The use of genomic and gene expression large-scale data for the analyses of sexual evolution

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colmea

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Kyoto Institute of Technology

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INSPER – São Paulo

Outline

1. Background in Sexual Evolution

2. Biological Problem

3. Large-scale Data

4. Statistical Approaches

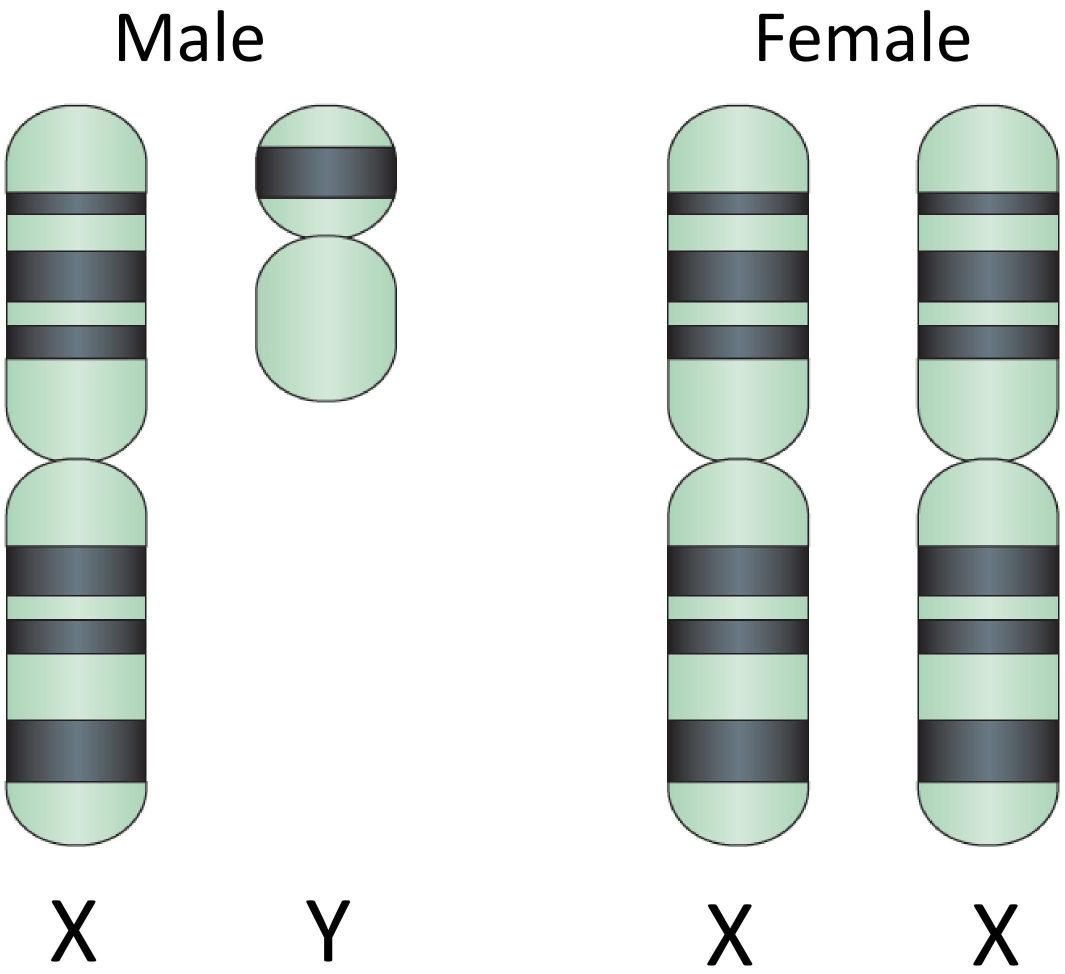
5. Ongoing Biological Problems

6. Perspectives

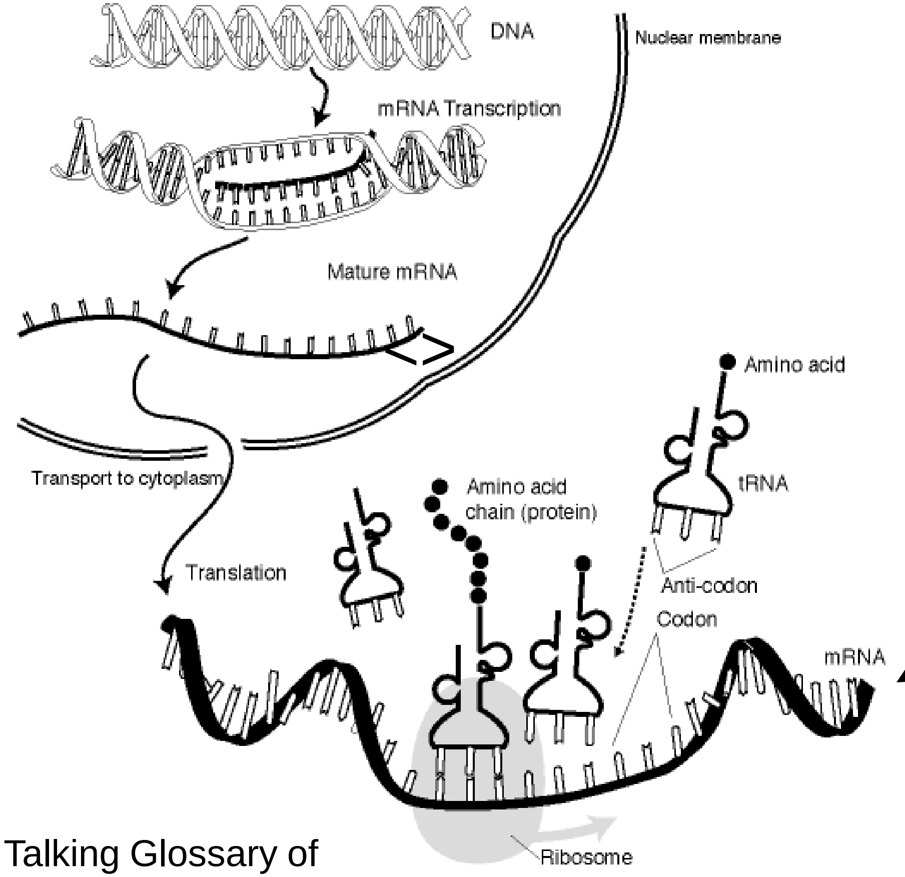
1. Background in Sexual Evolution

Why are there phenotypic differences between sexes?

Sex Chromosomes



1. Background in Sexual Evolution

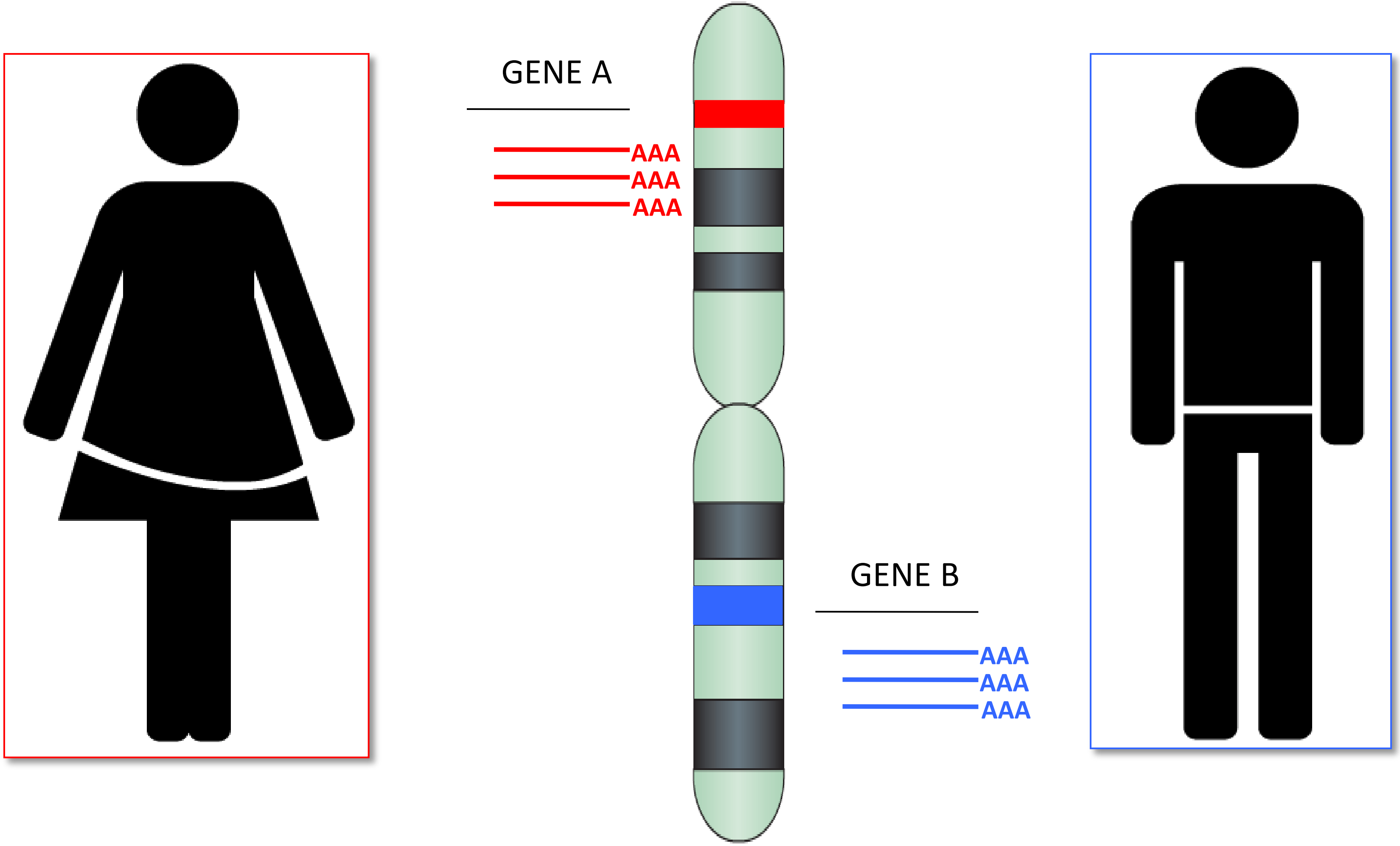


Measure the quantity of messenger RNA (mRNA) in the cell

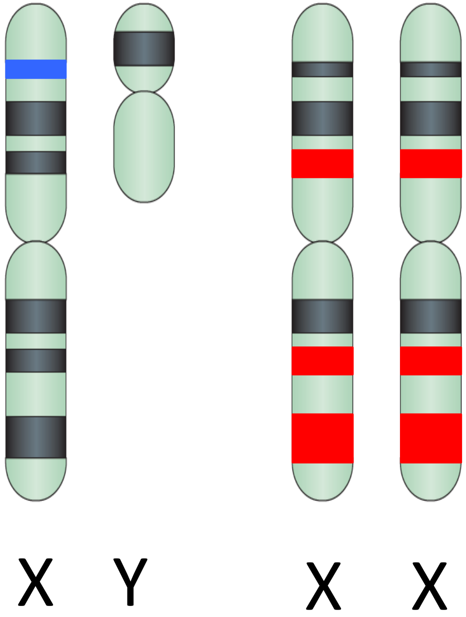
From Talking Glossary of Genetics

Gene activity = gene expression = number of mRNA molecules

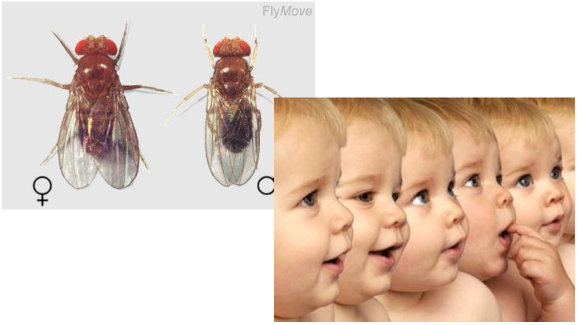
Sex-biased Gene Expression



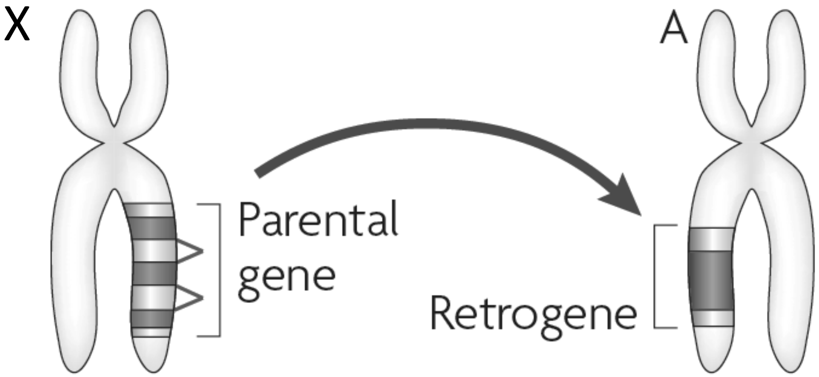
2. Biological Problem



What molecular mechanisms and genetics processes are involved?



XY systems

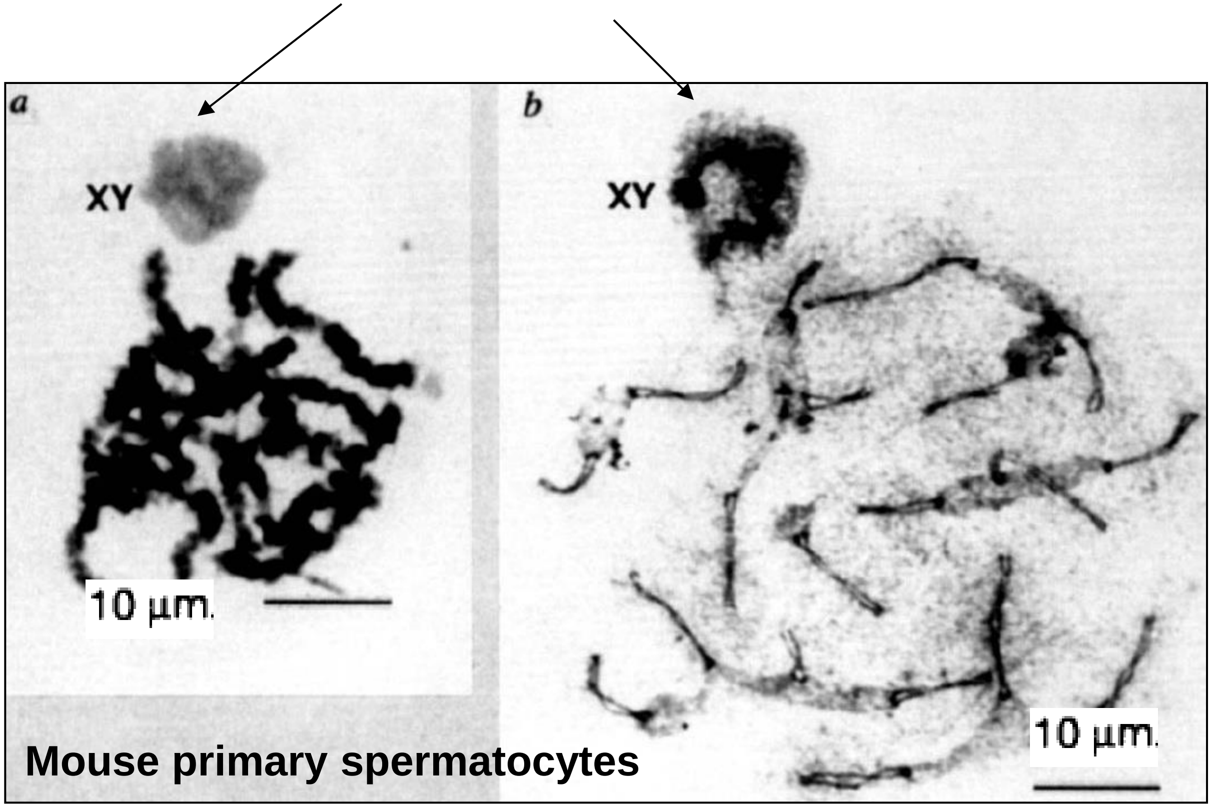


Testis bias

2. Biological Problem

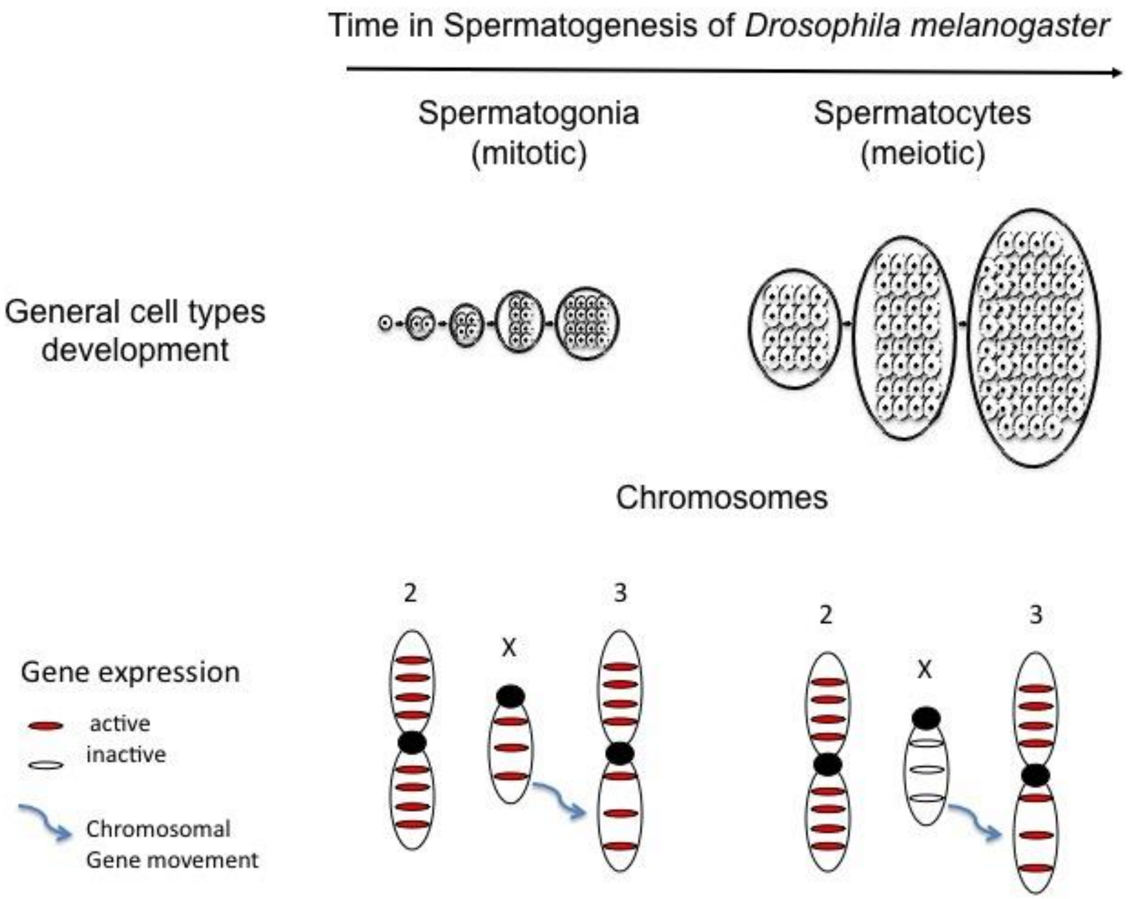
Meiotic Sex Chromosome Inactivation (MSCI)

Xist (X inactive-specific transcripts)



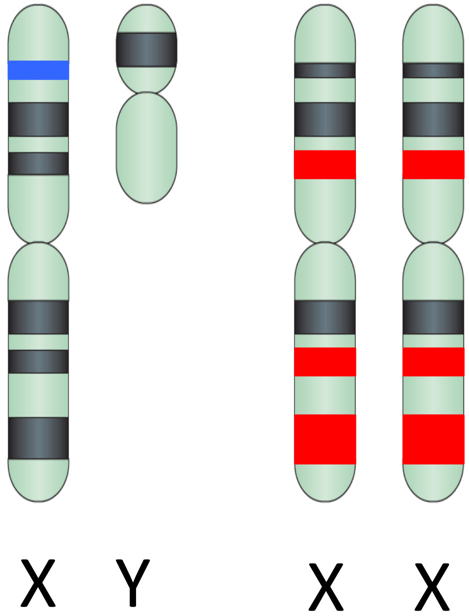
Richler C, Soreq H, Wahrman J, 1992. Nat Gene;
Ayoub N, Richler C, Wahrman J, 1997, Chromosoma

2. Biological Problem

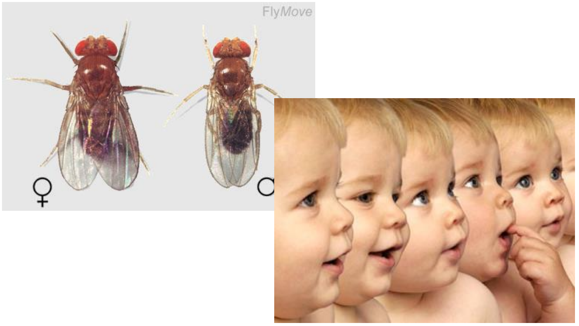

Time in Spermatogenesis of *Drosophila melanogaster*
Spermatogonia (mitotic)
Spermatocytes (meiotic)
General cell types development
Chromosomes
Gene expression
active
inactive
Chromosomal Gene movement
2
X
3
2
X
3

Lifschytz and Lindsley, 1972

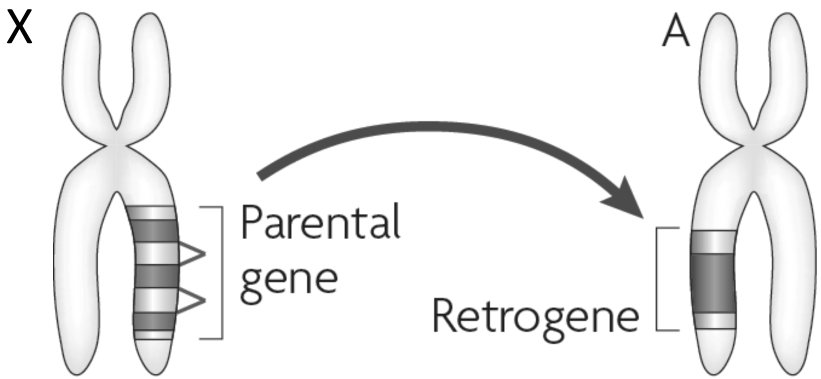
2. Biological Problem



- Does MSCI exist in *Drosophila*?
- Is MSCI involved in the distribution of sex-biased genes in the genome?



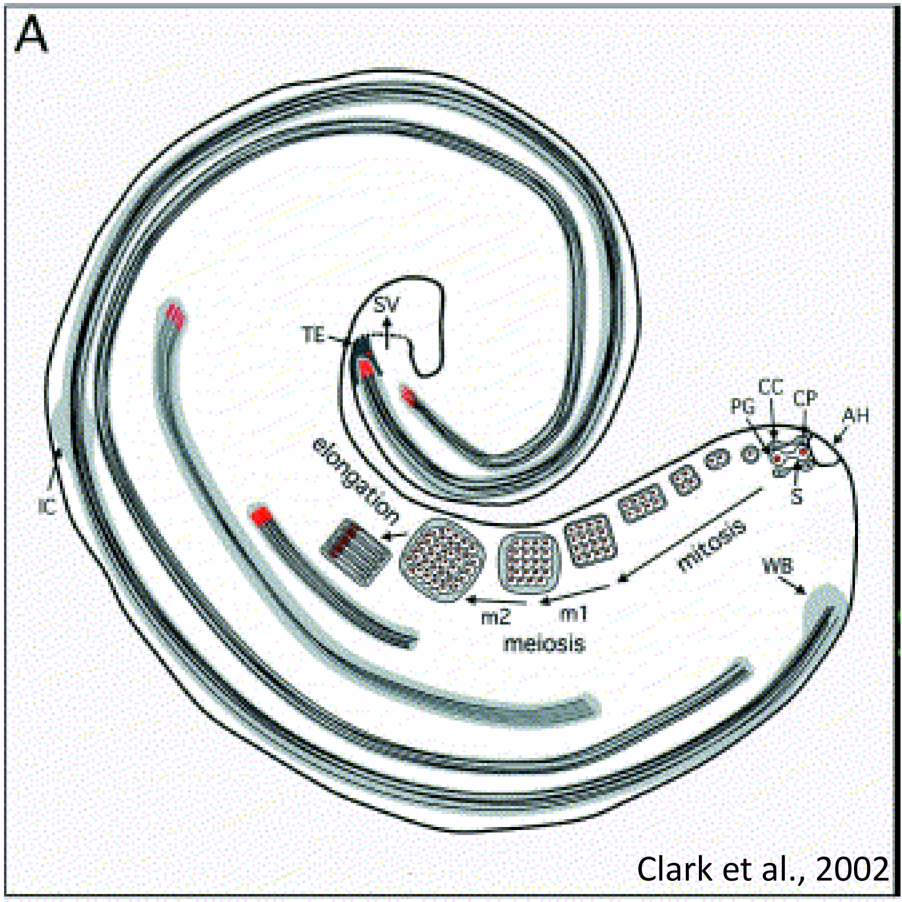
XY systems



Testis bias

3. Large-scale Data

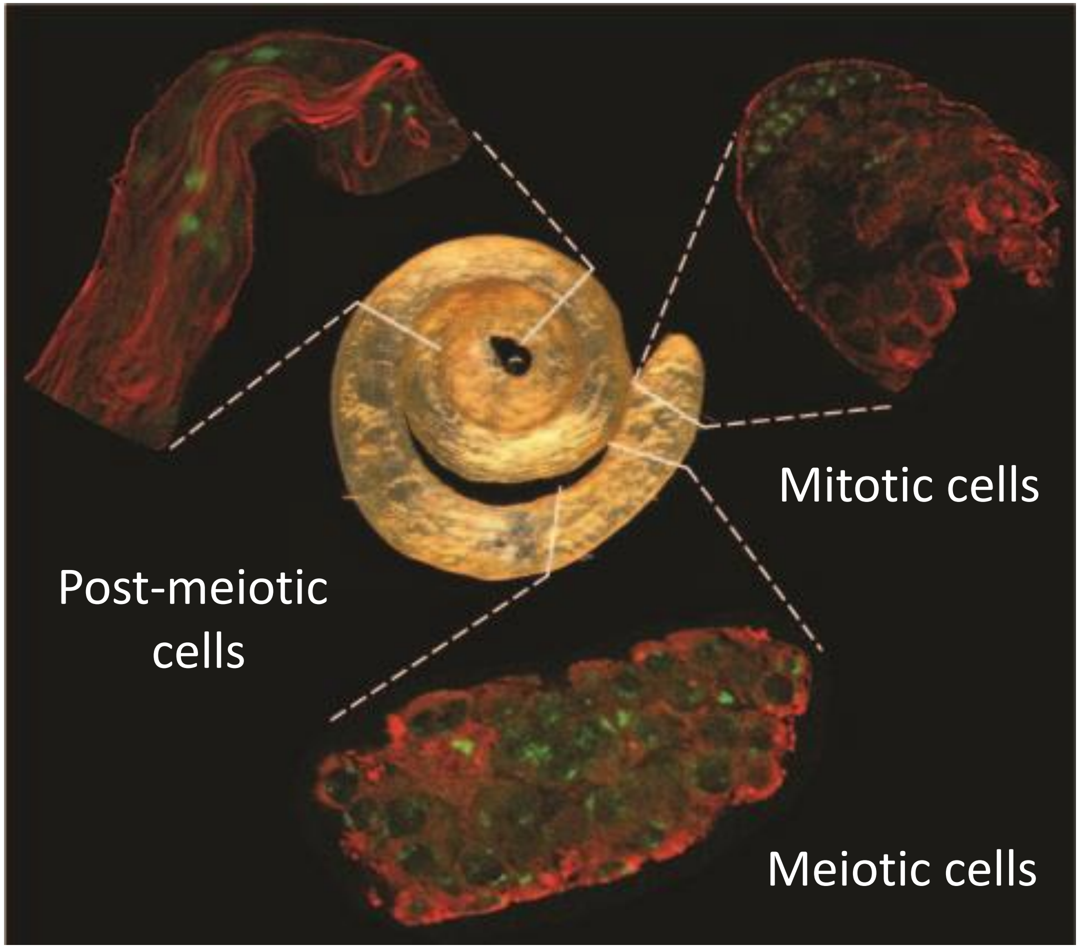
Drosophila melanogaster Spermatogenesis



Clark et al., 2002

3. Large-scale Data

Isolation of Spermatogenic Cells

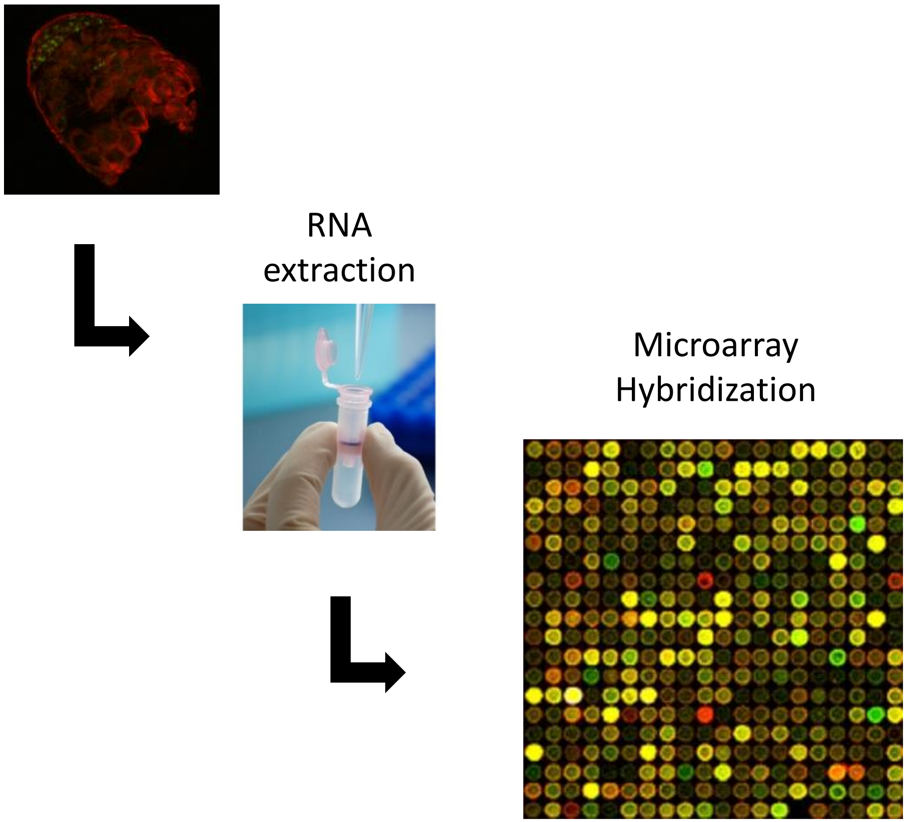


DNA

Cytoplasm

Vibranovski et al, 2009, PLoS Genetics

3. Large-scale Data



Tissue Isolation (n=3)

RNA extraction

Microarray Hybridization

Vibranovski et al, 2009, PLoS Genetics

3. Large-scale Data

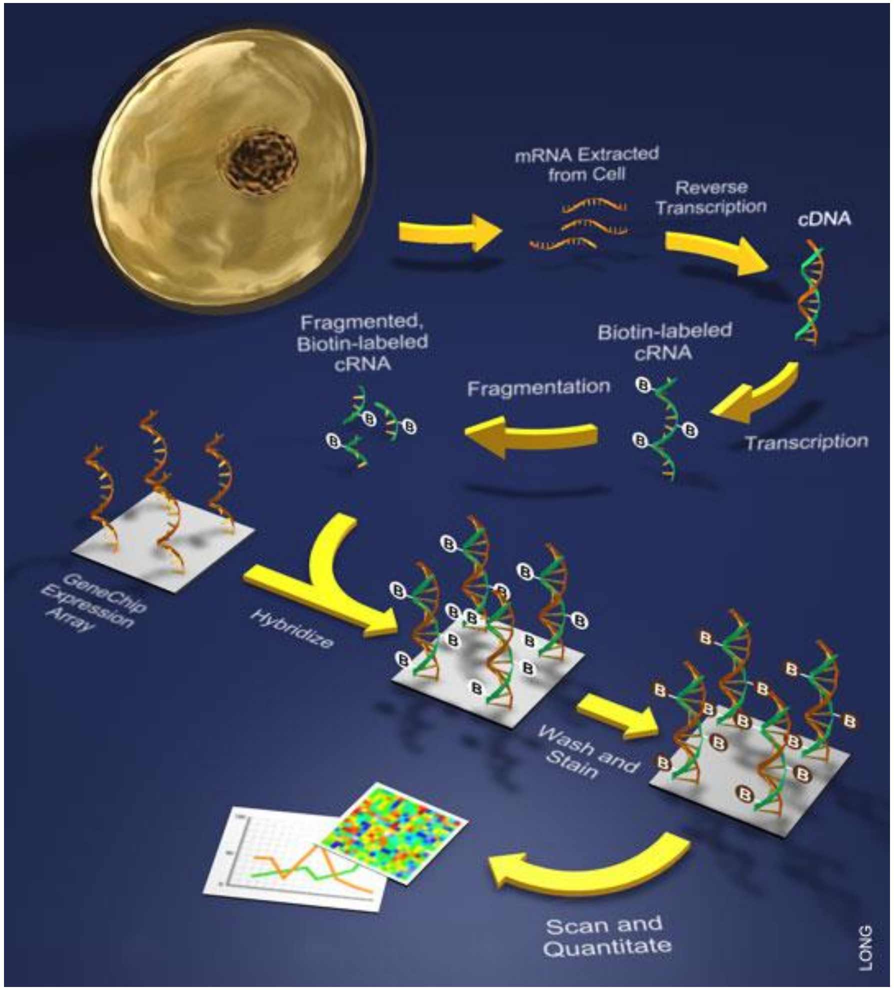
How to count the number of mRNA molecules for each gene in a cell?

Microarray: A chip containing small pieces of DNA corresponding to all *Drosophila* genes

→

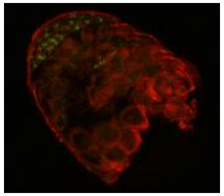
Microarray

Specific case for gene expression measure: the GeneChip array



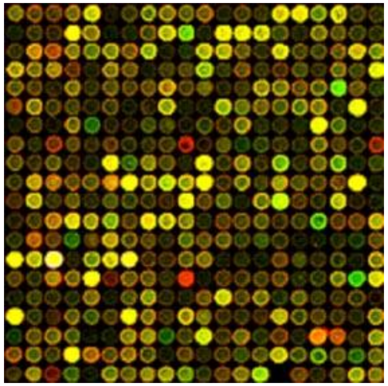
3. Large-scale Data

Tissue Isolation (n=3)

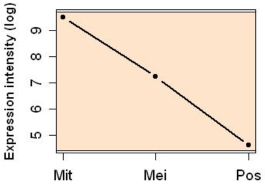


RNA extraction

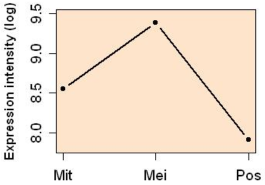
Microarray Hybridization



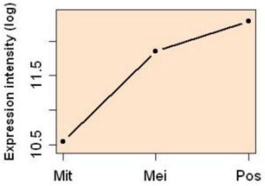
Gene profile



Bam



ms(3)K81

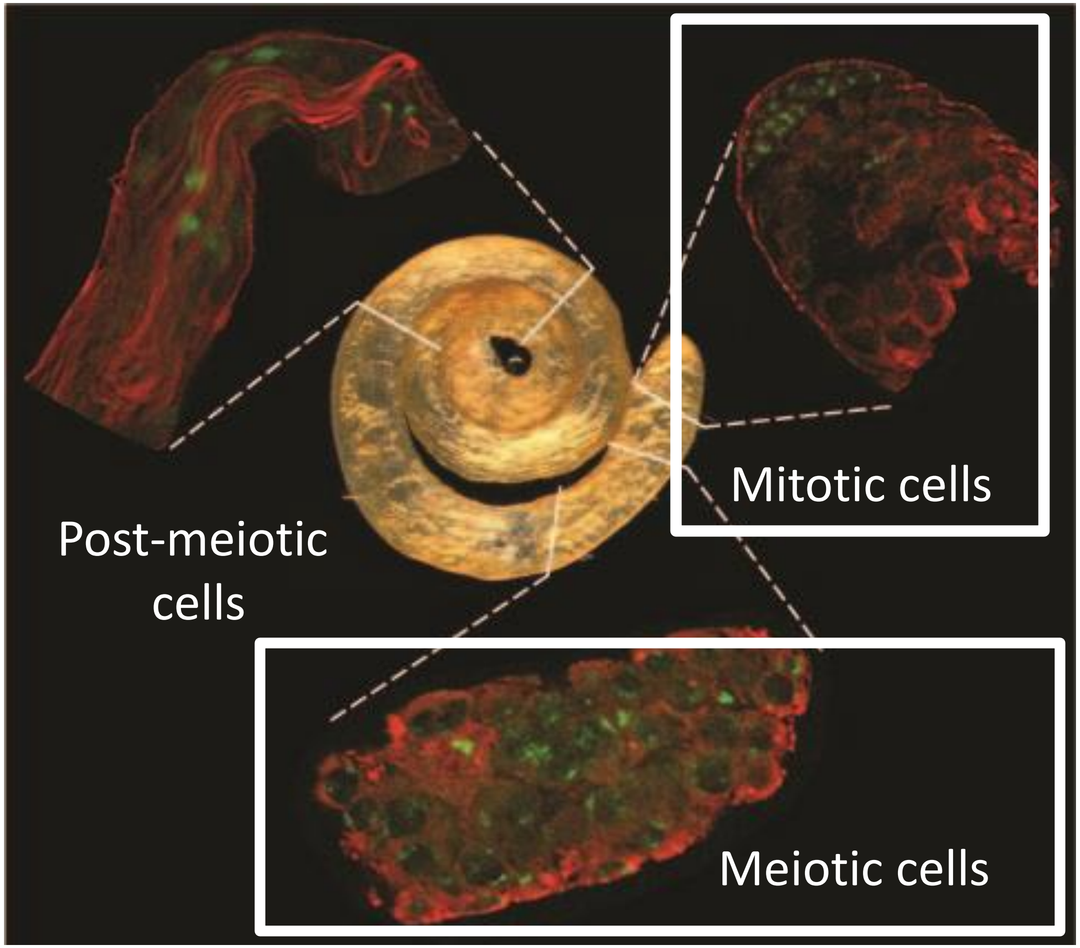


Mst35Bb

Vibranovski et al, 2009, PLoS Genetics

4. Statistical Approaches

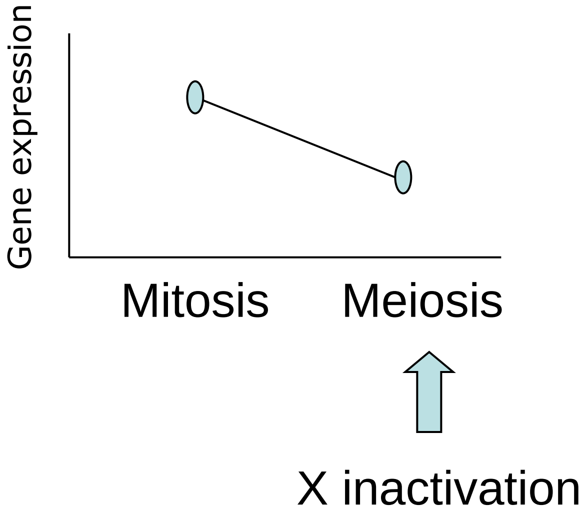
Meiotic Sex Chromosome Inactivation (MSCI)



Vibranovski et al, 2009, PLoS Genetics

4. Statistical Approaches

X inactivation occurs when the difference in activity (meiosis-mitosis) in X is lower than the difference in activity in autosome.



θ = *gene expression /activity*

$$\theta^{X}_{meiosis} - \theta^{X}_{mitosis} < \theta^{A}_{meiosis} - \theta^{A}_{mitosis}$$

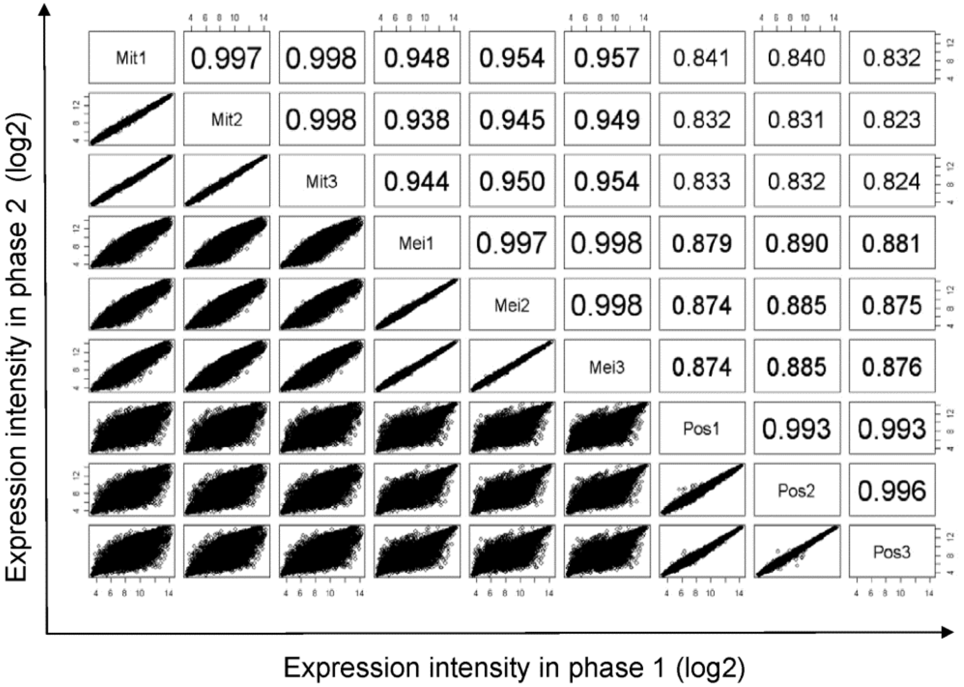
4. Statistical Approaches

Gene intensity

2982 genes (X chromosomes)
15099 genes (autosomes)

Spermatogenic replicates

3 mitotic
3 meiotic
3 post-meiotic



Mit1	0.997	0.998	0.948	0.954	0.957	0.841	0.840	0.832
	Mit2	0.998	0.938	0.945	0.949	0.832	0.831	0.823
		Mit3	0.944	0.950	0.954	0.833	0.832	0.824
			Mei1	0.997	0.998	0.879	0.890	0.881
				Mei2	0.998	0.874	0.885	0.875
					Mei3	0.874	0.885	0.876
						Pos1	0.993	0.993
							Pos2	0.996
								Pos3

Gene	Mit1	Mit2	Mit3	Mei1	Mei2	Mei3
292	10.182	10.199	10.395	9.798	9.880	9.862
792	5.273	5.357	5.509	5.548	5.577	5.587
1966	5.924	5.914	5.945	6.297	6.779	6.643
2811	5.177	5.197	5.168	5.092	5.166	5.169

Gene	Mit1	Mit2	Mit3	Mei1	Mei2	Mei3
2869	9.884	9.872	9.758	11.468	11.174	11.213
3939	4.833	4.755	4.775	4.827	4.839	4.804
10541	4.932	4.932	4.969	5.073	4.876	4.986
12928	10.331	10.485	10.524	11.480	11.628	11.514

4. Statistical Approaches

For chromosome i (X or A) and gene l, 3 replicates are measured

Mitosis: $mit_{i1l}, mit_{i2l}, mit_{i3l}$ $\qquad$ $\mathrm{E}(mit_{ikl}) = \theta_{il}^{mit}$

Meiosis: $mei_{i1l}, mei_{i2l}, mei_{i3l}$ $\qquad$ $\mathrm{E}(mei_{ikl}) = \theta_{il}^{mei}$

The objective is to learn whether genes are

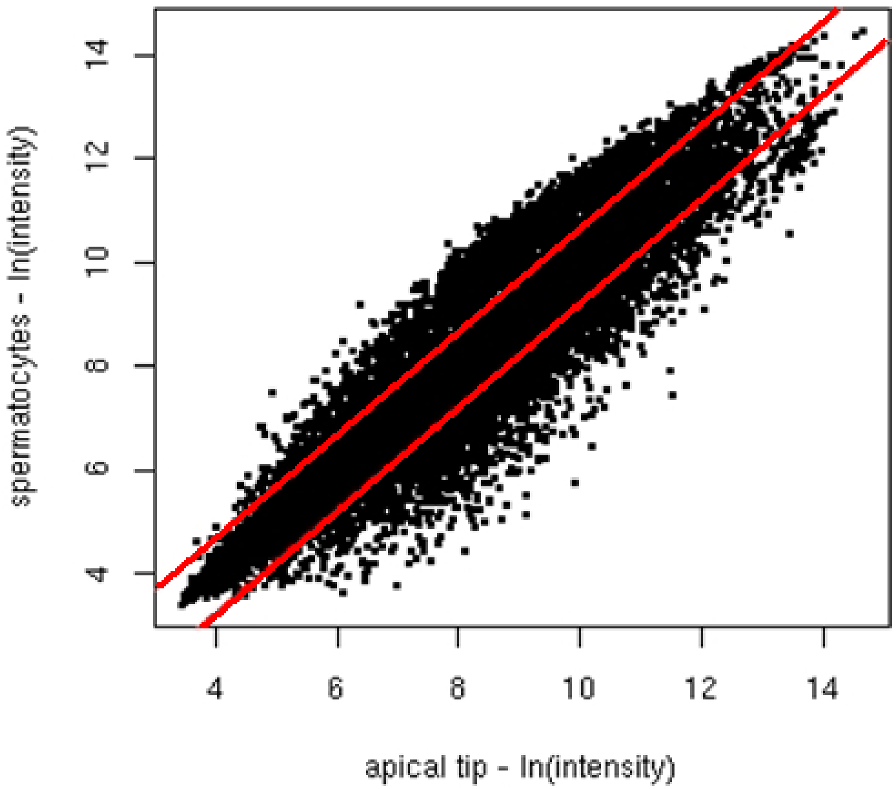
Differently expressed: $\begin{cases} H_{1,il}: \theta_{il}^{mei} > \theta_{il}^{mit} & \text{(OVER)} \\ H_{2,il}: \theta_{il}^{mei} < \theta_{il}^{mit} & \text{(UNDER)} \end{cases}$

or

Equally expressed: $H_{3,il}: \theta_{il}^{mei} = \theta_{il}^{mit}$ (EQUAL).

4. Statistical Approaches

How do biologists usually approach this problem?



APPROACH 1:
Identify differently expressed genes based on 2 fold intensity differences (based on homotypic experiments);

APPROACH 2:
Identify differently expressed genes by controlling type I error;

APPROACH 3:
Identify differently expressed genes by controlling false discovery rates;

APPROACH 4:
Hierarchical Bayes.

Storey and Tibshirani (2003) Statistical significance for genomewide studies. PNAS, 100(16), 9440-9445.

4. Statistical Approaches

How do biologists usually approach this problem?

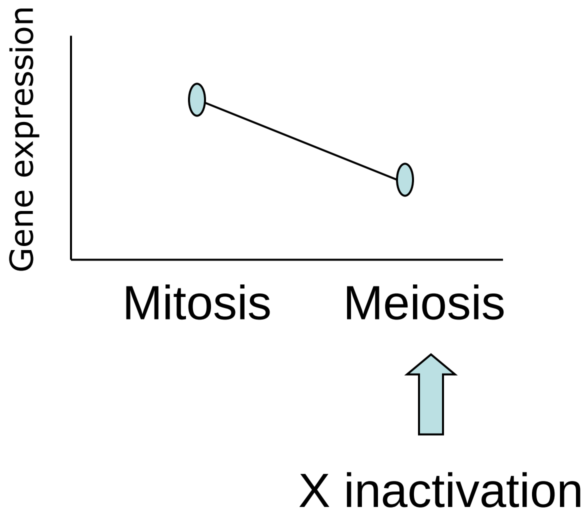
APPROACH 1, 2 or 3
Identify differently expressed genes

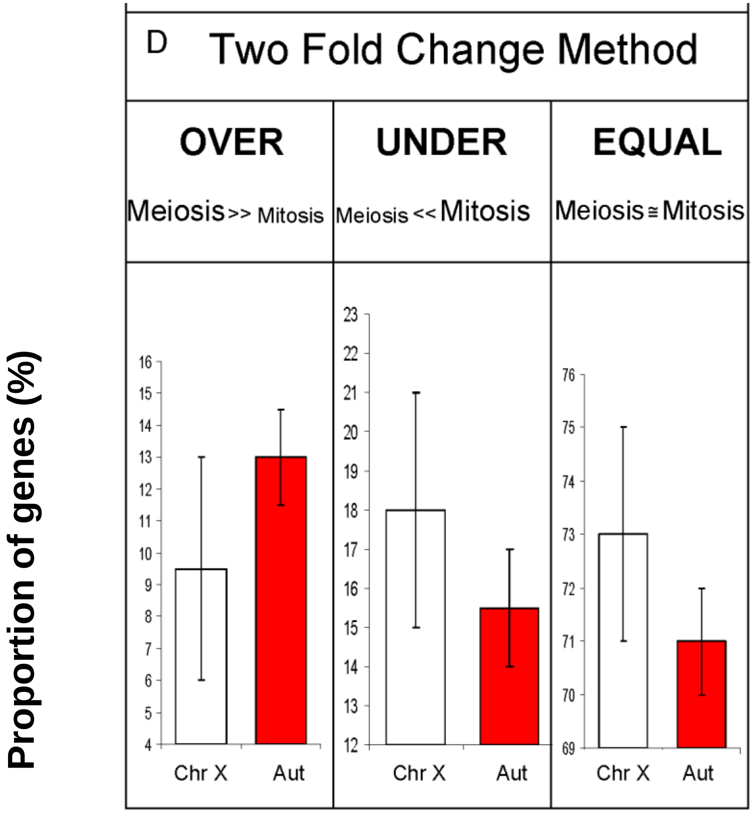
↓

Counts

Expression	ChrX	ChrA
Under expressed	537(18%)	2416(16%)
Equally expressed	2147(72%)	10720(71%)
Over expressed	298(10%)	1963(13%)
Total	2982	15099

4. Statistical Approaches





4. Statistical Approaches

Approach 2: *False positive rate (FPR)*

	(E) Evidence against H	(NE)No evidence against H	Total
(A) Over/under expressed	τ	$\eta_1-\tau$	η_1
(H) Equally expressed	η	$\eta_0-\eta$	η_0
Total	x	n-x	n

Measures error rate (evidence against H) when H is true: $E(\eta/\eta_0)$

FPR=0.05 => $E(\eta)<0.05E(\eta_0)$

<u>Bonferroni correction</u>

When evidence is measure by {p-value<0.05/n}, then

$$\Pr(\eta>0)<0.05$$

Controlling $E(\eta)<0.05n$ is too liberal!

Controlling $\Pr(\eta>0)<0.05$ is too conservative!

4. Statistical Approaches

Approach 3: *False discovery rate (FDR)*

	(E) Evidence against H	(NE)No evidence against H	Total
(A) Over/under expressed	τ	$\eta_1-\tau$	η_1
(H) Equally expressed	η	$\eta_0-\eta$	η_0
Total	x	n-x	n

$$FDR(\alpha) = E\left(\frac{\eta(\alpha)}{x(\alpha)}\right) \approx \frac{E(\eta(\alpha))}{E(x(\alpha))} = \frac{\eta_0 \alpha}{E(x(\alpha))} \approx \frac{n\hat{\pi}_0 \alpha}{\sum_{i=1}^{n} 1(p_i < \alpha)}$$

$$\hat{\pi}_0 = \lim_{\lambda \to 1} \frac{\sum_{i=1}^{n} 1(p_i > \lambda)}{n(1-\lambda)}$$ (estimate of true nulls)

$$FDR(\alpha) \approx \frac{\pi_0 \Pr(E \mid H)}{\Pr(E)} = \Pr(H \mid E)$$ (Looks Bayesian!!)

Approach 4: mixture model for differences

Because σ_X^2 and σ_A^2 (within gene variability) in the hierarchical model are negligible when compared τ^2 (between genes variability), the next model we implemented is a mixture of normals model that accounts for the excess of intensities around zero:

Model:

$$d_l = (\overline{x}_l^{mei} - \overline{x}_l^{mit}) \sim \pi N(\theta_1, \tau_1^2) + (1-\pi) N(\theta_2, \tau_2^2) \qquad l = 1, \ldots, n$$

Prior:

$$p(\pi, \theta_1, \theta_2, \tau_1^2, \tau_2^2) \propto \tau_1^{-2} \tau_2^{-2}$$

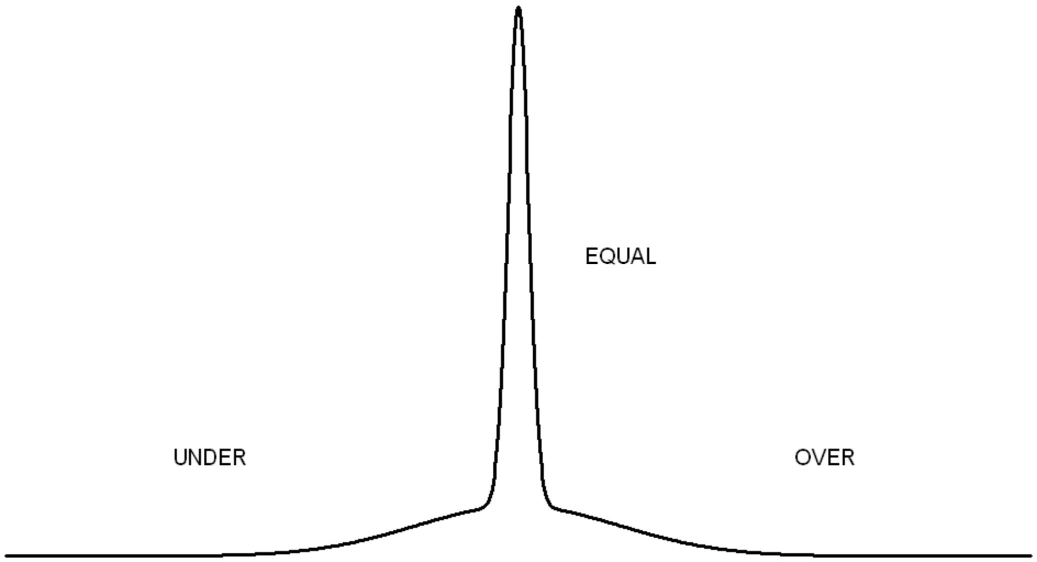
Approach 4: mixture model for differences

- Bayesian Mixture Model classifies genes as over or under expressed

  - Detect differential gene expression while comparing chromosomes distributions

  - Avoids the arbitrariness of folding

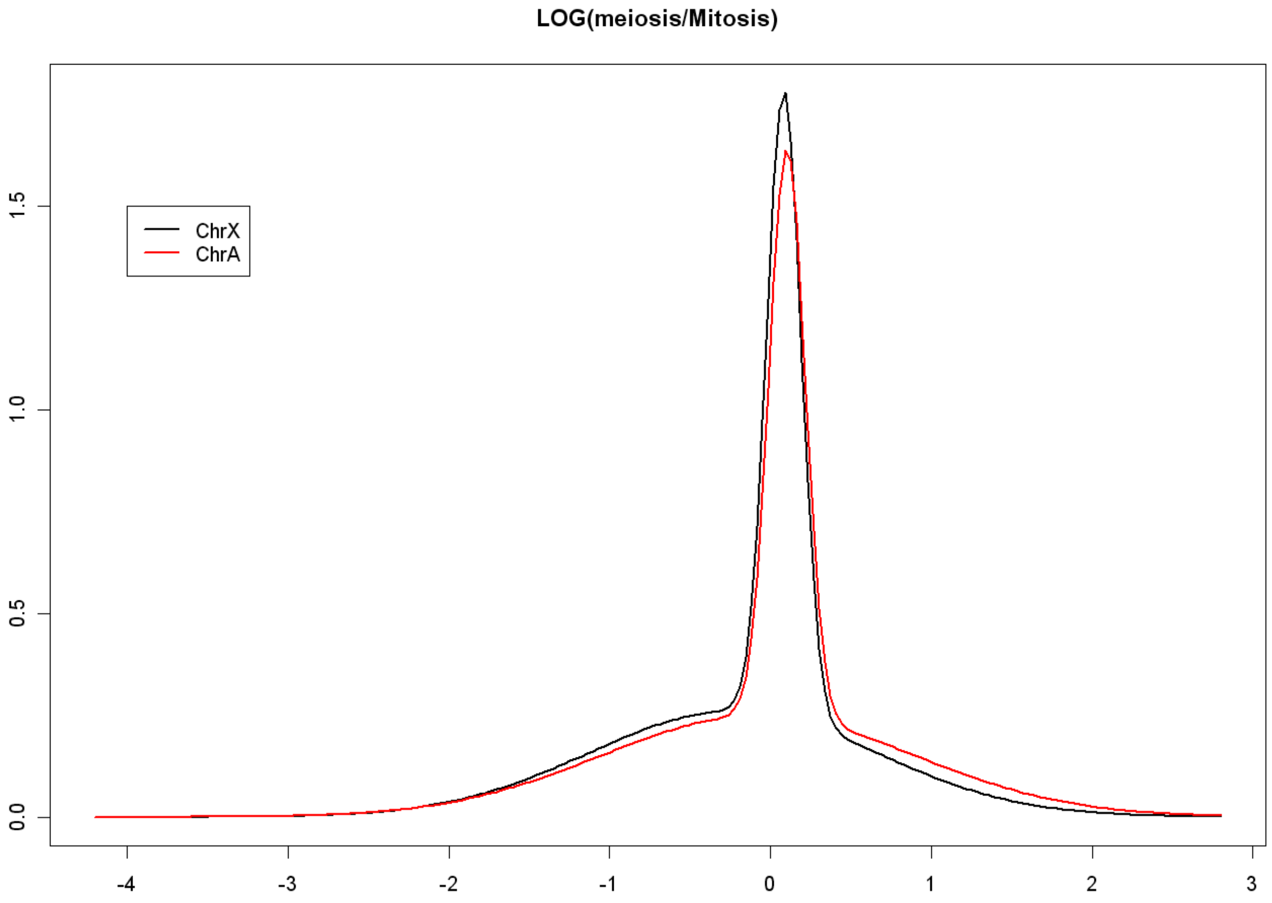
  - Avoids multiple testing distortions



Lopes, Mueller and Rosner (2003)

Approach 4: mixture model for differences

$$p(d \mid data) = \int \Big(\pi f_N(d;\theta_1,\tau_1^2) + (1-\pi) f_N(d;\theta_2,\tau_2^2) \Big) p(\pi,\theta_1,\theta_2,\tau_1^2,\tau_2^2 \mid data)\, d\pi\, d\theta_1\, d\theta_2\, d\tau_1^2\, d\tau_2^2$$



Approach 4: mixture model for differences

Classification (Prior): $Z_l \sim Ber(\pi)$

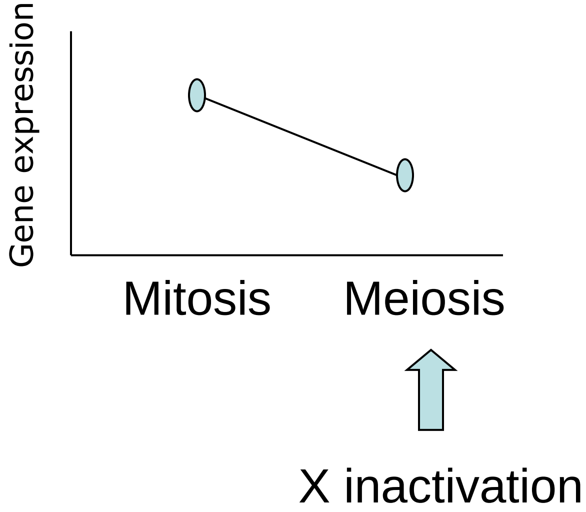
Classification (Posterior):

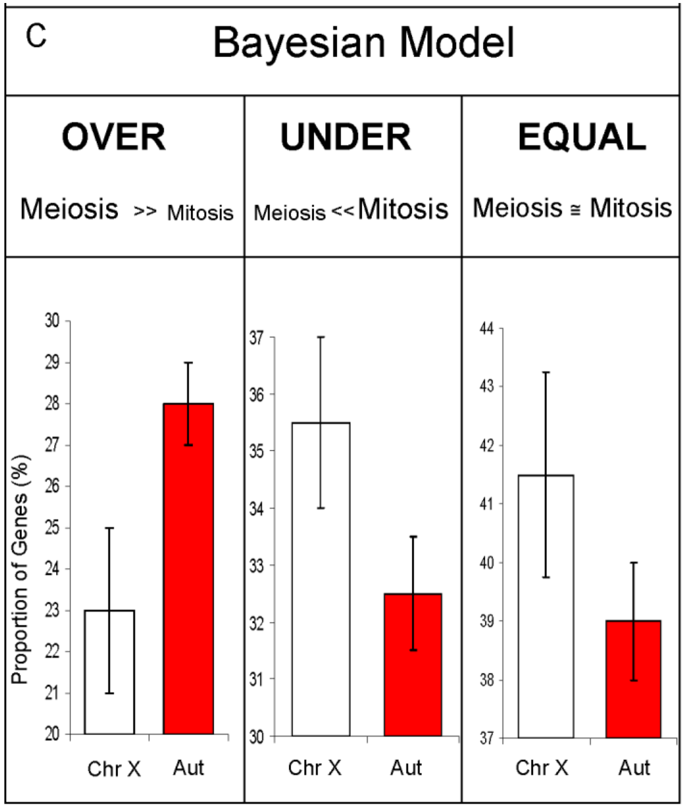
$(Z_l = 1)\,\&\,(d_l > 0)$ – over expressed

$(Z_l = 1)\,\&\,(d_l < 0)$ – under expressed

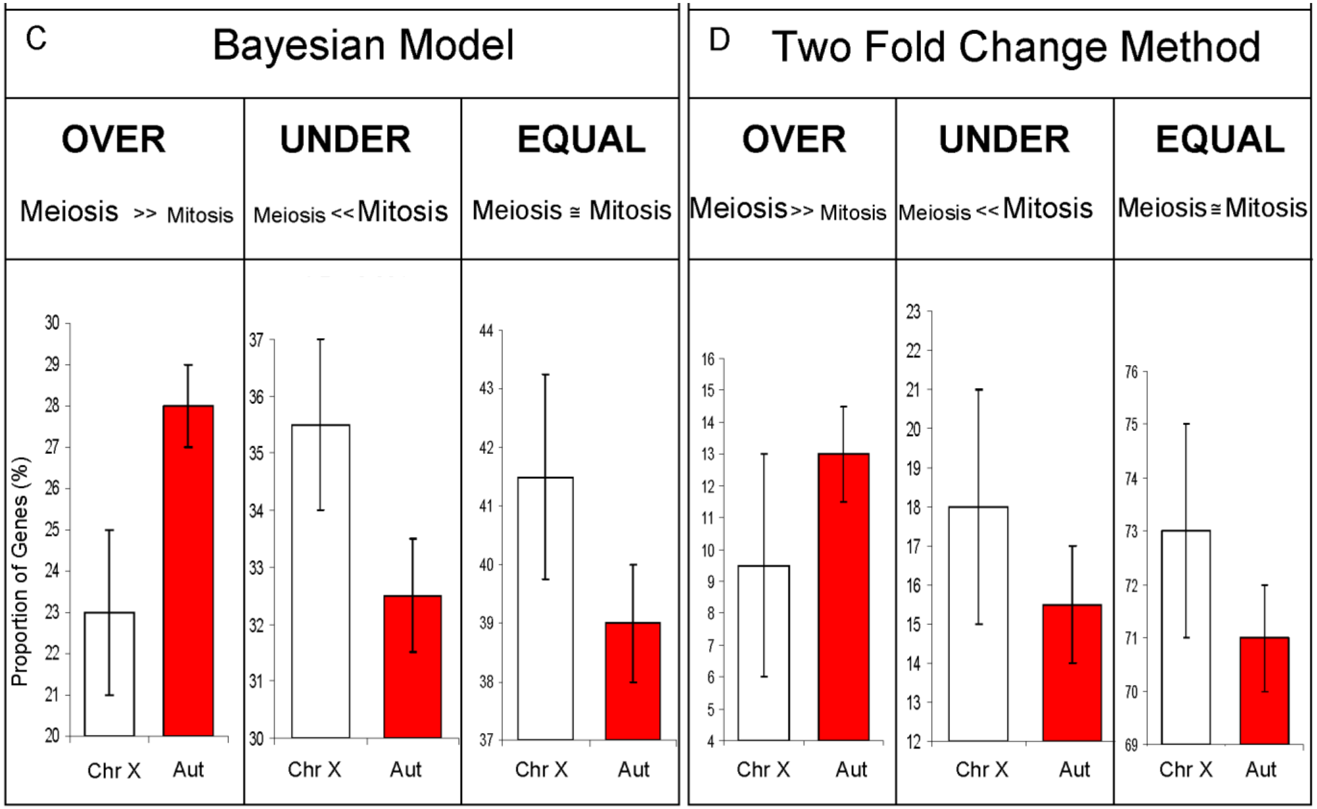
$(Z_l = 0)$ – equally expressed

Approach 4: Bayesian mixture model for differences



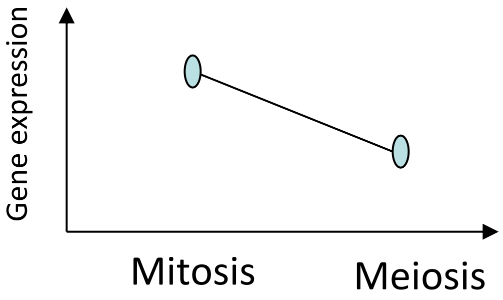


4. Statistical Approaches

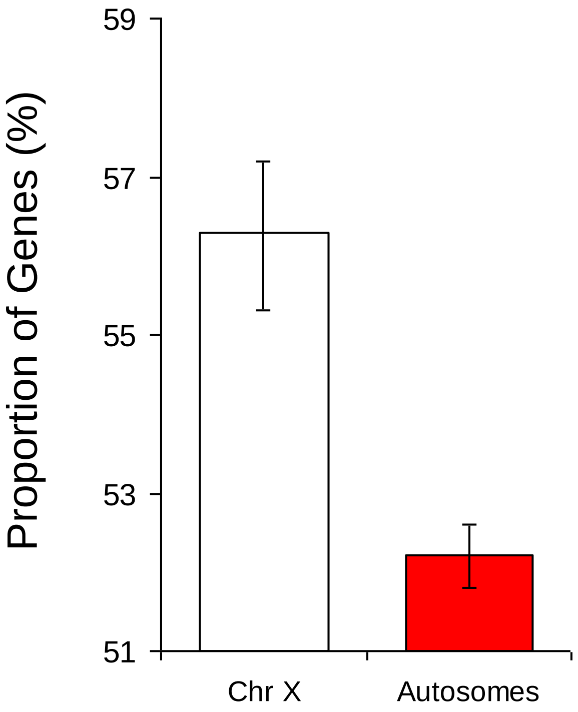


4. Statistical Approaches

Meiotic Sex Chromosome Inactivation (MSCI)



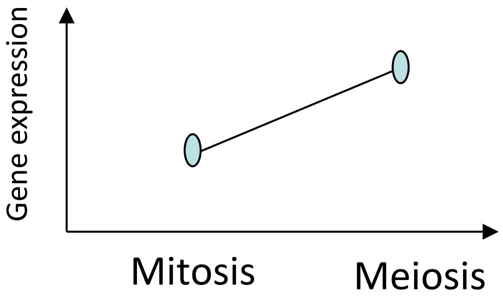
Inactivation

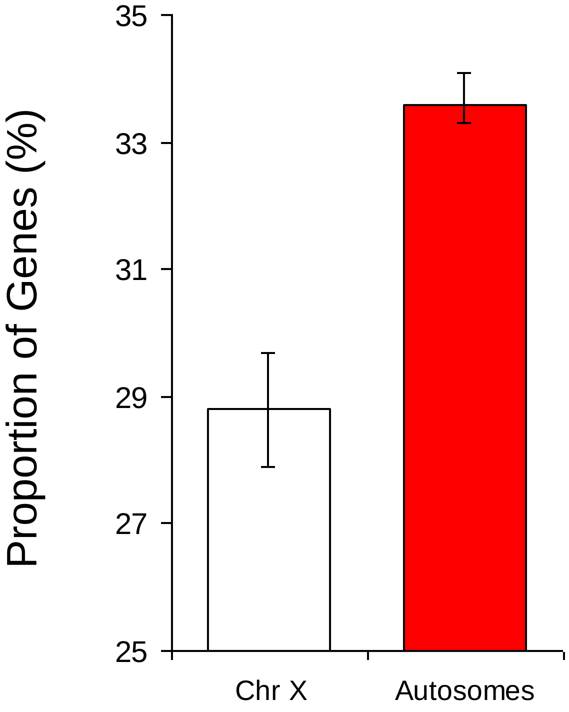


Vibranovski et al, 2009, PLoS Genetics

4. Statistical Approaches

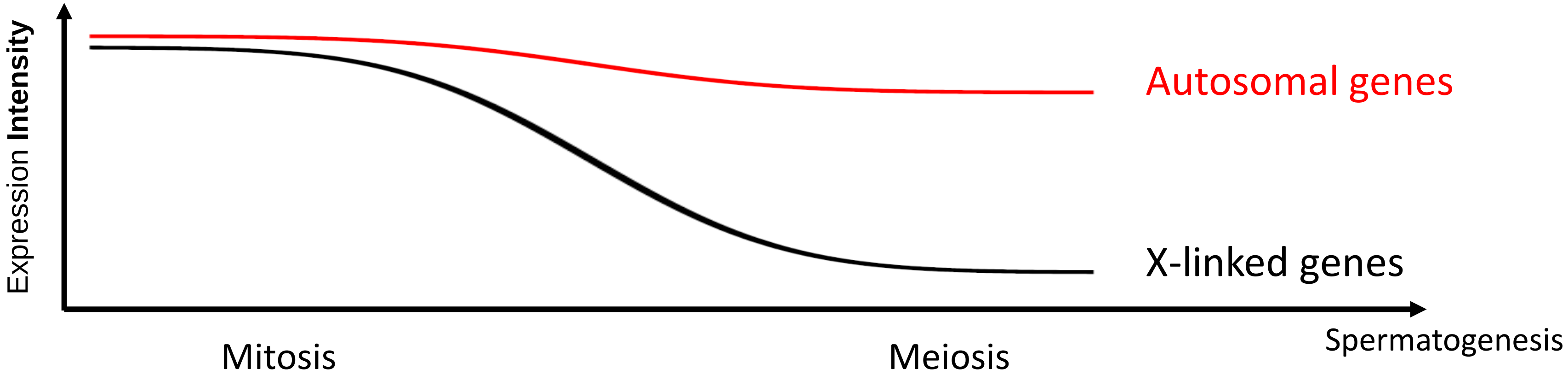
Meiotic Sex Chromosome Inactivation (MSCI)





Vibranovski et al, 2009, PLoS Genetics

2. Biological Problem



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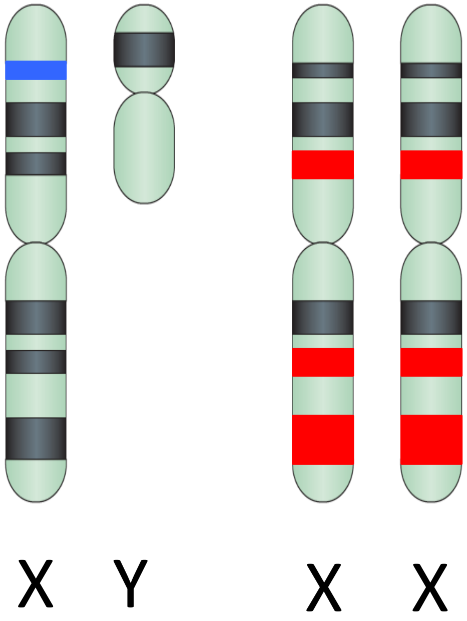
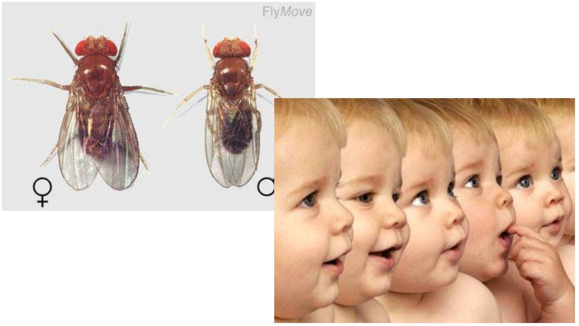
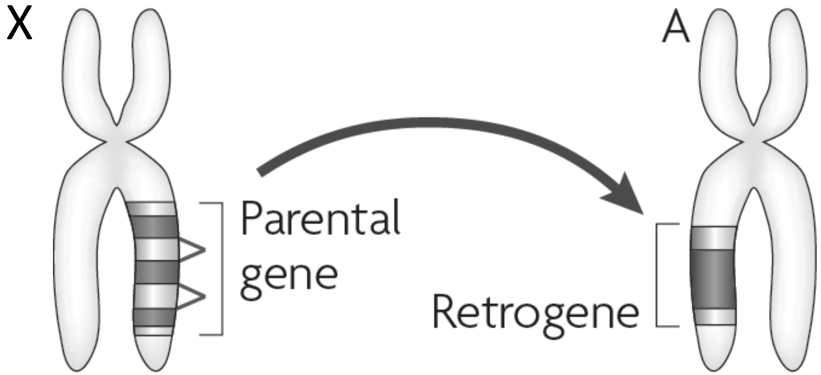
2. Biological Problem



- Does MSCI exist in *Drosophila*? YES
- Is MSCI involved in the distribution of sex-biased genes in the genome?

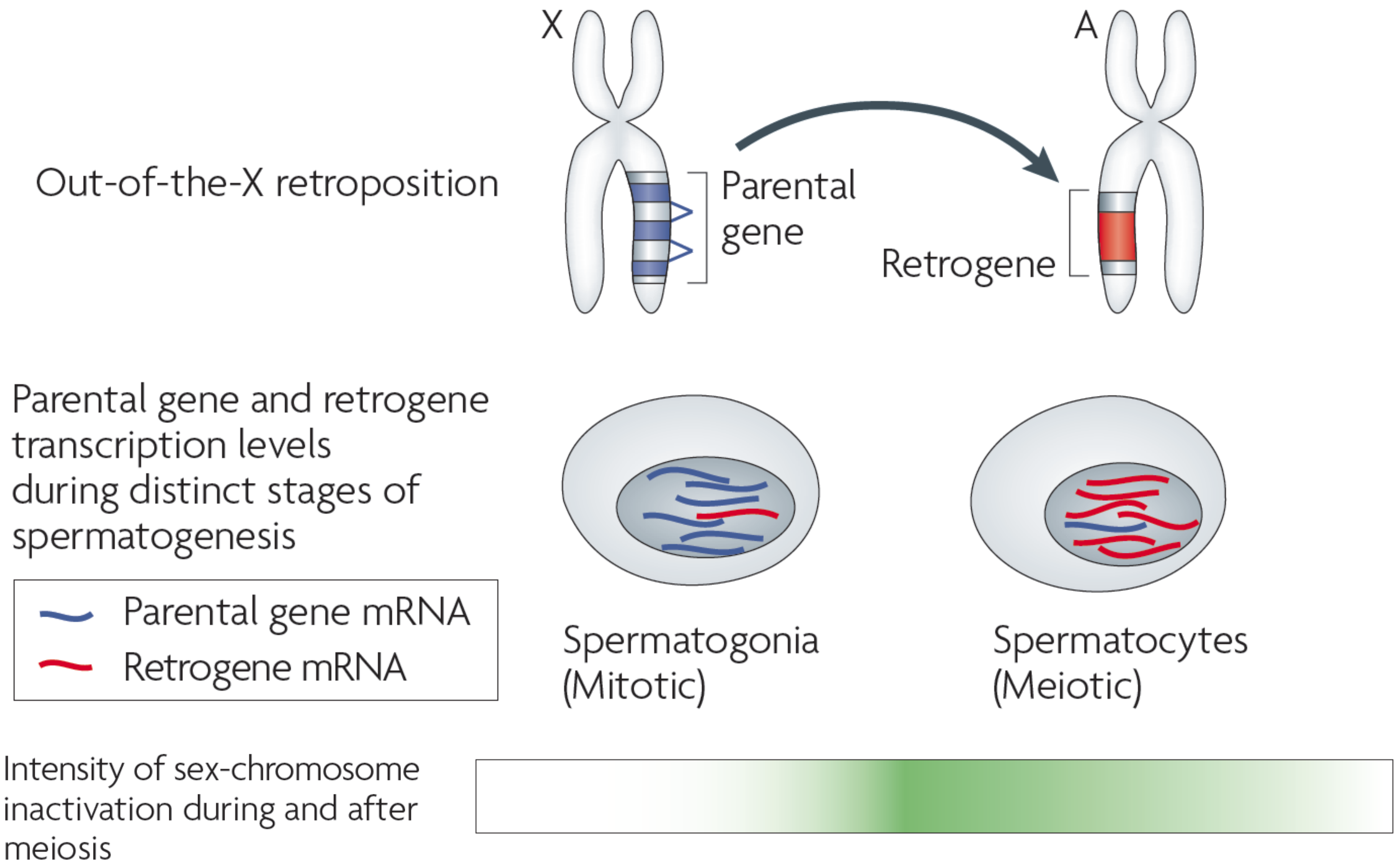


XY systems



Testis bias

2. Biological Problem


Out-of-the-X retroposition
X
Parental gene
A
Retrogene
Parental gene and retrogene transcription levels during distinct stages of spermatogenesis
Parental gene mRNA
Retrogene mRNA
Spermatogonia (Mitotic)
Spermatocytes (Meiotic)
Intensity of sex-chromosome inactivation during and after meiosis

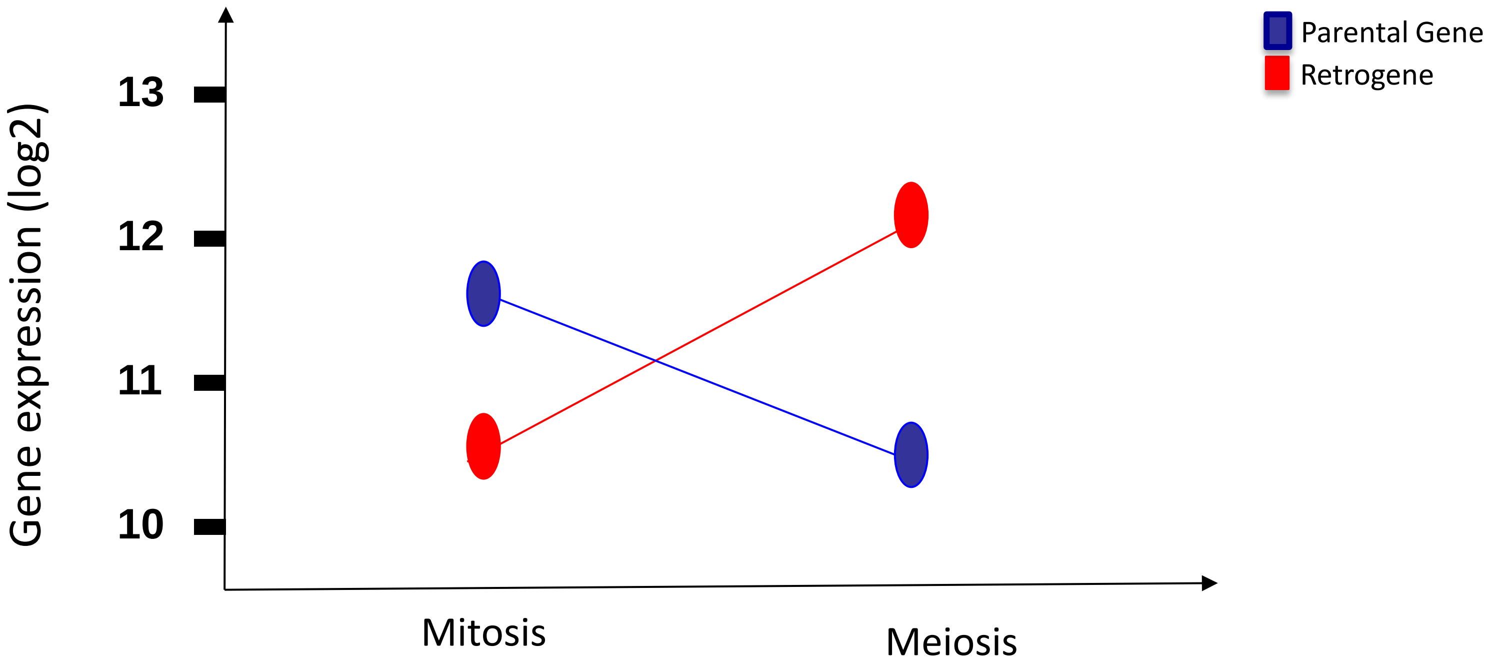
Kaessmann et al, 2009, Nat Rev Genetics

2. Biological Problem

Complementary expression

Tom40: Translocase of outer membrane 40 / Chr X

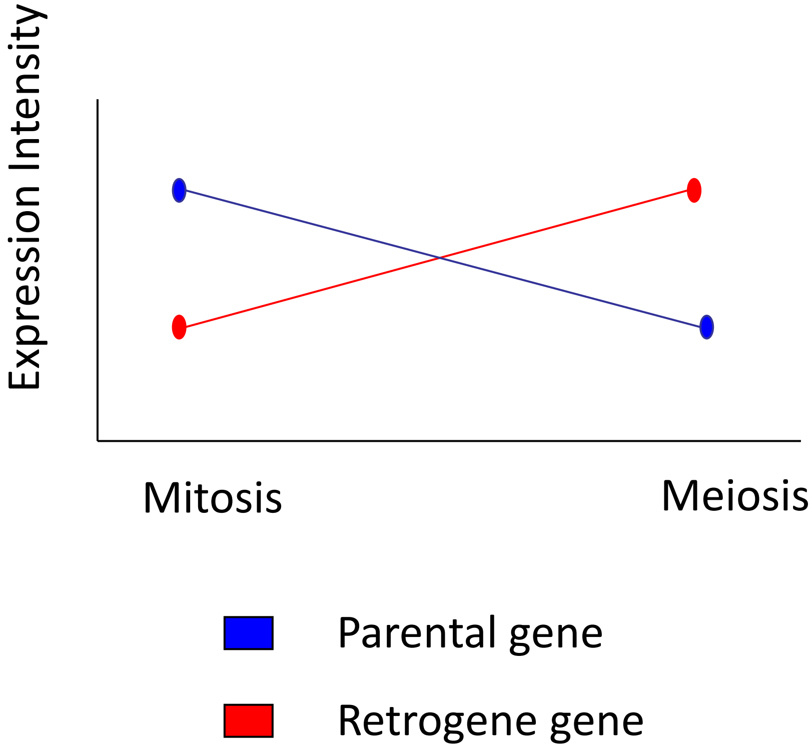
Tomboy40: protein transmembrane transporter activity / Chr 2R



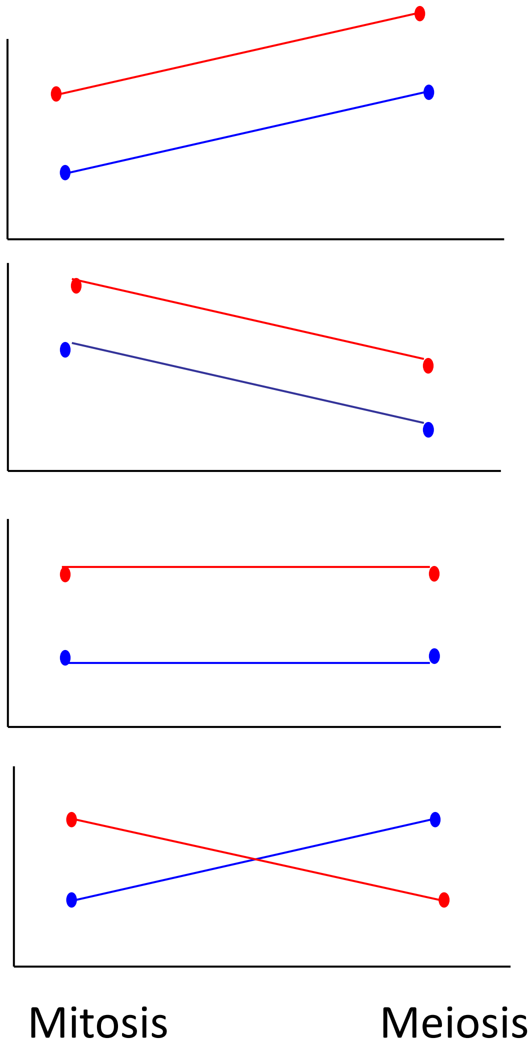
Vibranovski et al, 2009, PLoS Genetics

2. Biological Problem

Complementary expression

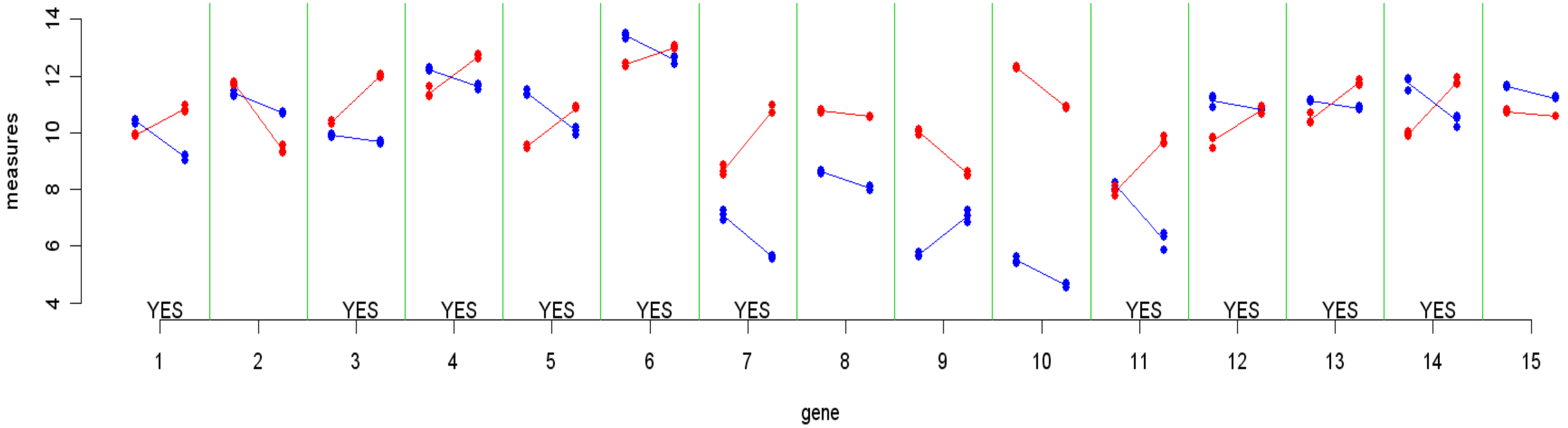


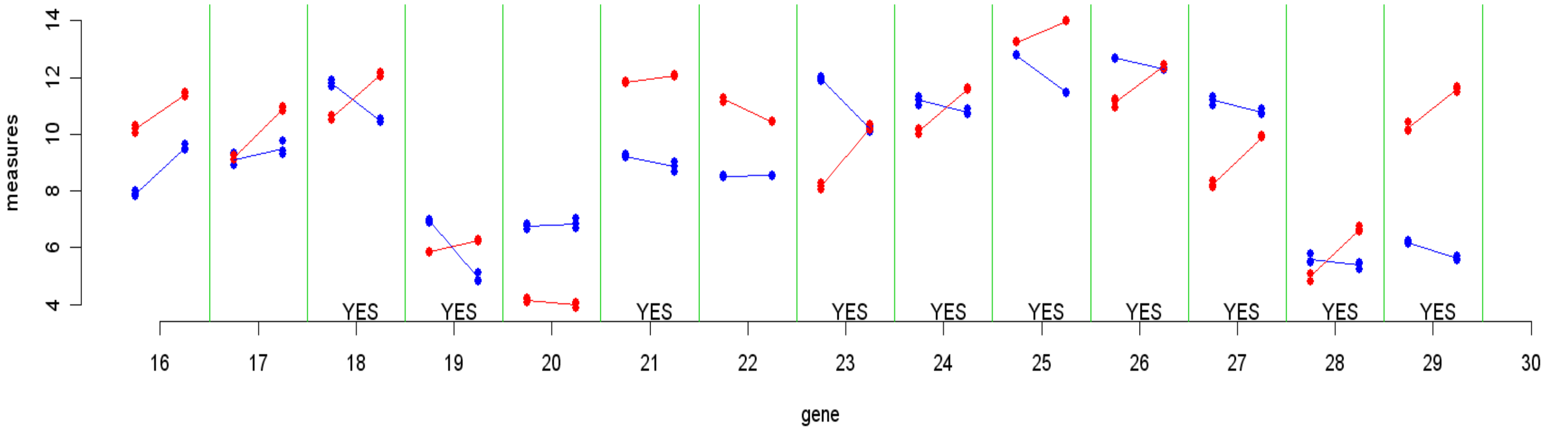
No Complementary expression



4. Statistical Approaches

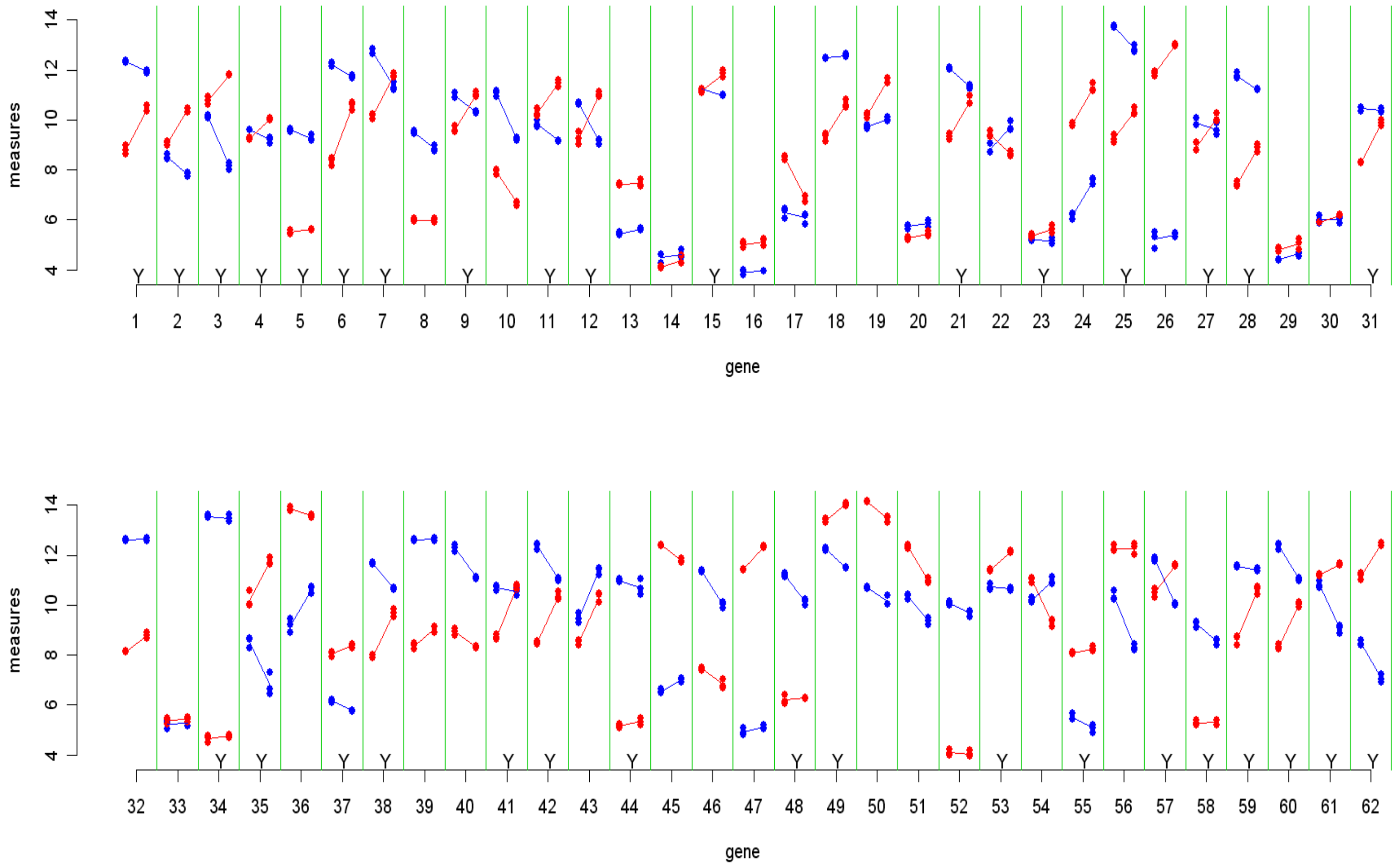
Out of X





4. Statistical Approaches

Out of A



4. Statistical Approaches

Approach 1: Counting YESES and NOS

Counts	NO	YES
Out of X	9	20
Out of A	28	34

Proportions	NO	YES
Out of X	0.31	0.69
Out of A	0.45	0.55

Fisher's Exact Test for Count Data

Null hypothesis: true odds ratio = 1

Alternative hypothesis: true odds ratio is not equal to 1

p-value = 0.2546

Estimated odds ratio=0.55 (roughly (34/28)/(20/9)=0.546)

95% confidence interval: (0.1888,1.5086)

95% confidence interval for YES (normal approximation)

Out of X: (0.518,0.862)

Out of A: (0.424,0.676)

Approach 2: Controling for FDR

Considering only significant change of expression 2 fold difference p<0.05, q < 0.05

Counts	NO	YES
Out of X	9	18
Out of A	37	25

Proportions	NO	YES
Out of X	0.33	0.66
Out of A	0.60	0.40

Fisher's Exact Test for Count Data

Null hypothesis: true odds ratio = 1

Alternative hypothesis: true odds ratio is not equal to 1

p-value = 0.03687

Estimated odds ratio=0.342 (roughly (25/37)/(18/9)=0.339)

95% confidence interval: (0.11,0.95)

95% confidence interval for YES (normal approximation)

Out of X: (0.478,0.842)

Out of A: (0.276,0.524)

Approach 3: Bayesian hierarchical model

$$\begin{pmatrix} mit_{ijkl} \\ mei_{ijkl} \end{pmatrix} \sim N\left[\begin{pmatrix} \theta_{ijl}^{mit} \\ \theta_{ijl}^{mei} \end{pmatrix}, \sigma_i^2 I_2\right]$$

$$\begin{pmatrix} \theta_{ijl}^{mit} \\ \theta_{ijl}^{mei} \end{pmatrix} \sim N\left[\begin{pmatrix} \theta_{ij}^{mit} \\ \theta_{ij}^{mei} \end{pmatrix}, \begin{pmatrix} \tau_{mit}^2 & 0 \\ 0 & \tau_{mei}^2 \end{pmatrix}\right]$$

Pairs mit_{ijkl} and mei_{ijkl}, for each gene l, each classification group i (out of X, out of A) and gene type j (parental, offspring), have individual means θ_{ijl}^{mit} and θ_{ijl}^{mei}, respectively, and common classification group variances σ_i^2. Then, the objective is to compute,

$$\Pr\left[\mathrm{YES}_{\text{Out of X},l}\right] = \Pr\left[\left\{\theta^{mit}_{\text{Out of X},par,l} > \theta^{mei}_{\text{Out of X},\,par,l}\right\} \; \mathrm{I} \; \left\{\theta^{mit}_{\text{Out of X},off\,,l} < \theta^{mei}_{\text{Out of X},,off\,,l}\right\}\right]$$

$$\Pr\left[\mathrm{YES}_{\text{Out of A},l}\right] = \Pr\left[\left\{\theta^{mit}_{\text{Out of A},par,l} > \theta^{mei}_{\text{Out of A},par,l}\right\} \; \mathrm{I} \; \left\{\theta^{mit}_{\text{Out of A},,off\,,l} < \theta^{mei}_{\text{Out of A},,off\,,l}\right\}\right]$$

Approach 3: Bayesian hierarchical model

Hyperparameters

$$\Theta = (\theta_{11}^{mit}, \theta_{12}^{mit}, \theta_{21}^{mit}, \theta_{22}^{mit}, \theta_{11}^{mei}, \theta_{12}^{mei}, \theta_{21}^{mei}, \theta_{22}^{mei})$$

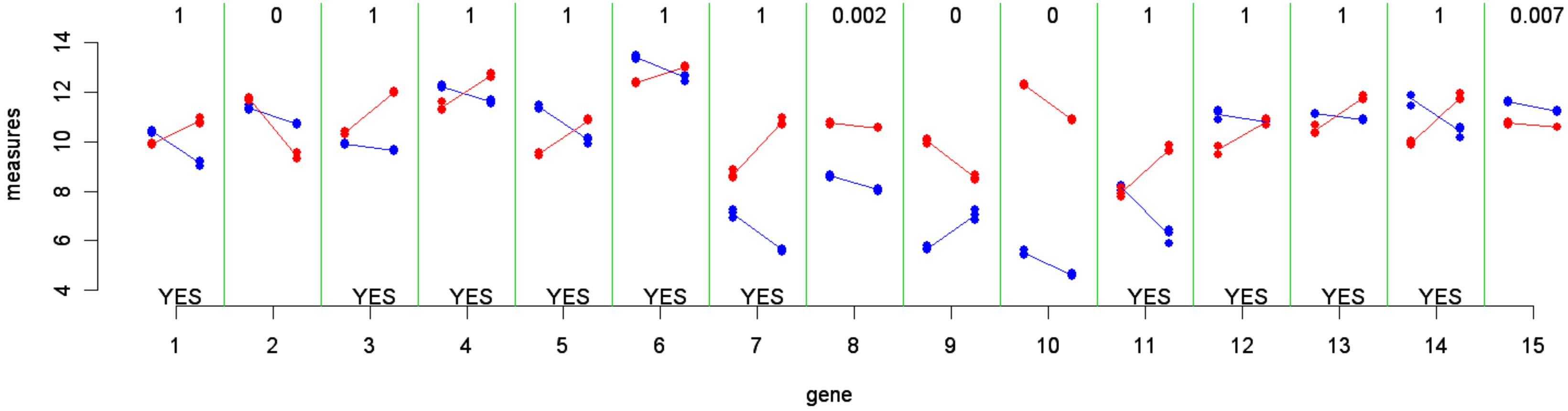
$$\Lambda = (\sigma_1^2, \sigma_2^2, \tau_{mit}^2, \tau_{mei}^2)$$

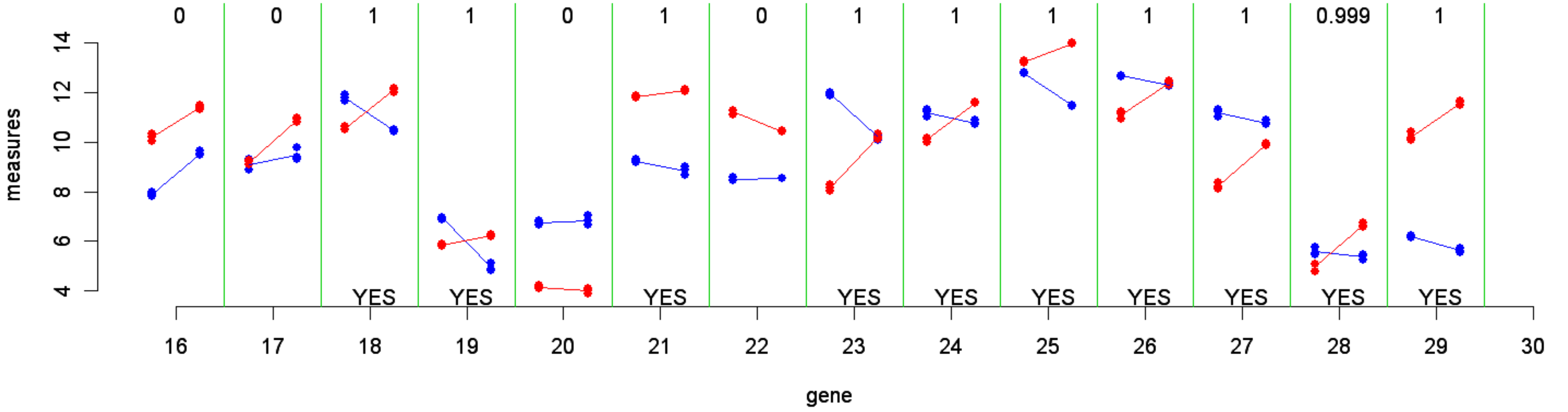
Prior distribution

$$p(\Theta, \Lambda) \propto \sigma_1^{-2}\sigma_2^{-2}\tau_{mit}^{-2}\tau_{mei}^{-2}$$

This represents vague/noninformative prior views.

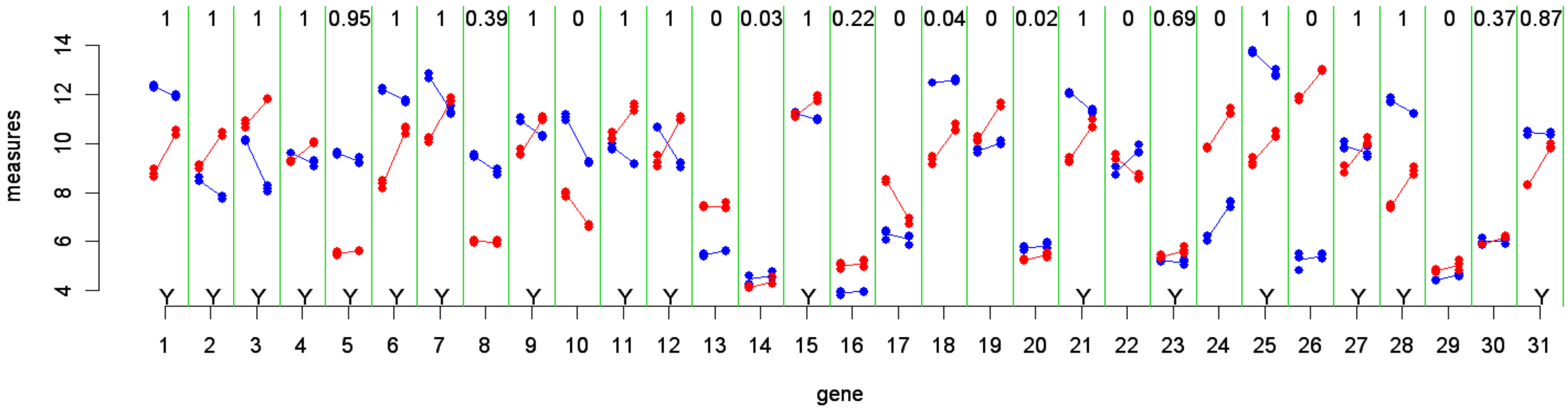
Approach 3: Bayesian hierarchical model

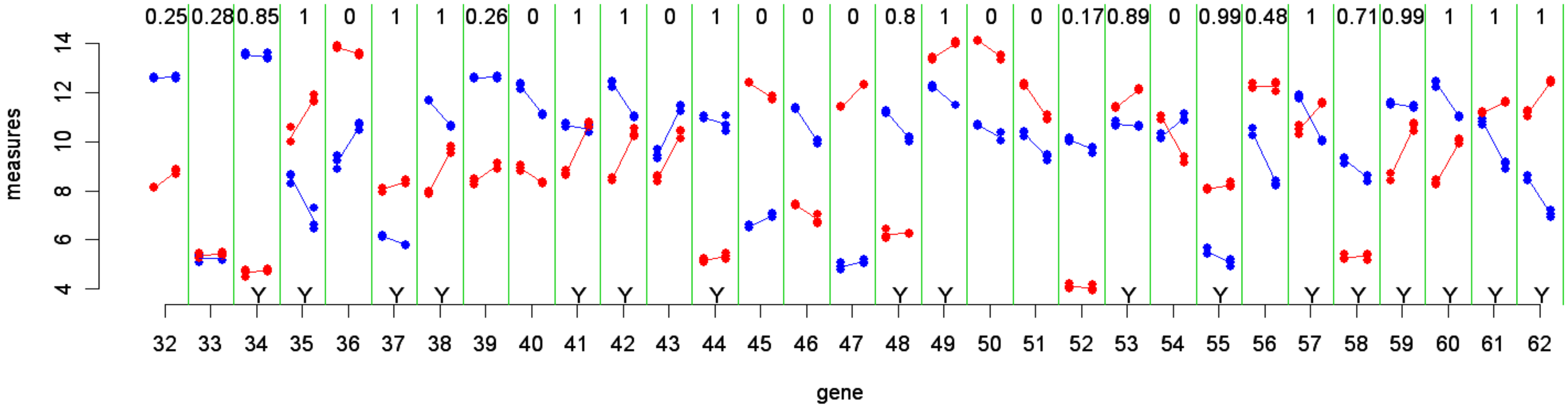




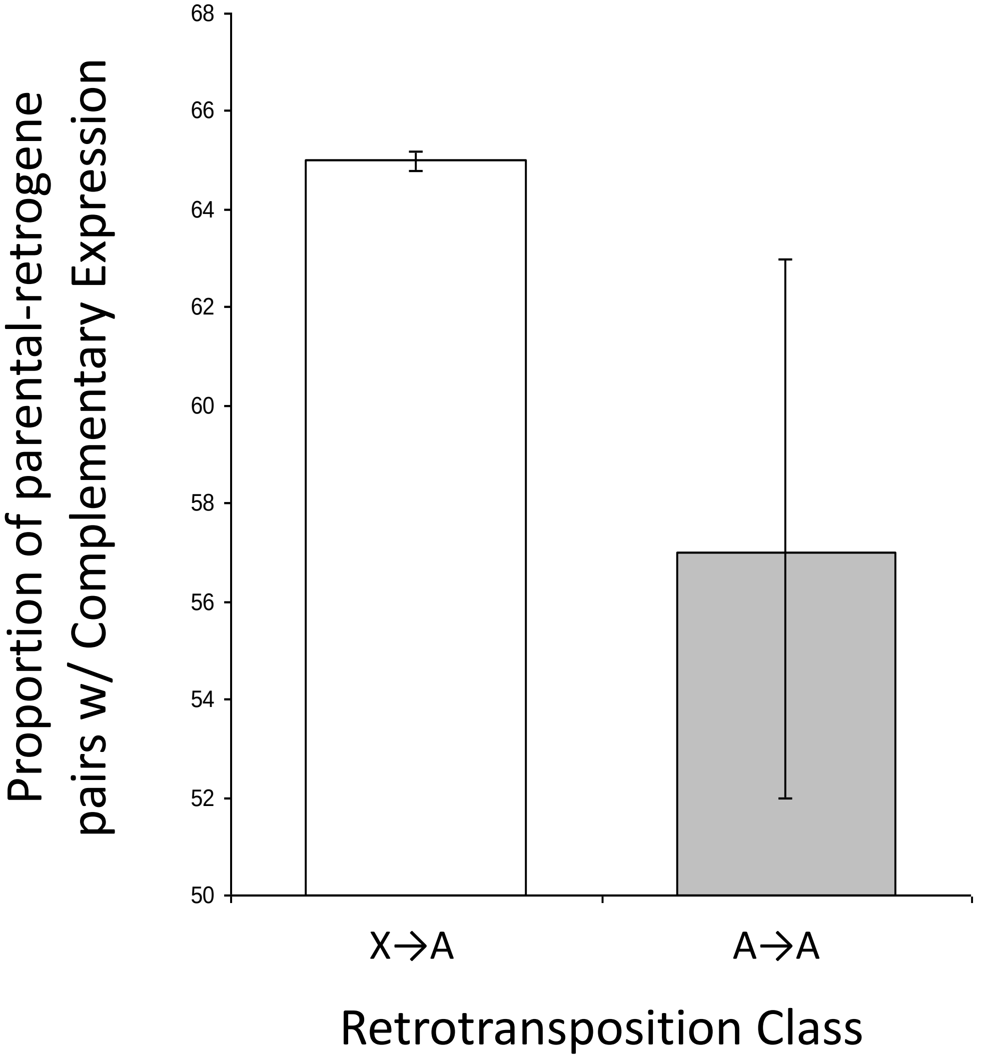
4. Statistical Approaches

Approach 3: Bayesian hierarchical model



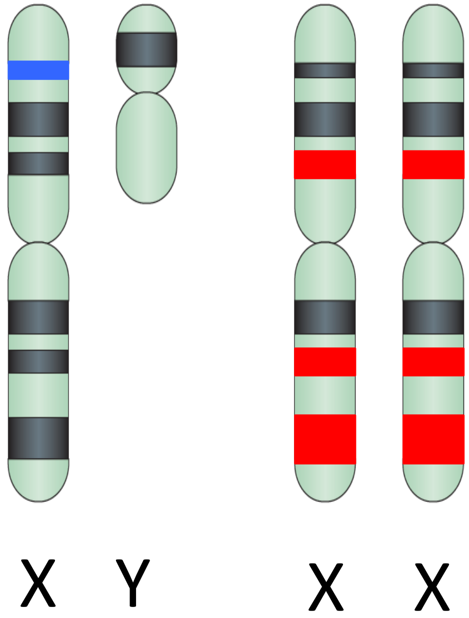


4. Statistical Approaches

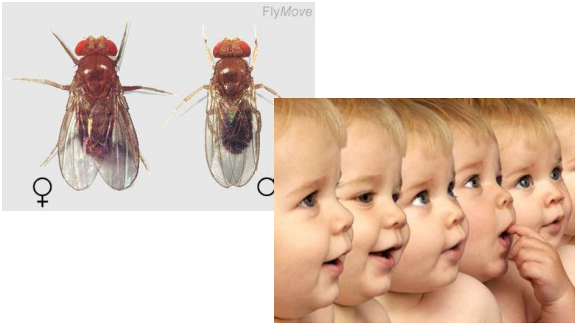


Vibranovski et al, 2009, PLoS Genetics

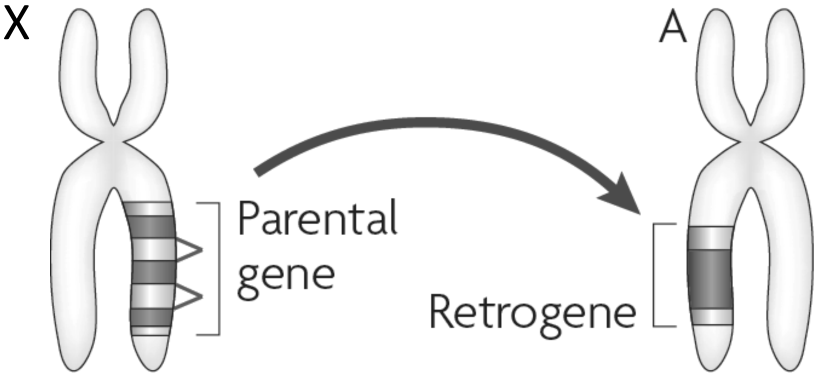
2. Biological Problem



- Does MSCI exist in *Drosophila*? YES
- Is MSCI involved in the distribution of sex-biased genes in the genome? YES

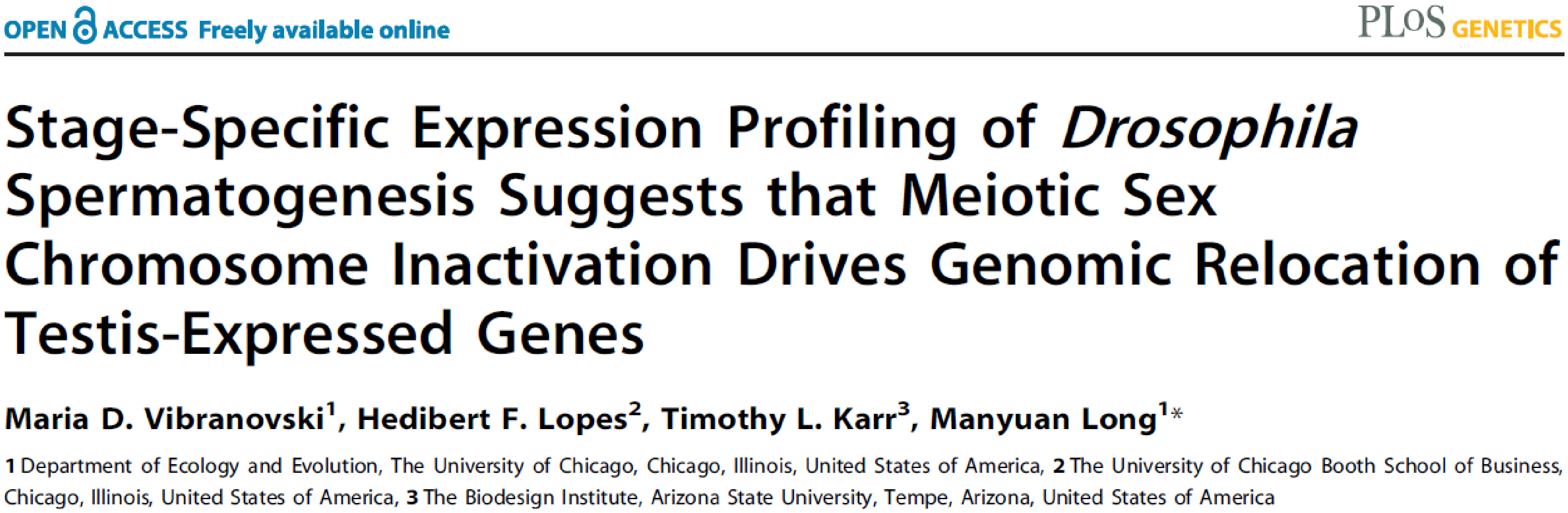


XY systems



Testis bias

2. Biological Problem



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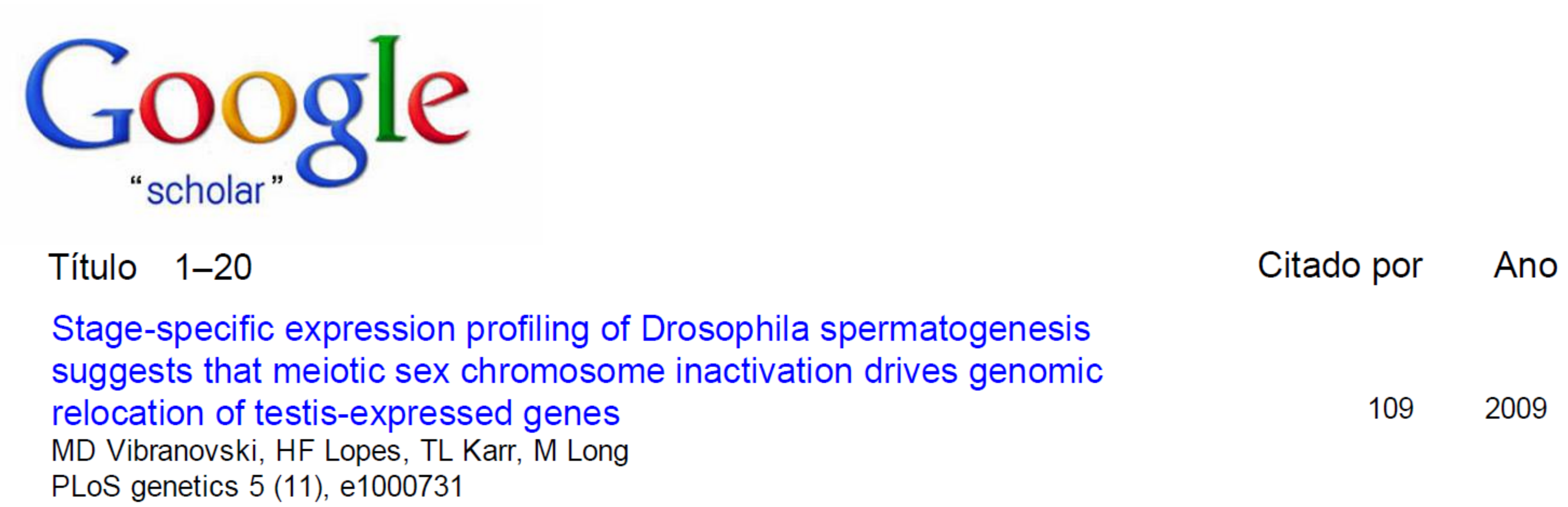
PLoS GENETICS

Stage-Specific Expression Profiling of *Drosophila* Spermatogenesis Suggests that Meiotic Sex Chromosome Inactivation Drives Genomic Relocation of Testis-Expressed Genes

Maria D. Vibranovski[1], Hedibert F. Lopes[2], Timothy L. Karr[3], Manyuan Long[1]*

[1] Department of Ecology and Evolution, The University of Chicago, Chicago, Illinois, United States of America, [2] The University of Chicago Booth School of Business, Chicago, Illinois, United States of America, [3] The Biodesign Institute, Arizona State University, Tempe, Arizona, United States of America

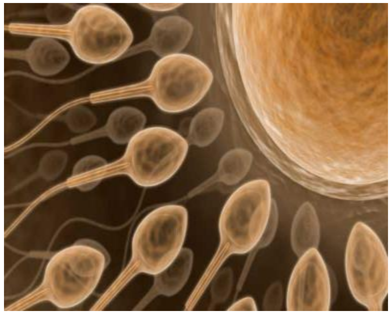


Google "scholar"

Título 1–20	Citado por	Ano
Stage-specific expression profiling of Drosophila spermatogenesis suggests that meiotic sex chromosome inactivation drives genomic relocation of testis-expressed genes MD Vibranovski, HF Lopes, TL Karr, M Long PLoS genetics 5 (11), e1000731	109	2009

2. Biological Problem

Male infertility



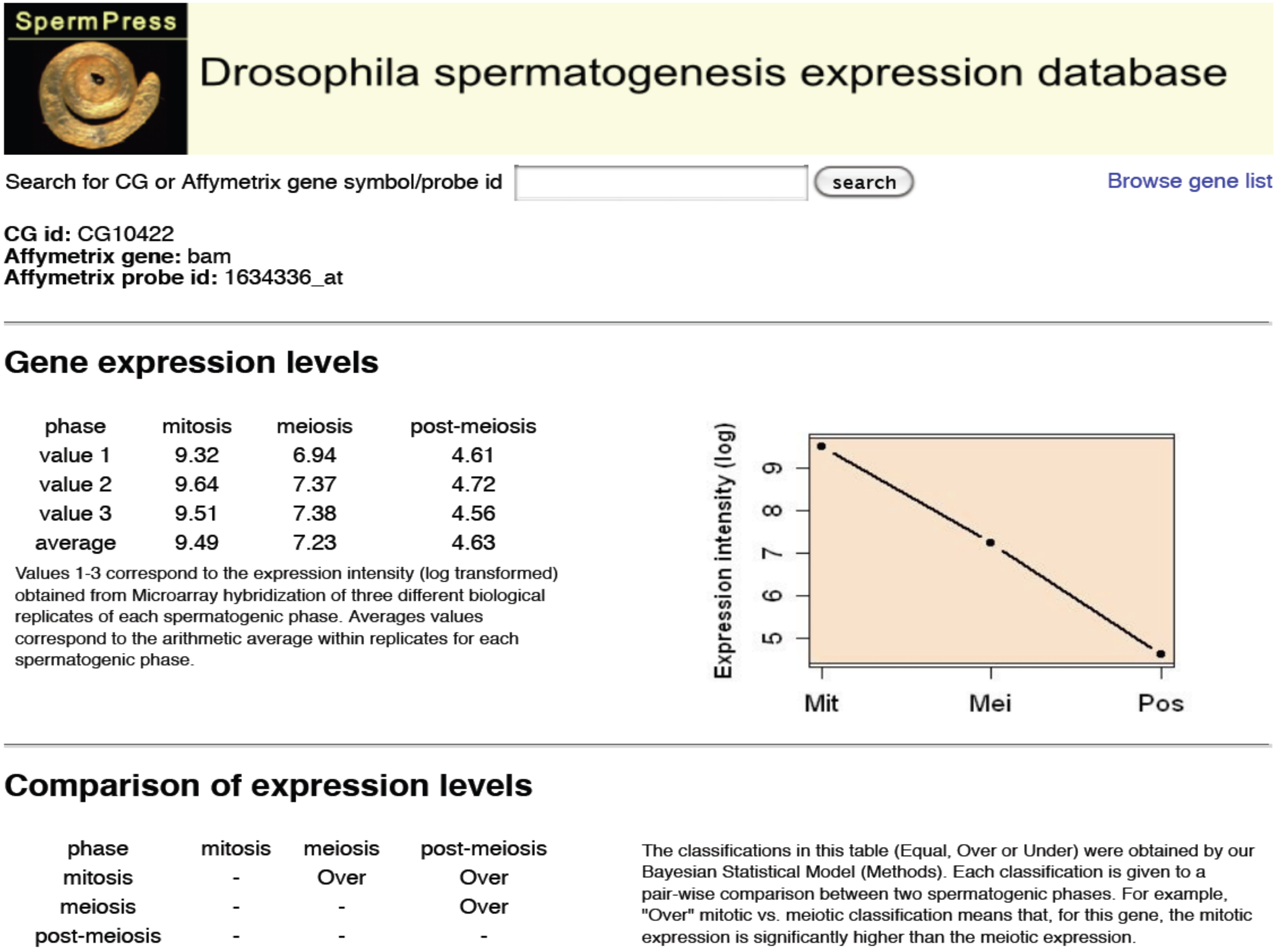
In Humans:

- 40% of infertility
- High cost treatment
- Associated with gene expression/function in spermatogenesis
- 30% of infertility are sperm deficiency

Drosophila Model:

- Spermatozoa is one of the few cell types that has homologous function for all sexual organism including humans
- Post-meiotic transcription

2. Biological Problem



Drosophila spermatogenesis expression database

Search for CG or Affymetrix gene symbol/probe id

CG id: CG10422
Affymetrix gene: bam
Affymetrix probe id: 1634336_at

Gene expression levels

phase	mitosis	meiosis	post-meiosis
value 1	9.32	6.94	4.61
value 2	9.64	7.37	4.72
value 3	9.51	7.38	4.56
average	9.49	7.23	4.63

Values 1-3 correspond to the expression intensity (log transformed) obtained from Microarray hybridization of three different biological replicates of each spermatogenic phase. Averages values correspond to the arithmetic average within replicates for each spermatogenic phase.

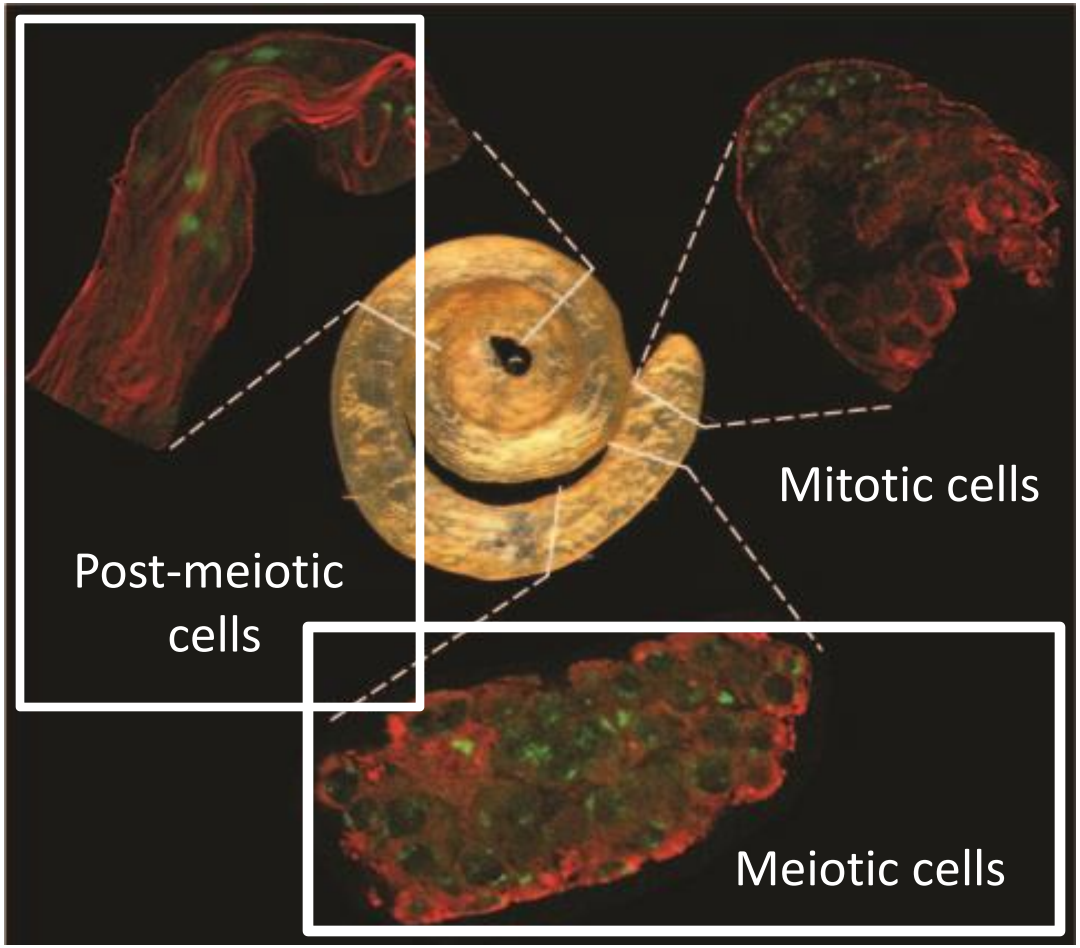
Comparison of expression levels

phase	mitosis	meiosis	post-meiosis
mitosis	-	Over	Over
meiosis	-	-	Over
post-meiosis	-	-	-

The classifications in this table (Equal, Over or Under) were obtained by our Bayesian Statistical Model (Methods). Each classification is given to a pair-wise comparison between two spermatogenic phases. For example, "Over" mitotic vs. meiotic classification means that, for this gene, the mitotic expression is significantly higher than the meiotic expression.

http://pondside.uchicago.edu/~longlab/spermpress/

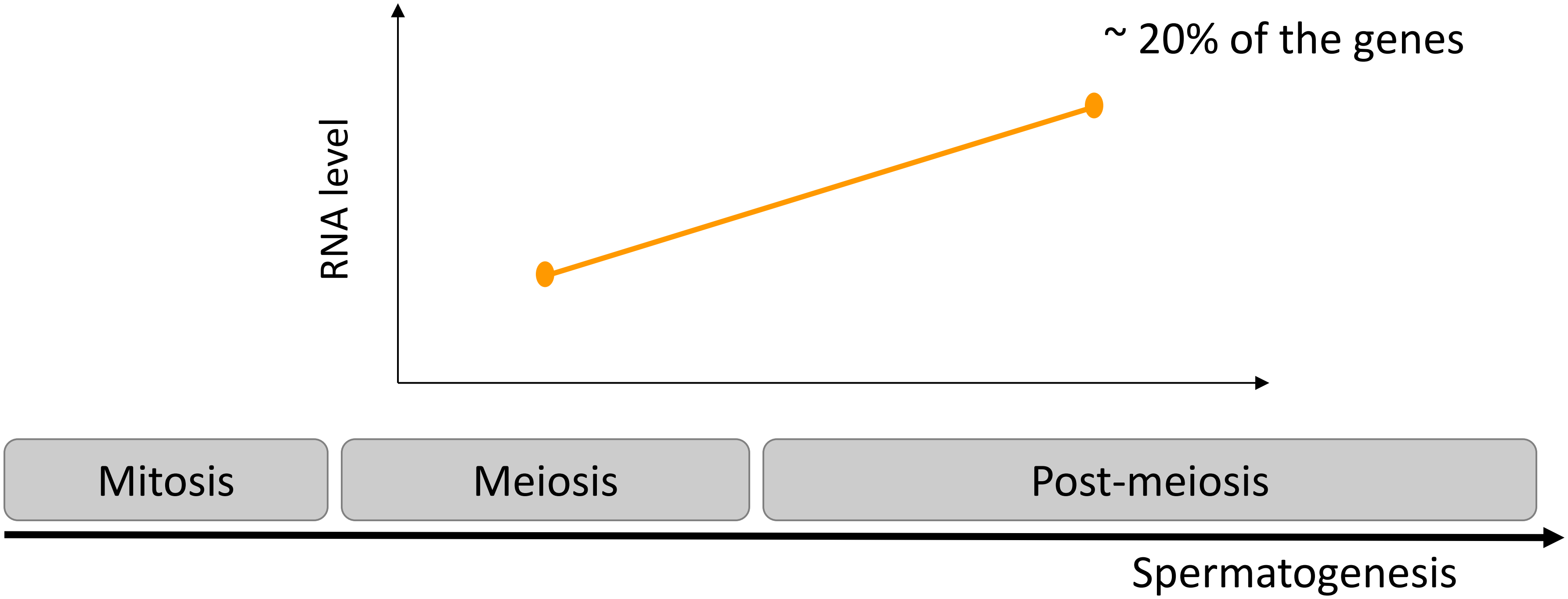
5. Ongoing Biological Problems



Vibranovski et al, 2009, PLoS Genetics

5. Ongoing Biological Problems

Post-meiosis Over-expression



~ 20% of the genes

RNA level

Mitosis

Meiosis

Post-meiosis

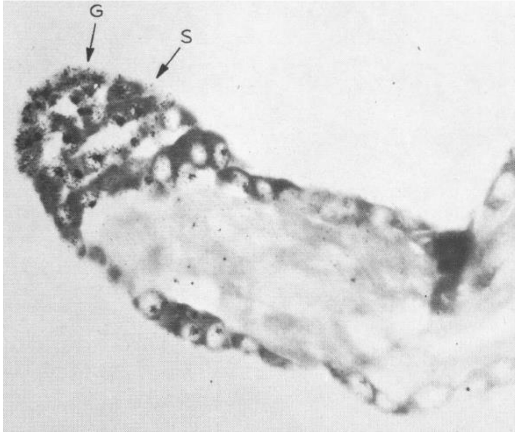
Spermatogenesis

Vibranovski et al, 2009, PLoS Genetics

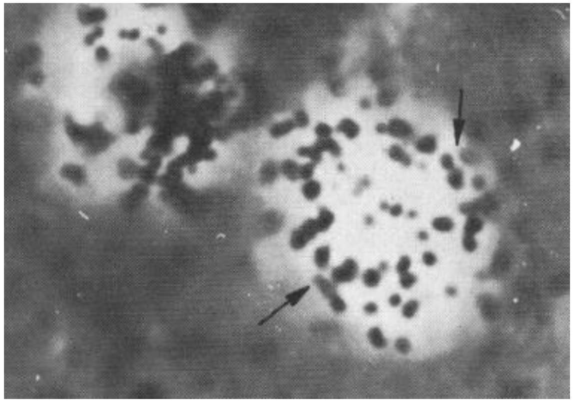
5. Ongoing Biological Problems

RNA synthesis does NOT occur in post-meiotic stages

Testis Apical region



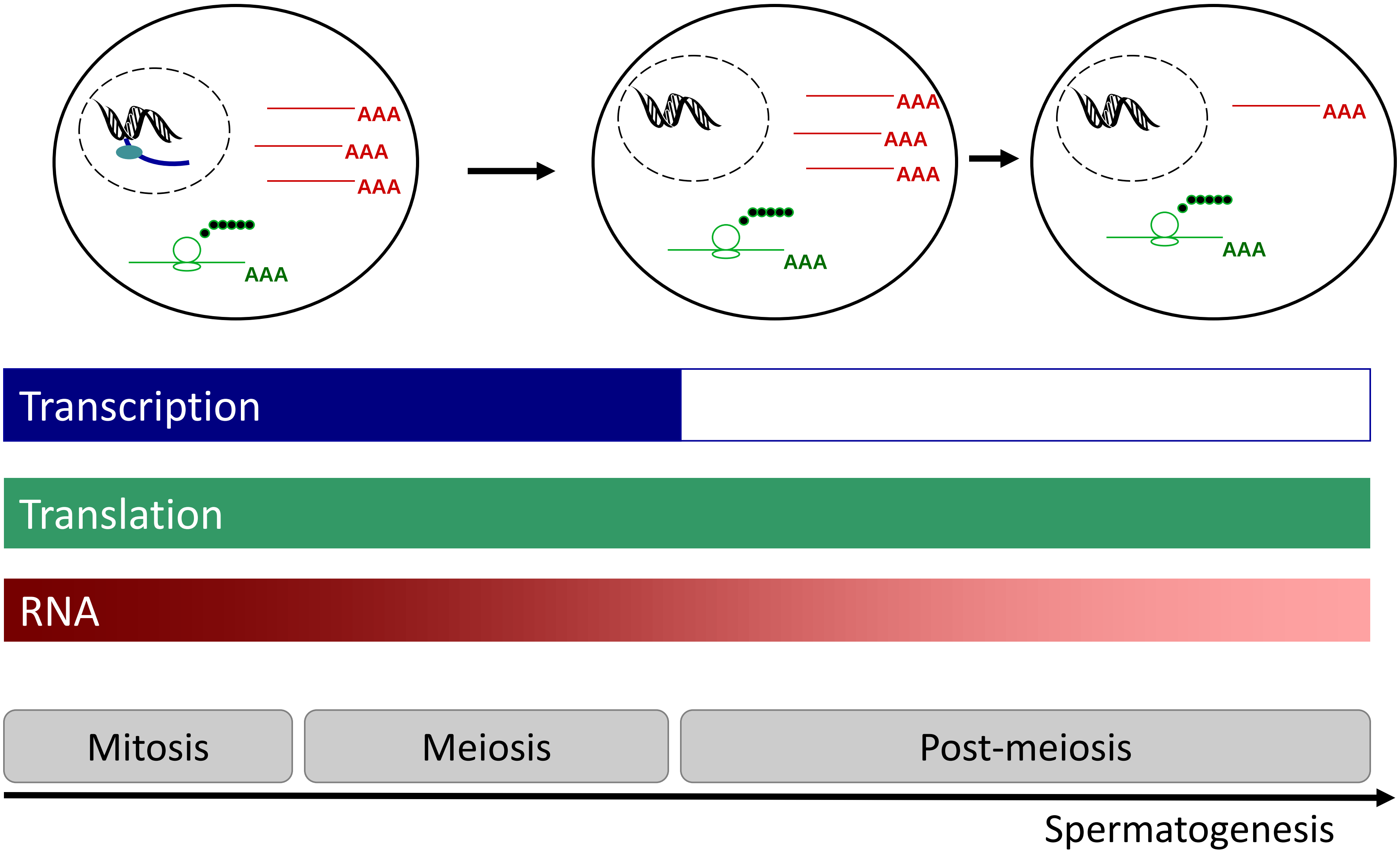
Spermatocytes



RNA synthesis: incorporation of [^{3}H]uridine in the *Drosophila melanogaster* testes has been studied by autoradiography.

Olivieri and Olivieri, 1965, Mutation Research

5. Ongoing Biological Problems



Transcription

Translation

RNA

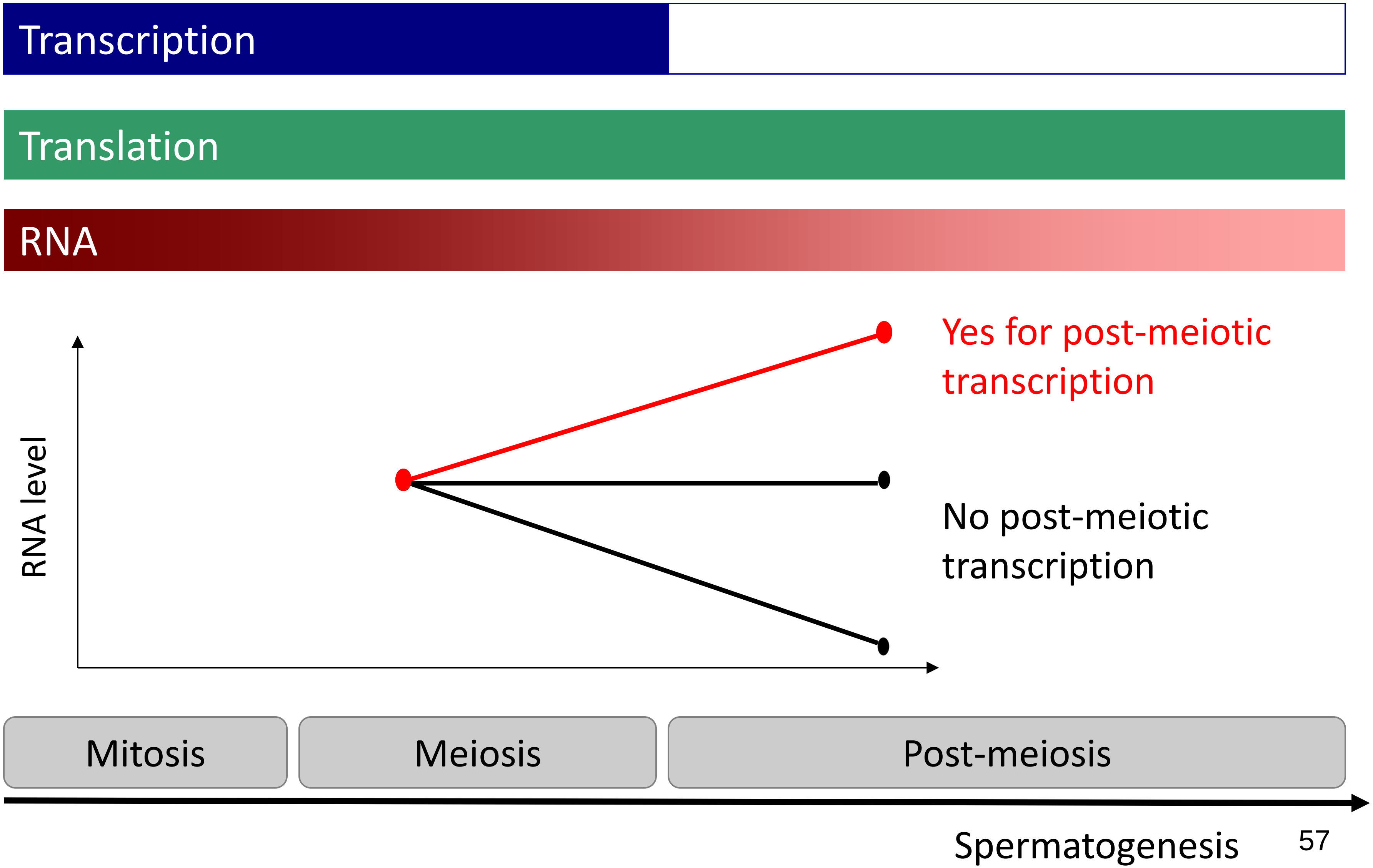
Mitosis

Meiosis

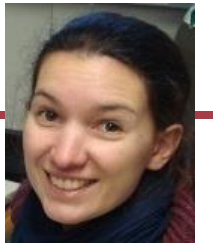
Post-meiosis

Spermatogenesis

5. Ongoing Biological Problems

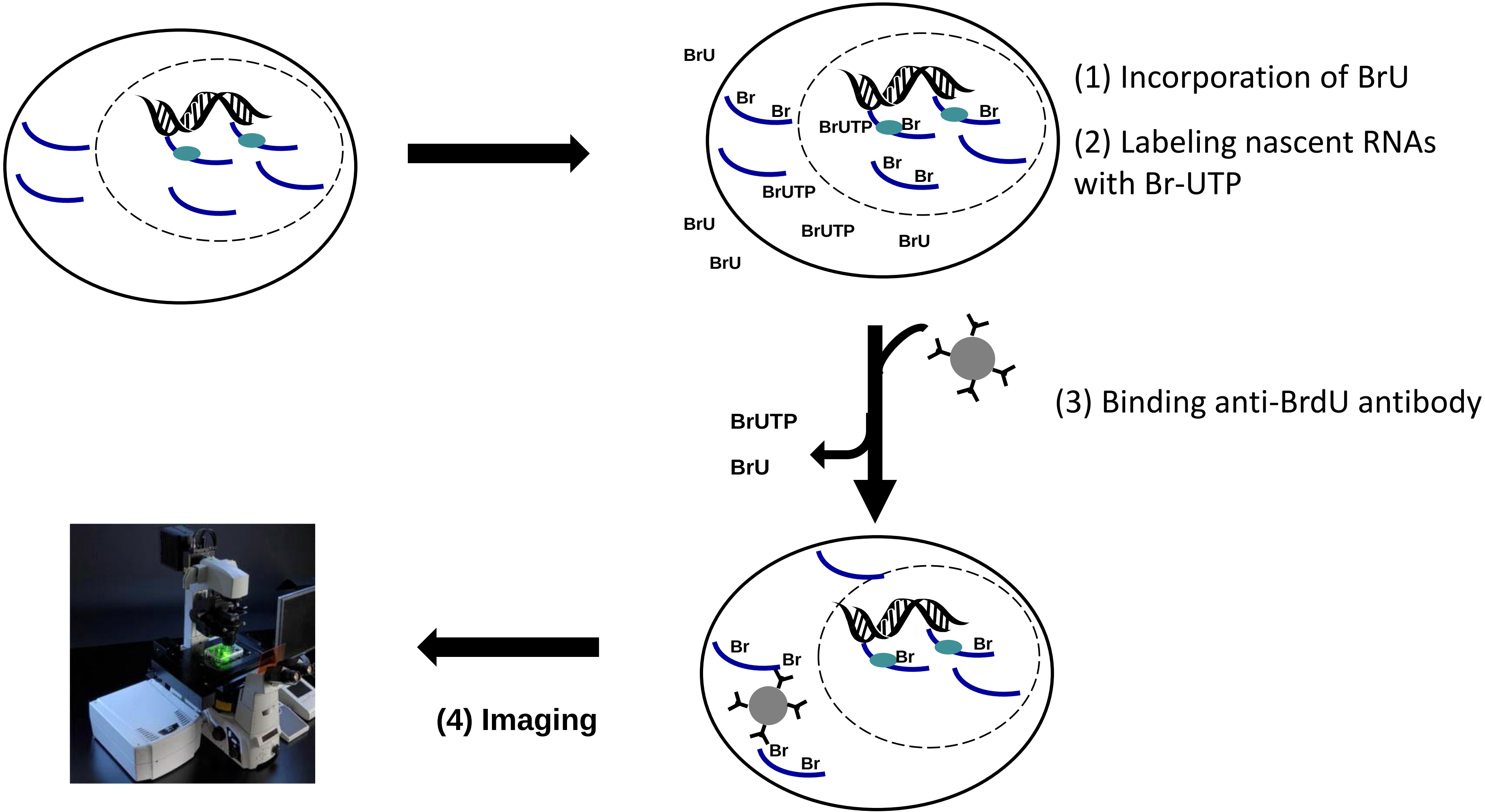


5. Ongoing Biological Problems



Domitille Chalopin

Bromo-uridine (BrU) incorporation: Direct evidence



(1) Incorporation of BrU

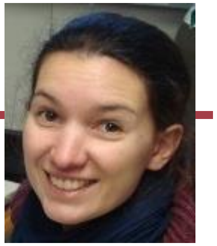
(2) Labeling nascent RNAs with Br-UTP

(3) Binding anti-BrdU antibody

(4) Imaging

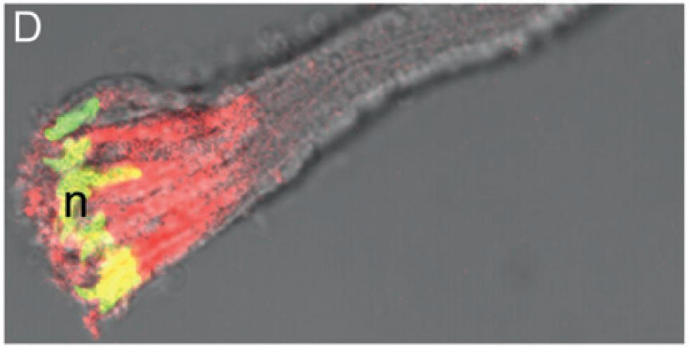
Vibranovski et al, 2010, Genetics

5. Ongoing Biological Problems



Domitille Chalopin

Bromo-uridine (BrU) incorporation:
Post-meiotic transcription - Direct evidence

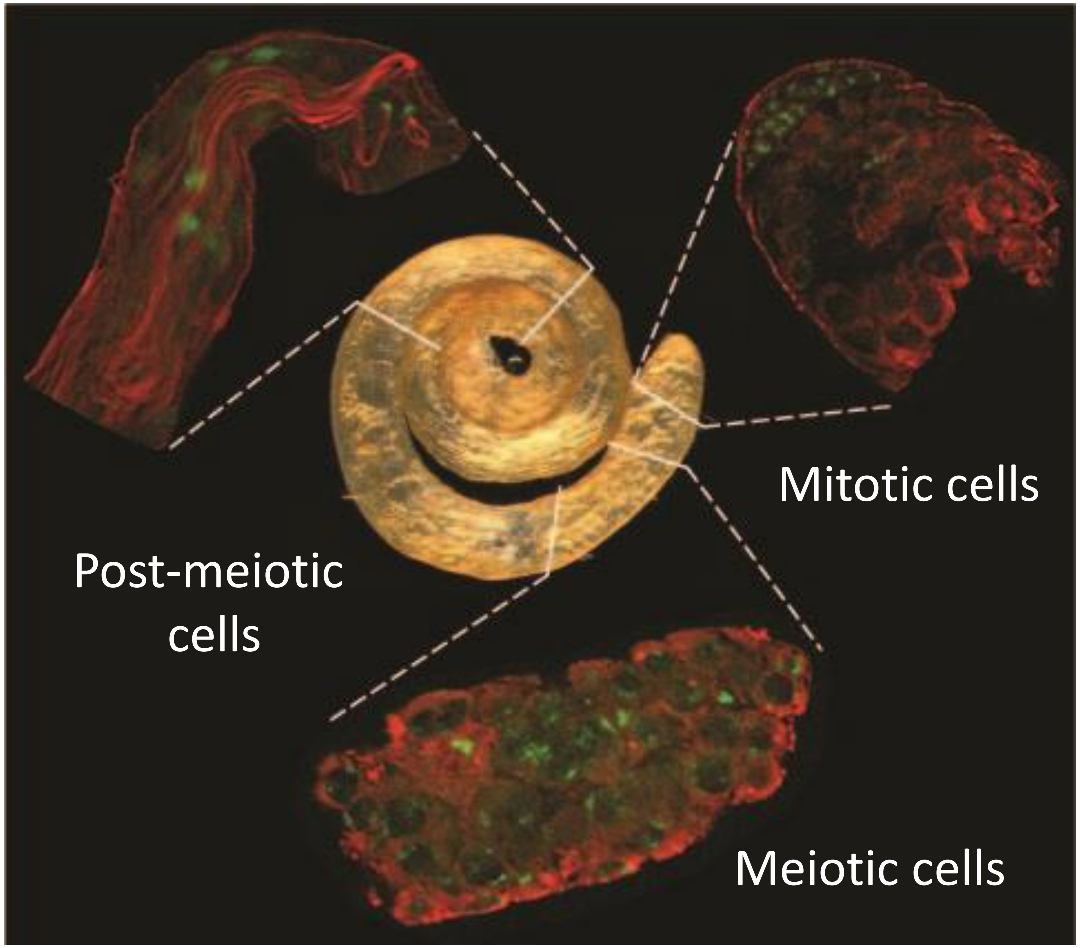


Spermatid cyst

BrU
DNA
n: nuclei

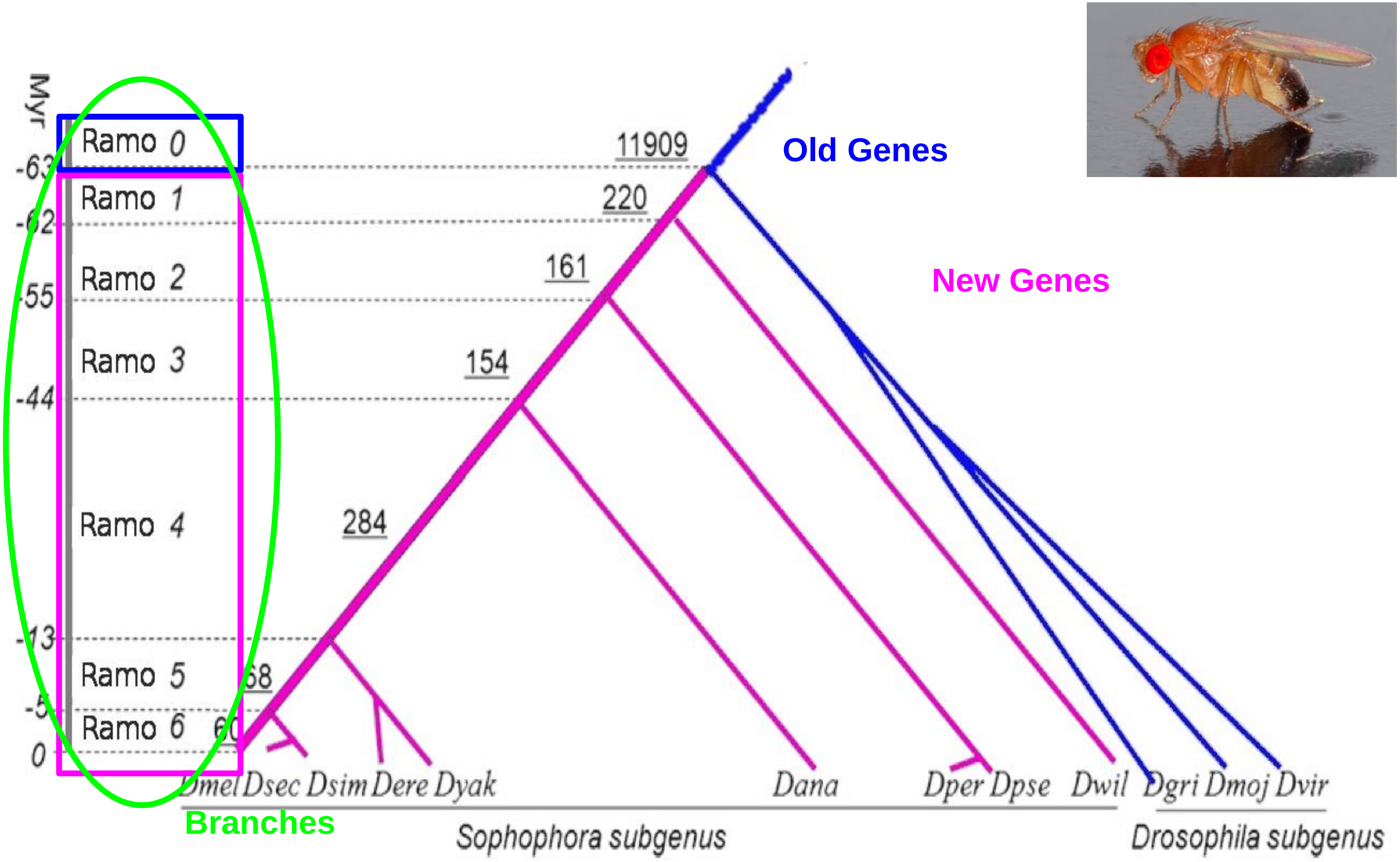
Vibranovski et al, 2010, Genetics

5. Ongoing Biological Problems



Vibranovski et al, 2009, PLoS Genetics

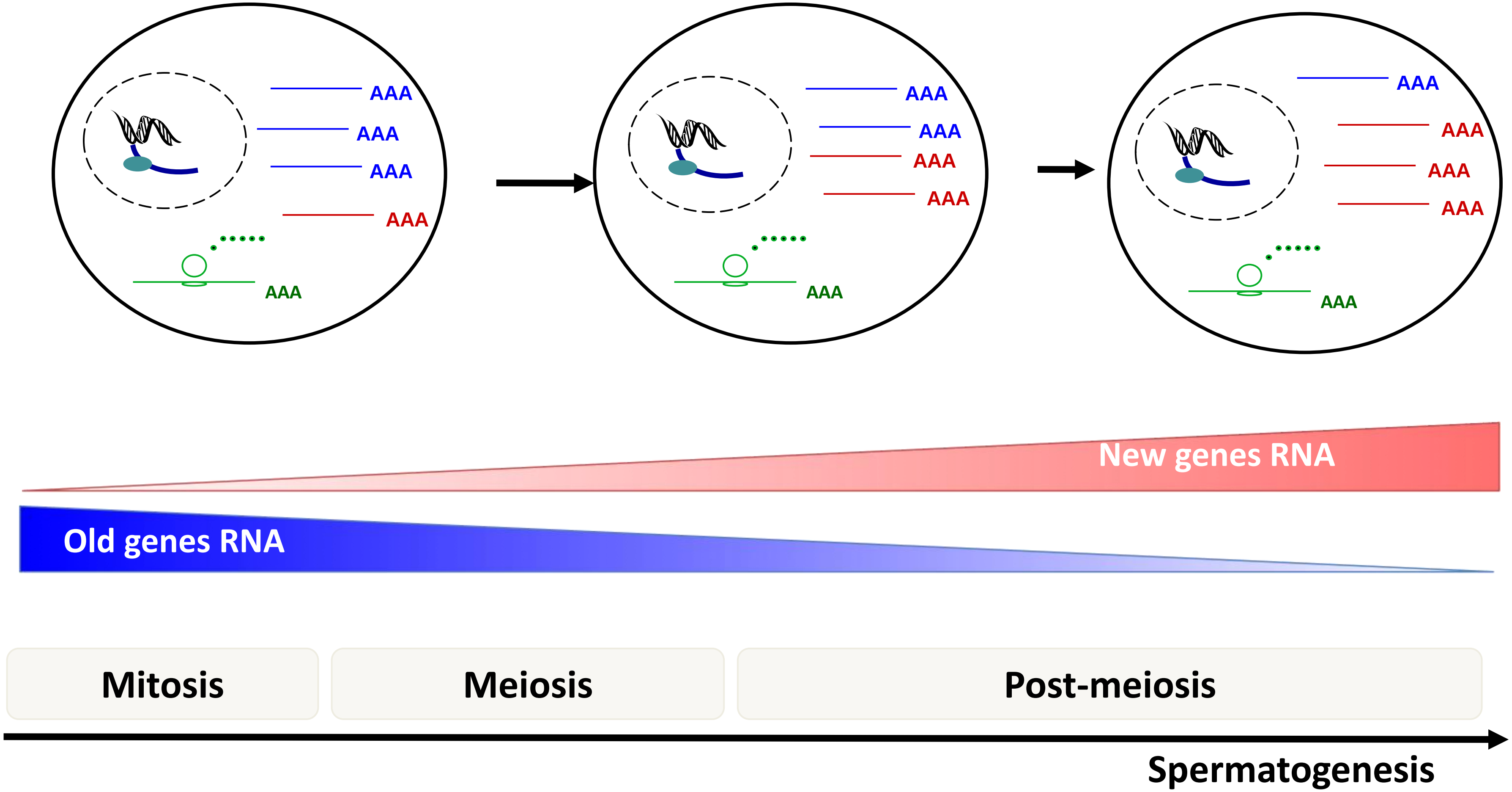
5. Ongoing Biological Problems



Zhang, Vibranovski, Krinsky and Long, 2010

5. Ongoing Biological Problems

Júlia Raices



New genes RNA

Old genes RNA

Mitosis

Meiosis

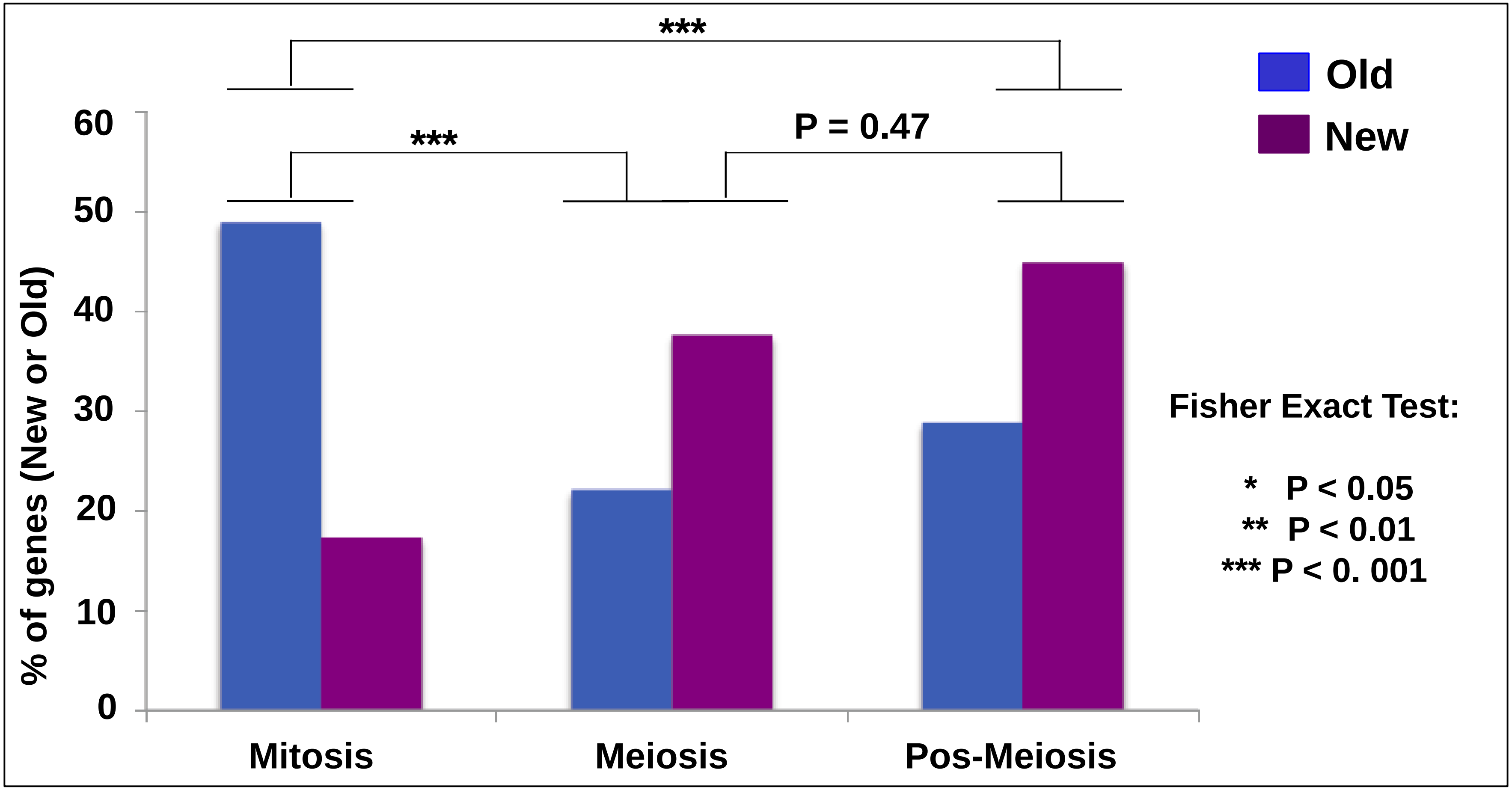
Post-meiosis

Spermatogenesis

5. Ongoing Biological Problems

Júlia Raices

Proportion of Genes by Spermatogenesis Phase and by age

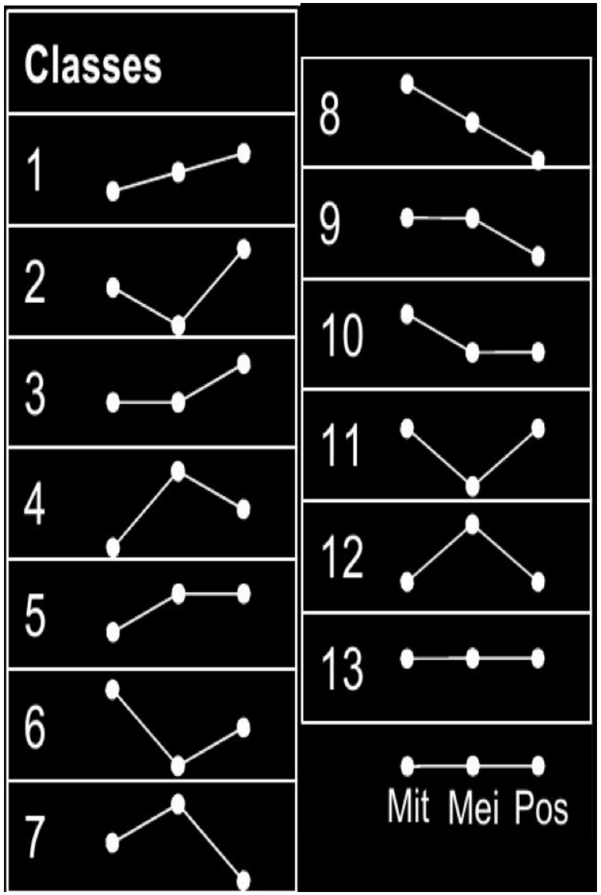


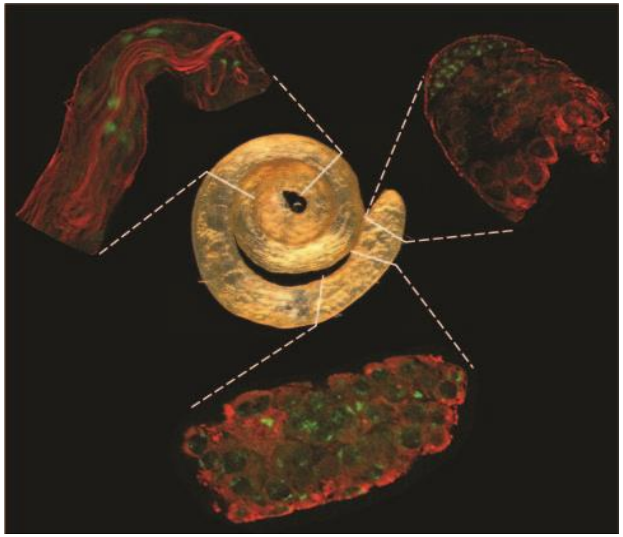
Fisher Exact Test:

* P < 0.05
** P < 0.01
*** P < 0. 001

5. Ongoing Biological Problems

Júlia Raices





5. Ongoing Biological Problems

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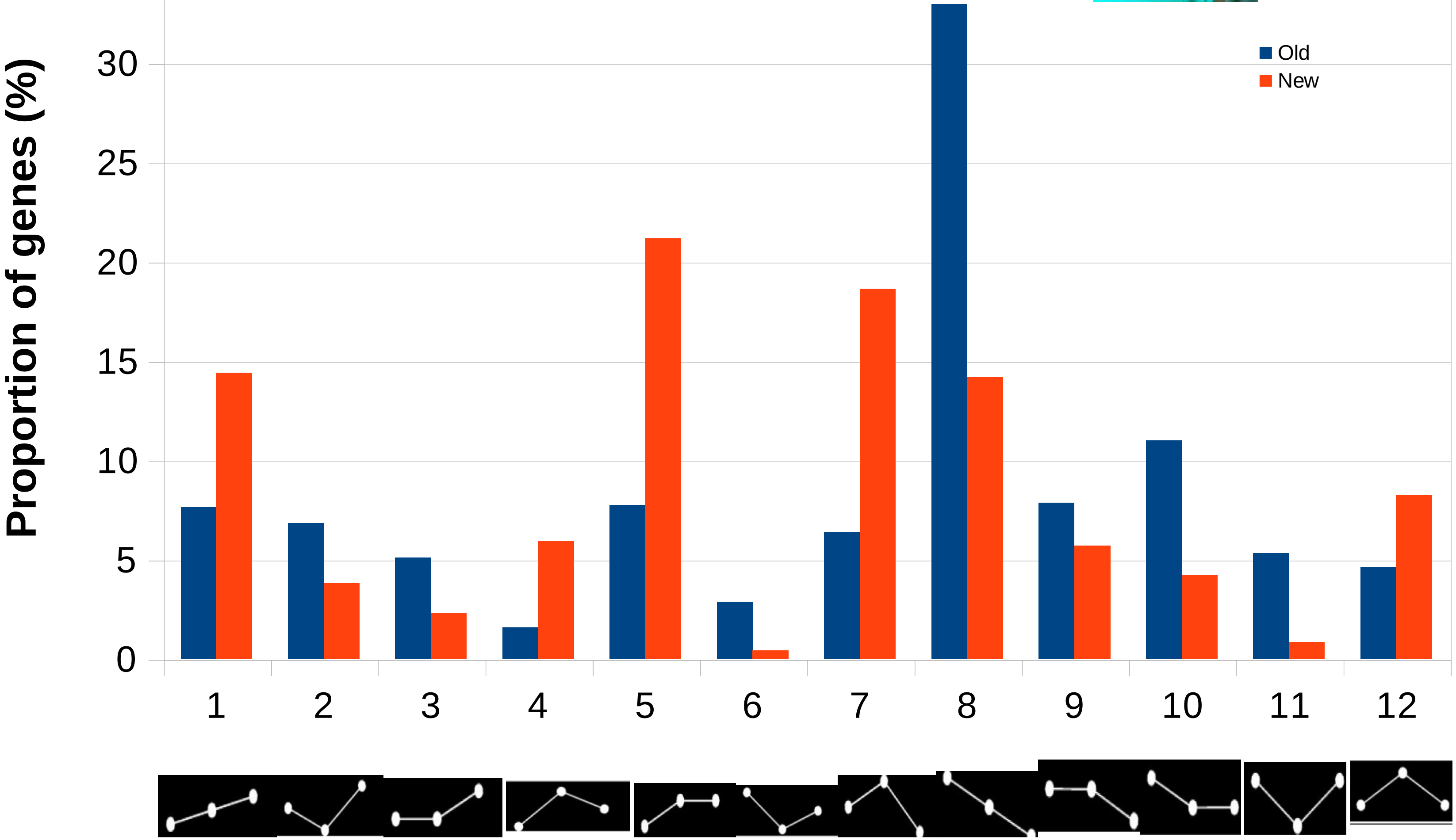
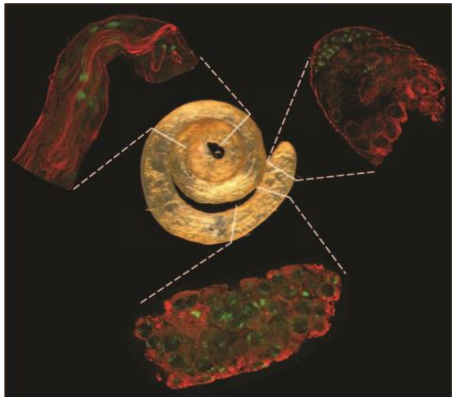
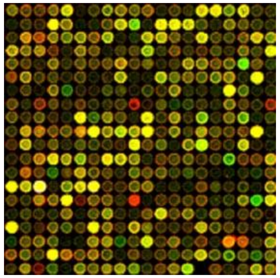


Perspectives

Development



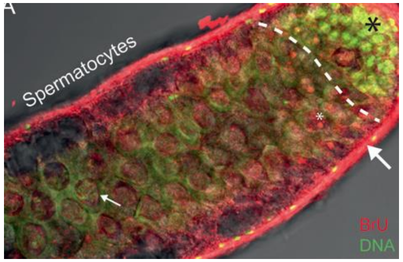
Molecular Biology



Bioinformatics

Genetics

Cell Biology



Statistics

Acknowledgments

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Post-meiotic transcrption

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Hedibert Lopes

Timothy Karr

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instituto de biociências

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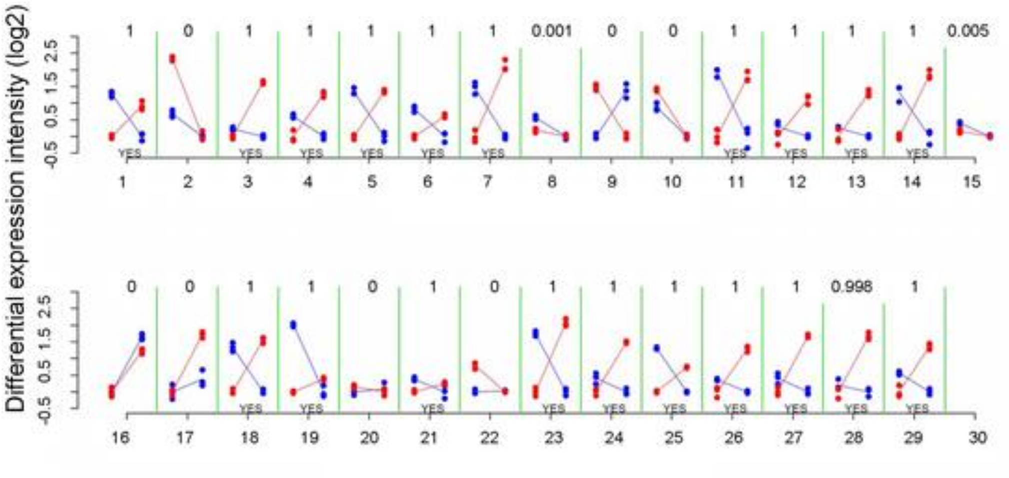
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

NATIONAL INSTITUTES OF HEALTH

NSF

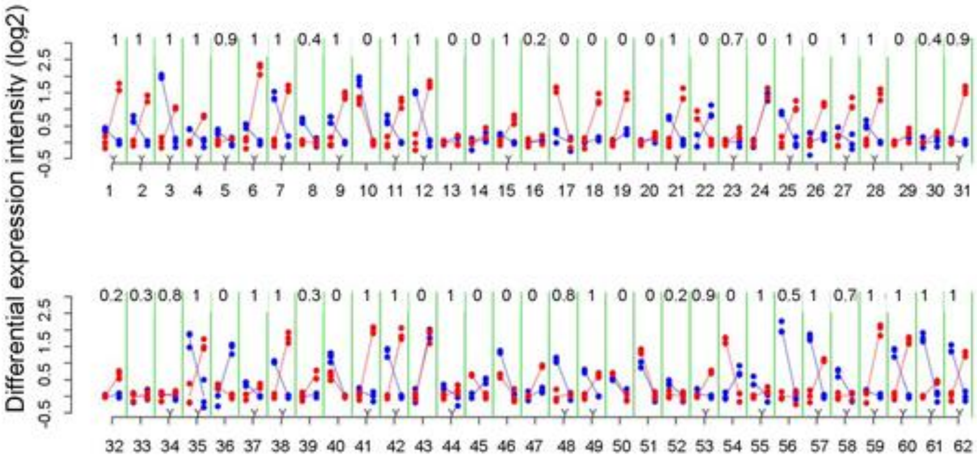
CHICAGO BOOTH The University of Chicago Booth School of Business

ASU BIODESIGN INSTITUTE ARIZONA STATE UNIVERSITY


Differential expression intensity (log2)
-0.5
0.5
1.5
2.5
1 0 1 1 1 1 1 0.001 0 0 1 1 1 1 0.005
YES
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 0 1 1 0 1 0 1 1 1 1 1 0.998 1
YES
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

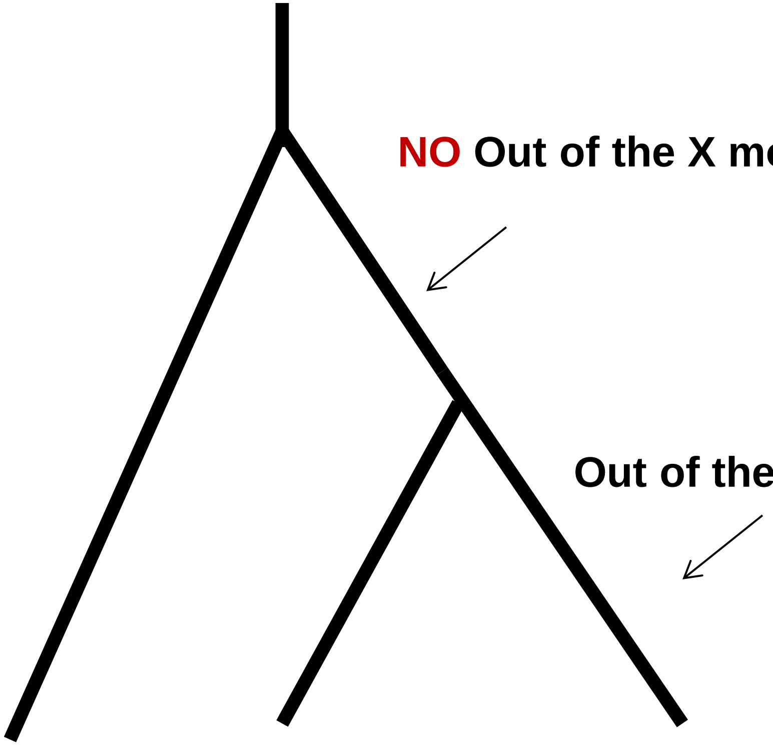
X➔A

A


Differential expression intensity (log2)
-0.5
0.5
1.5
2.5
1 1 1 1 0.9 1 1 0.4 1 0 1 1 0 0 1 0.2 0 0 0 0 1 0 0.7 0 1 0 1 1 0 0.4 0.9
Y
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
0.2 0.3 0.8 1 0 1 1 0.3 0 1 1 0 1 0 0 0 0.8 1 0 0 0.2 0.9 0 1 0.5 1 0.7 1 1 1 1
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62

A(A

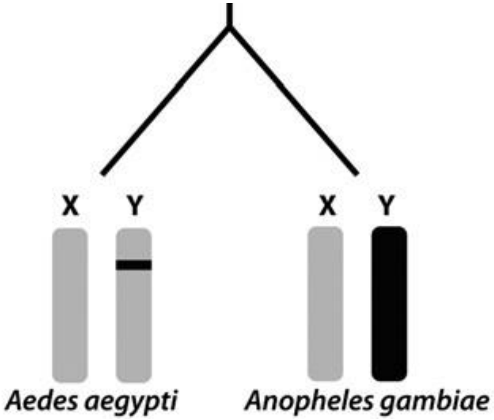
B



NO Out of the X movement

Out of the X movement

D. melanogaster *Aedes aegypti* *Anopheles gambiae*



Toups and Hahn, 2010, Genetics