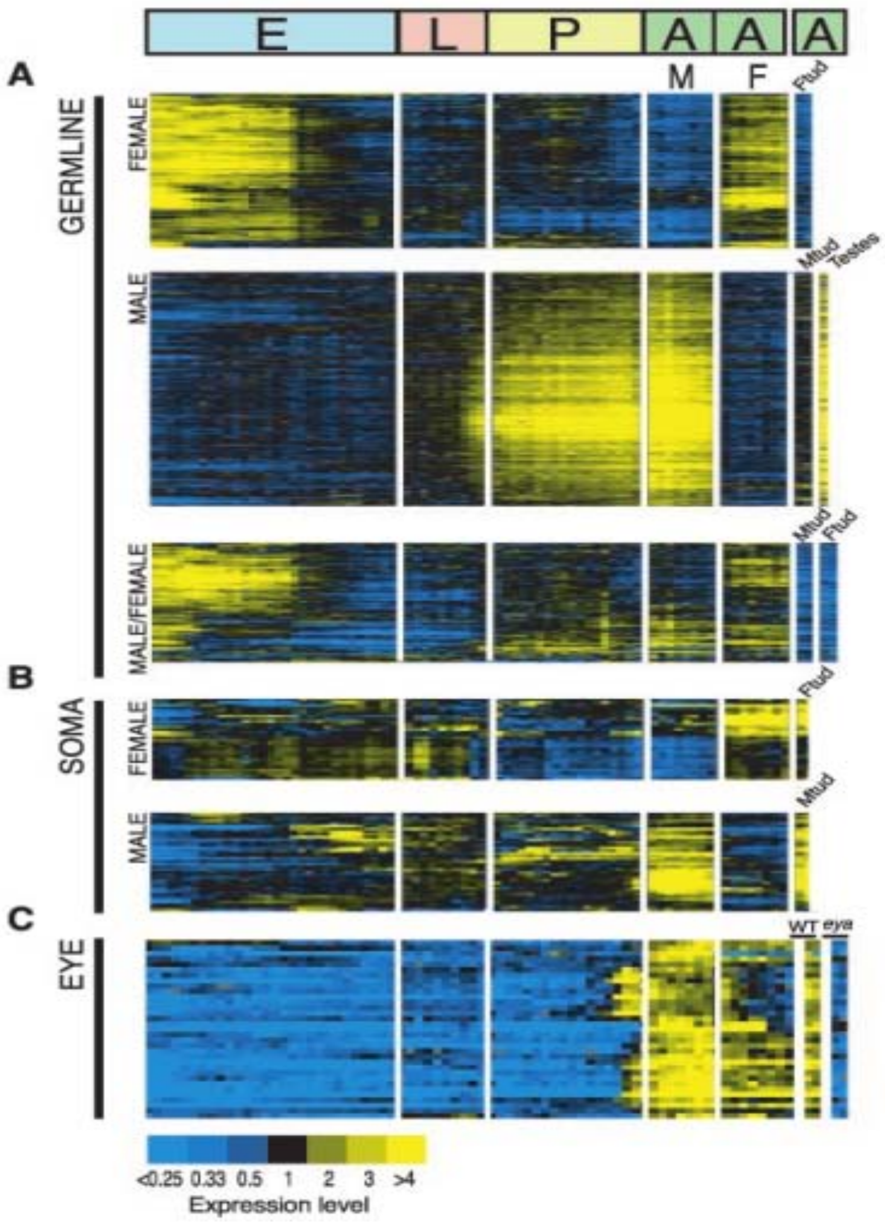
Patterns of gene expression through the Drosophila life cycle





Michelle N. Arbeitman, et al.Science.2002

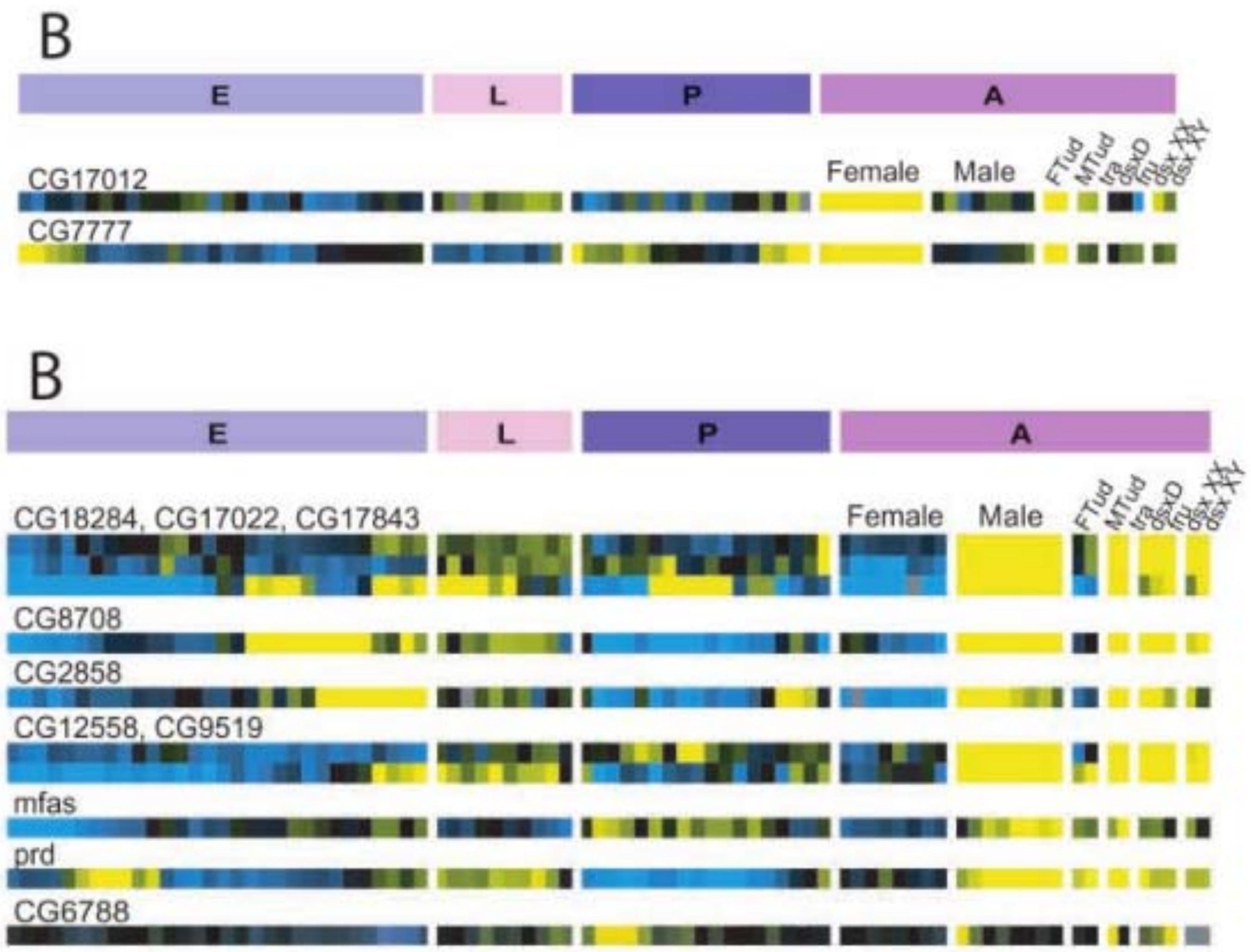
Sex difference in the transcriptional programs in *Drosophila melanogaster*

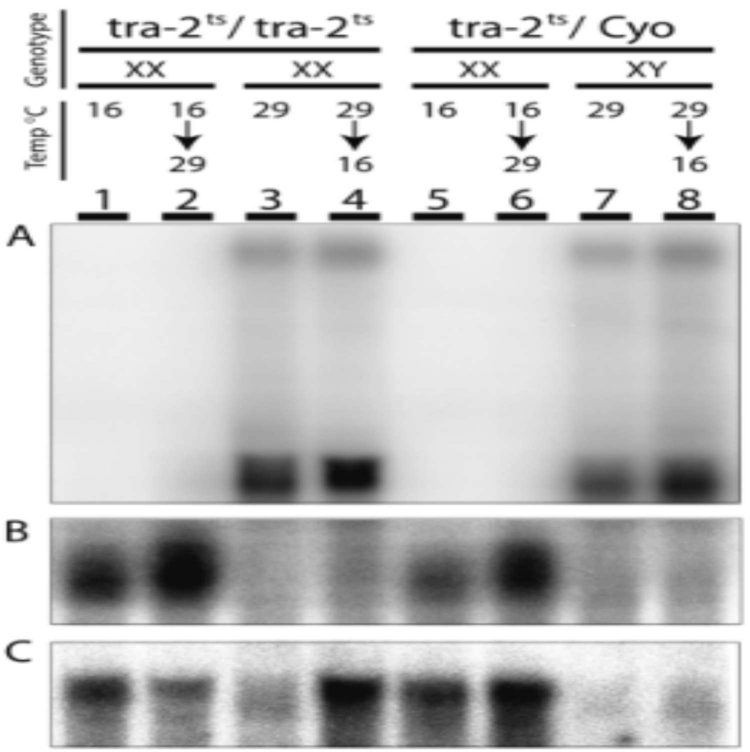


- ➢111 genes were expressed in both male and female germ lines.
- ➢31 genes had significantly higher expression in the soma of adult females compared with 37 genes in males.

Michelle N. Arbeitman, et al.Science.2002

Temporal regulation of sex-differential adult gene expression by the hierarchy





CG18284

CG7777

YP1 (control)

Sex-differential expression of all 11 genes is the consequence of the developmental action of the sex hierarchy and is independent of the hierarchy during adult stages.

Michelle N. Arbeitman, et al.Development.2007

Modes of dsx regulation of sex-differentially expressed genes

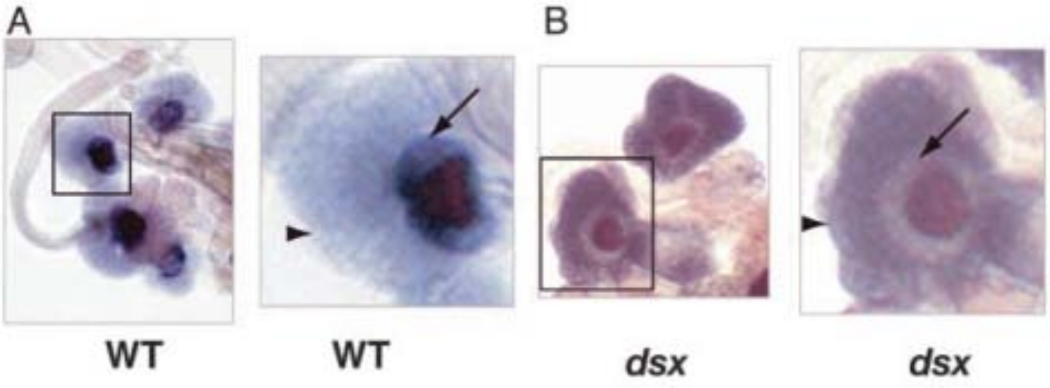


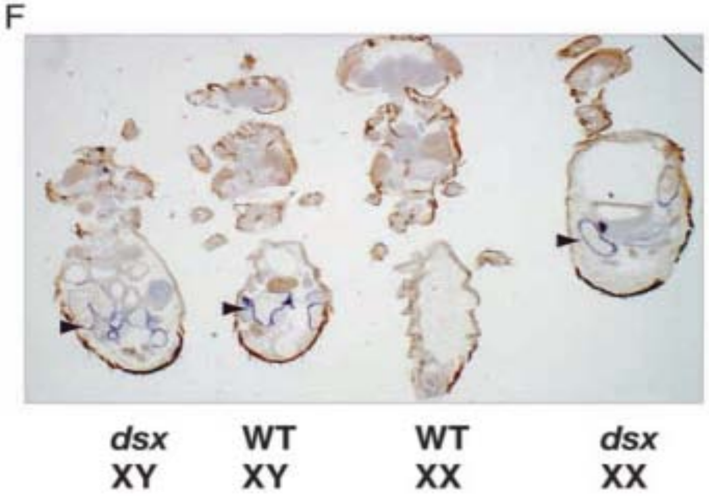
Table 3. Modes of regulation by *dsx*

Gene	Expression localized to	Genotype / DSX phenotype: XY wt DSX^M	XX wt DSX^F	XY *dsx* –	XX *dsx* –	XX *dsx^D* DSX^M	XX *tra* DSX^M	Suggested mode of regulation: DSX^M	DSX^F
*Yp1**	Female fat body							–	+
CG17012	Spermathecae	–1.29	4.64	–0.95	–0.15	–1.34	–1.89	0	+
CG17843	Male AG	1.31	–3.16	1.08	0.09	0.80	0.55	0	–
CG17022	Male AG	3.29	–2.69	3.83	2.28	3.86	3.75	0	–
CG18284	Male AG	2.92	–1.60	2.46	0.43	3.33	2.39	0	–
prd	Male AG	0.40	–0.30	0.62	0.61	1.31	0.94	0	–
CG2858	EB	1.10	–2.07	–0.68	0.35	1.24	1.01	+	–
CG8708	AED	1.15	–1.07	0.50	0.05	1.79	2.00	+	–

Values given in table are log-transformed microarray ratios.
*Mode of regulation previously established (reviewed by Christiansen et al., 2002).
AG, accessory gland; EB, ejaculatory bulb; AED, anterior ejaculatory duct; wt, wild type; 0, no effect; +, positive effect; –, negative effect.

- DSX^F positively regulates sex-specific expression of CG17012.

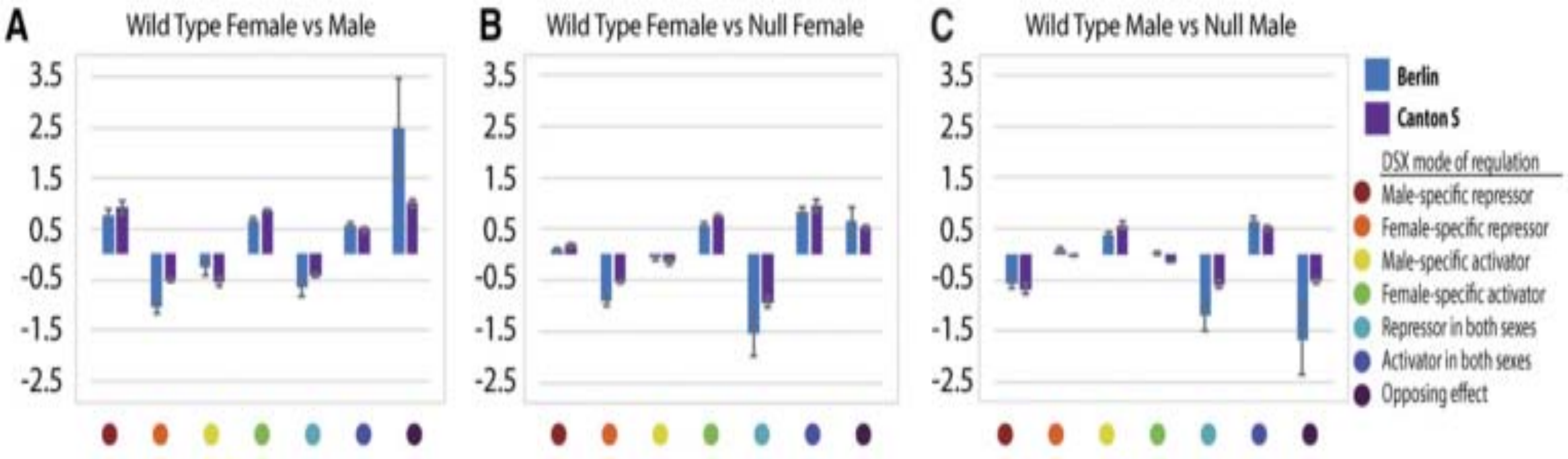
- Male-specific expression of *prd* is a negative control by DSX^F to prevent the formation of male accessory glands.



Michelle N. Arbeitman, et al.Development.2007 a new consensus DSX-binding site

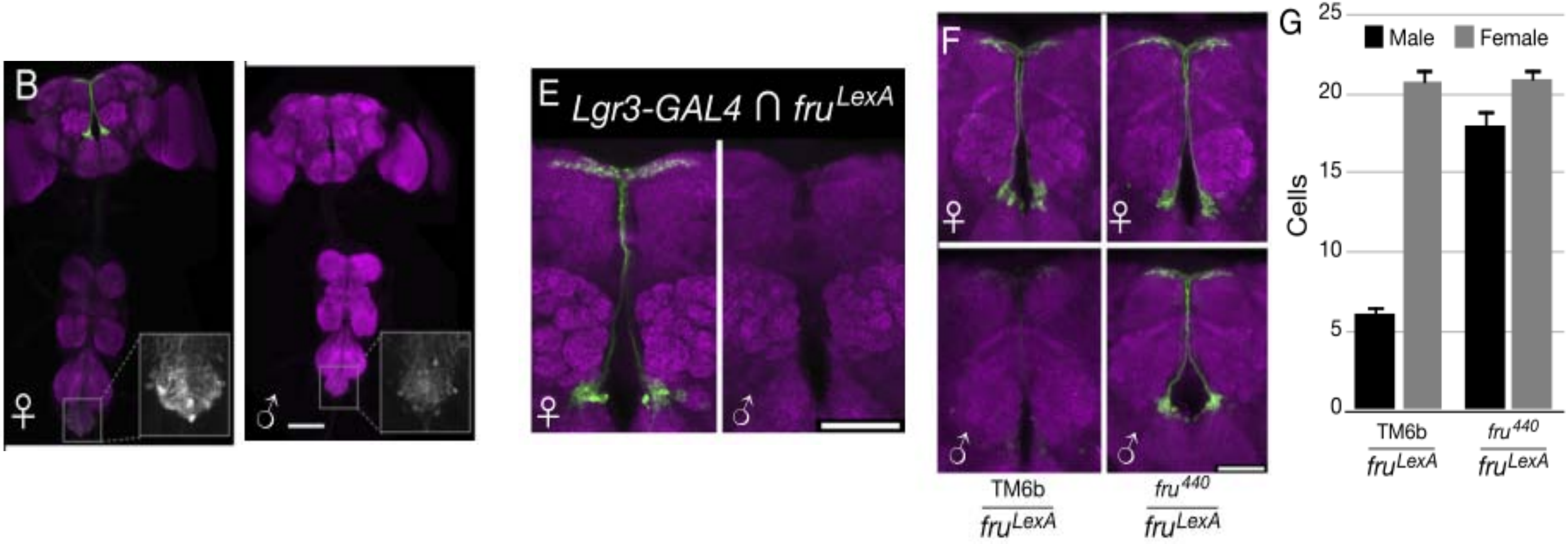
Shengzhan D. Luo,et al. Development.2011

The effect of different DSX modes on regulation of expression



M. N. Arbeitman, et al.Genetics.2016

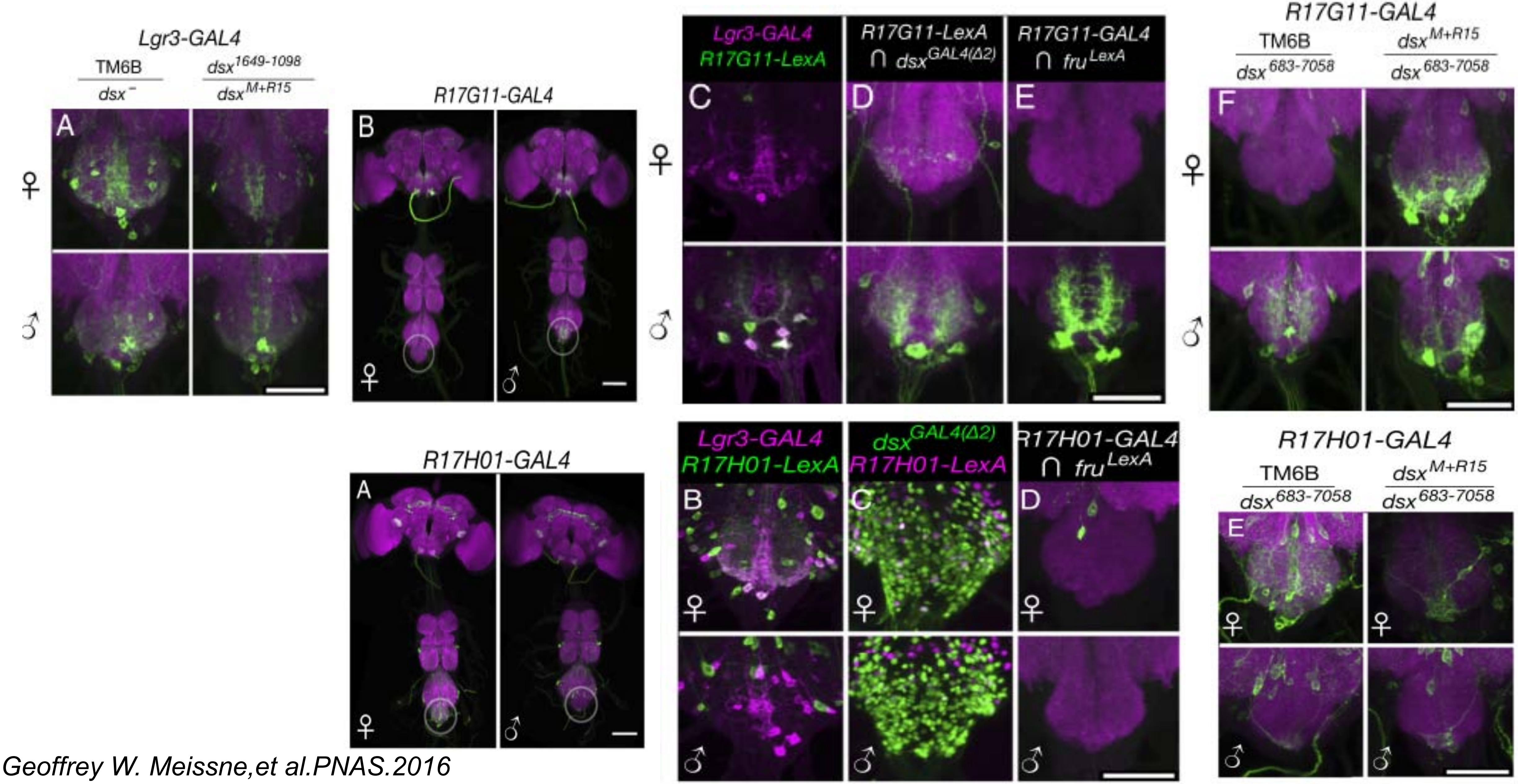
Drosophila *Lgr3* is regulated by Fru and Dsx in separate populations of neurons



Fru^{M} inhibits expression of Lgr3 in the male median bundle.

Geoffrey W. Meissne,et al.PNAS.2016

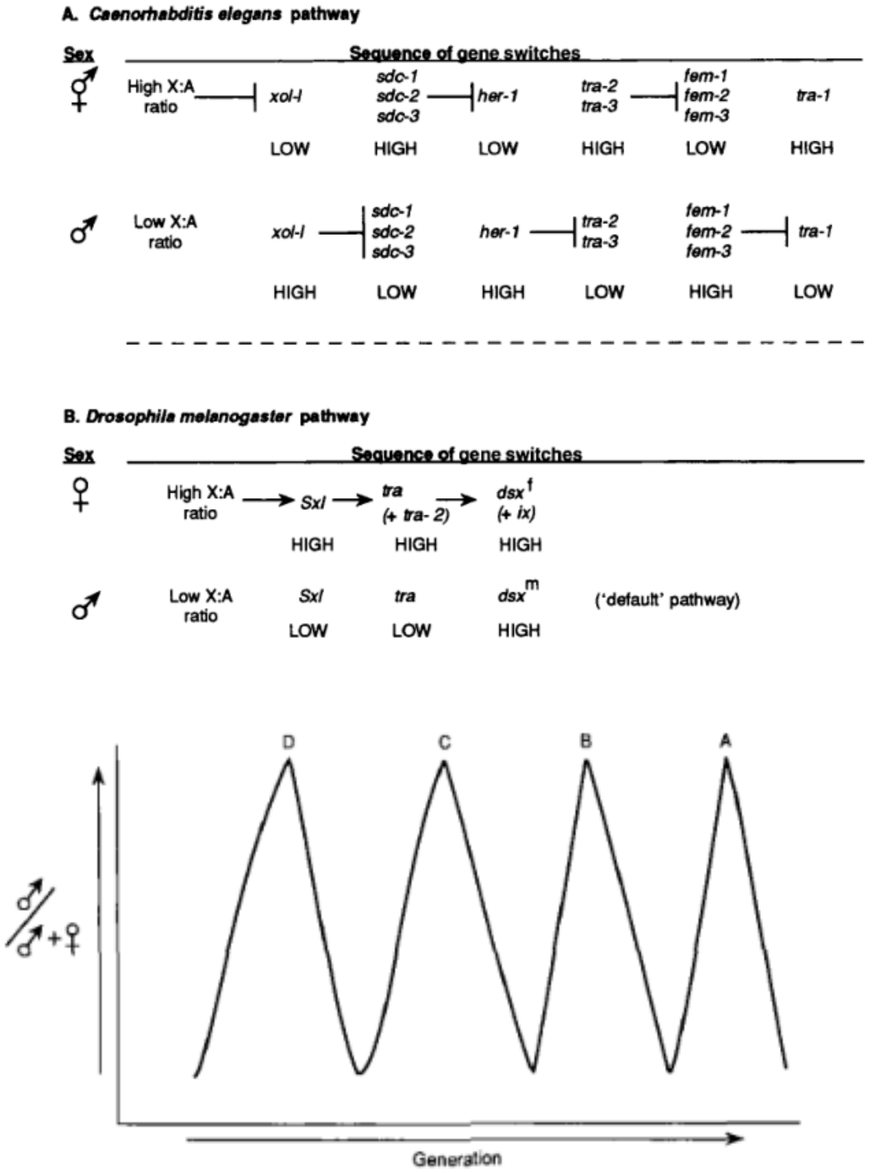
Dsx^F activates and inhibits Lgr3 expression in different abdominal ganglion neurons



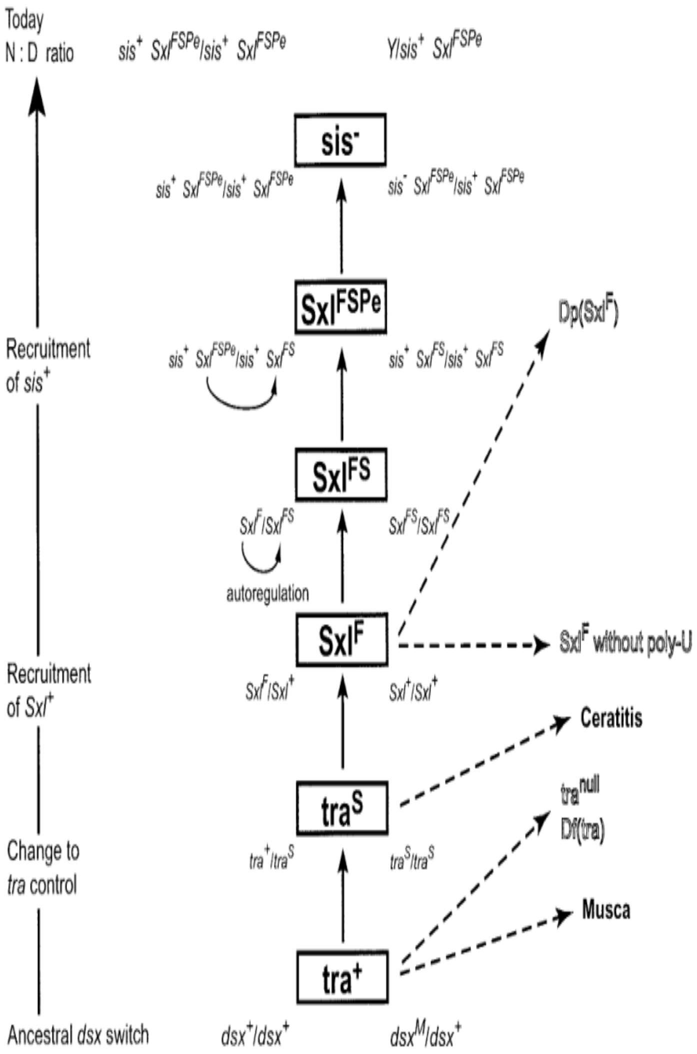
Geoffrey W. Meissne,et al.PNAS.2016

PART3:the evolution of sex differences

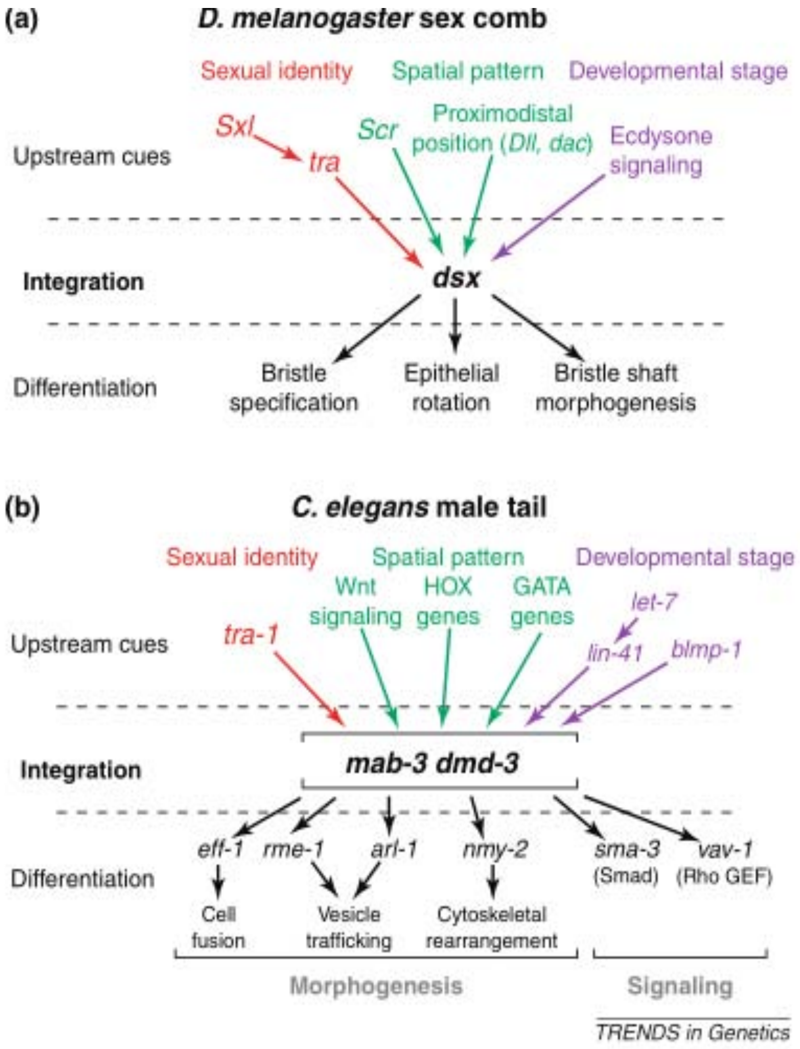
Dmrt gene



A.S. Wilkins.Bioessays.1994



A.Pomiankowski,et al. Genetics.2004



A.Kopp. Trends in Genetics.2012

References

- Chen E.H and Baker B.S.1997 Compartmental organization of the Drosophila genital imaginal discs. Development 124, 205-218
- Keisman E.L., et al.,2001 The Sex Determination Gene doublesex Regulates the A/P Organizer to Direct Sex-Specific Patterns of Growth in the Drosophila Genital Imaginal Disc. Developmental Cell, 215–225
- Baker B. S and S.M, 2002 Sex-Specific Deployment of FGF Signaling in Drosophila Recruits Mesodermal Cells into the Male Genital Imaginal Disc. Cell(109):651-661.
- Christiansen A.E., et al.2002 Sex comes in from the cold:the integration of sex and pattern. TRENDS in Genetics 18(10)
- Arbeitman A.E., et al, 2002 Gene Expression During the Life Cycle of Drosophila melanogaster. Science.297(27)
- Arbeitman A.E., et al, 2004 A genomic analysis of Drosophila somatic sexual differentiation
- and its regulation.Development 131.2007-2021
- Arbeitman A.E., et al. 2016 Sex Differences in Drosophila Somatic Gene Expression: Variation and Regulation by doublesex.Genetics
- Shengzhan D. Luo. et al. 2011 Direct targets of the D. melanogaster DSX F protein and the evolution of sexual development. Development 138, 2761-2771
- Meissner G.M et.al., 2016 Sex-specific regulation of Lgr3 in Drosophila neurons. PNAS.1256-1265
- Pomiankowski A. 2003 The Evolution of the Drosophila Sex-Determination Pathway. Genetics 166: 1761–1773
- Wilkin A.S. et al.1994 Moving up the hierarchy:a hypothesis on the evolution of a genetic sex determination pathway.Bioessay.17(01)
- Kopp A.2012 Dmrt genes in the development and evolution of sexual dimorphism. Trends in Genetics.28(4)

Sex Behavior Meets the Sex Determination Regulatory Hierarchy: The Genetic Control of Sexual Behavior

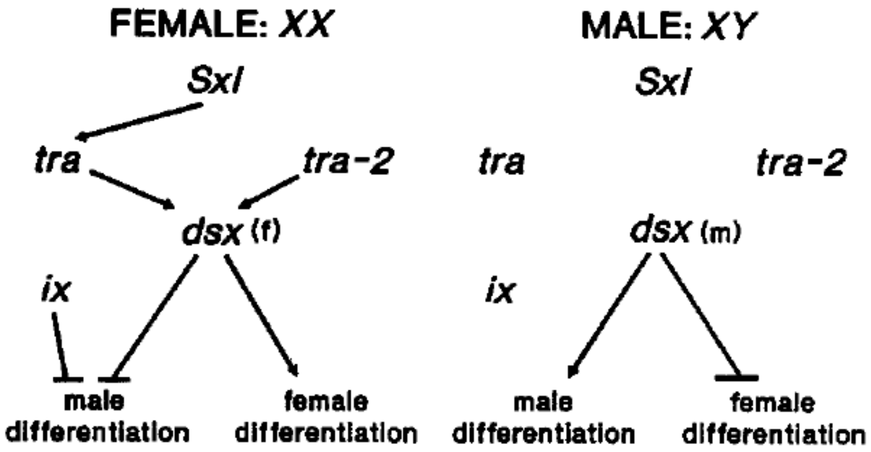
邢丽敏

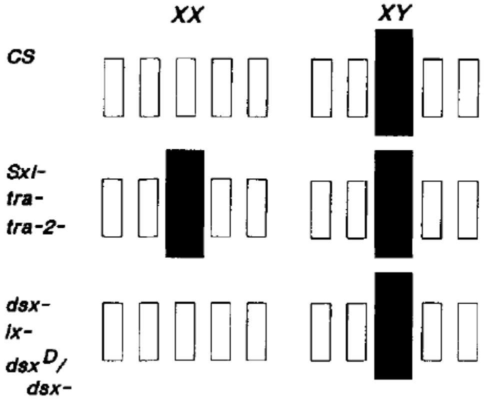
- Bruce’ s entry into the genetics of sexual behavior came from the discovery of a new branch in the sex determination- *fruitless* gene

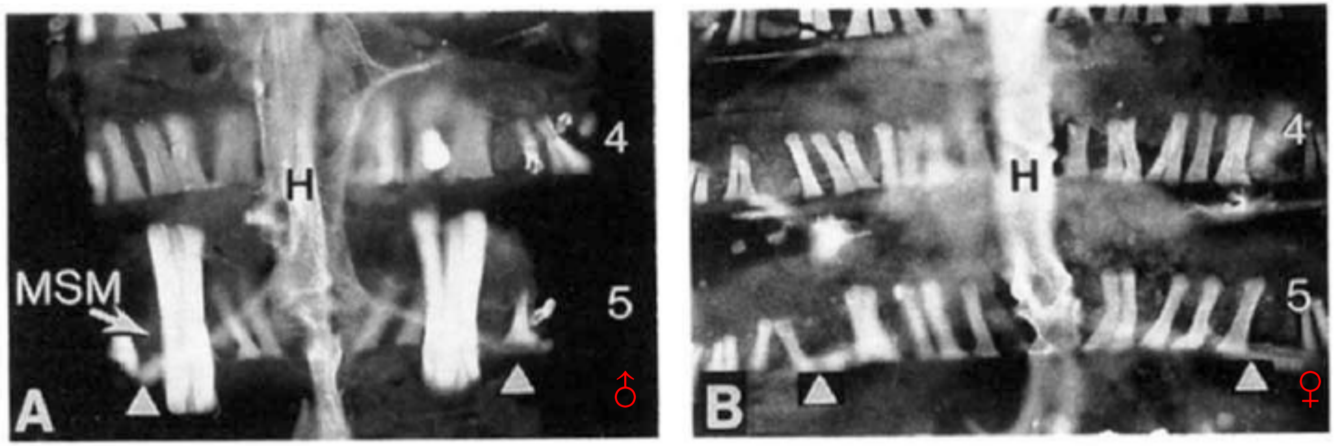
- The expression pattern of *fruitless* and *doublesex* and their regulation to innate behaviors

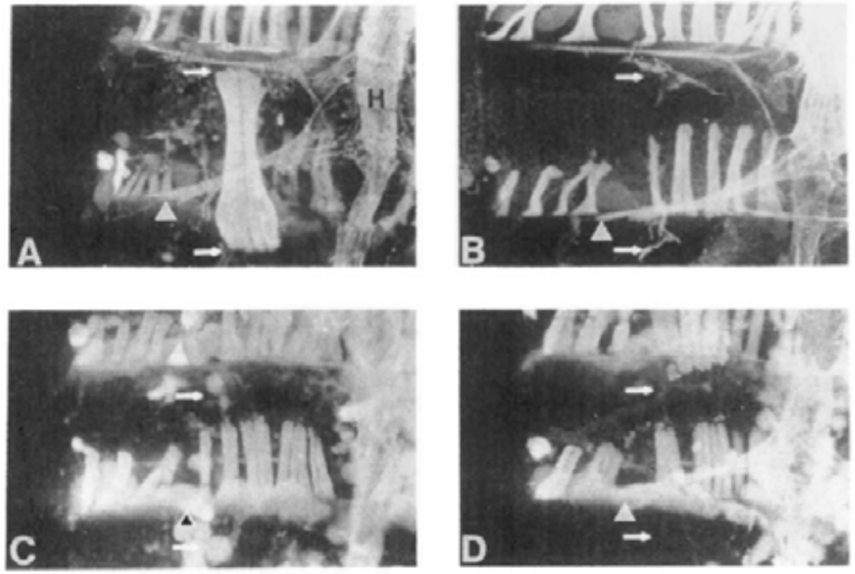
- A deeper exploration of the neuronal mechanisms underlying sexual behavior

The Muscle of Lawrence (MOL) depended on the function of *sxl, tra* and *tra-2*, but not *doublesex* or *intersex*



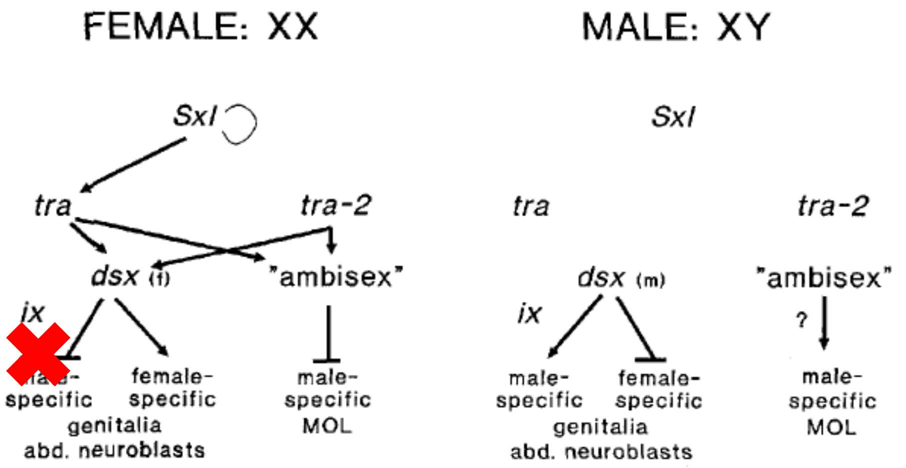


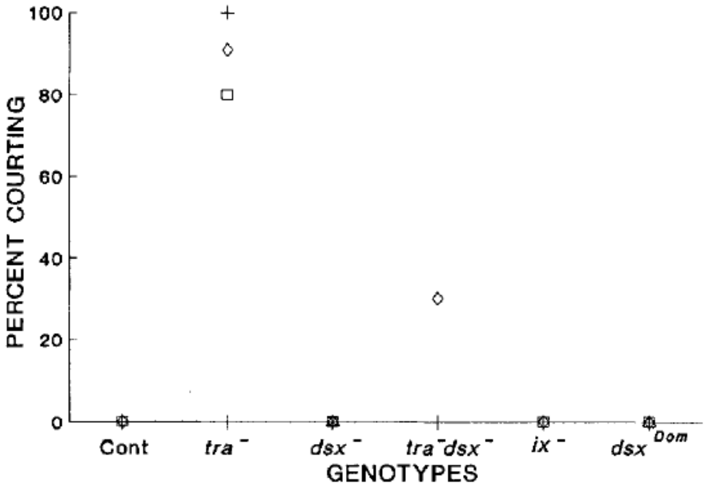




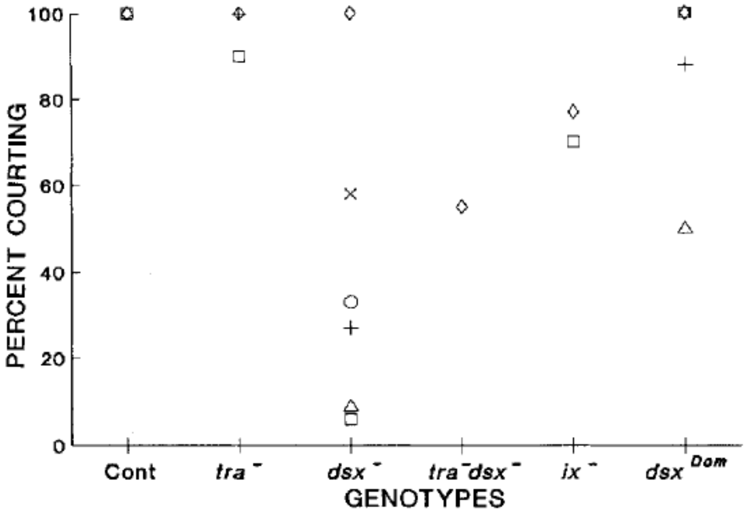
(A)*X/X;* $tra^{5}/Df(3L)st^{J7}$;
(B)*X/X ;* $dsx^{1}/Df(3R)dsx^{15}$;
(C)*X/X;* $ix'/Df(2R)en^{B}$;
(D)*X/X;* $ix^{1}/ix^{1}; dsr^{D} dsx+$.

Taylor., Genetics. 1992





female flies

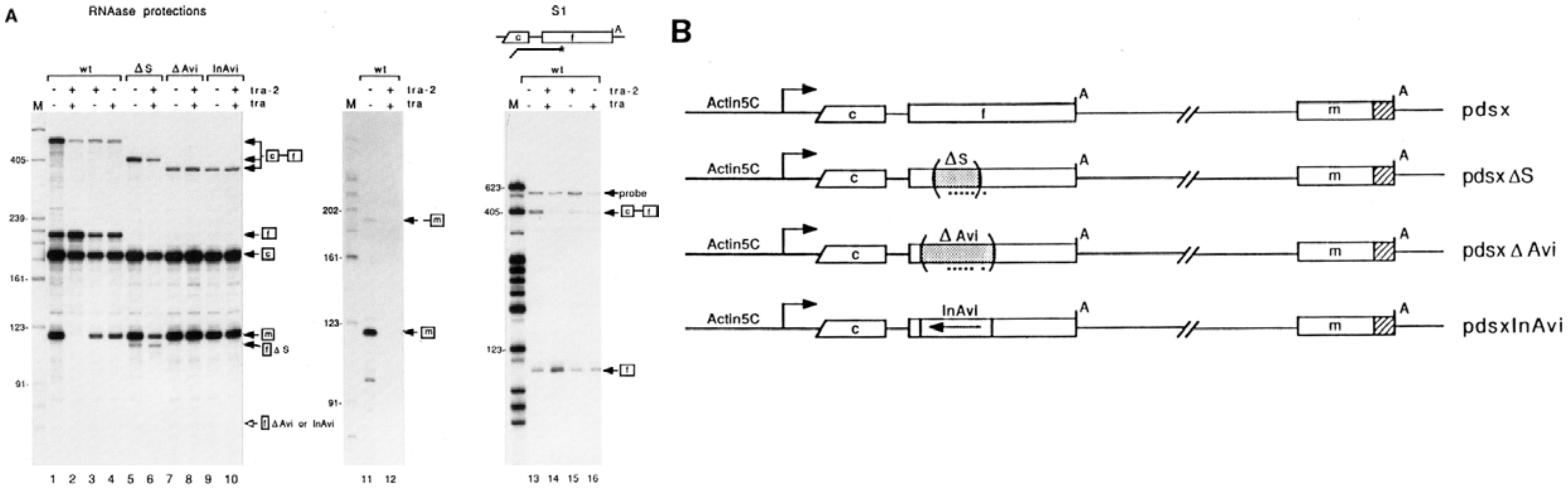


male flies

These findings suggested the existence of a previously unrecognized branch in the sex-determination hierarchy

Taylor, et al., Genes Dev. 1992

A 13-nt sequence, repeated six times in *dsx* in a noncoding region, was necessary and sufficient to direct sex-specific splicing



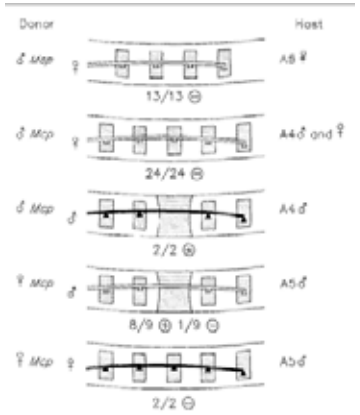
dsx, tra, tra-2, and Sxl in S-L2 cells

Fished out another genomic region by the 13-nt repeat sequences, which is the *fru* gene

Ryner, L. C., and B. S. Baker, Genes Dev. 1991

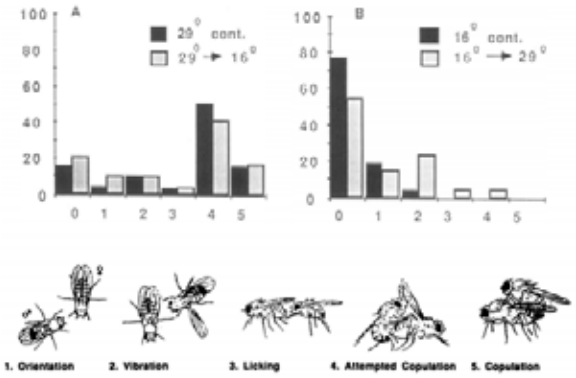
How does *fru* become a good candidate gene residing at the top of the new branch in the sex determination regulatory hierarchy?

evelopment of the MOL was dependent on the sex of the innervating neurons



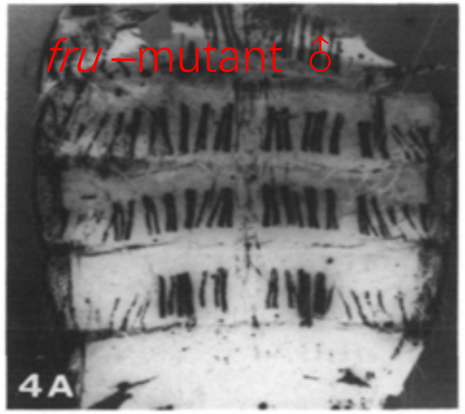
Lawrence and Johnston 1986

tra-2 is required in adult females to block male-specific courtship behaviors



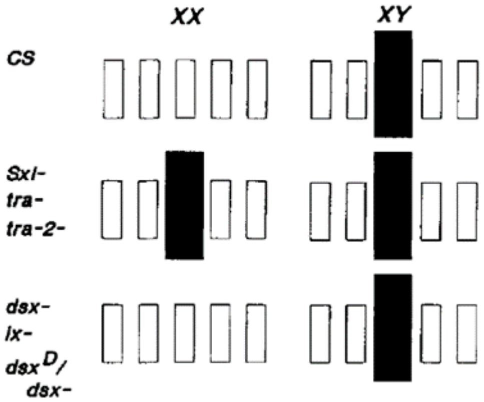
Belote and Baker., 1987

fru was known to be involved in the development of MOL



Gailey., et al. 1991

the Muscle of Lawrence (MOL) is sex-specific development and depended on the function of *tra* and *tra-2*, but not *dsx*

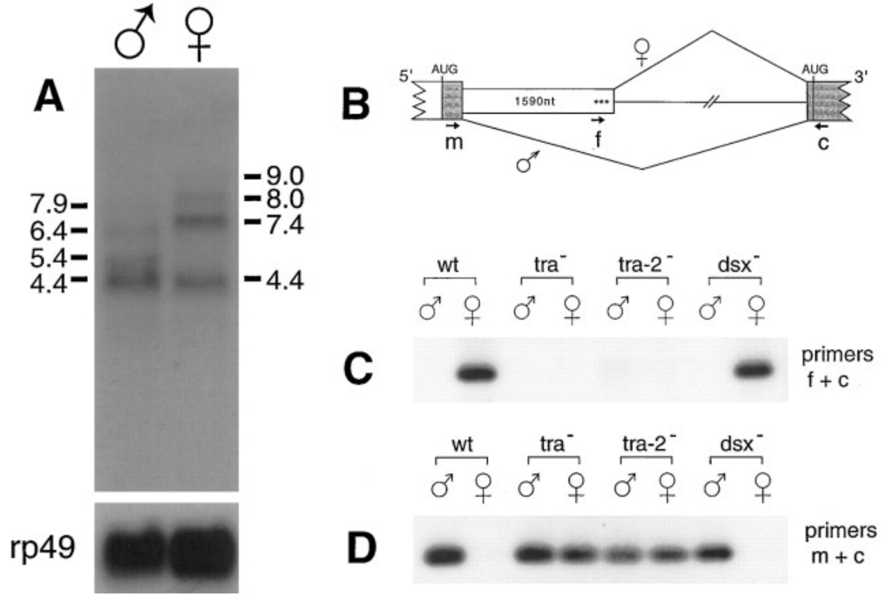


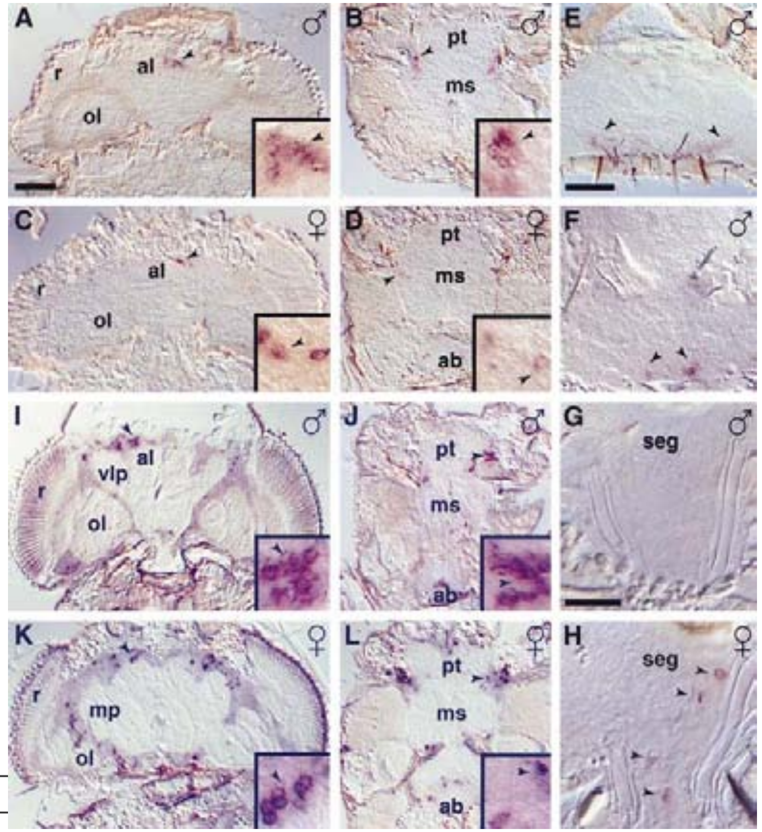
Taylor., 1992

Cell, Vol. 87, 1079–1089, December 13, 1996, Copyright ©1996 by Cell Press

Control of Male Sexual Behavior and Sexual Orientation in Drosophila by the *fruitless* Gene

fru' s sex-specific transcripts were only expressed in the adult nervous system and in a very distinct pattern





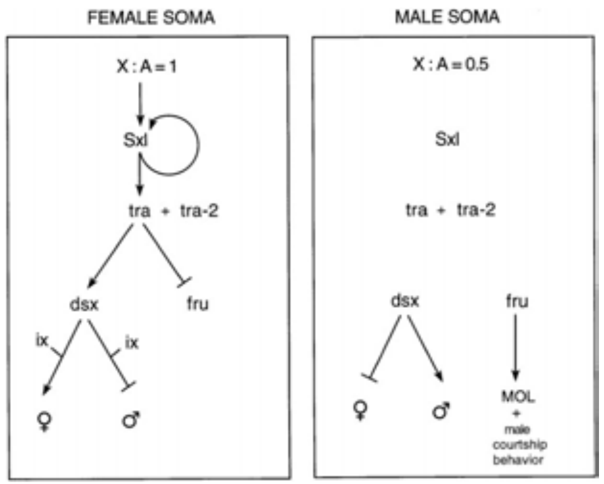


Table 1. Courtship Behavior of *fru* Mutants

	male + male			male + female		
Genotype	All courtship CI* (%)	Wing ext. (%)	N	All courtship CI* (%)	Wing ext. (%)	N
wild-type	4 ± 1	0 ± 0	10	84 ± 3	46 ± 7	7
*fru*¹/*fru*¹	51 ± 4	21 ± 3	25	61 ± 5	35 ± 4	21
*fru*³/*fru*³	32 ± 5	1 ± 0	31	15 ± 5	0 ± 0	20
*fru*⁴/*fru*⁴	41 ± 6	2 ± 1	25	29 ± 7	2 ± 1	20
*fru*³/*fru*⁴	42 ± 7	1 ± 0	16	22 ± 8	1 ± 1	13
*fru*³/*fru*ʷ¹²	1 ± 0	0 ± 0	9	1 ± 1	0 ± 0	10
*fru*⁴/*fru*ʷ¹²	8 ± 5	1 ± 1	9	7 ± 5	0 ± 0	12
*fru*ʷ¹²/*Cha*ᴹ⁵	2 ± 1	0 ± 0	14	0 ± 0	0 ± 0	6
*fru*ʷ²⁷/*Cha*ᴹ⁵	2 ± 2	0 ± 0	15	0 ± 0	0 ± 0	7

* CI, courtship index.

Sexual orientation and courtship behavior in *Drosophila* are regulated by *fruitless* (*fru*), the first gene in branch of the sex-determination hierarchy.

Ryner, *et al*. Cell. 1996

This discovery was the critical finding that led Bruce' s laboratory into the study of behavioral genetics

Fruitless Consortium

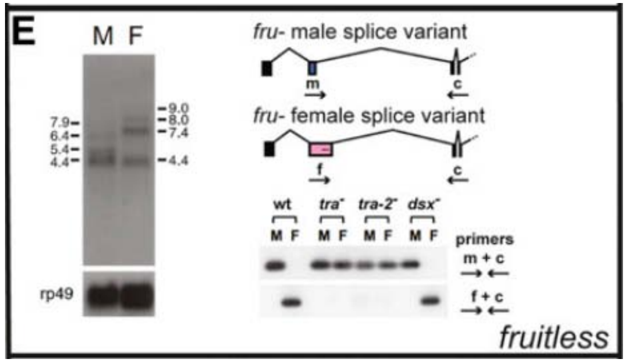
fru’ s role in male courtship behavior

fru’ s sex-specific transcripts

the role of *fru* in MOL developmen t

fru alleles ;

the clones spanning the *fru* genomic interval



Bruce Baker VS Manichean

- The combined work of the consortium had built a very strong case that a single gene, fru, had highly specific control of *Drosophila* sexual orientation and sex behavior（Ryner et al. 1996）.

- Whether or not behavioral repertoires could in fact be genetically specified, or were predominantly shaped by environmental factors

.

Cell, Vol. 105, 13–24, April 6, 2001, Copyright ©2001 by Cell Press

Are Complex Behaviors Specified by Dedicated Regulatory Genes? Reasoning from *Drosophila*

Review

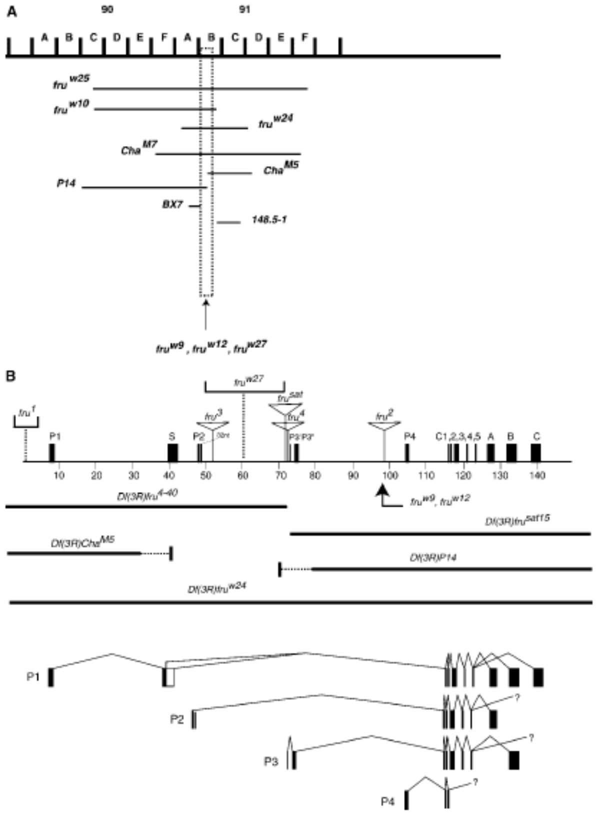
Bruce S. Baker,*§ Barbara J. Taylor,† and Jeffrey C. Hall‡

(Ridley, 1995). Such a definition includes species-specific behaviors studied extensively by ethologists (e.g., court-

Fruitless Consortium- sex-specific transcripts of the *fru*

Molecular Genetic Dissection of the Sex-Specific and Vital Functions of the *Drosophila melanogaster* Sex Determination Gene *fruitless*

Anuranjan Anand,*,† Adriana Villella,‡ Lisa C. Ryner,* Troy Carlo,‡ Stephen F. Goodwin,‡,§
Ho … Morales,‡ Jeffrey C. Hall,‡ Bruce S. Baker*
… J. Taylor**



fru's vit

TABLE 2

Summary of transcript classes expressed by *fru* alleles

fru allele	Transcript class P1	P2	P3	P4
$fru^{1\,a}$	+	+	+	+
$fru^{2\,a}$	(+)	(+)	(+)	+
$fru^{3\,a}$	(+)	(+)	+	+
$fru^{4\,a}$	(+)	(+)	+	+
$Cha^{M5\,b}$	$(+)^{d}$	+	+	+
$fru^{4\text{-}40\,b}$	–	–	+	+
$fru^{w27\,b,e}$	–	–	+	+
$fru^{w12\,b}$	–	–	–	+
$fru^{w9\,c}$	–	–	–	+
$fru^{w13\,c}$	–	–	–	+
$fru^{sat15\,b}$	–	–	–	–
$fru^{w24\,b}$	–	–	–	–
				–

TABLE 5

Courtship behavior of *fru* mutant males

Genotypes	CI (m → m)	WEI (m → m)	CI (m → f)	WEI (m → f)	ChI

TABLE 7

Courtship song summary for *fru* mutants

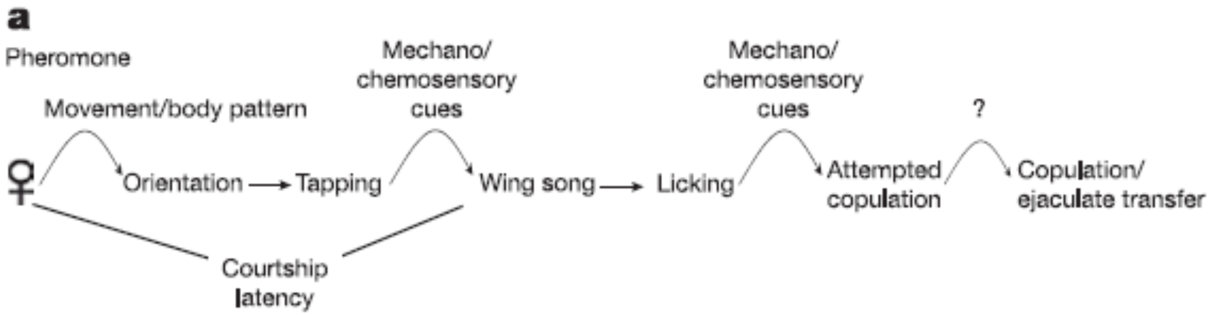
Genotype	*N* wing extension	*N*	IPI	CPP	Frequency	Width

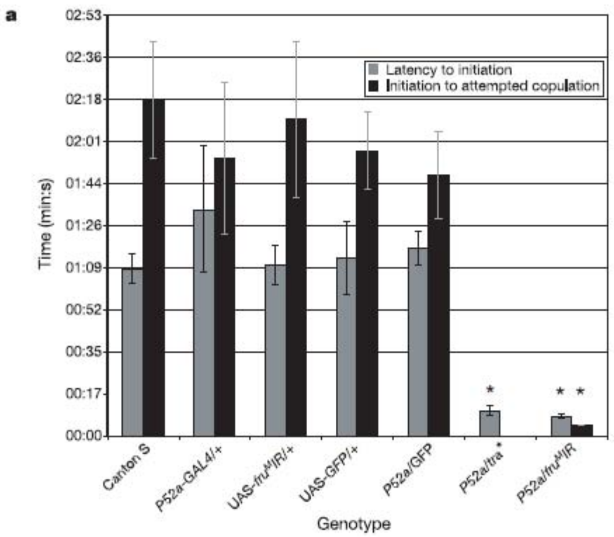
TABLE 8

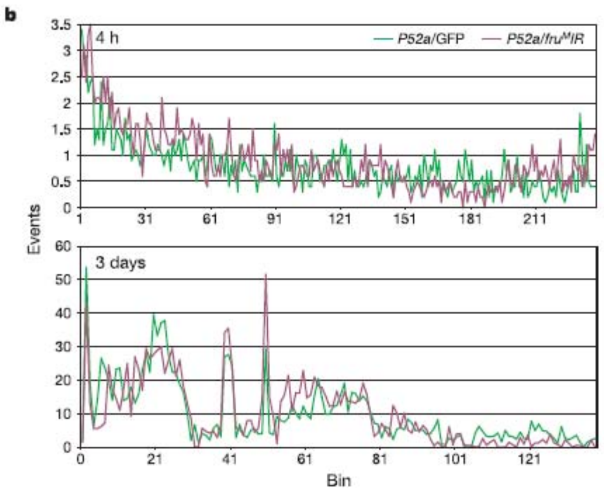
***fru* mutations disrupt the muscle of Lawrence development**

- the consortium showed that *fru* encodes transcription factors whose sex-specific transcripts are generated by alternative splicing controlled by the sex hierarchy proteins Tra and Tra-2。This splicing was shown to be necessary for male-specific courtship behavior.

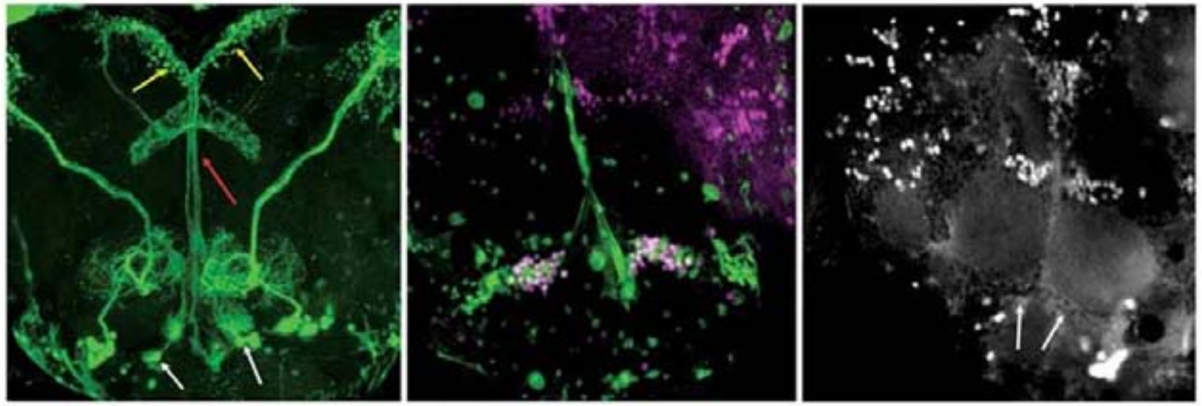
A small group of *fru*^{M} in the median bundle neurons appropriately trigger the sequential execution of the courtship ritual in *Drosophila*

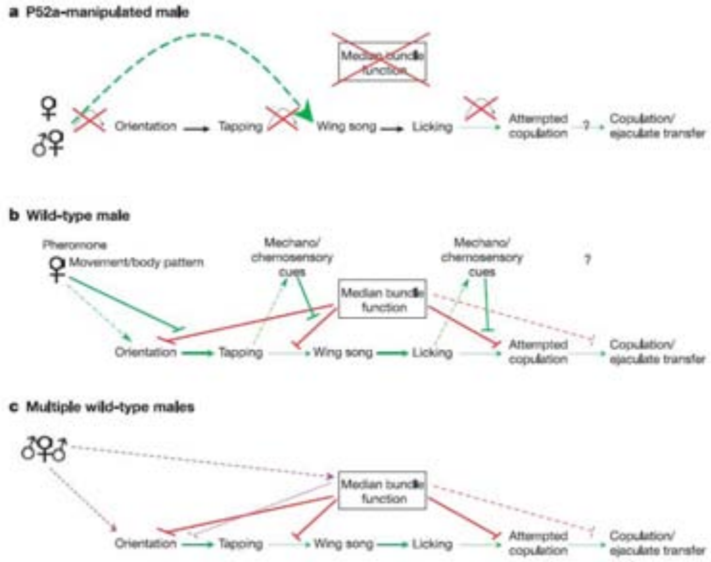






locomotion

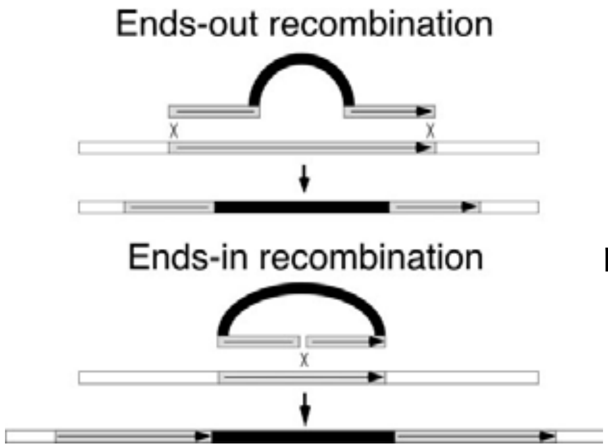


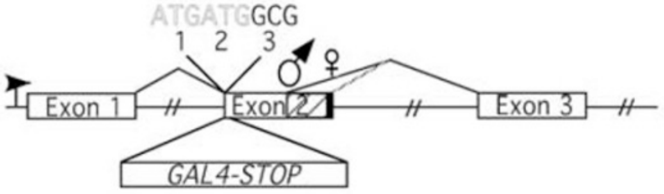


P52a-GAL4 expression in *Fru*^{M} neurons of the median bundle. *P52a*-GAL4 directs inhibition of *Fru*^{M} expression by a UAS-*fru*^{MIR} transgene.

Manoli, D. S. and B. S. Baker., Nature. 2004

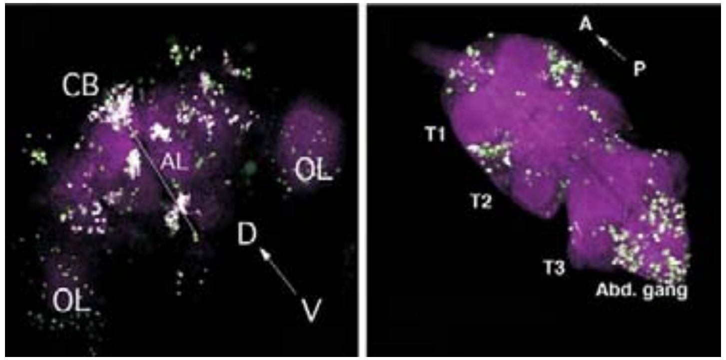
Fru^M proteins specify the neural substrates of male courtship.

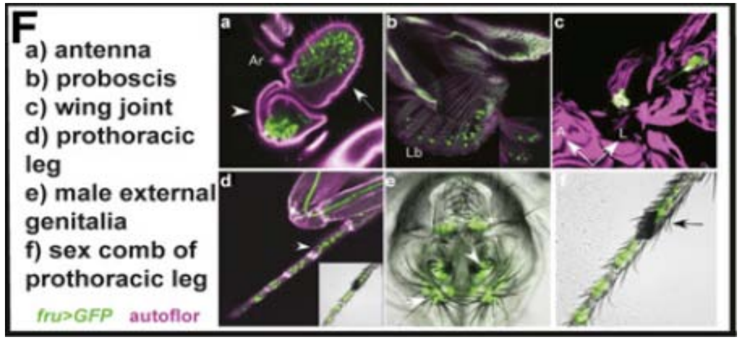


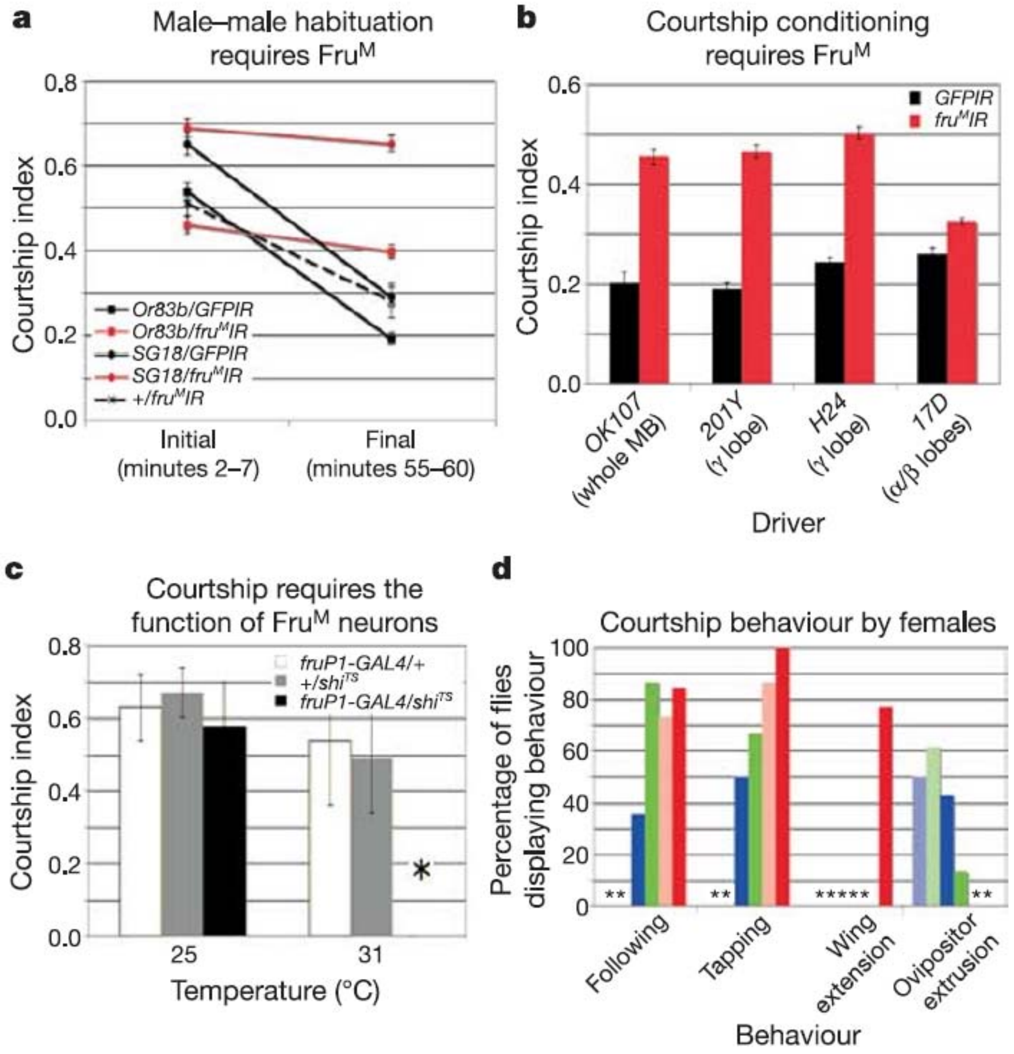


nsert the Gal4 coding region into *fru*

同源重组，GAL4替代P1启动子，
只有在UAS驱动时可以表达并
定位*fru*^{M}



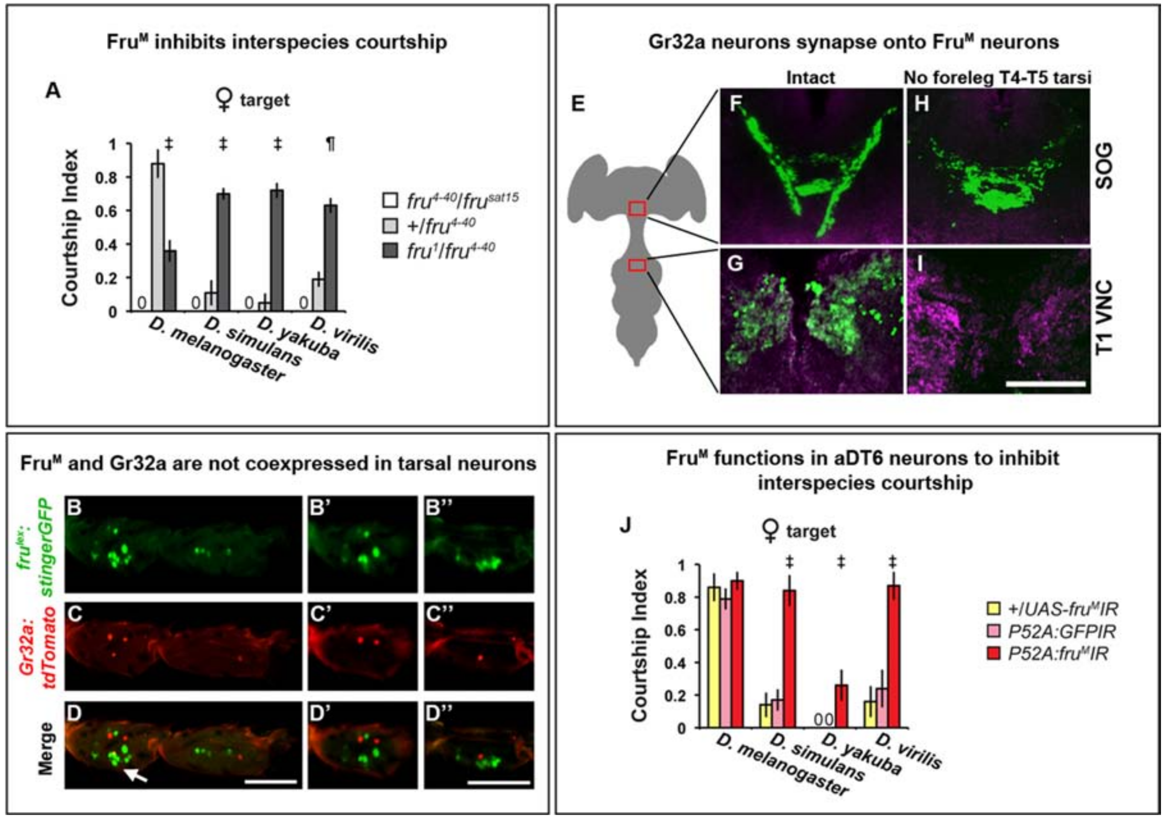




fru^M is important for the detection of ethologically relevant sensory information and for transmission of this information to the central nervous system.

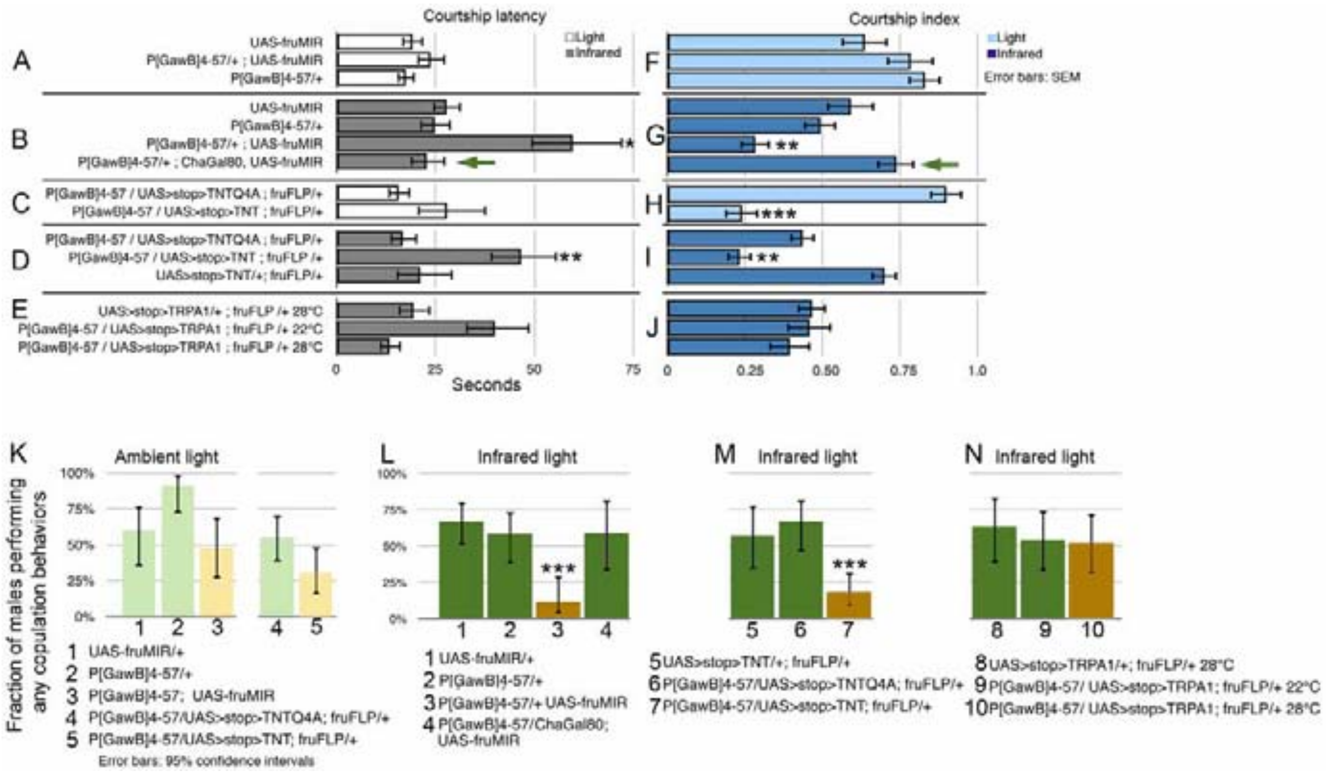
Gong and Goloc., 2003 Manoli et al., Nature., 2005

Gr32a and Fru^M inhibit interspecies courtship by Synaptic connections



Fan, P.,et al. Cell. 2013

A small subset of *fruitless* subesophageal neurons modulate early courtship in *Drosophila*



Tran, D. H., et al. PLoS One. 2014

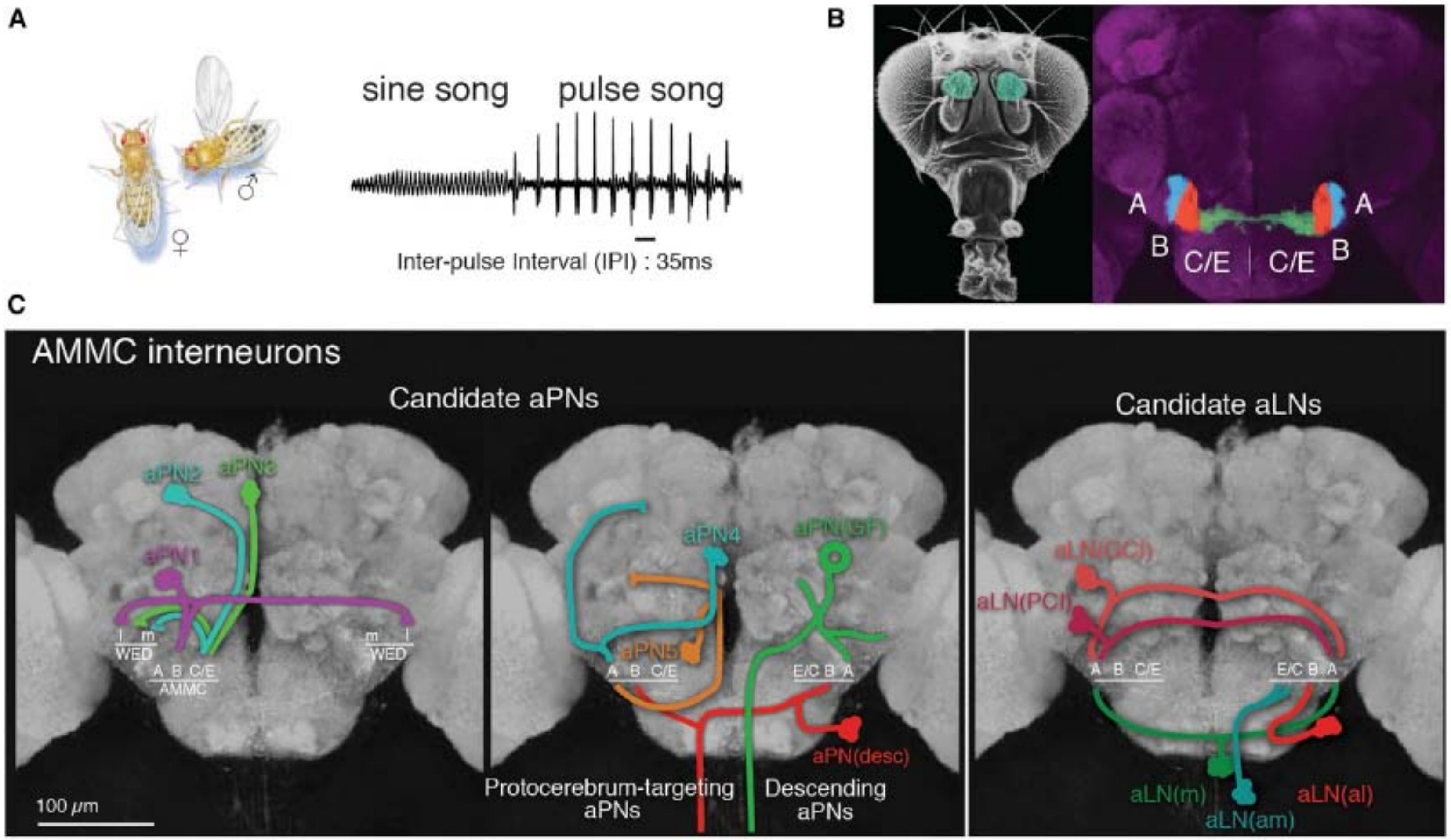
Current Biology 24, 1039–1049, May 19, 2014 http://dx.doi.org/10.1016/j.cub.2014.03.048

Article

This song is perceived via mechanosensory neurons in the antennal Johnston's organ

Neural Pathways for the Detection and Discrimination of Conspecific Song in *D. melanogaster*

Alexander G. Vaughan,[1] Chuan Zhou,[1] Devanand S. Manoli,[2] and Bruce S. Baker[1,*]
[1]Janelia Farm Research Campus, HHMI, Ashburn, VA 20147, USA
[2]Department of Psychology, UCSF, San Francisco, CA 94122, USA



two classes of interneurons are necessary for song responses—the aPN1 and GABAergic interneuron aLN(al).

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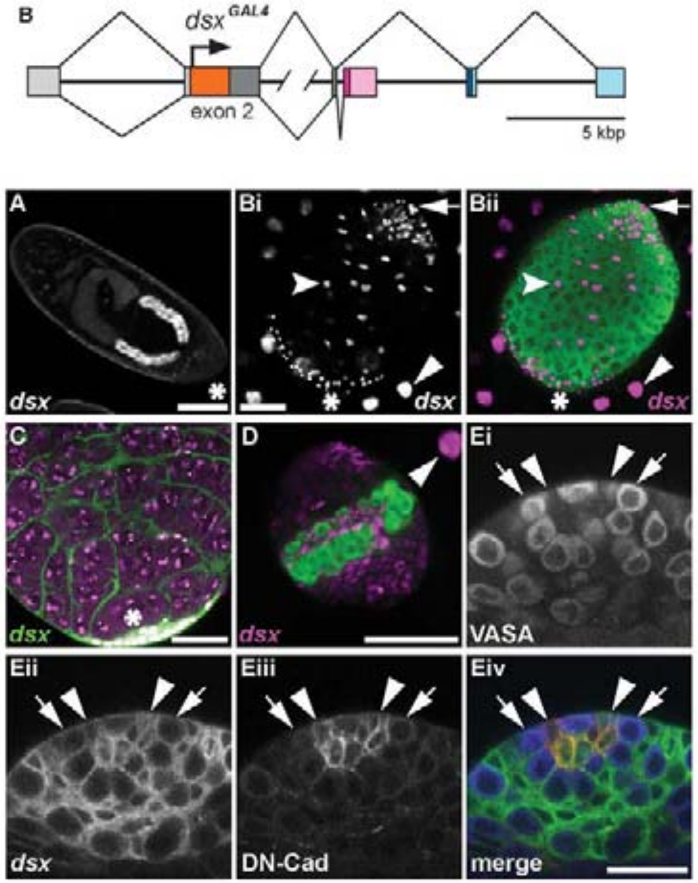
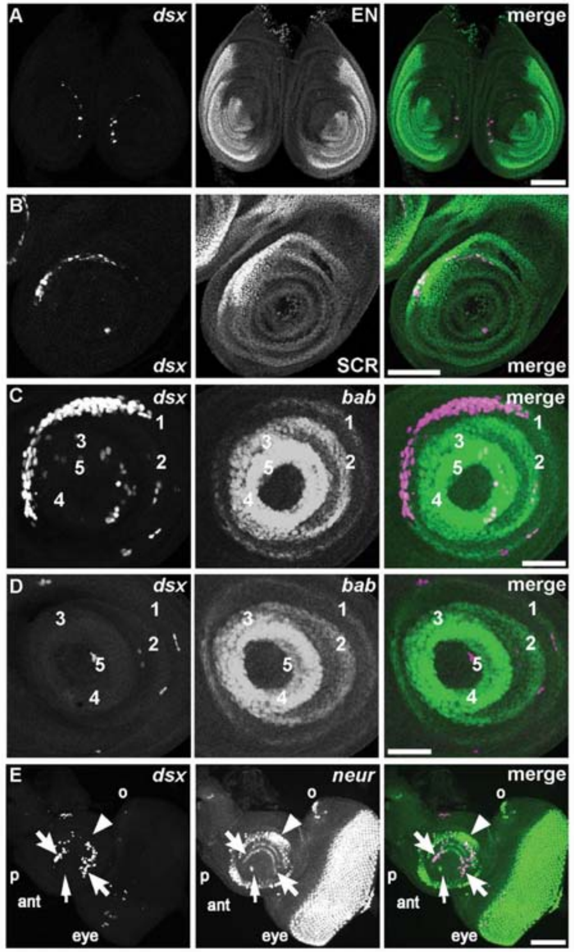
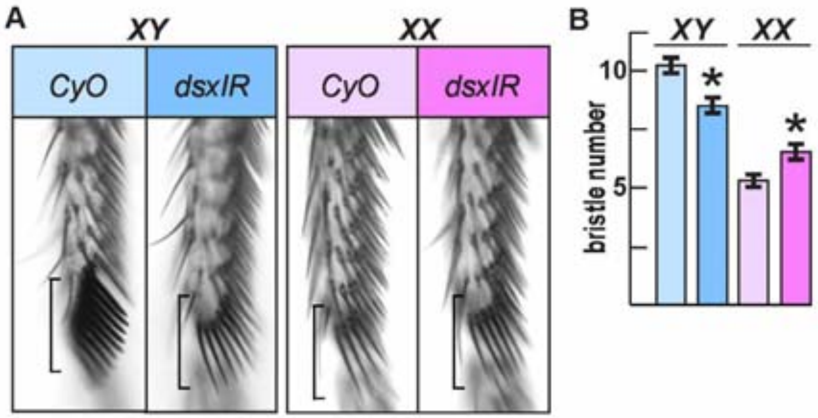
Sex and the Single Cell. II. There Is a Time and Place for Sex

Carmen C. Robinett[1¤], Alexander G. Vaughan[1¤], Jon-Michael Knapp[1,2¤], Bruce S. Baker[1,2*¤]

1 Biology Department, Stanford University, Stanford, California, United States of America, 2 Neuroscience Program, Stanford University, Stanford, California, United States of America







the developing gonads in stage 13–17 embryos

imaginal discs

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Sex and the Single Cell. II. There Is a Time and Place for Sex

Carmen C. Robinett[1¤], Alexander G. Vaughan[1¤], Jon-Michael Knapp[1,2¤], Bruce S. Baker[1,2*¤]

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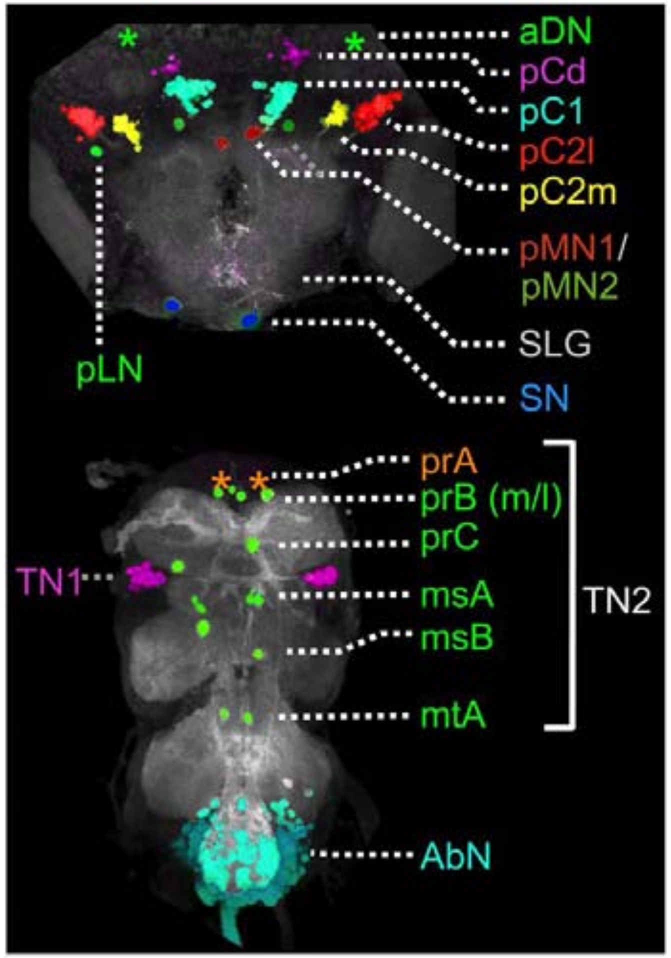
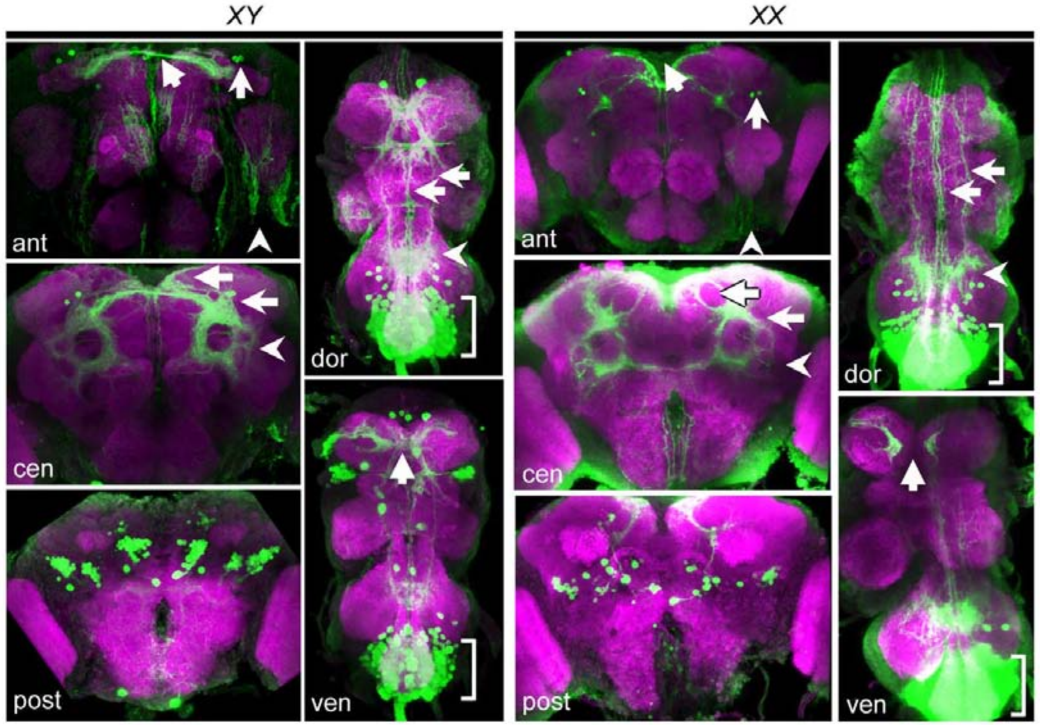
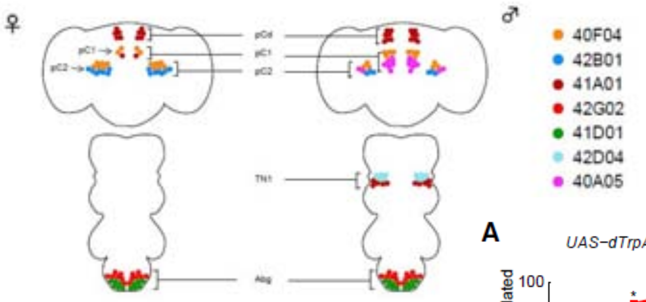
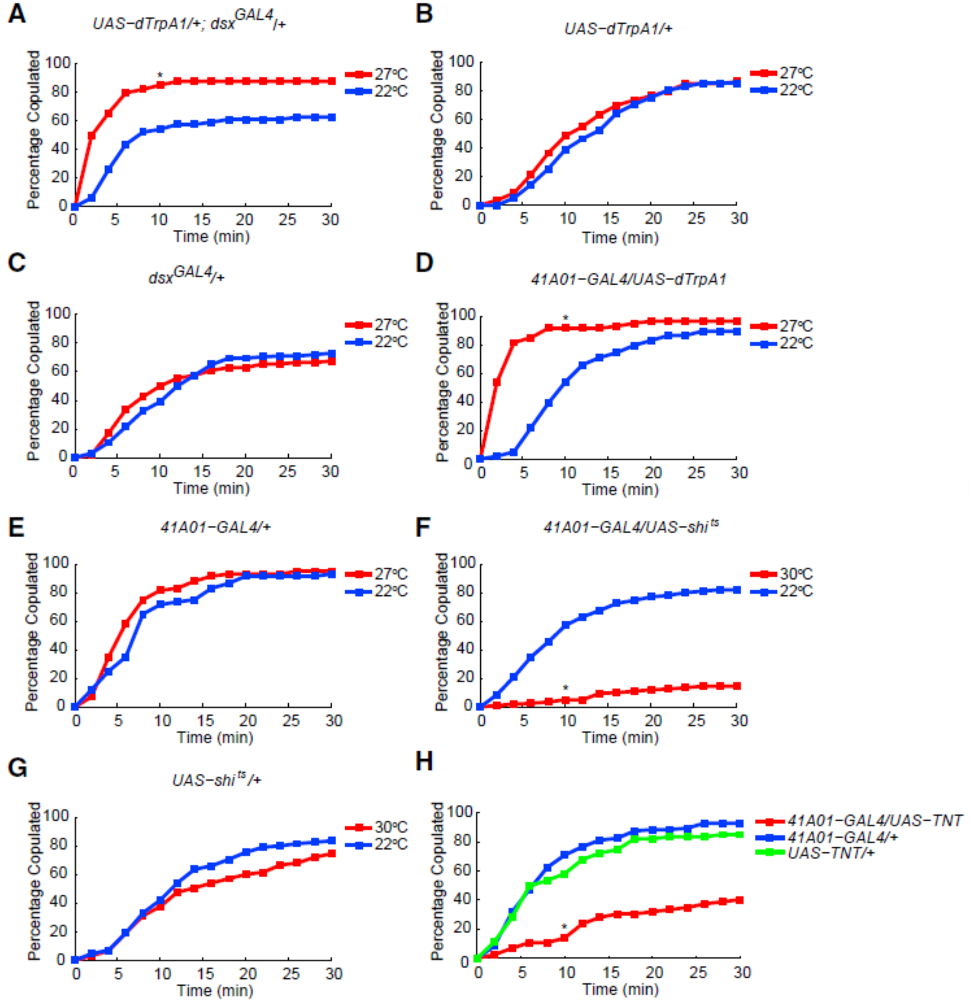
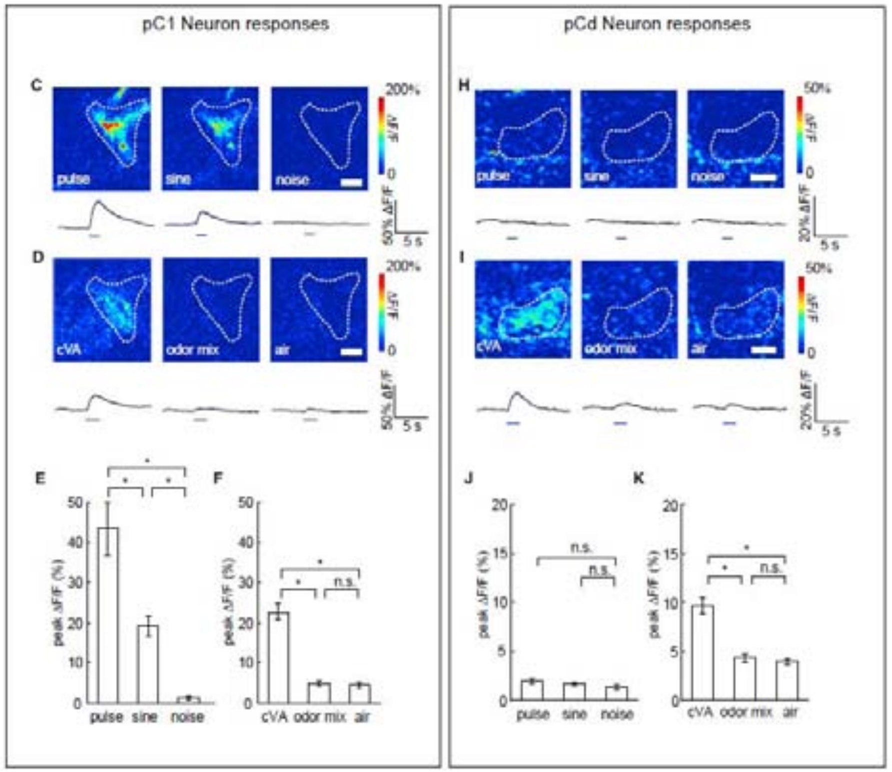


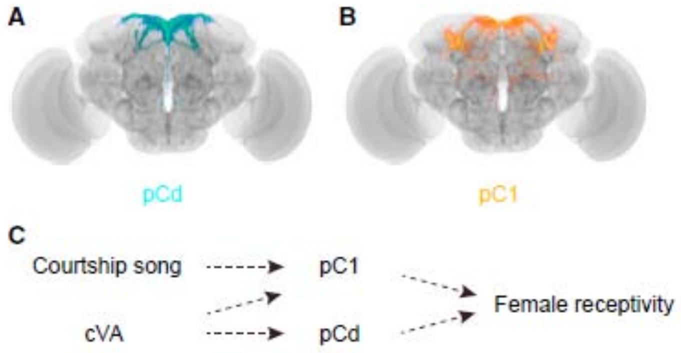


dsx-expressing neurons mediate virgin female receptivity to courting males



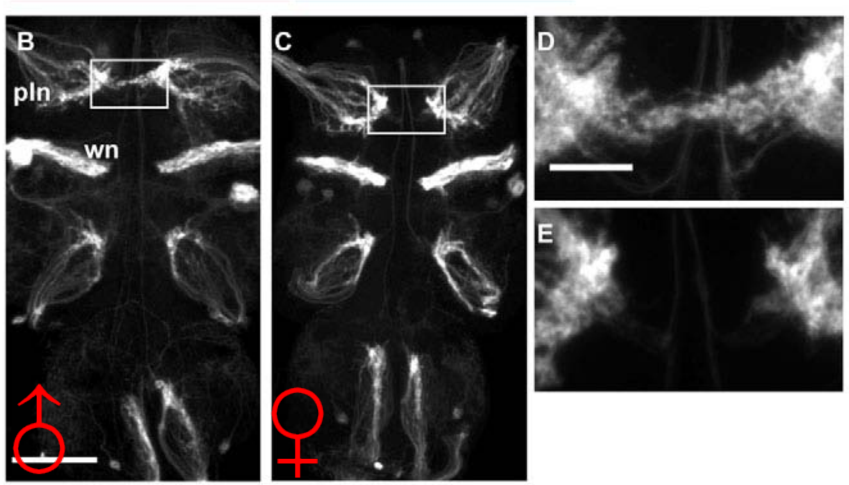




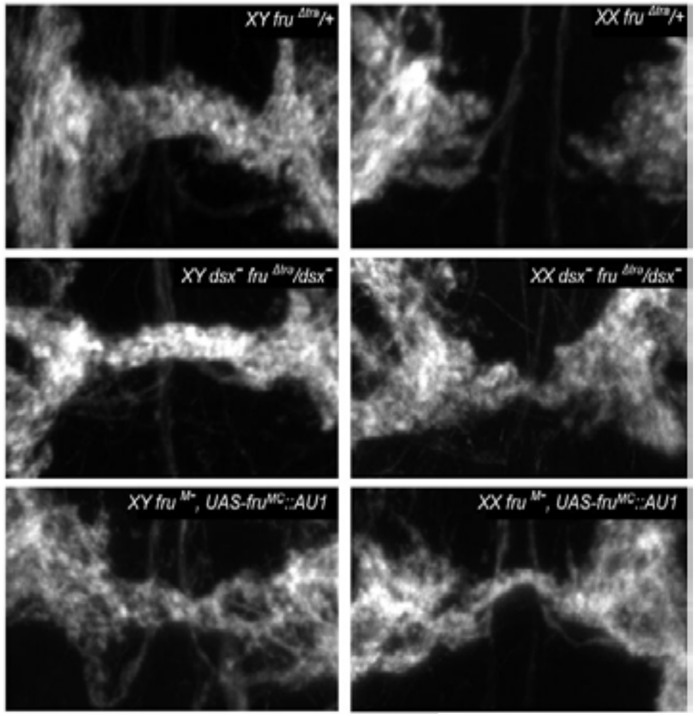


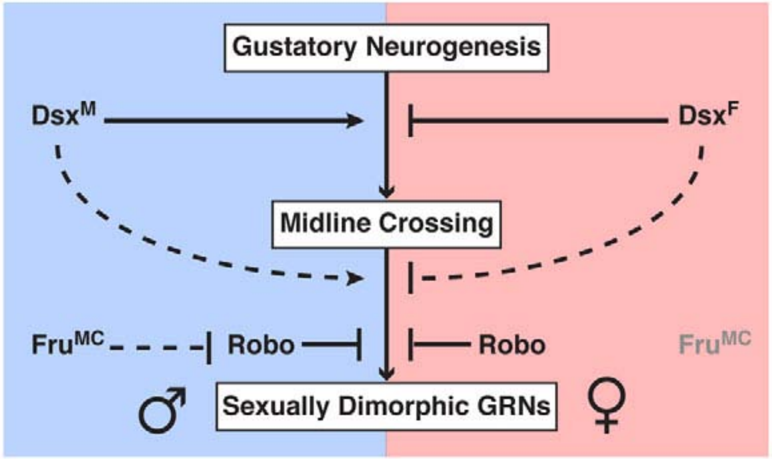
Zhou et al., Neuron., 2014

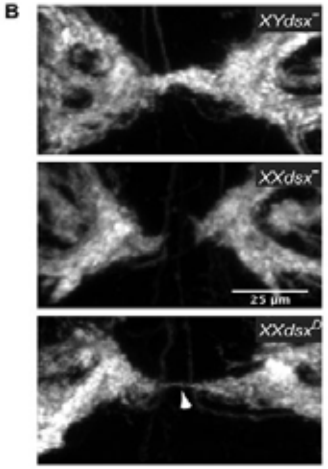
The *dsx* and *fru* branches of the sex hierarchy cooperate in controlling anatomical sex differences in neural circuitry

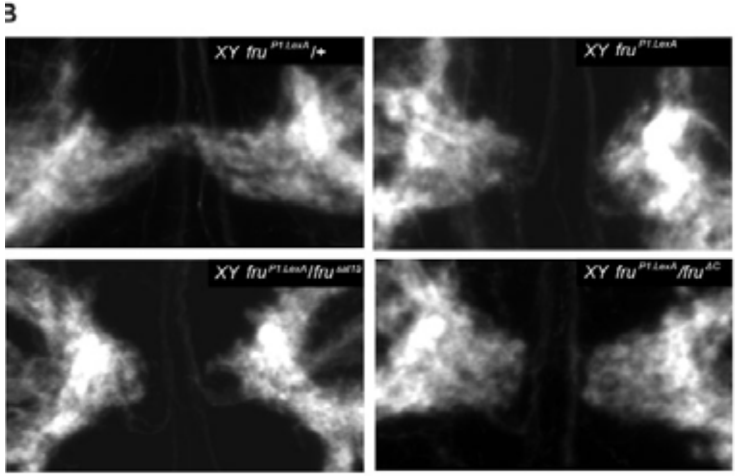


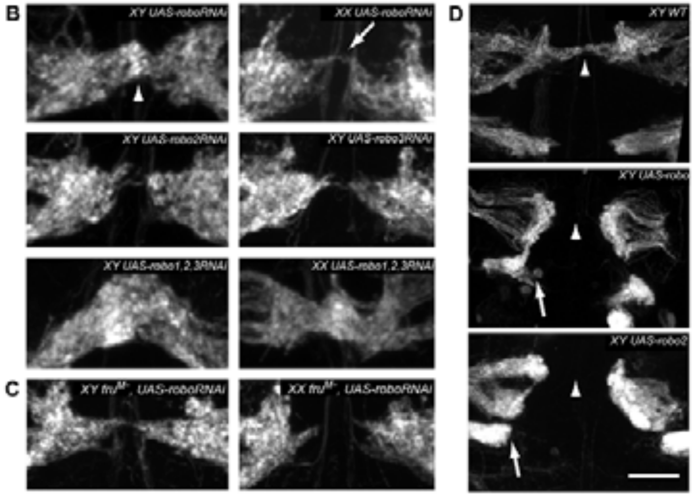
poxn-Gal4 (expressed in GRNs)
-driven UAS-mCD8::GFP







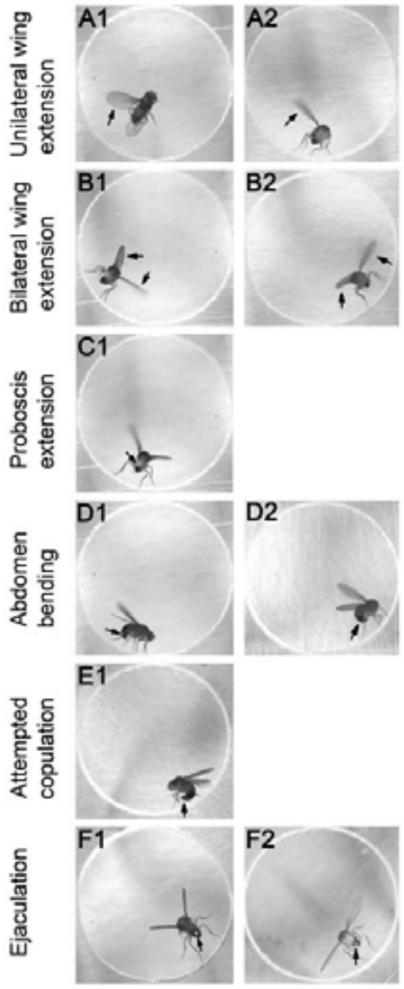




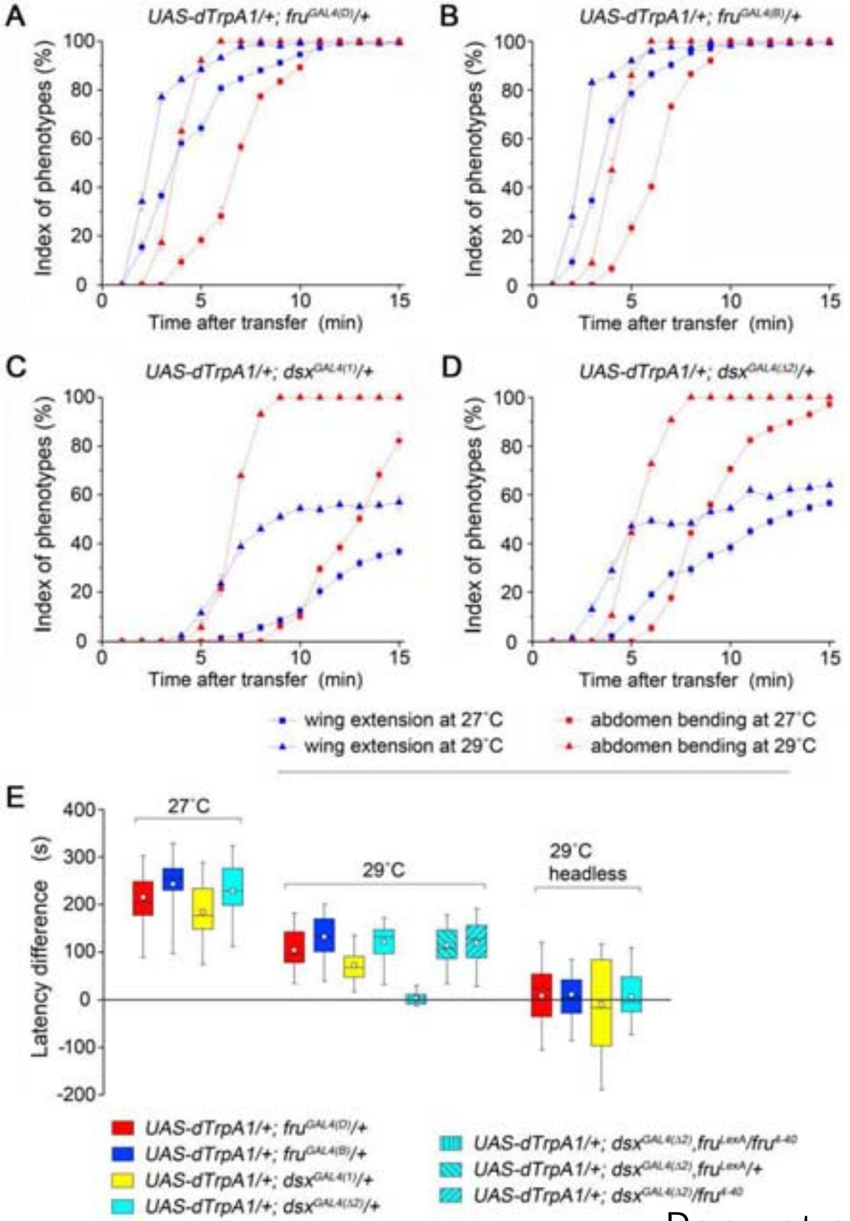
males lacking either (1) all Fru^{M} proteins or (2) Fru^{MC} proteins only do not form contralateral gustatory receptor neuron (GRN) projections

Mellert,. et al. Development. 2010

Activation of either all *fru*^{M} or all *dsx* neurons in solitary males can induce almost all steps of courtship

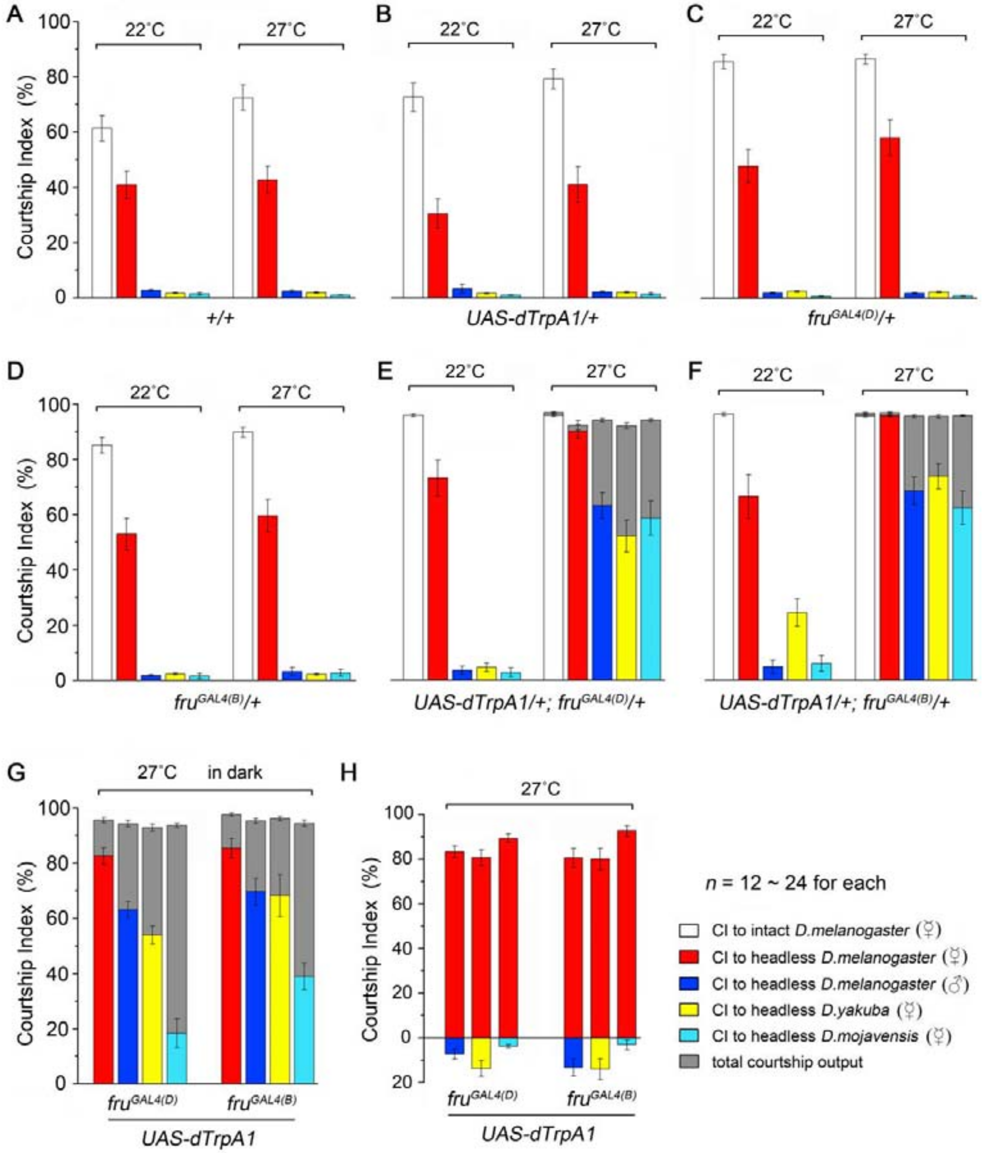


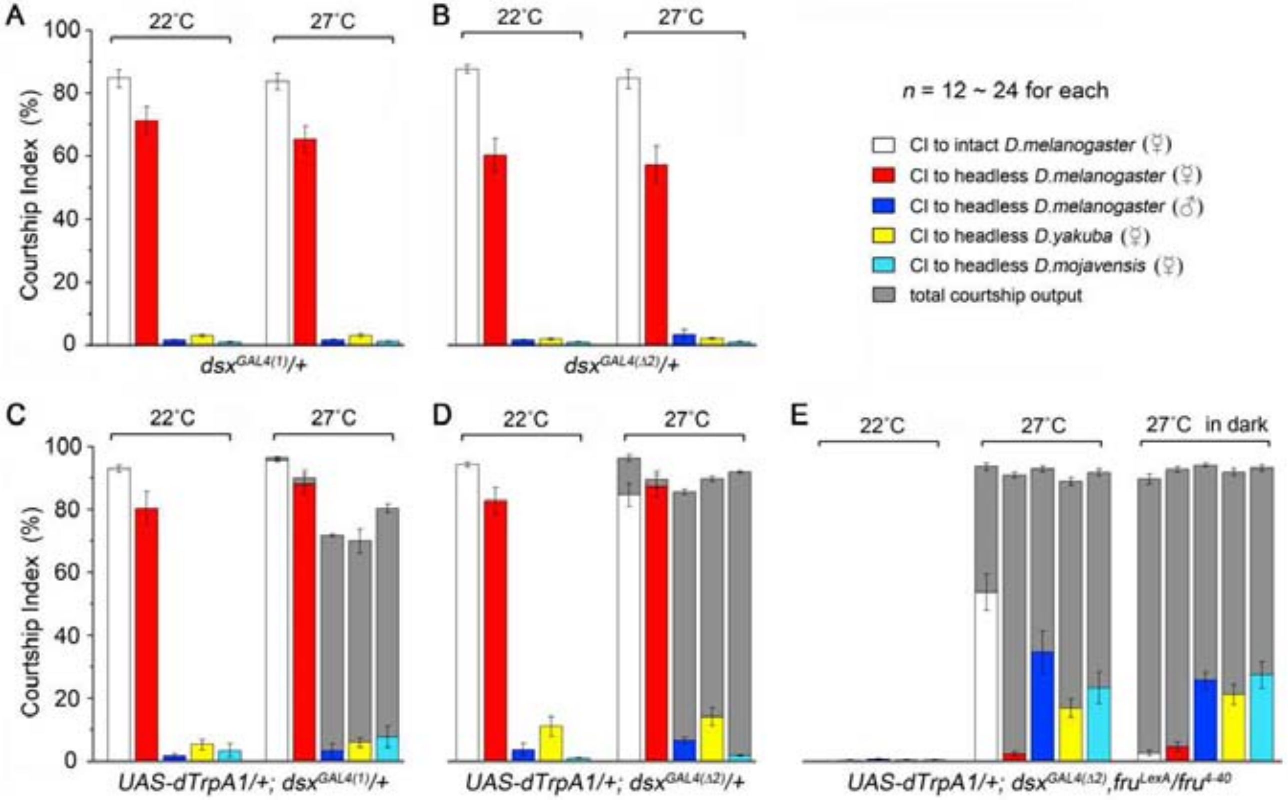
	Activated *fru*^{M} neurons		Activated *dsx* neurons		*wtcs*
	fru$^{GAL4(D)}$	*fru*$^{GAL4(B)}$	*dsx*$^{GAL4(1)}$	*dsx*$^{GAL4(\Delta 2)}$	
A1:	24/24	24/24	24/24	24/24	0/36
A2:	24/24	24/24	24/24	24/24	0/24
B1:	24/24	24/24	24/24	24/24	0/36
B2:	24/24	24/24	24/24	24/24	0/24
C1:	24/24	24/24	24/24	24/24	0/24
D1:	24/24	24/24	24/24	24/24	0/36
D2:	24/24	24/24	24/24	24/24	0/24
E1:	24/24	24/24	10/24	24/24	0/36
F1:	16/24	24/24	3/12(30min)	4/12(30min)	0/36
F2:	11/24	19/24	4/24(30min)	5/24(30min)	0/24



Pan., et al. PLoS One. 2011

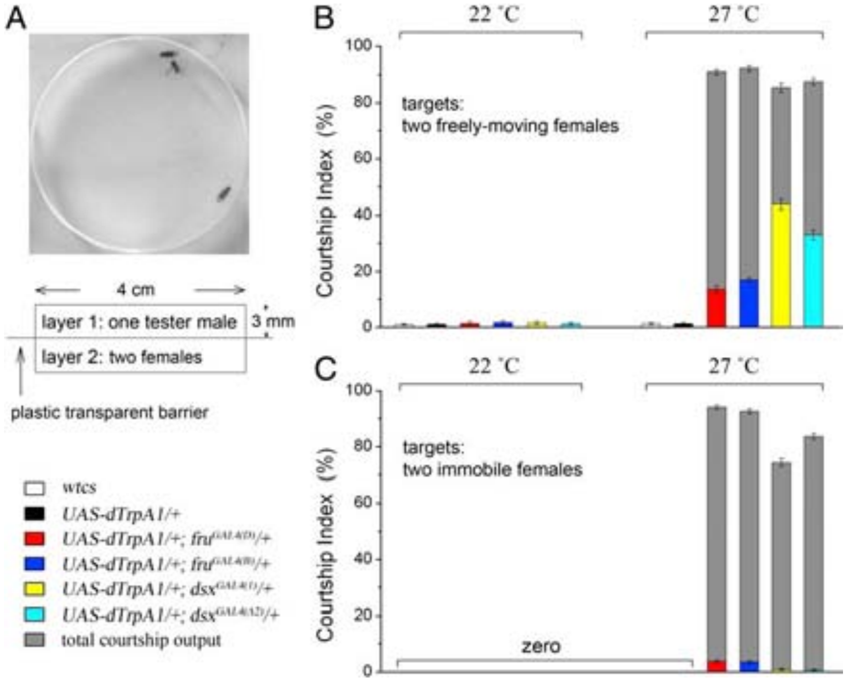
fru^{M} neurons involve in courtship promotion and recognition and the *fru*^{M}-independent courtship pathway is primarily vision dependent

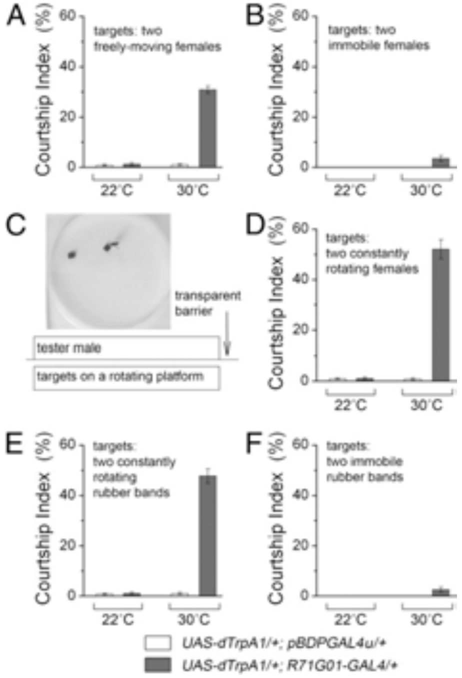


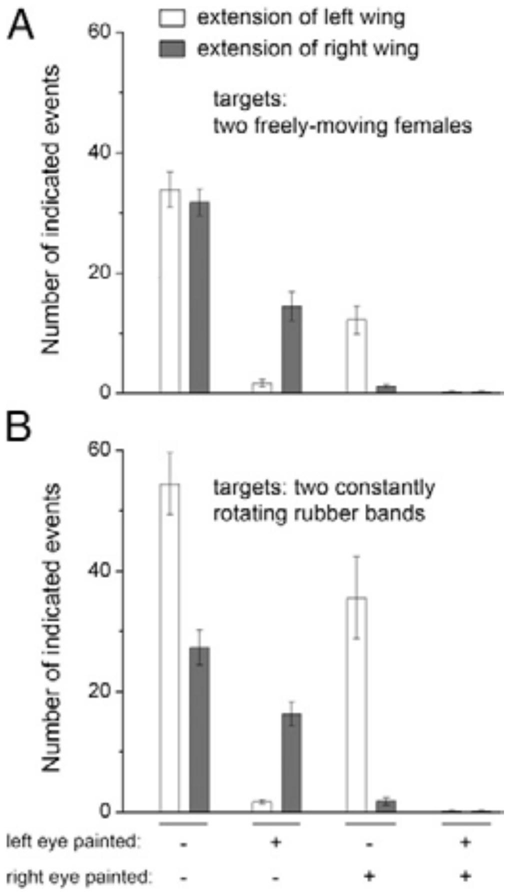


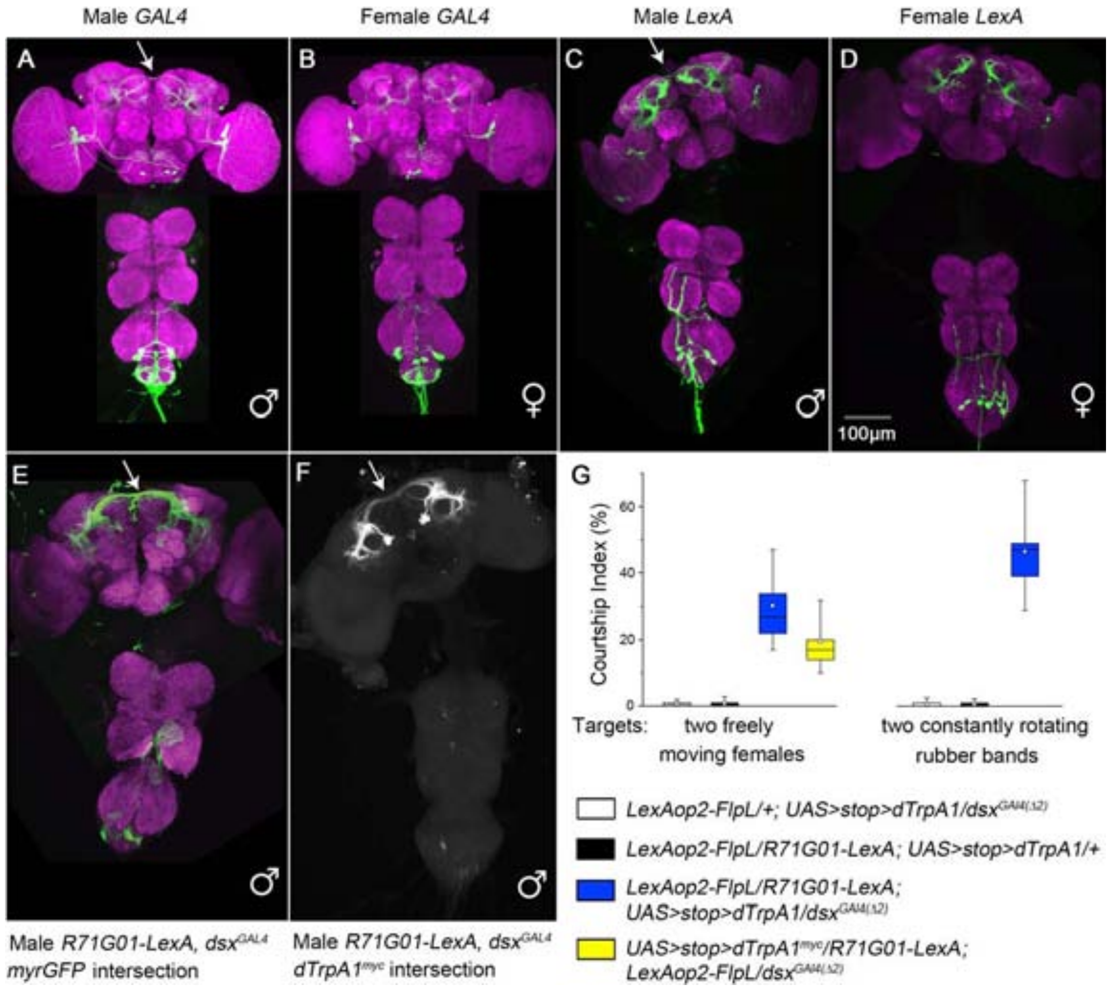
Pan., et al. PLoS One. 2011

Motion input and activation of P1 neurons are individually necessary, and jointly sufficient to elicit early courtship behaviors



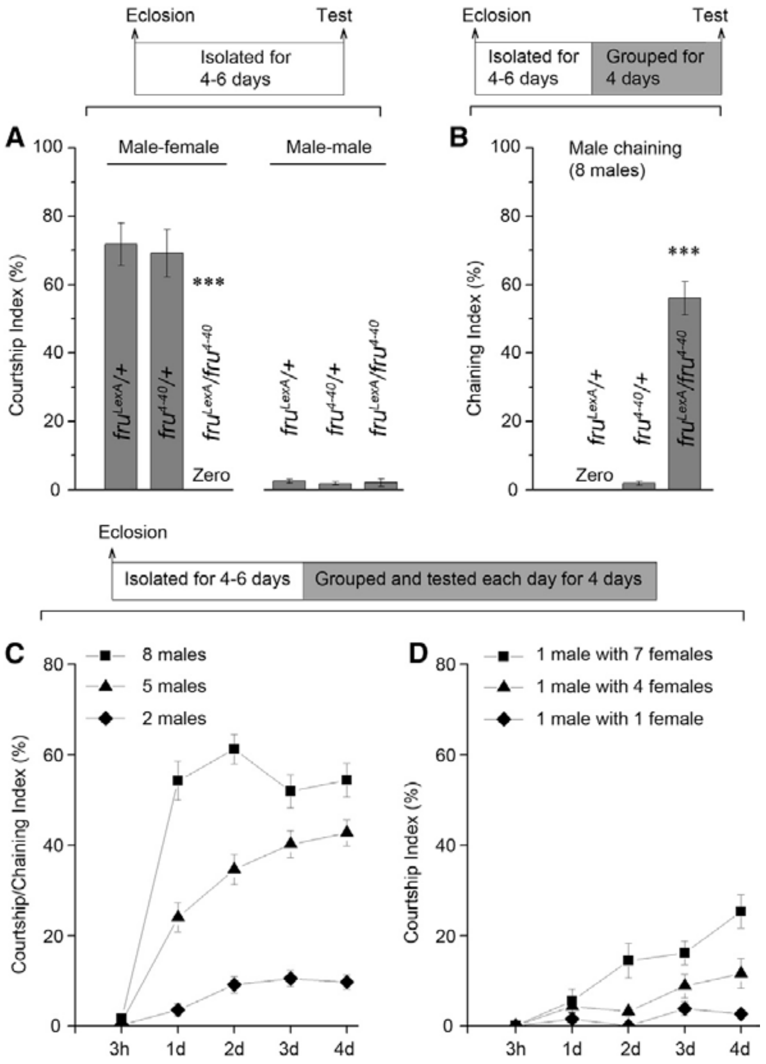


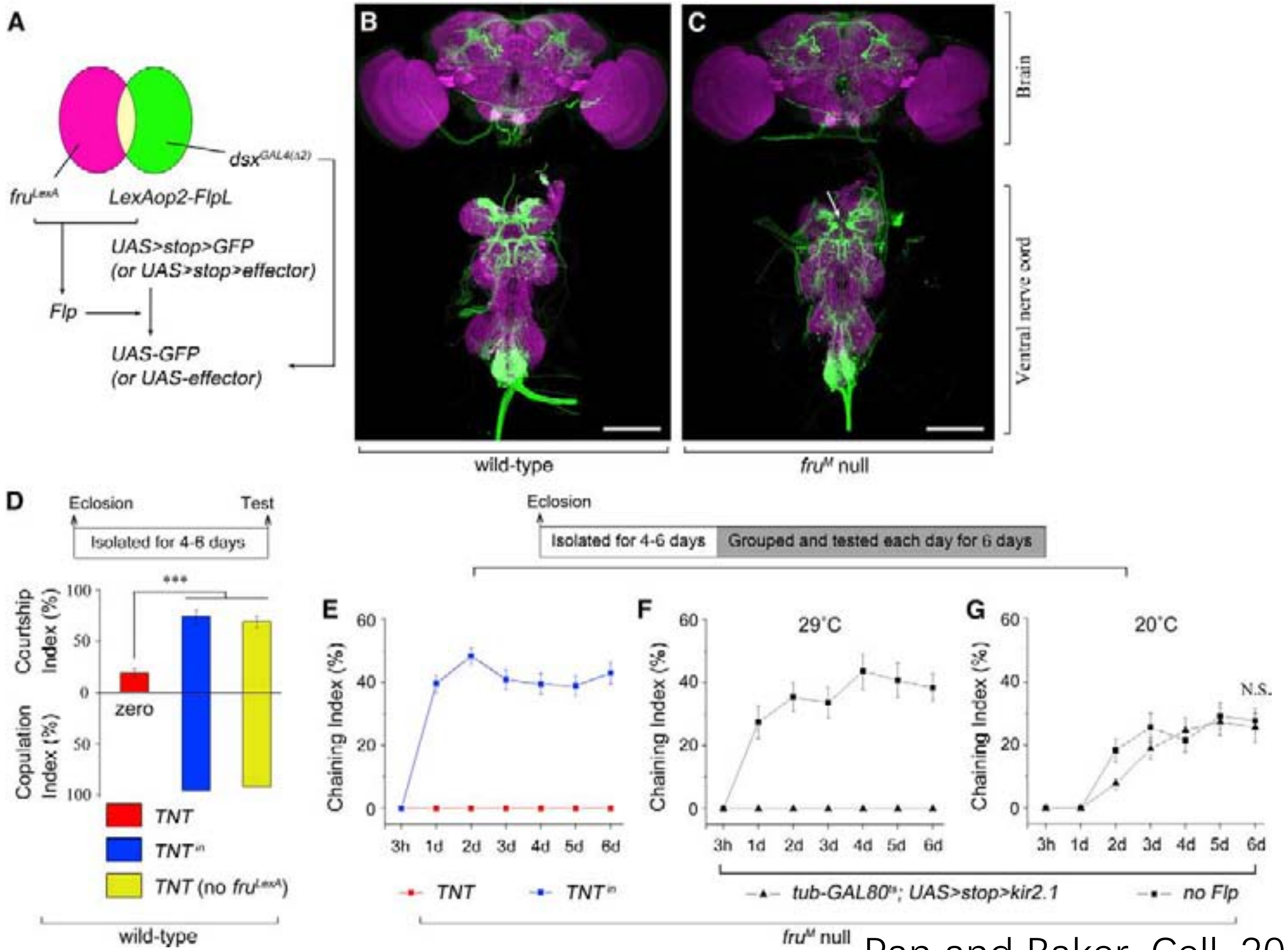




Pan., et al. PNAS. 2012

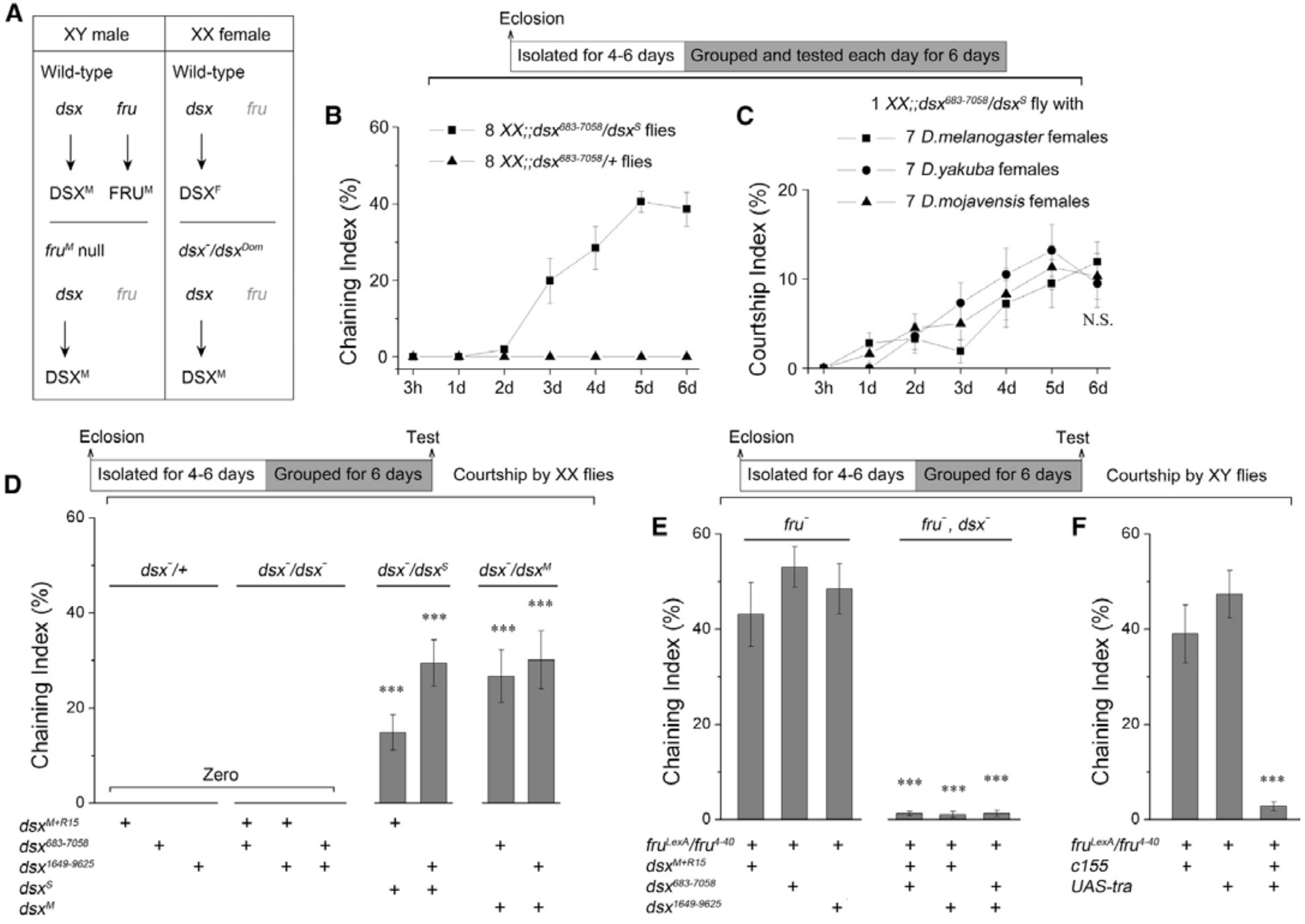
Activity of *fru^M* and *dsx* overlapping neurons is necessary during adulthood for courtship acquisition in *fru^M*-null males.

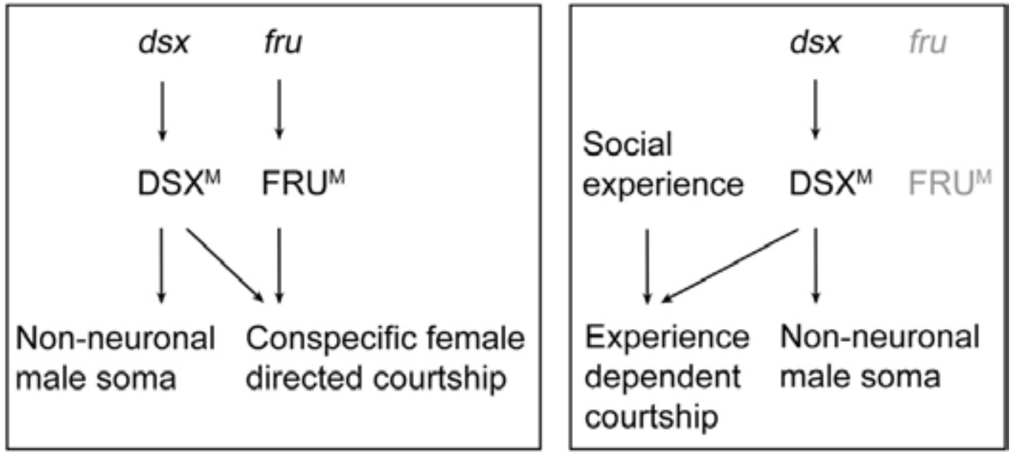




Pan and Baker. Cell. 2014

dsx^M is necessary and sufficient for the acquisition of the potential for such experience-dependent courtship.

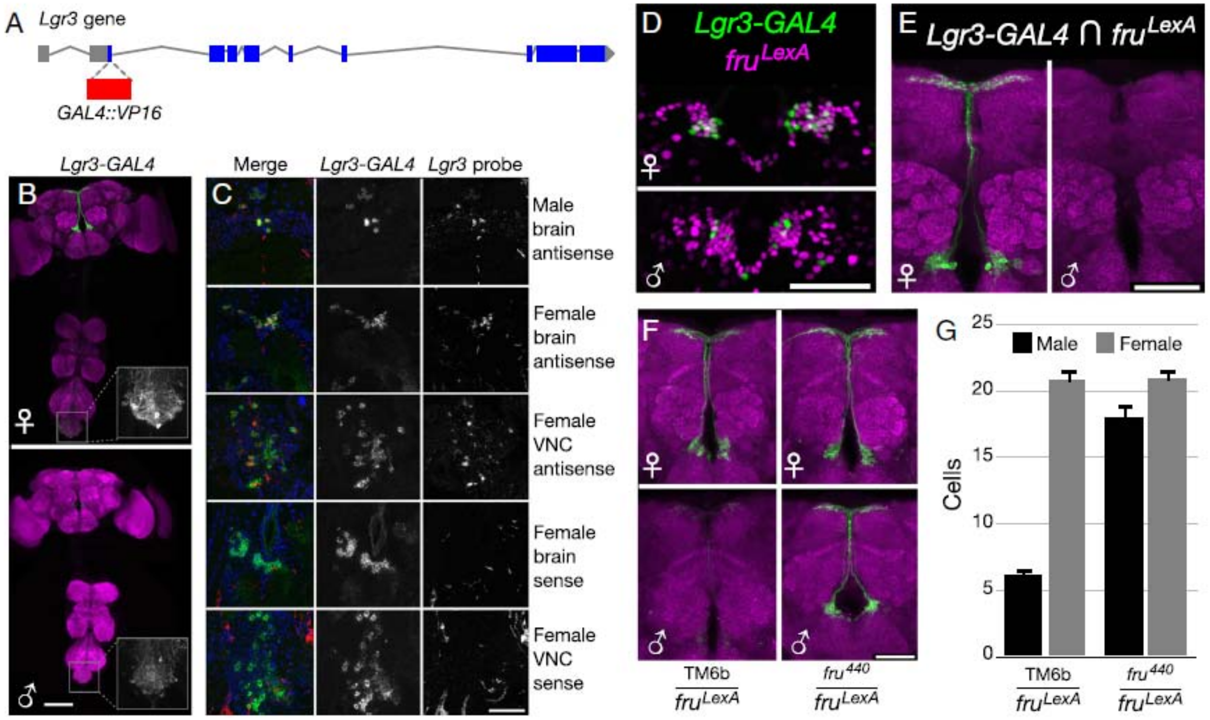


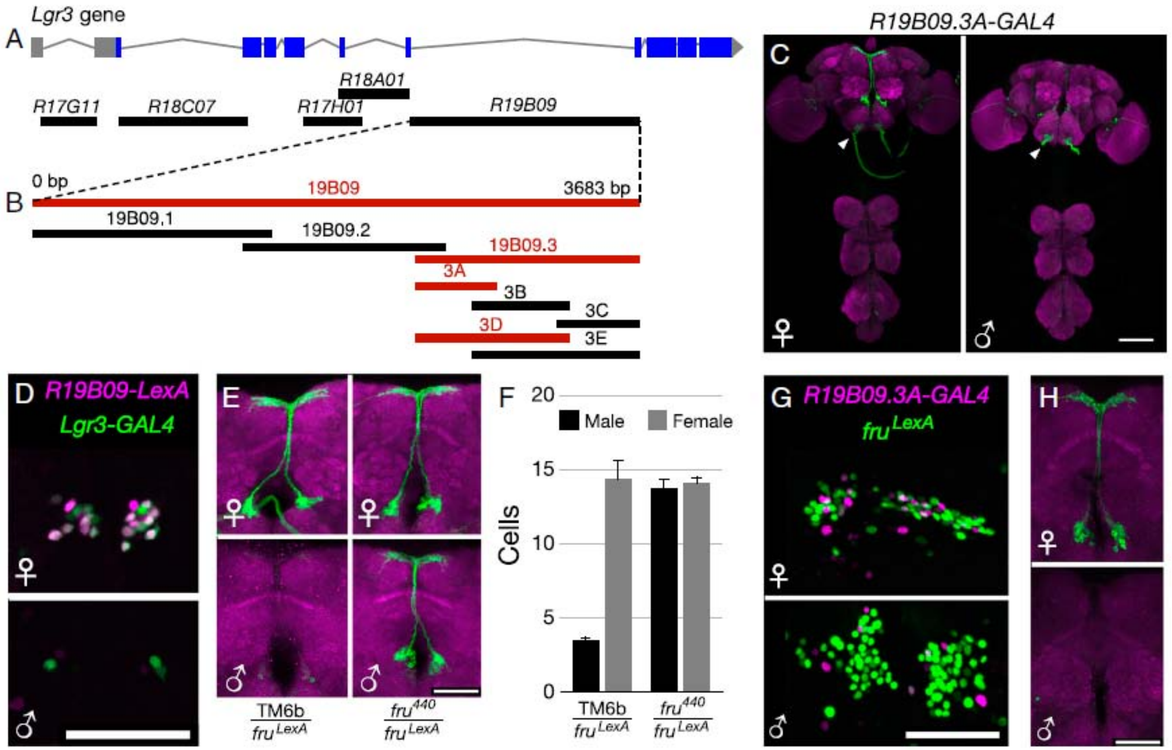


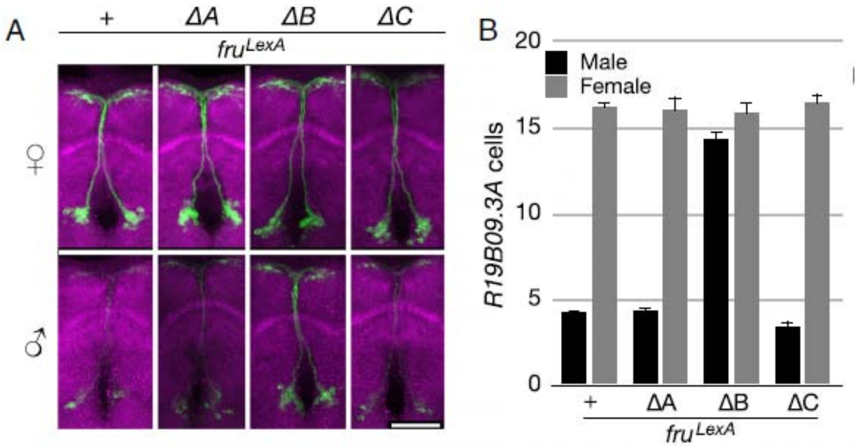
Pan and Baker. Cell. 2014

FruM Inhibits Expression of *Lgr3* in the Male Median Bundle, and Fru^M Acts Through a Small Region of an *Lgr3* Intron.

Lgr3 is a member of the leucine-rich repeat G-protein-coupled receptor (Lgr) family



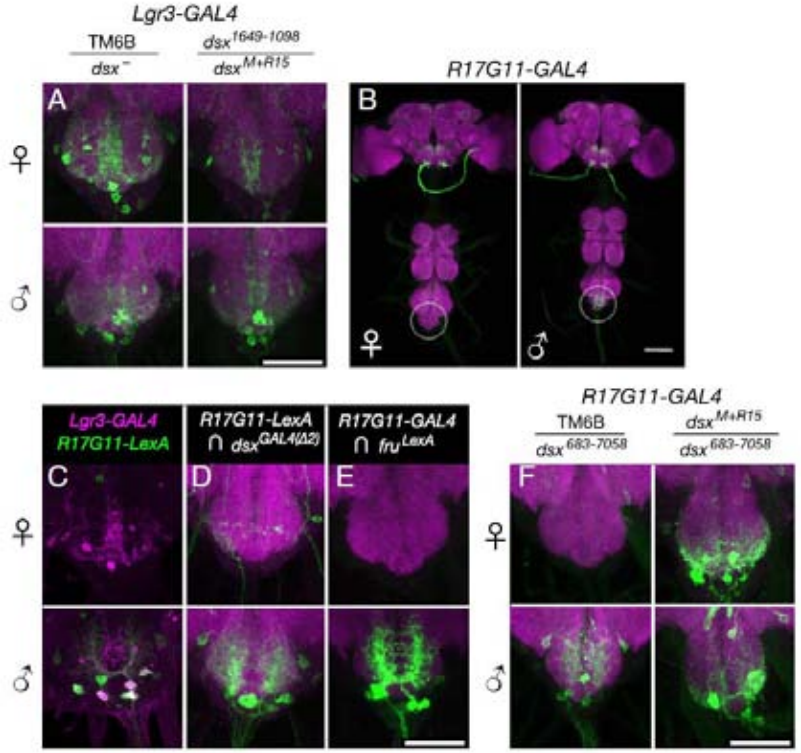


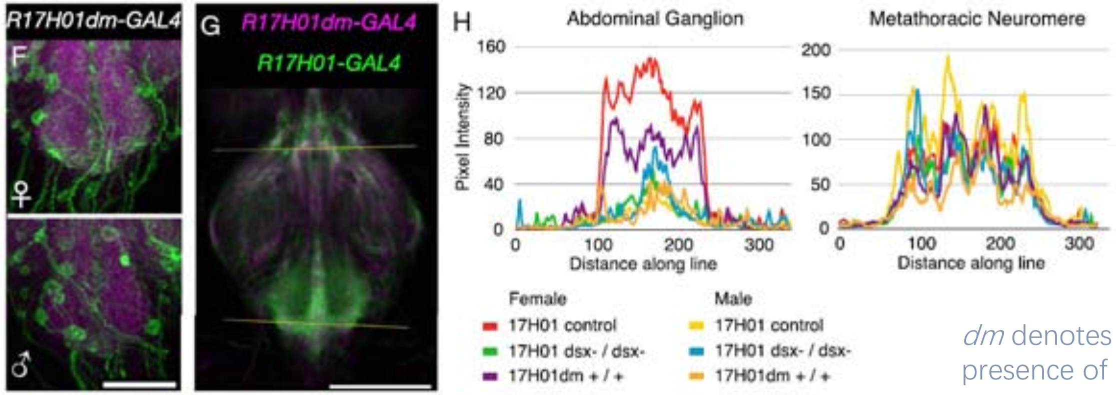


the B isoform of Fru, whose DNA binding domain interacts with a short region of an Lgr3 intron.

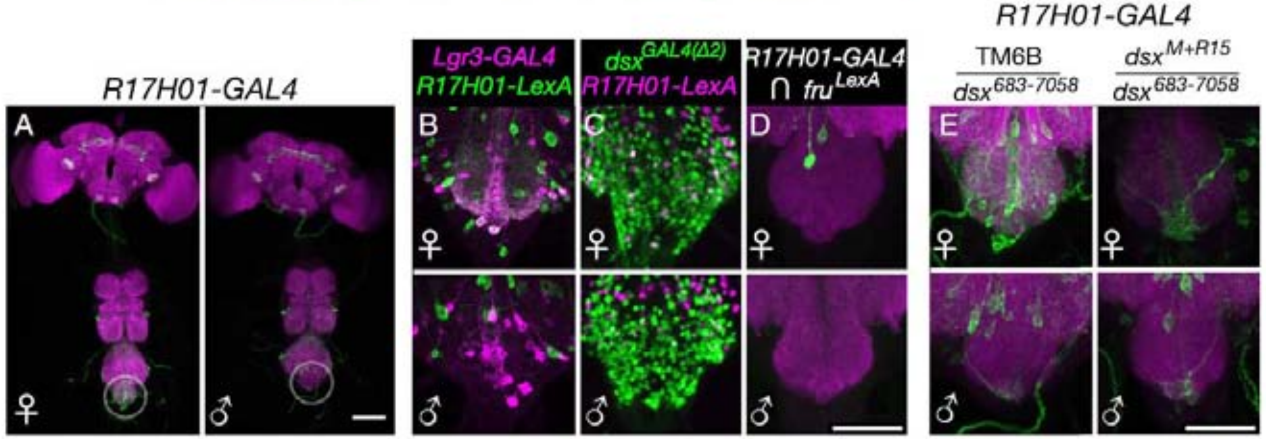
Meissner, G. W., et al. PNAS. 2016

DsxF plays the direct controlling role, it activates *R17H01* expression in females, but inhibits *R17G11*,

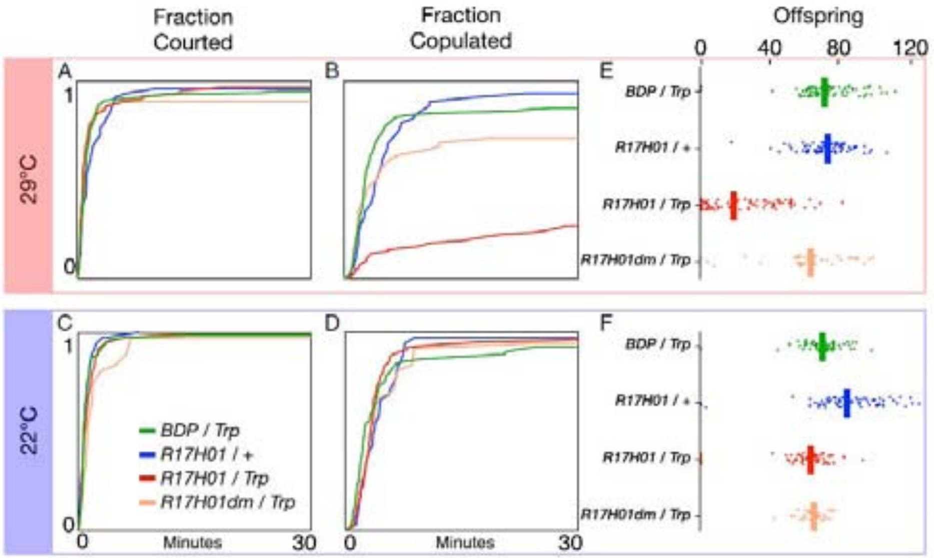




dm denotes the presence of mutated DSX binding sites.



Activation of *R17H01*-GAL4 neurons reduces female receptivity and fecundity.



Meissner, G. W., et al. PNAS. 2016

Literature Cited

1. Taylor, B. J., 1992 Differentiation of a male-specific muscle in Drosophila-Melanogaster does not require the sex-determining genes doublesex or intersex. Genetics 132: 179–191.
2. Gong, W. J., and K. G. Golic, 2003 Ends-out, or replacement, genetargeting in Drosophila. Proc. Natl. Acad. Sci. USA 100: 2556–2561. https://doi.org/10.1073/pnas.0535280100
3. Gailey, D. A., B. J. Taylor, and J. C. Hall, 1991 Elements of the fruitless locus regulate development of the muscle of Lawrence, a male-specific structure in the abdomen of drosophilamelanogaster adults. Development 113: 879–890.
4. Manoli, D. S., M. Foss, A. Villella, B. J. Taylor, J. C. Hall et al., 2005 Male-specific fruitless specifies the neural substrates of Drosophila courtship behaviour. Nature 436: 395–400. https://doi.org/10.1038/nature03859
5. Manoli, D. S., G. W. Meissner, and B. S. Baker, 2006 Blueprints for behavior: genetic specification of neural circuitry for innate behaviors. Trends Neurosci. 29: 444–451. https://doi.org/10.1016/j.tins.2006.06.006
6. Ryner, L. C., S. F. Goodwin, D. H. Castrillon, A. Anand, A. Villella et al., 1996 Control of male sexual behavior and sexual orientation in Drosophila by the fruitless gene. Cell 87: 1079– 1089. https://doi.org/10.1016/S0092-8674(00)81802-4
7. Manoli, D. S., M. Foss, A. Villella, B. J. Taylor, J. C. Hall et al., 2005 Male-specific fruitless specifies the neural substrates of Drosophila courtship behaviour. Nature 436: 395–400. https://doi.org/10.1038/nature03859
8. Vaughan, A. G., C. Zhou, D. S. Manoli, and B. S. Baker, 2014 Neural pathways for the detection and discrimination of conspecific song in D. melanogaster. Curr. Biol. 24: 1039– 1049. https://doi.org/10.1016/j.cub.2014.03.048
9. Zhou, C., Y. Pan, C. C. Robinett, G. W. Meissner, and B. S. Baker, 2014 Central brain neurons expressing doublesex regulate female receptivity in Drosophila. Neuron 83: 149–163. https://doi.org/10.1016/j.neuron.2014.05.038
10. Zhou, C., R. Franconville, A. G. Vaughan, C. C. Robinett, V. Jayaraman et al., 2015 Central neural circuitry mediating courtship song perception in male Drosophila. Elife 4: e08477. https://doi.org/10.7554/eLife.08477
11. Mellert, D. J., J. M. Knapp, D. S. Manoli, G. W. Meissner, and B. S. Baker, 2010 Midline crossing by gustatory receptor neuron axons is regulated by fruitless, doublesex and the Roundabout receptors. Development 137: 323–332. https://doi.org/10.1242/dev.045047
12. Mellert, D. J., C. C. Robinett, and B. S. Baker, 2012 Doublesex functions early and late in gustatory sense organ development. PLoS One 7: e51489. https://doi.org/10.1371/journal.pone.0051489
13. Fan, P.,D. S. Manoli, O. M. Ahmed, Y. Chen, N. Agarwal et al., 2013 Genetic and neural mechanisms that inhibit Drosophila from mating with other species. Cell. 2013154: 89–102. https://doi.org/10.1016/j.cell.2013.06.008
14. Tran, D. H., G. W. Meissner, R. L. French, and B. S. Baker, 2014 A small subset of fruitless subesophageal neurons modulate early courtship in Drosophila. PLoS One. 2014 9: e95472. https://doi.org/ 10.1371/journal.pone.0095472
15. Manoli, D. S., and B. S. Baker,. Median bundle neurons coordinate behaviours during Drosophila male courtship. Nature. 2004. 430: 564–569. https://doi.org/10.1038/nature02713
16. Mellert, D. J., J. M. Knapp, D. S. Manoli, G. W. Meissner, and B. S. Baker, 2010 Midline crossing by gustatory receptor neuron axons is regulated by fruitless, doublesex and the Roundabout receptors. Development 137: 323–332. https://doi.org/10.1242/ dev.045047
17. Pan, Y., and B. S. Baker, 2014 Genetic identification and separation of innate and experience-dependent courtship behaviors in Drosophila. Cell 156: 236–248. https://doi.org/10.1016/j.cell.2013.11.041
18. Pan, Y., C. C. Robinett, and B. S. Baker, 2011 Turning males on: activation of male courtship behavior in Drosophila melanogaster. PLoS One 6: e21144. https://doi.org/10.1371/journal.pone.0021144
19. Pan, Y., G. W. Meissner, and B. S. Baker, 2012 Joint control of Drosophila male courtship behavior by motion cues and activation of male-specific P1 neurons. Proc. Natl. Acad. Sci. USA 109: 10065–10070. https://doi.org/10.1073/pnas.1207107109
20. Meissner, G. W., S. D. Luo, B. G. Dias, M. J. Texada, and B. S. Baker, 2016 Sex-specific regulation of Lgr3 in Drosophila neurons. Proc. Natl. Acad. Sci. USA 113: E1256–E1265. https://doi.org/10.1073/pnas.1600241113

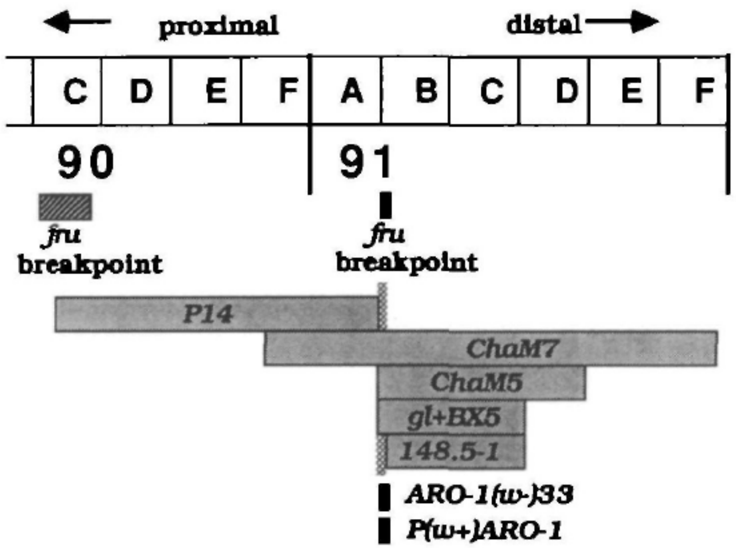
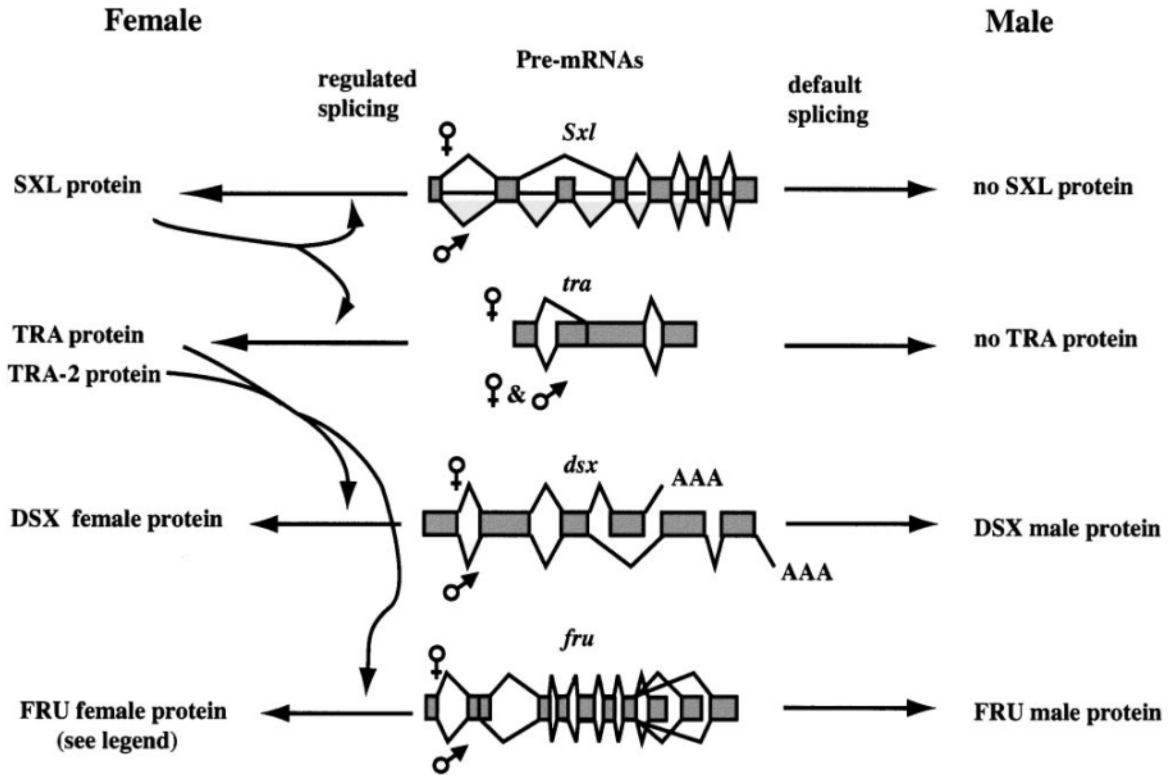


Fig. 1. Approximate breakpoint cytology of chromosomal



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