The Abdus Salam International Centre for Theoretical Physics

2415-15

Winter School on Quantitative Systems Biology

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Of flies and men: Facets of biological systems

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Genomics of Systems

Epigenetic pathways

Epigenetics

- piRNA pathway and defense of the genome
- The Wolbachia.
- Sex determination

NON-CODING RNAS

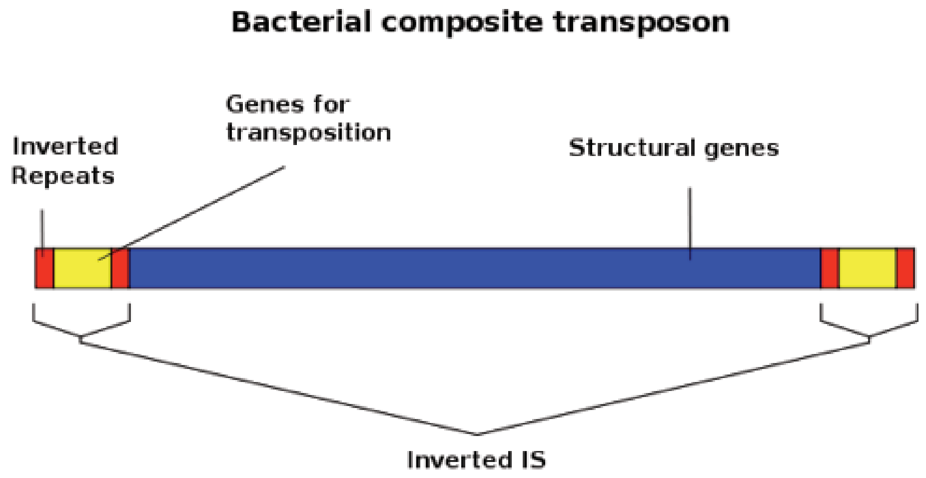
- The age of non-coding RNAs
- Implicated in a wide-variety of phenomena: Transposon silencing, genetic network controls, epigenetics.
- The field is thriving because of massively parallel sequencing.

Parasitic elements

- Viruses: insert themselves into the genome.
- Transposons: maybe remnants of viruses, that can jump around, selfish DNA elements that live in the genome.

Orgel LE, **Crick** FH: Selfish DNA: the ultimate parasite. Nature 1980

Transposons



- **Transposable element** (**TE**) DNA sequence that can jump around in the genome. Transposition often results in duplication of the TE. Barbara McClintok's jumping genes.
- TEs make up a large fraction of the C-value of eukaryotic cells. Junk DNA.

Transposon types

- Class I TE (retrotransposons) "copy and paste". Retroviruses are in this class, but have added ability to escape cell through their RNA phase. DNA-> RNA transcription, RNA->DNA reverse transcription through a reverse transcriptase encoded in TE. Three orders of retrotransposons:
  - Those with long terminal repeats (LTRs): encode reverse transcriptase, similar to retroviruses;
  - LINES: encode reverse transcriptase, lack LTRs, transcribed by RNA pol II.
  - SINES: parasitic transposons. no reverse transcriptase, transcribed by RNA pol III.
- Class II TE (DNA transposons), "cut and paste". No RNA intermediate. Cut-and-paste can result in duplication if transposition occurs during S phase of cell cycle.
  - catalyzed by transposase.
  - replicative transposition (helitron) is another mode.

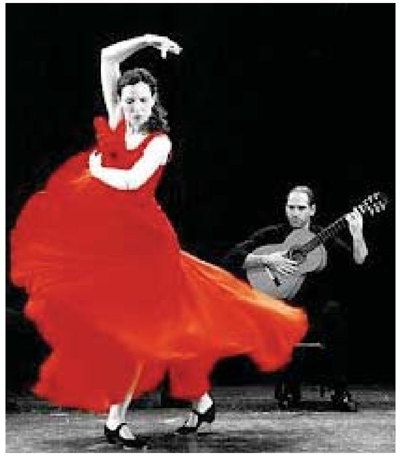
P-element

- Drosophila melanogaster specific transposon
- Discovered in 1970s,
- Not present in the Morgan flies at Columbia University (from 1905)
- Class II transposon, codes for P-transposase, which recognizes the inverted repeats (31 bp) on the flanks.
- P element is active in germ line, which has factors for splicing that are not present in the somatic cells.
- Hybrid dysgenesis.

Pest Control

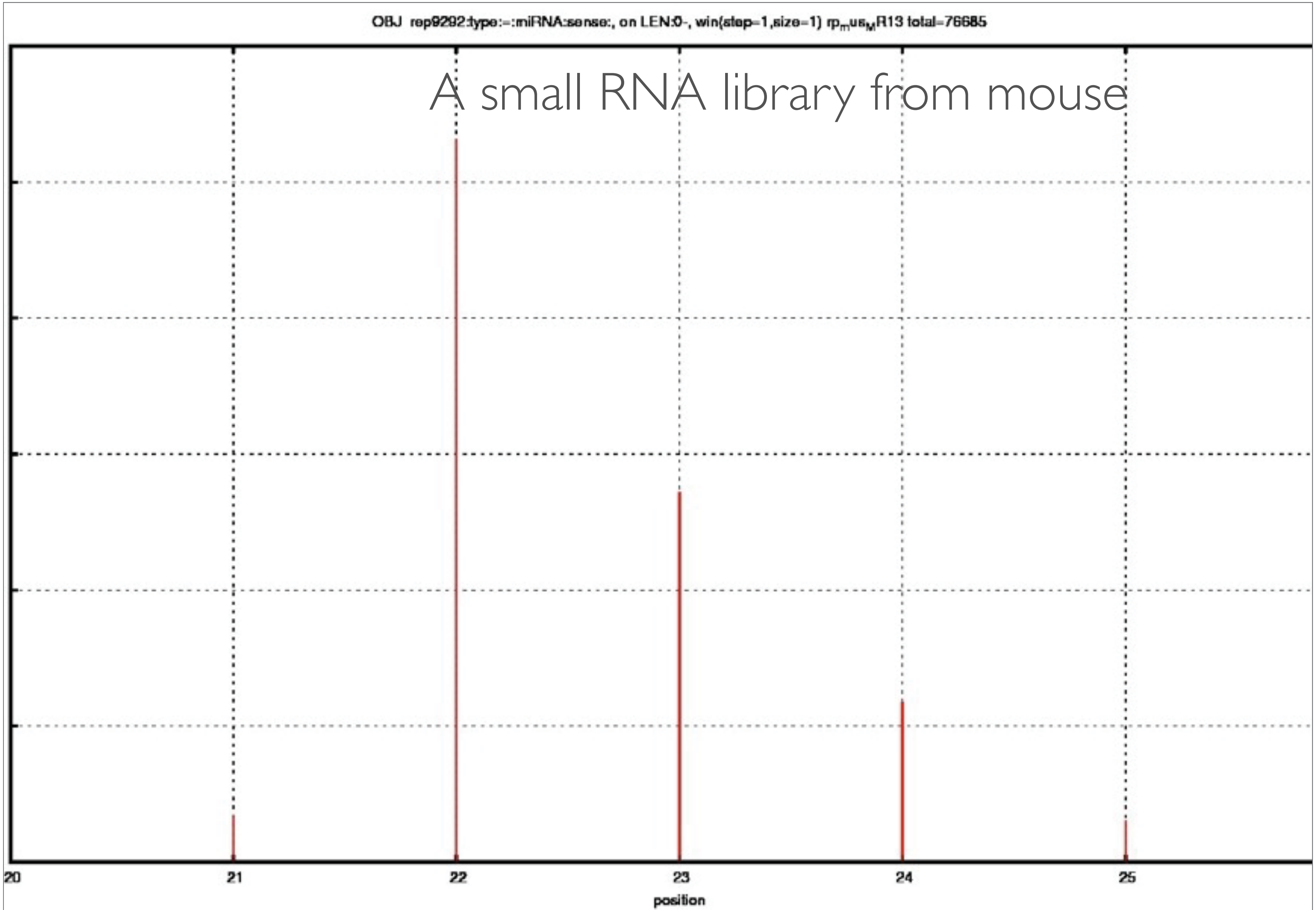
- How do you control them ?
- How do you get rid of them ?
- Against viruses and bacteria, Innate and adaptive immunity
- Against selfish elements, we need an adaptive mechanism, which is provided by the piRNA pathway.

Flamenco locus



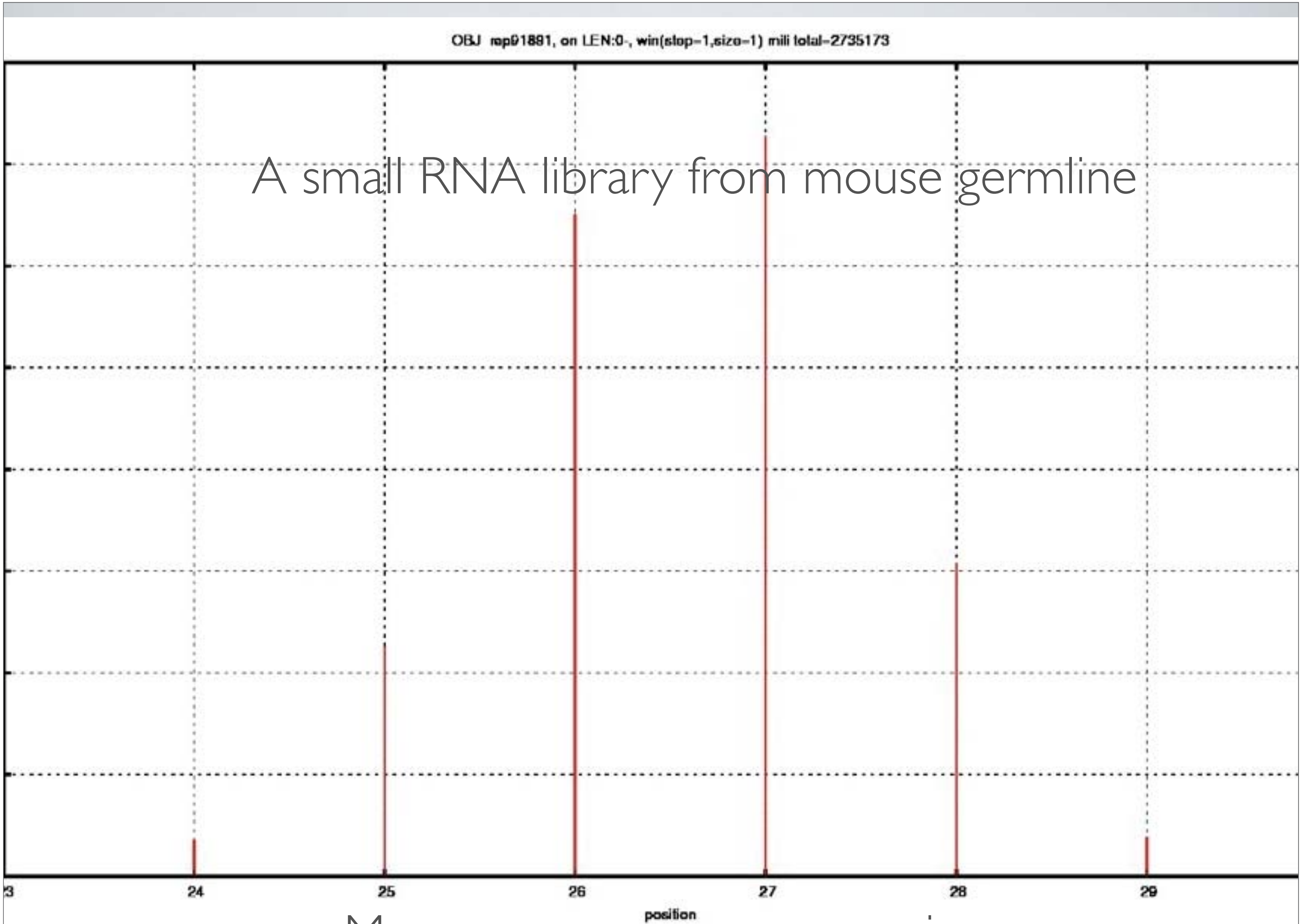
- A repeat rich region of the genome on chr X, far from any coding genes.
- Disrupting it allowed certain transposons (Idefix, Gypsy and Zam) to get active.
- Long futile search for a gene or cause for its potent effect.
- piRNAs solved this mystery.

A small RNA library from mouse



Many sequences, few species

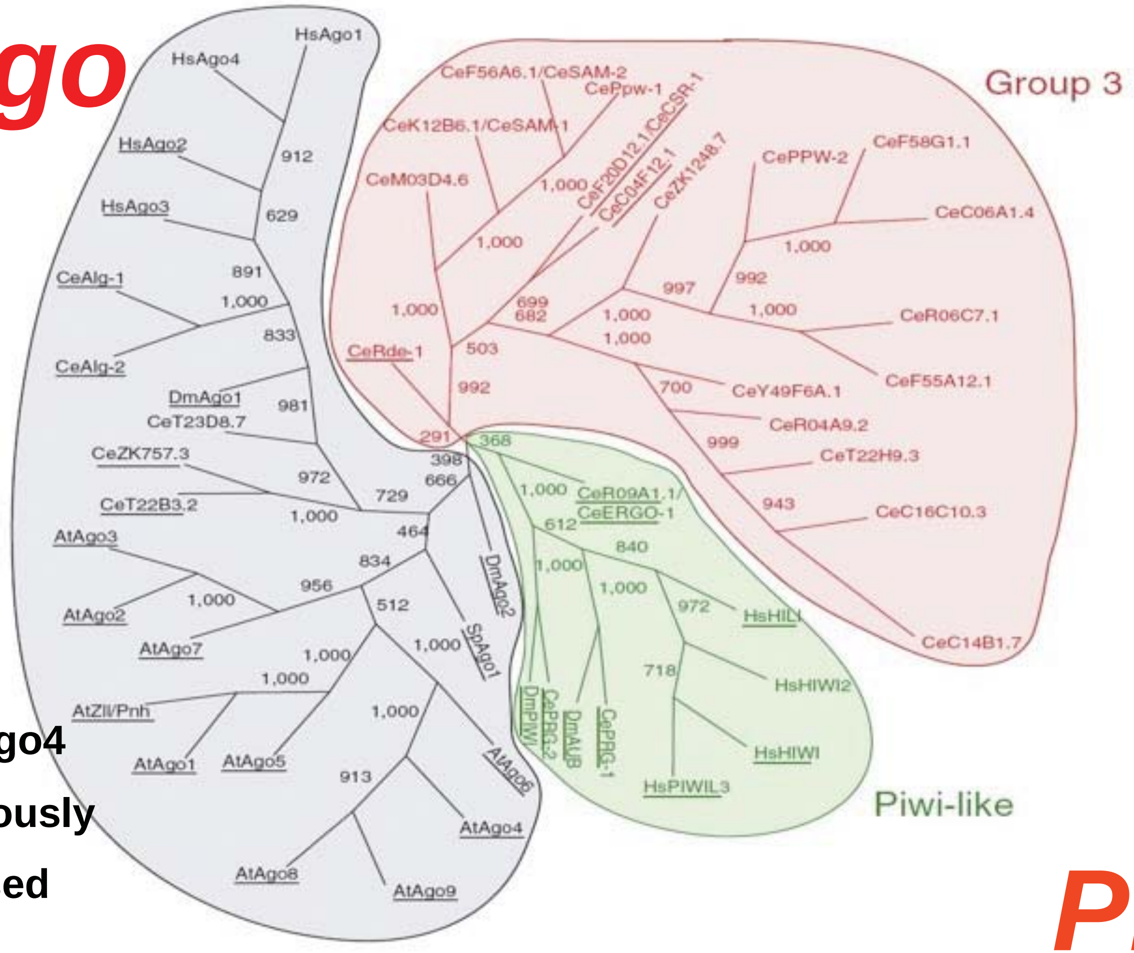
A small RNA library from mouse germline

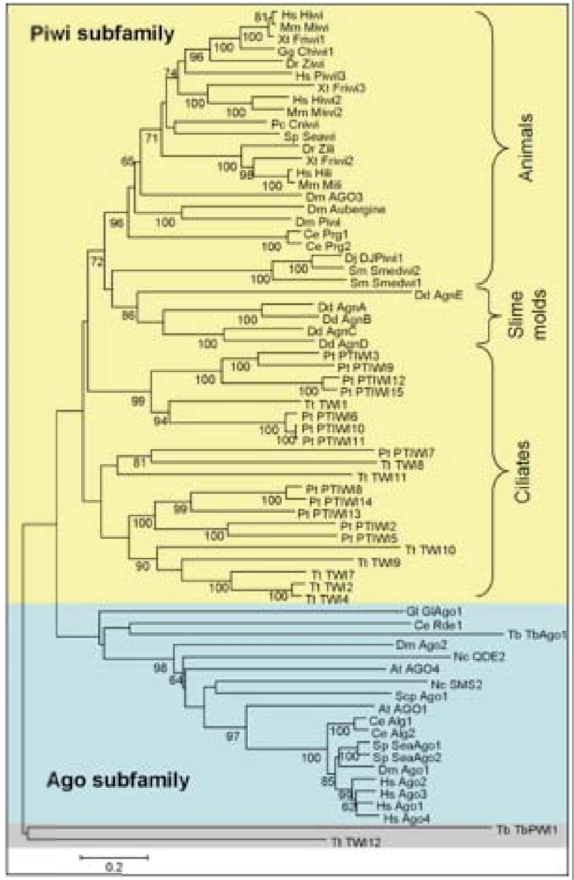


Many sequences, many species

Argonaute family

Sasaki *et al.* 2003





Ago1-Ago4

Ubiquitously

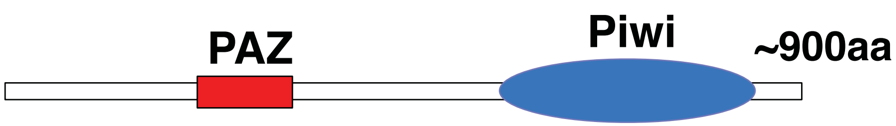
expressed

Piwi

human: hPiwil 1- 4

mice: Miwi, Mili, Miwi2

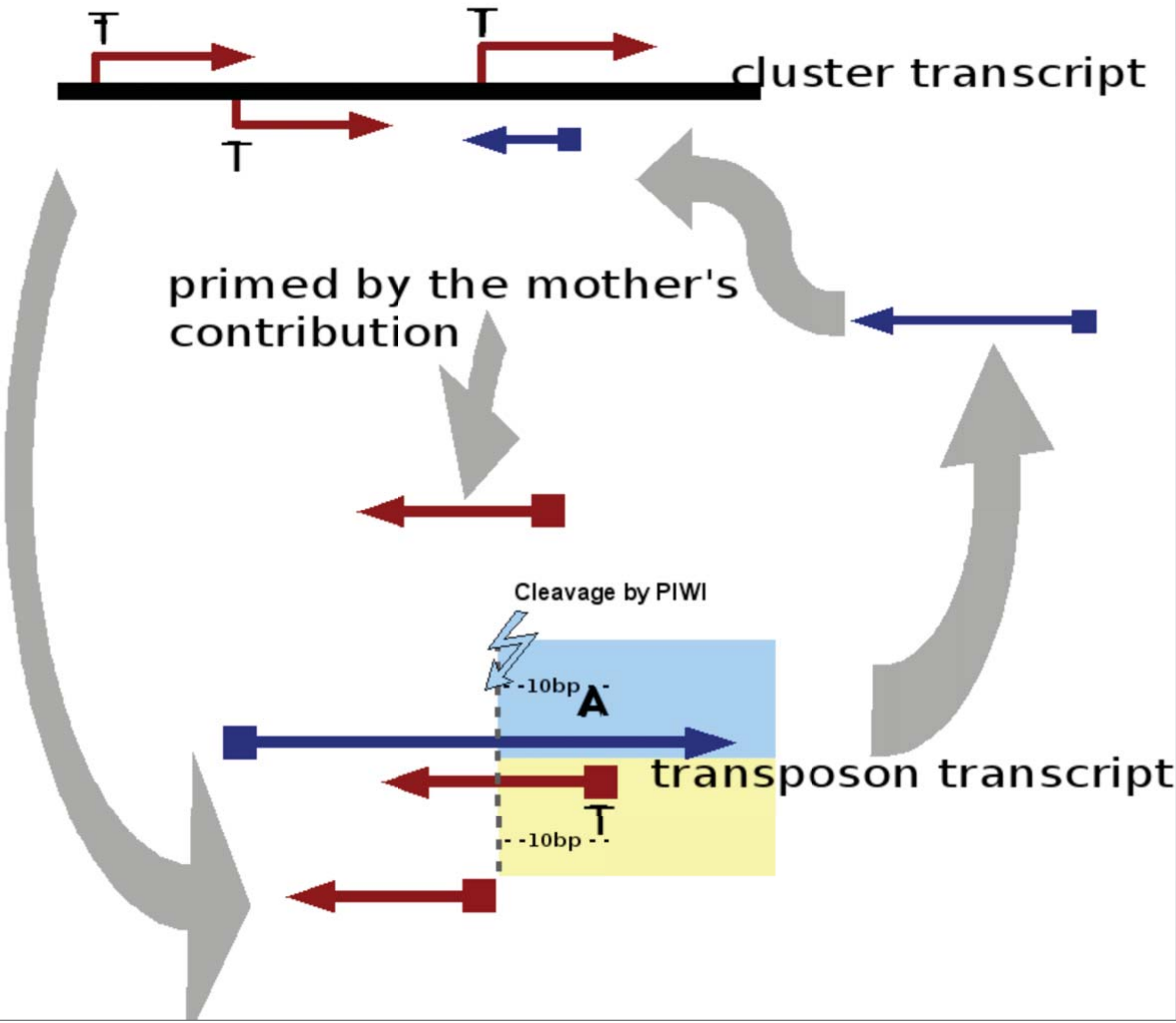
Germline-specific

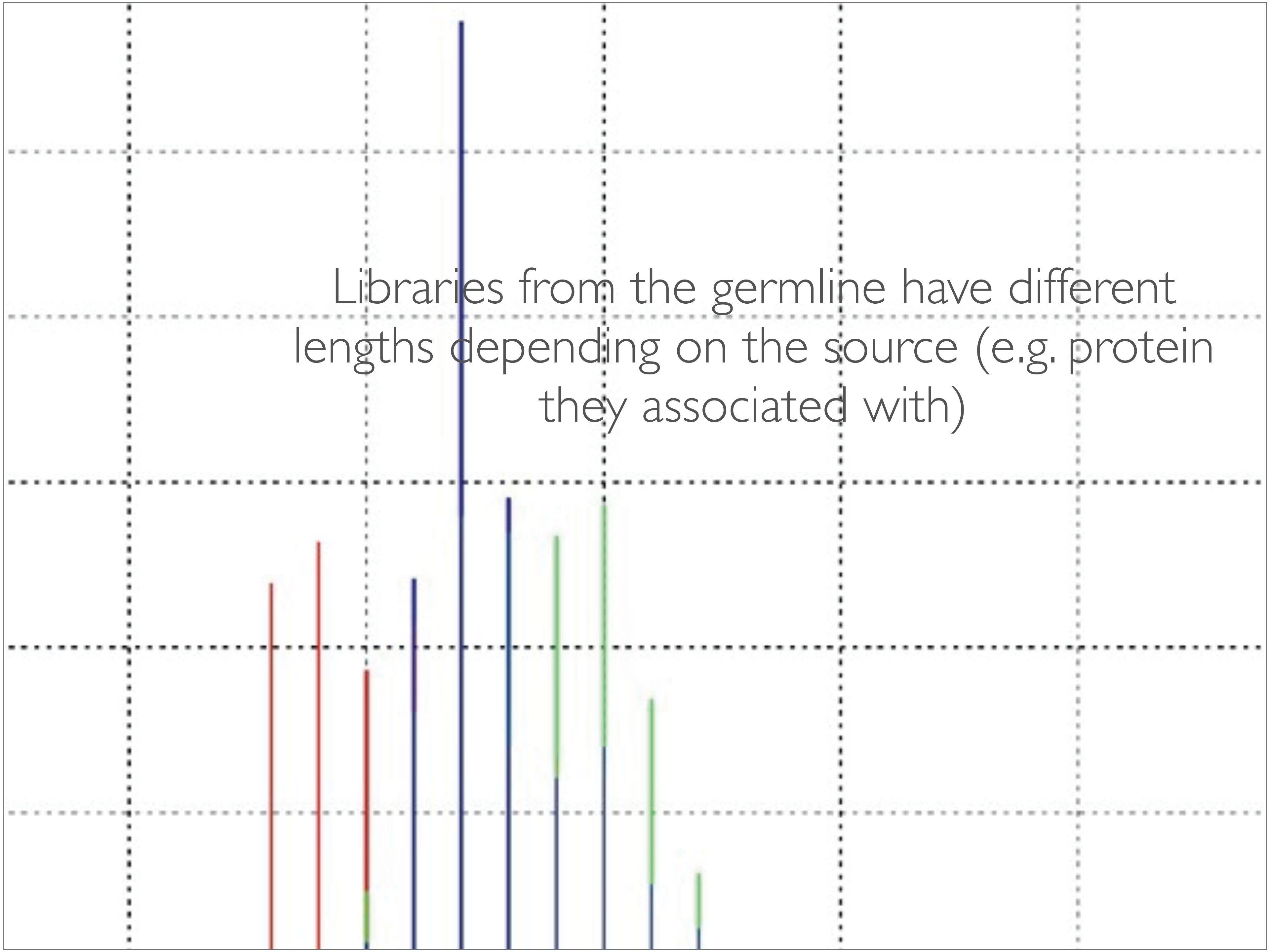


CASE STUDIES

- Libraries from small RNAs associated with AGO3, AUB and PIWI proteins in the drosophila female germline.

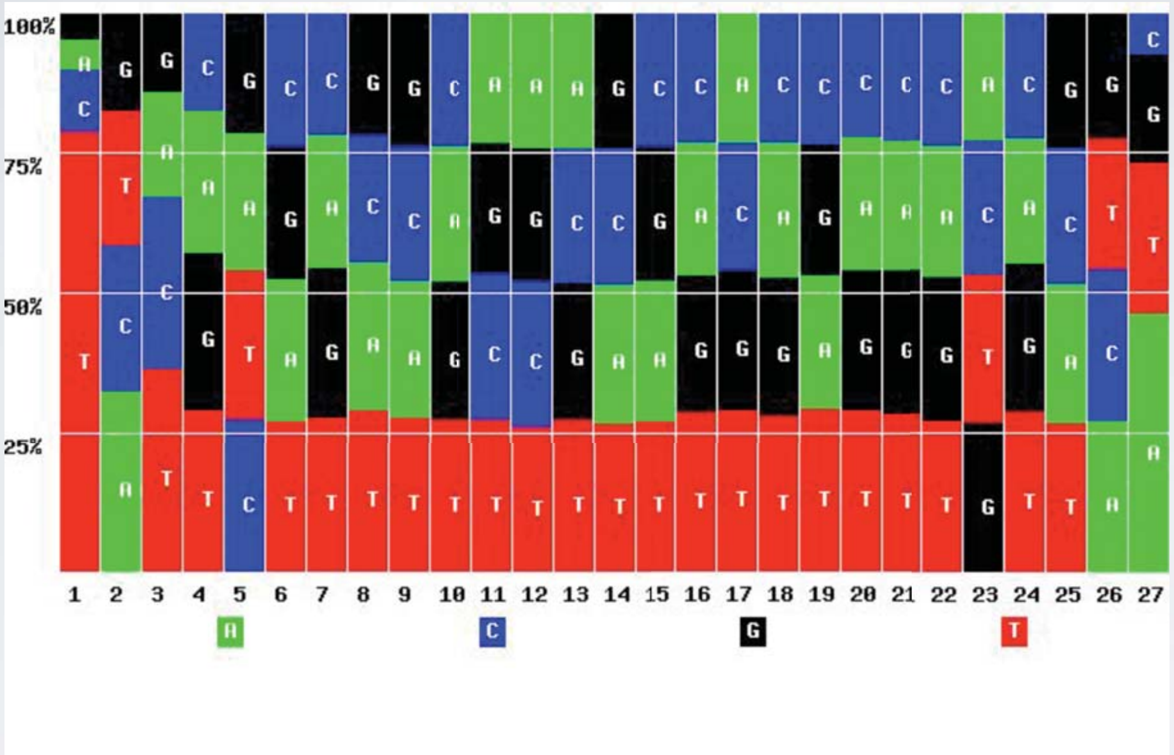
THE PING-PONG MECHANISM





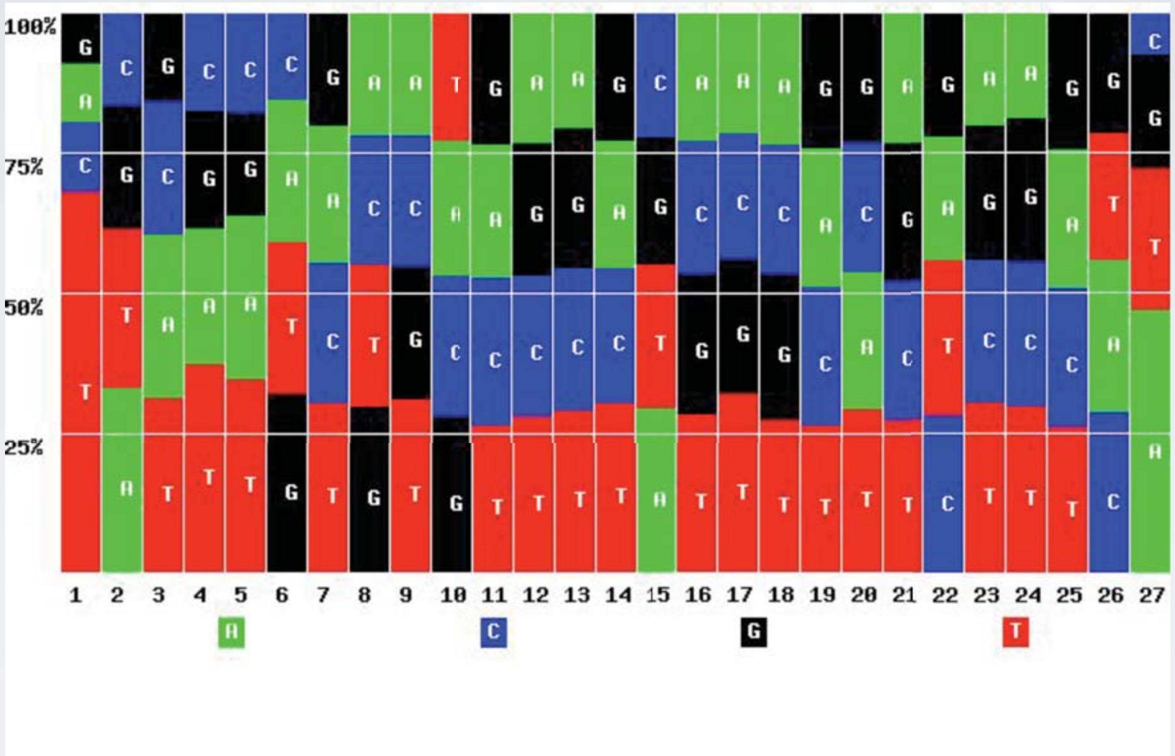
Libraries from the germline have different lengths depending on the source (e.g. protein they associated with)

PIWI ASSOCIATED PIRNAS

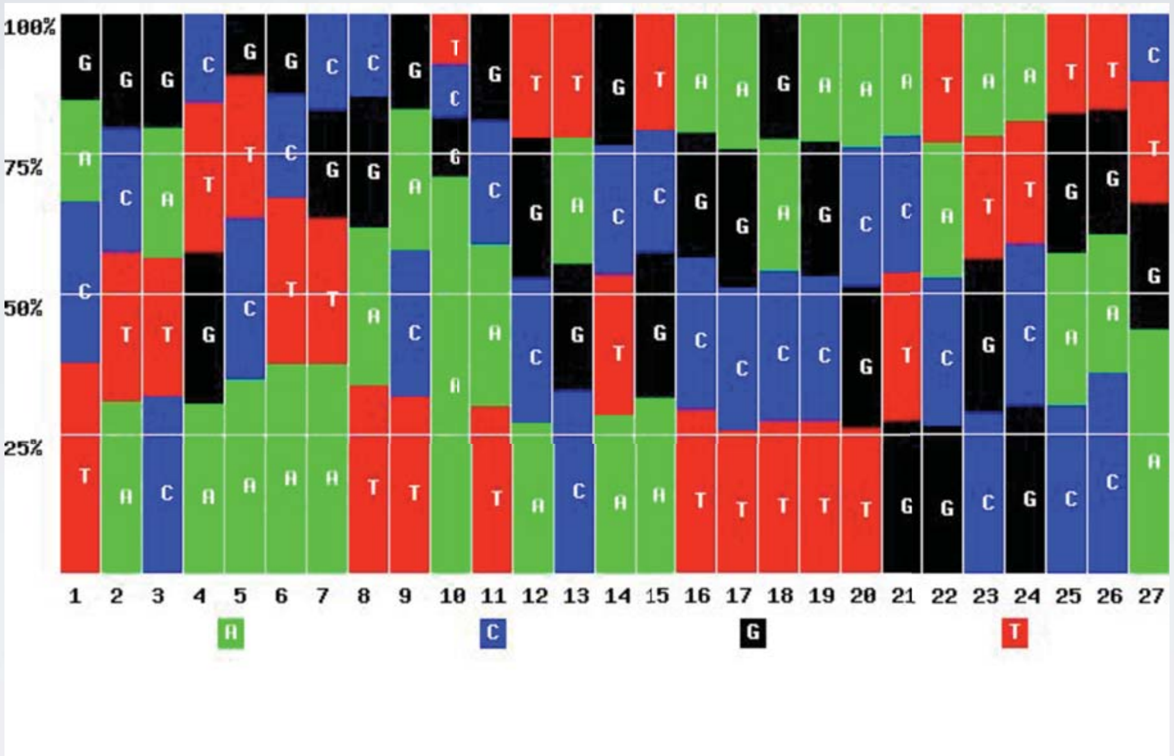


Nucleotide biases at different positions of the piRNA

AUB ASSOCIATED PIRNAS



AGO3 ASSOCIATED PIRNAS



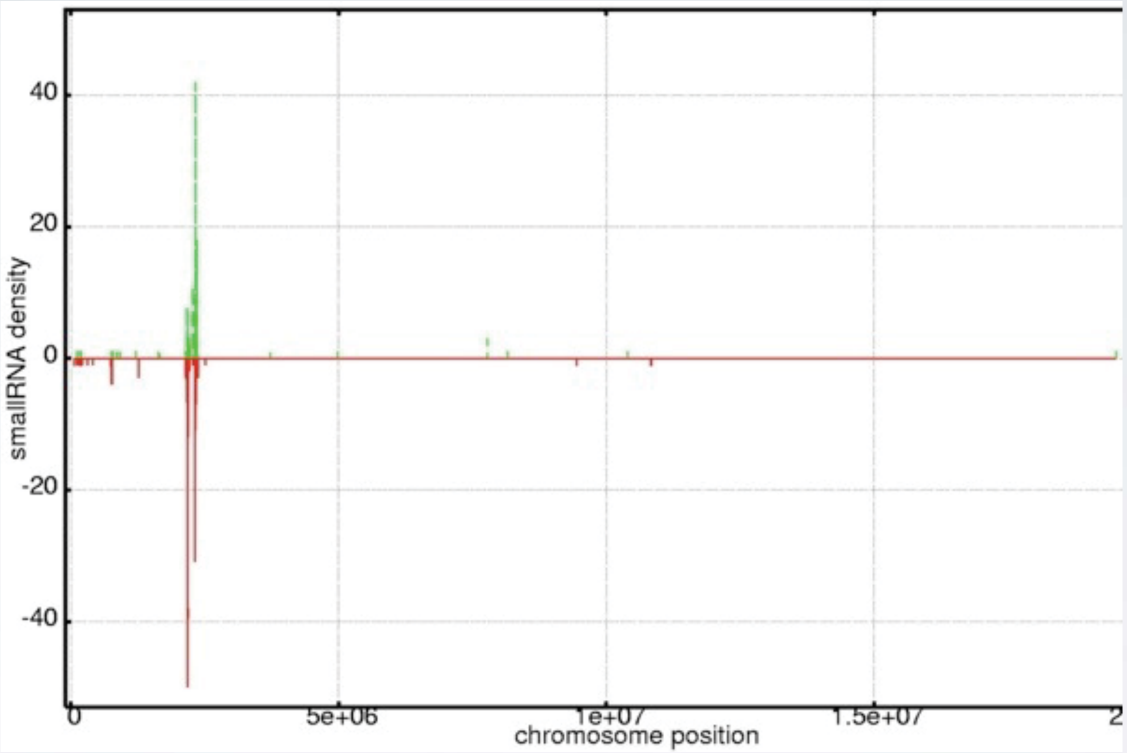
CLASSIFICATION BY TYPE

Annotations	AGO3 piRNAs	AUB piRNAs	PIWI piRNAs
type	IP_AGO3_mult	IP_Aub_mult	IP_Piwi_mult
repeat_antisense	34661	139960	134521
repeat_sense	100447	44396	40739
none	9815	17880	21311
gene_exon_sense	2216	13838	7723
rRNA_antisense	4984	11057	3731
rRNA_sense	2892	6456	4435
tRNA_sense	518	1773	1751
miRNA_sense	2455	1249	817
gene_intron_sense	271	809	1765
snoRNA_sense	186	561	533
type	IP_AGO3_mult	IP_Aub_mult	IP_Piwi_mult

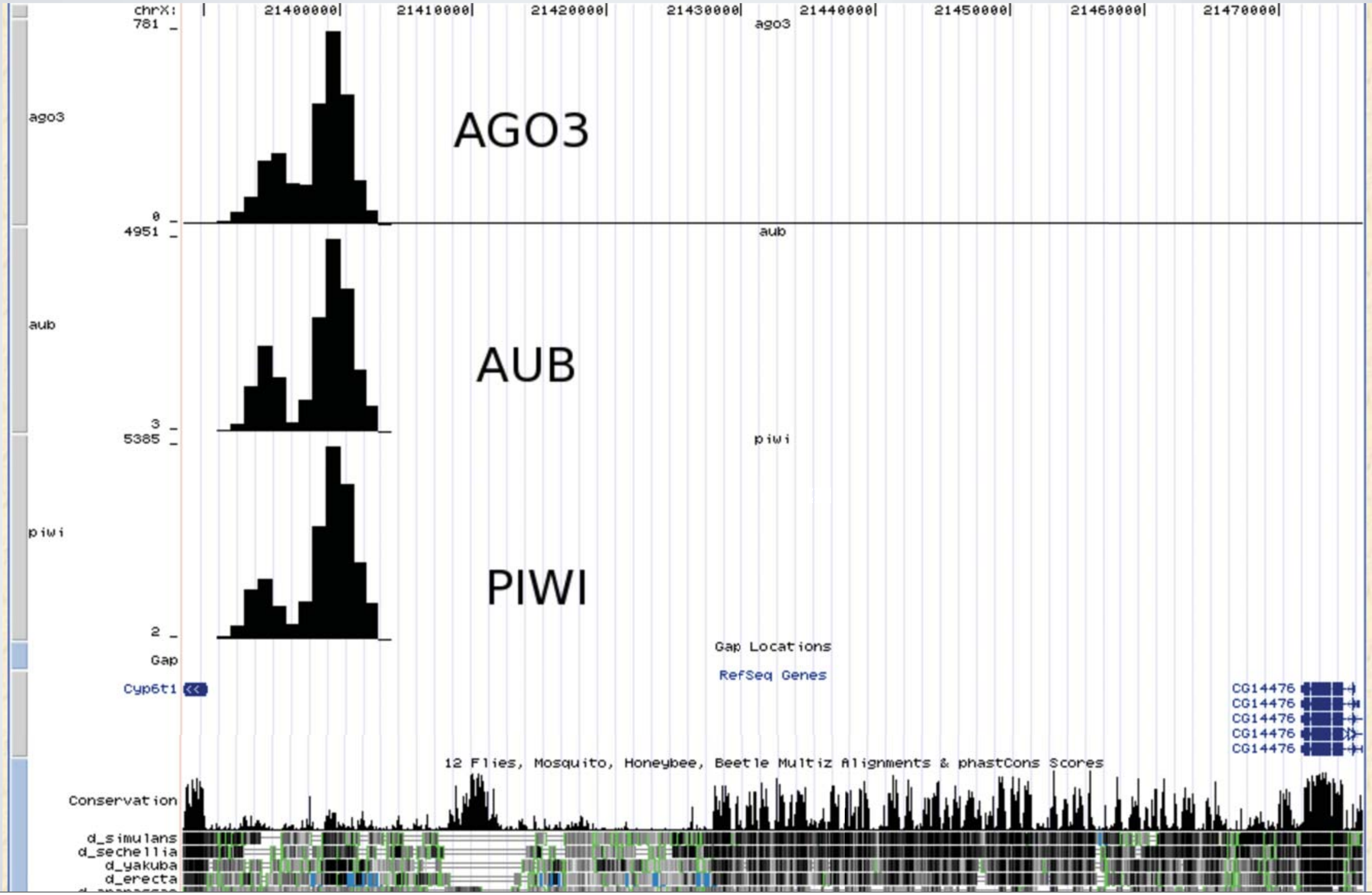
AUB CLUSTERS ON GENOME

chr	chr_start	chr_end	density_plus	density_minus	tot_density	gene_range
arm_X	21391000	21404001	4036	1	4037	ucsc - omim - genbank <------------ *(81430)*-------------> ucsc - omim - genbank
arm_2R	2304000	2360001	1675	1562	3237	ucsc - omim - genbank <------------ *(124995)*-------------> ucsc - omim - genbank
arm_2R	2142000	2199001	901	2045	2946	bin3 (ucsc - omim - genbank)<-- *(5724)* -- Pld (ucsc - omim - genbank) -- *(2251806)* --> CG40958 (ucsc - omim - genbank)
arm_3L	23277000	23312001	739	202	941	CG34031 (ucsc - omim - genbank)<-- *(25225)* -- nAcRalpha-80B (ucsc - omim - genbank) -- *(23332425)* --> CG32230 (ucsc - omim - genbank)

AUB DENSITY ON ARM_2R

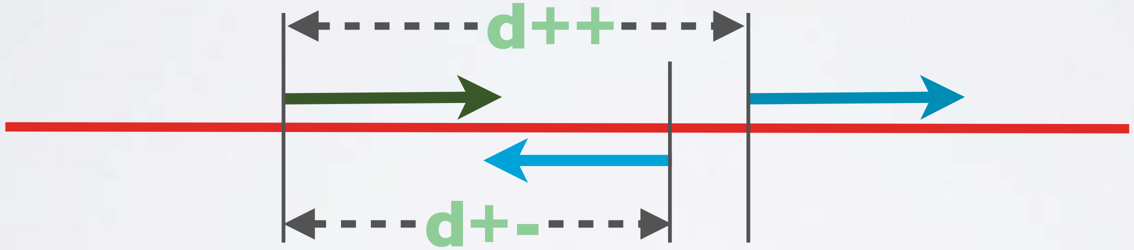


CHRX LOCUS

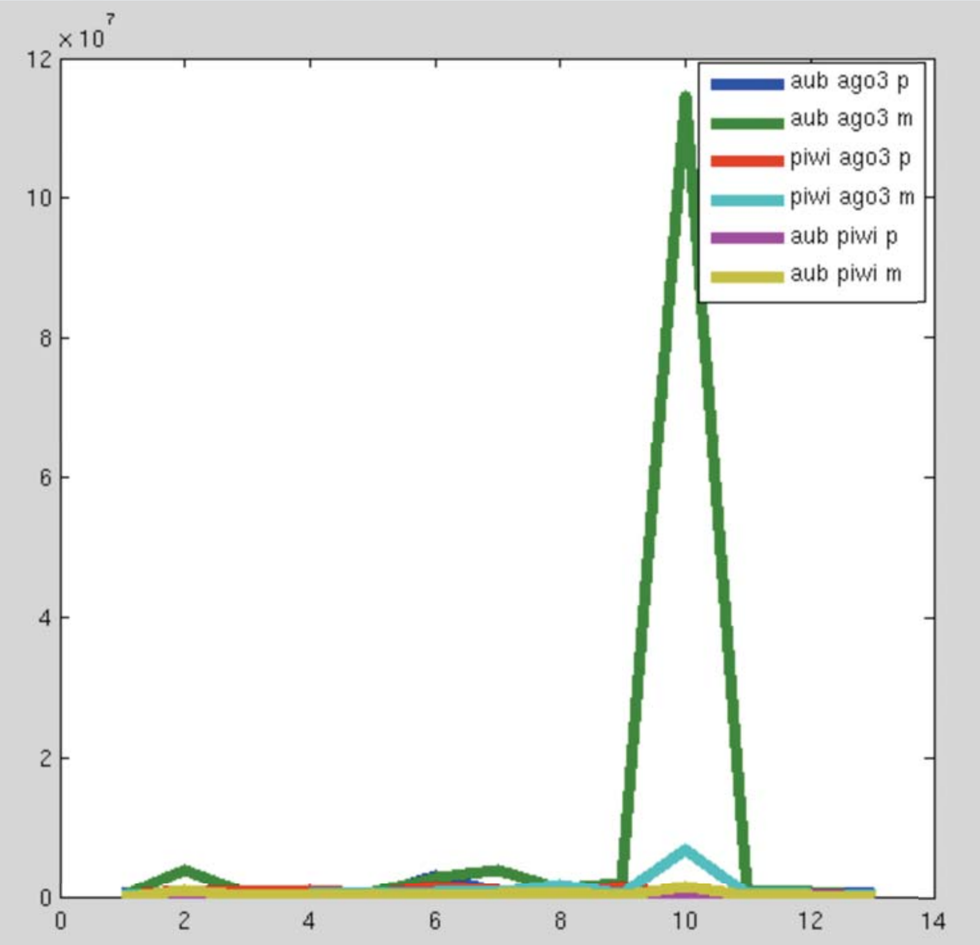

chrX:
21400000
21410000
21420000
21430000
21440000
21450000
21460000
21470000
ago3
781
0
AGO3
aub
4951
3
AUB
piwi
5385
2
PIWI
Gap Locations
Gap
RefSeq Genes
Cyp6t1
CG14476
CG14476
CG14476
CG14476
CG14476
12 Flies, Mosquito, Honeybee, Beetle Multiz Alignments & phastCons Scores
Conservation
d_simulans
d_sechellia
d_yakuba
d_erecta

CORRELATIONS

- Relationship between pairs of libraries.
- A measure spatial relationship, between reads of different orientations.

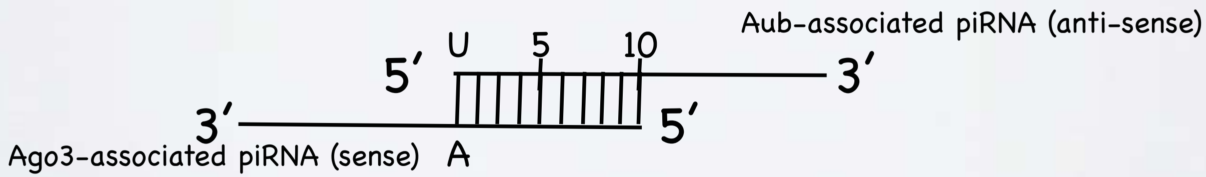


CORRELATIONS BETWEEN LIBRARIES

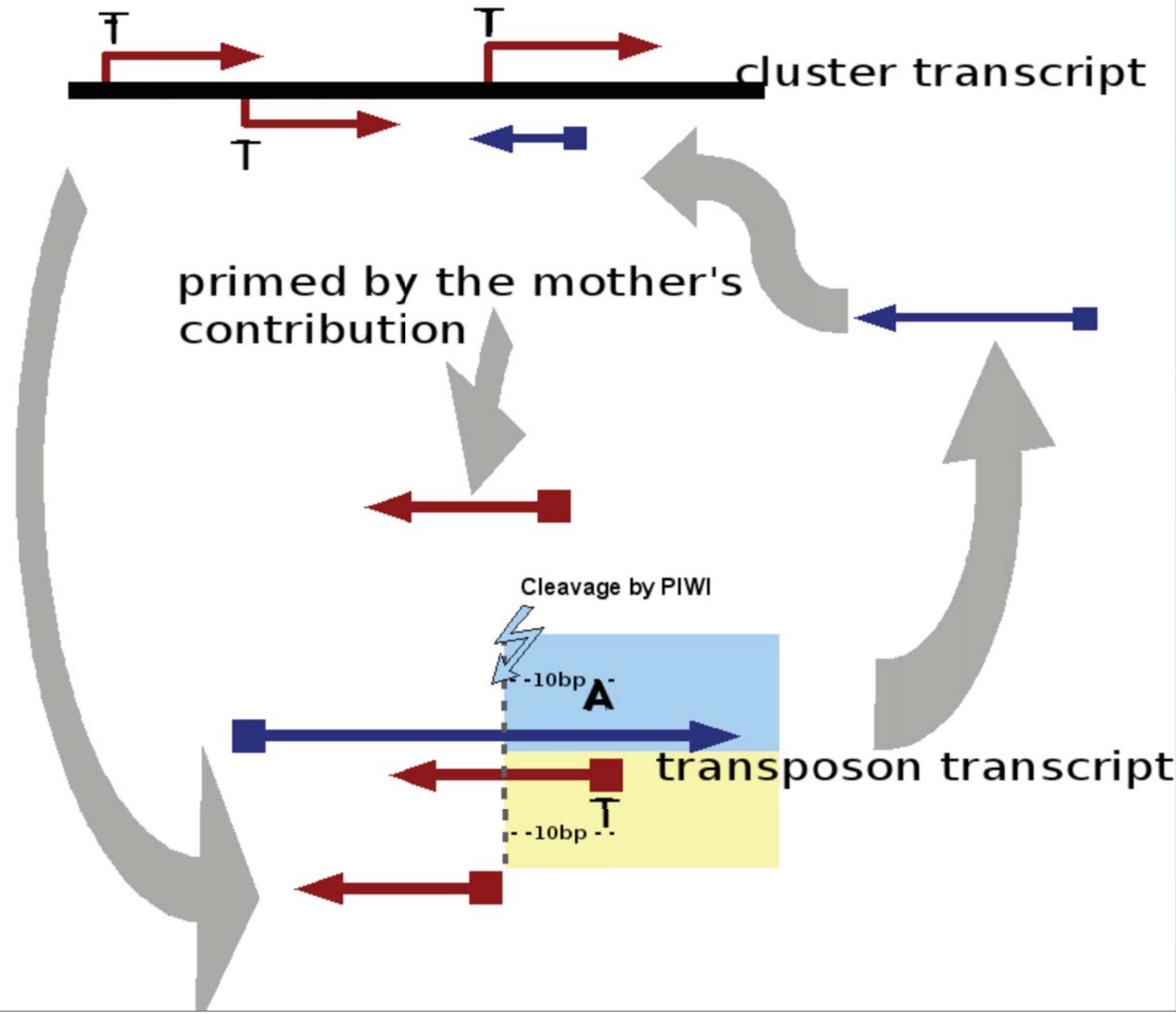


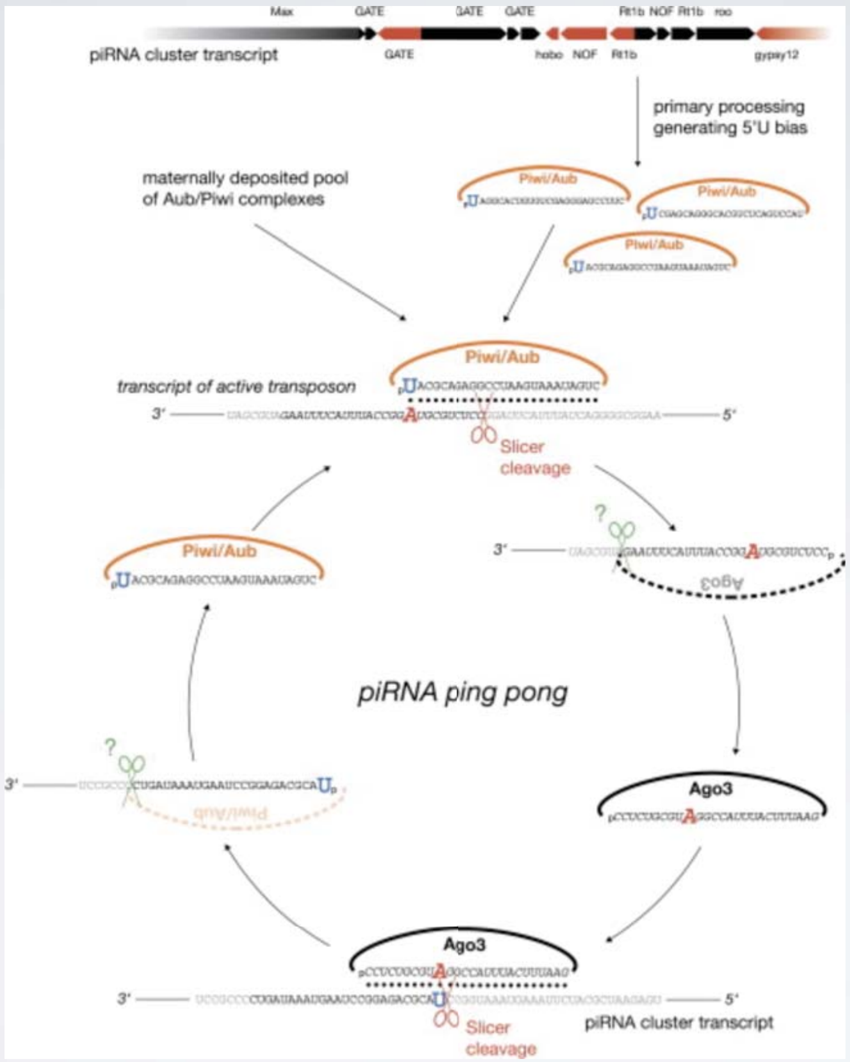
PING-PONG SILENCING OF TRANSPOSONS

- Aubergine associated piRNAs (rasiRNAs) are antisense to transposons.
- The 10-nt offset rasiRNAs (piRNAs) associated with Ago3 are sense to transposons.
- 10-nt is the magic number associated with argonaute's enzymatic activity.

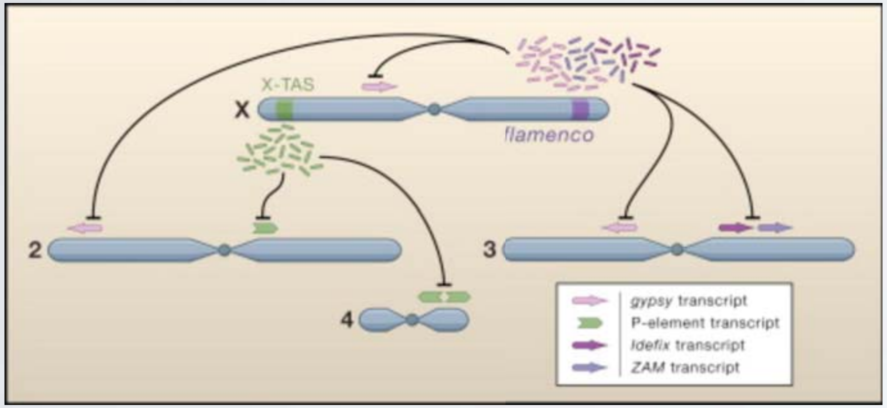


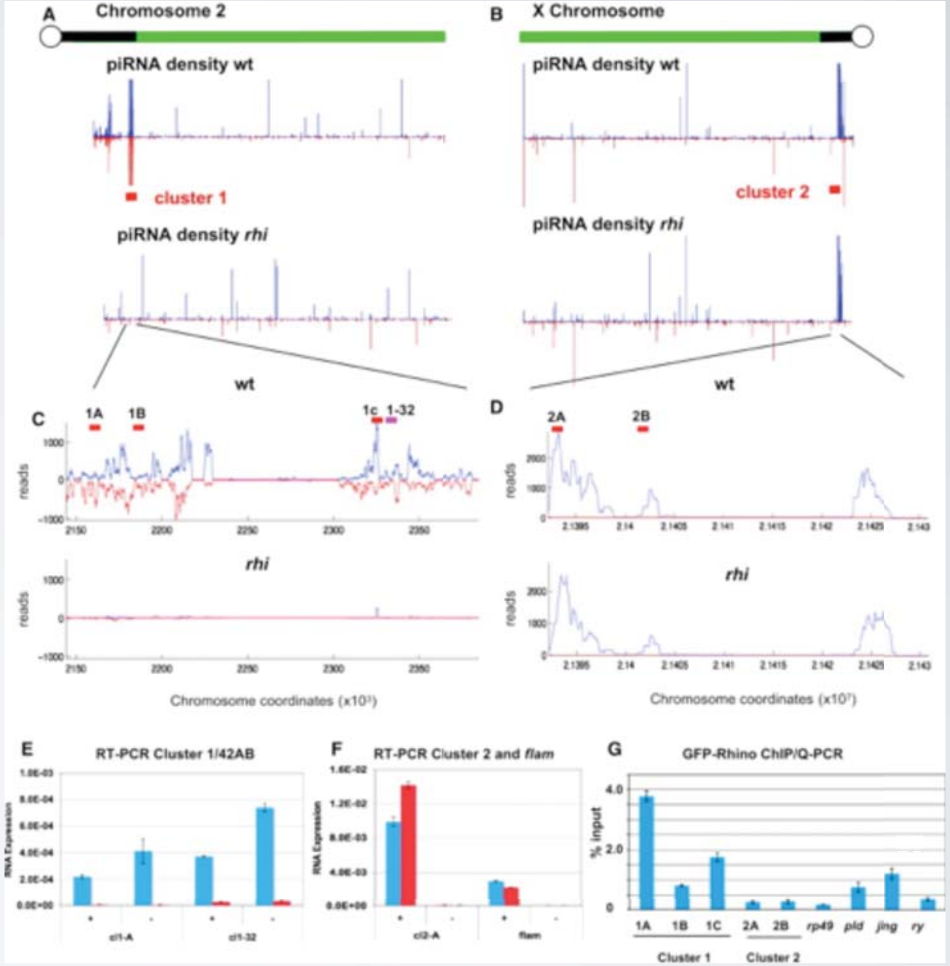
THE PING-PONG MECHANISM





Max
GATE
GATE
GATE
GATE
hobo
NOF
Rt1b
NOF
Rt1b
Rt1b
roo
gypsy12
piRNA cluster transcript
primary processing
generating 5'U bias
maternally deposited pool
of Aub/Piwi complexes
Piwi/Aub
Piwi/Aub
Piwi/Aub
Piwi/Aub
transcript of active transposon
3'
5'
Slicer
cleavage
?
Ago3
Piwi/Aub
piRNA ping pong
?
Piwi/Aub
Ago3
Ago3
3'
5'
piRNA cluster transcript
Slicer
cleavage


X-TAS
X
flamenco
2
3
4
gypsy transcript
P-element transcript
Idefix transcript
ZAM transcript

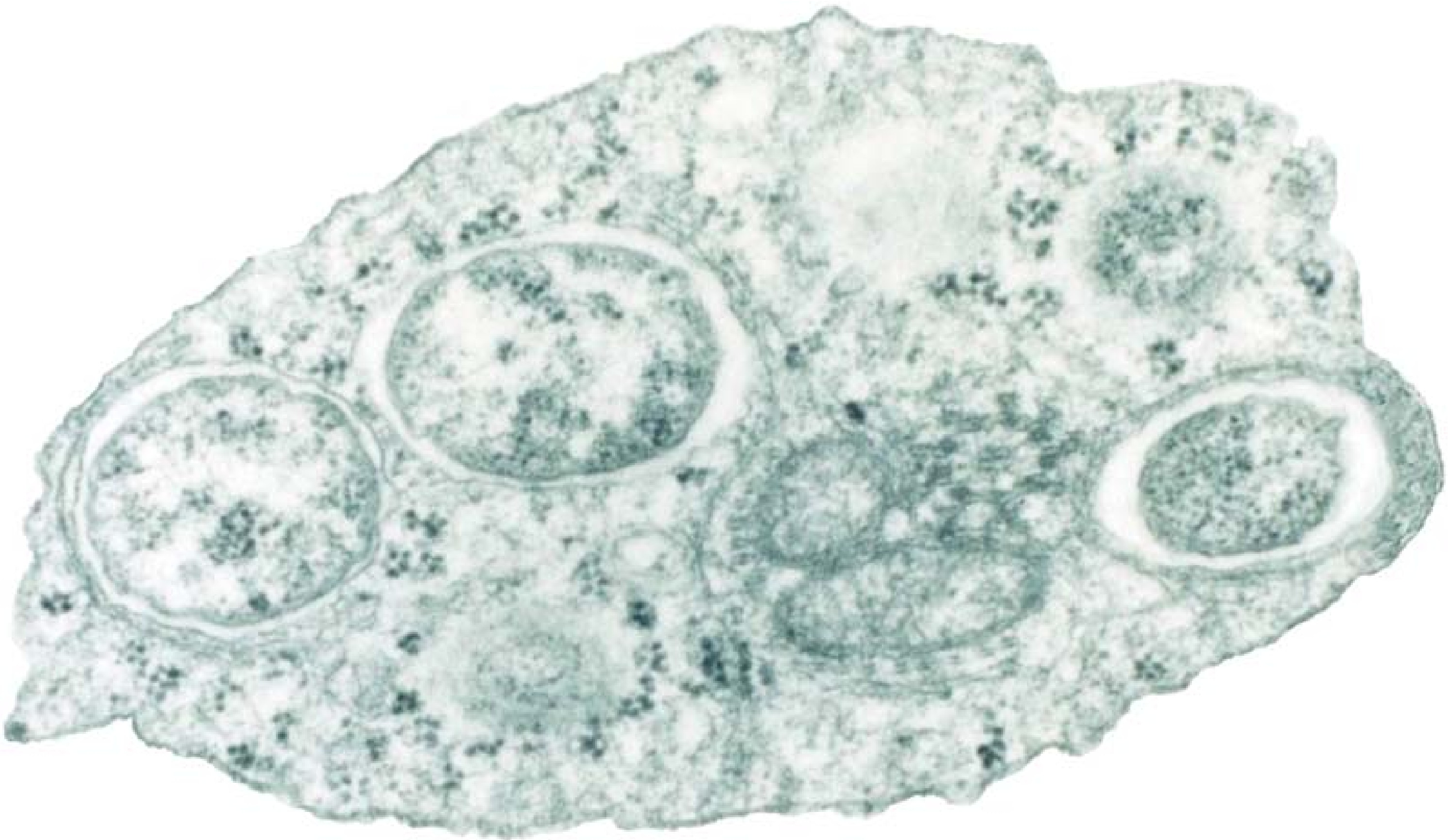

A
Chromosome 2
B
X Chromosome
piRNA density wt
cluster 1
cluster 2
piRNA density rhi
wt
C
D
1A
1B
1c
1-32
2A
2B
reads
rhi
Chromosome coordinates (x10³)
Chromosome coordinates (x10⁷)
E
RT-PCR Cluster 1/42AB
F
RT-PCR Cluster 2 and flam
G
GFP-Rhino ChIP/Q-PCR
% input
1A
1B
1C
2A
2B
rp49
pld
jing
ry
Cluster 1
Cluster 2

CONCLUSIONS

- Massively parallel sequencing generates large amounts of data, but simple-minded analyses is often sufficient to derive biology.
- There is room for data-mining and pattern recognition, only the surface has been scratched.
- There are software and hardware challenges, for data management as well as user-friendly browser-based tools.

Wolbachia

- One of the most abundant and successful endo-symbiont/parasite
- Controls host behaviors and reproductive success



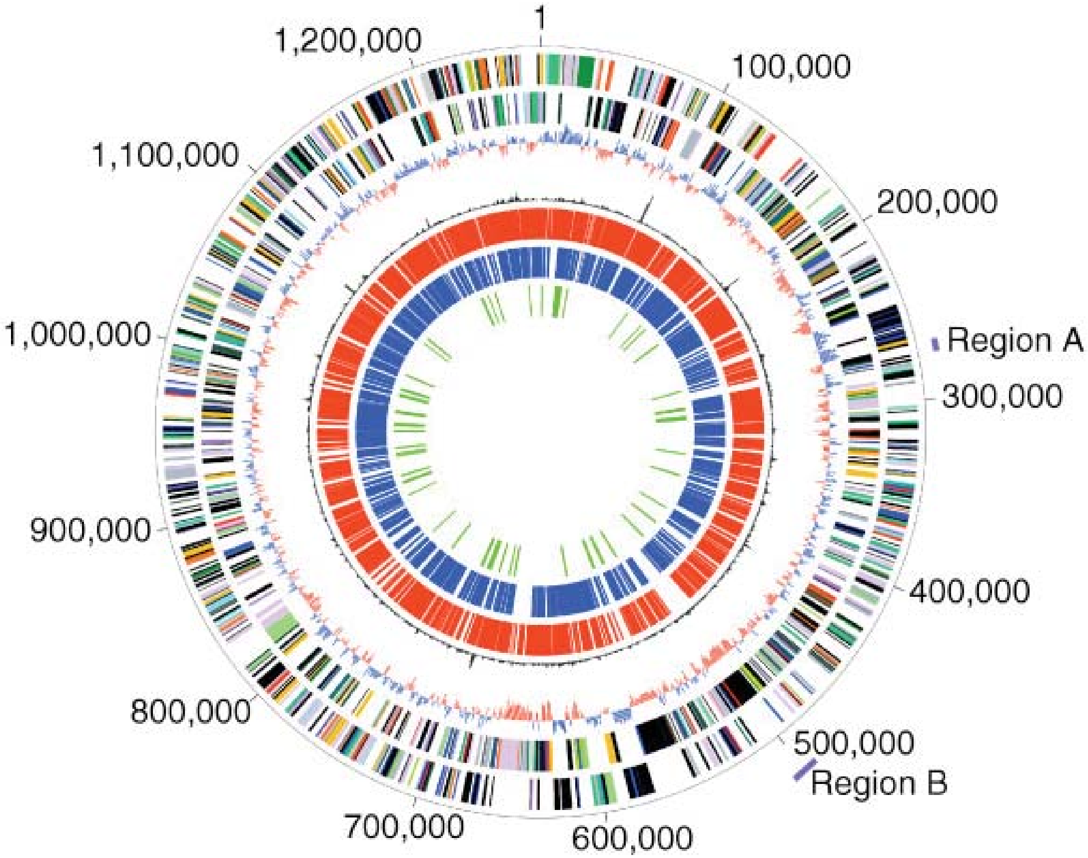
Transmission electron micrograph of Wolbachia within an insect cell.

Sequenced un-intentionally by the D. melanogaster genome project

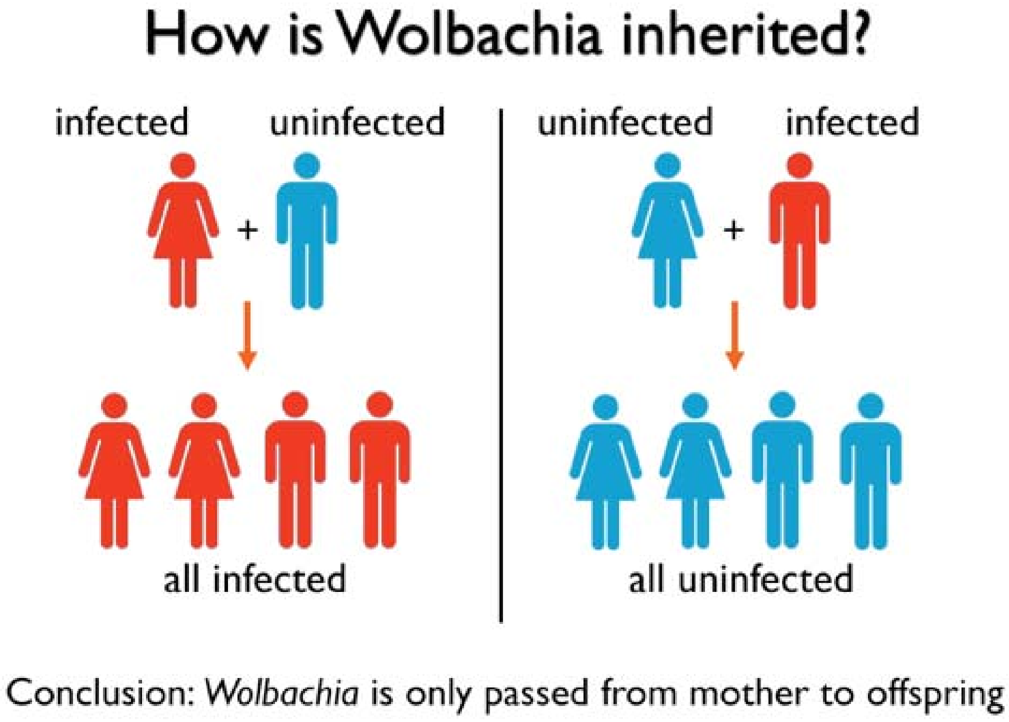
Genome Biol. 2005; 6(3): R23.
Published online 2005 February 22. doi: 10.1186/gb-2005-6-3-r23
PMCID: PMC1088942

Serendipitous discovery of *Wolbachia* genomes in multiple *Drosophila* species

Steven L Salzberg,[1] Julie C Dunning Hotopp,[1] Arthur L Delcher,[1] Mihai Pop,[1] Douglas R Smith,[2] Michael B Eisen,[3] and William C Nelson[1]



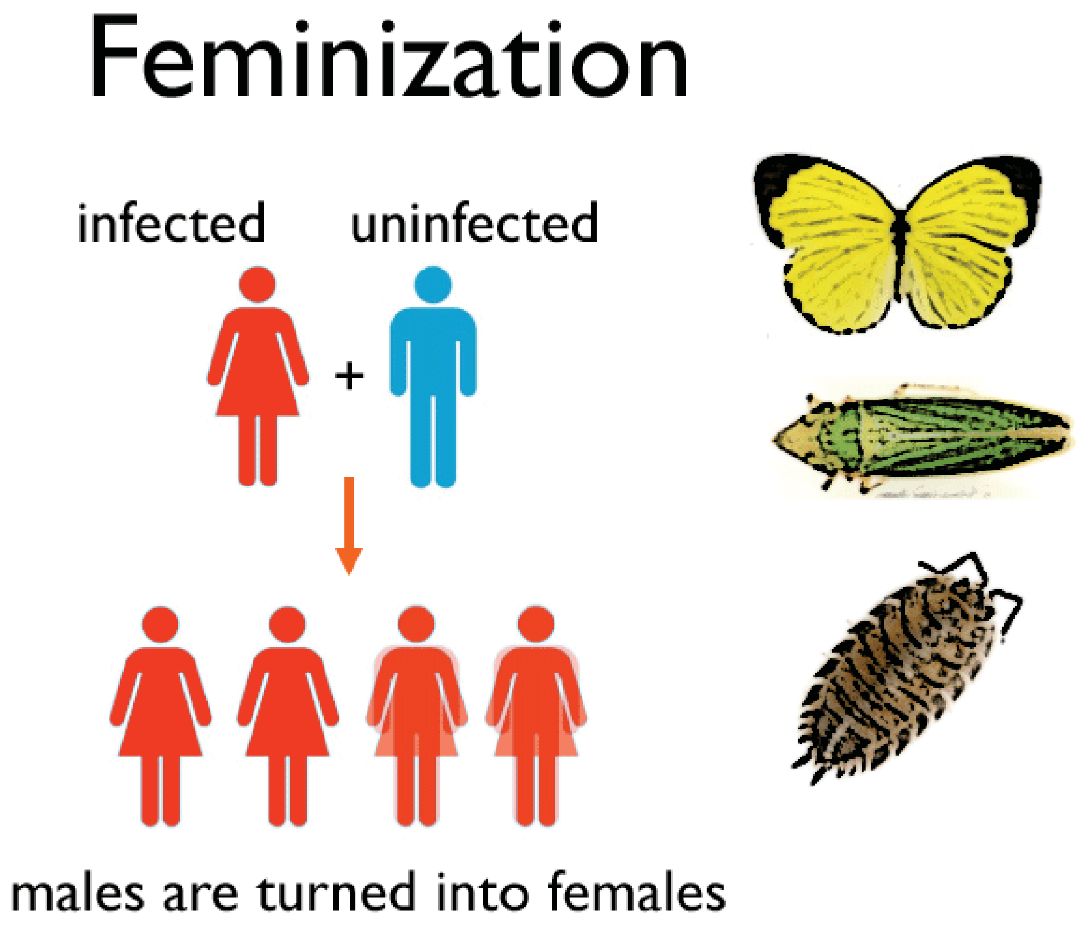
Circular map comparing the *w*Mel genome with the *w*Ana, *w*Sim and *w*Moj assemblies. Ring 1 (outermost ring): forward strand genes; ring 2: reverse strand genes; ring 3: GC-skew plot; ring 4: X2 analysis of trinucleotide composition, with peaks indicating atypical regions; ring 5: *w*Mel genes present in wAna assembly; ring 6: *w*Mel genes present in the wSim assembly; ring 7: *w*Mel genes present in *w*Moj assembly. Large regions on the *w*Mel genome that were not recovered in the *w*Ana or *w*Sim assemblies are marked on the outside (regions A, B).



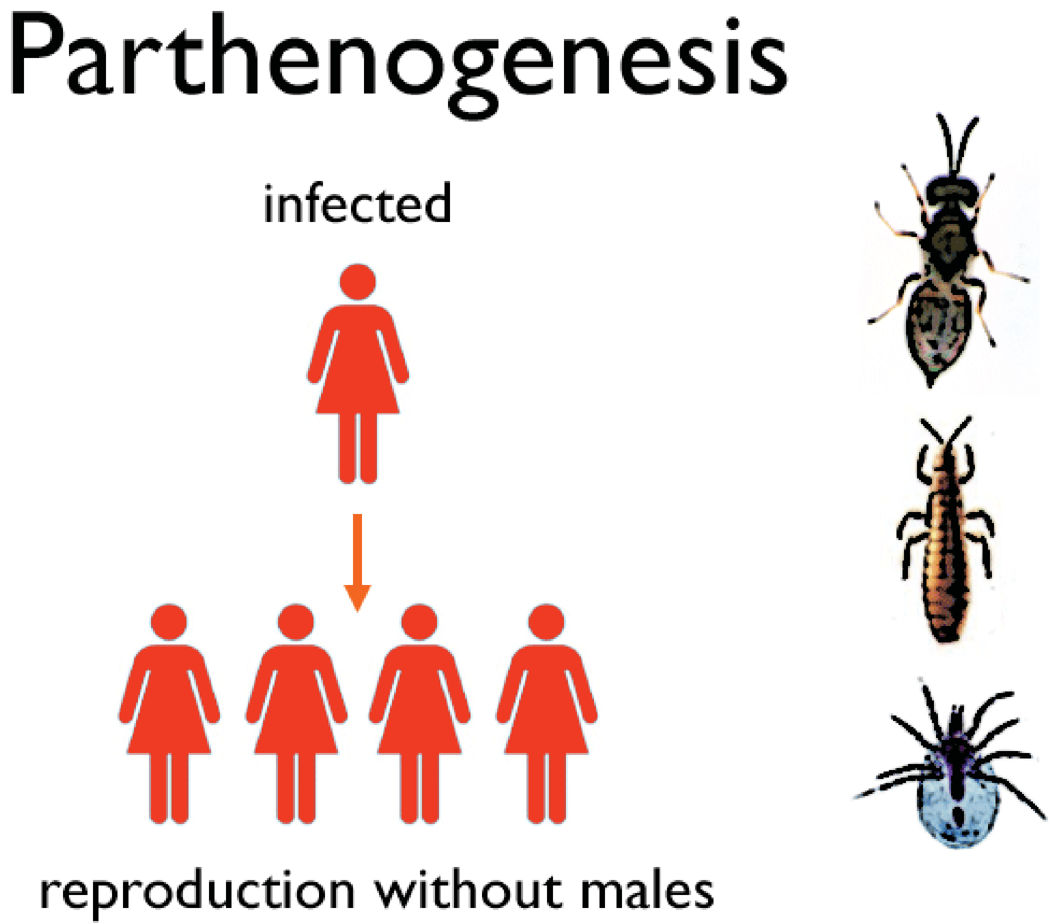
Wolbachia Biology

Within arthropods Wolbachia has evolved four main modes of manipulating host reproductive biology in order to increase it own transmission. Critical to understanding Wolbachia biology and evolution is its mode of transmission. Wolbachia are no easily transmitted from one host to another. Instead, Wolbachia is almost exclusively transmitted from mother to offspring vi infected eggs. Males can be infected with Wolbachia but males DO NOT transmit Wolbachia to offspring or any other hosts The reproductive manipulation of hosts by Wolbachia include 1) feminization of infected males (turning genetic males int females). 2) Induced parthenogenesis (reproduction without males) 3) killing of infected males and 4) Cytoplasmi Incompatibility (CI), the modification of sperm from infected males resulting embryonic defects and death when sperm fertiliz eggs not similarly infected.

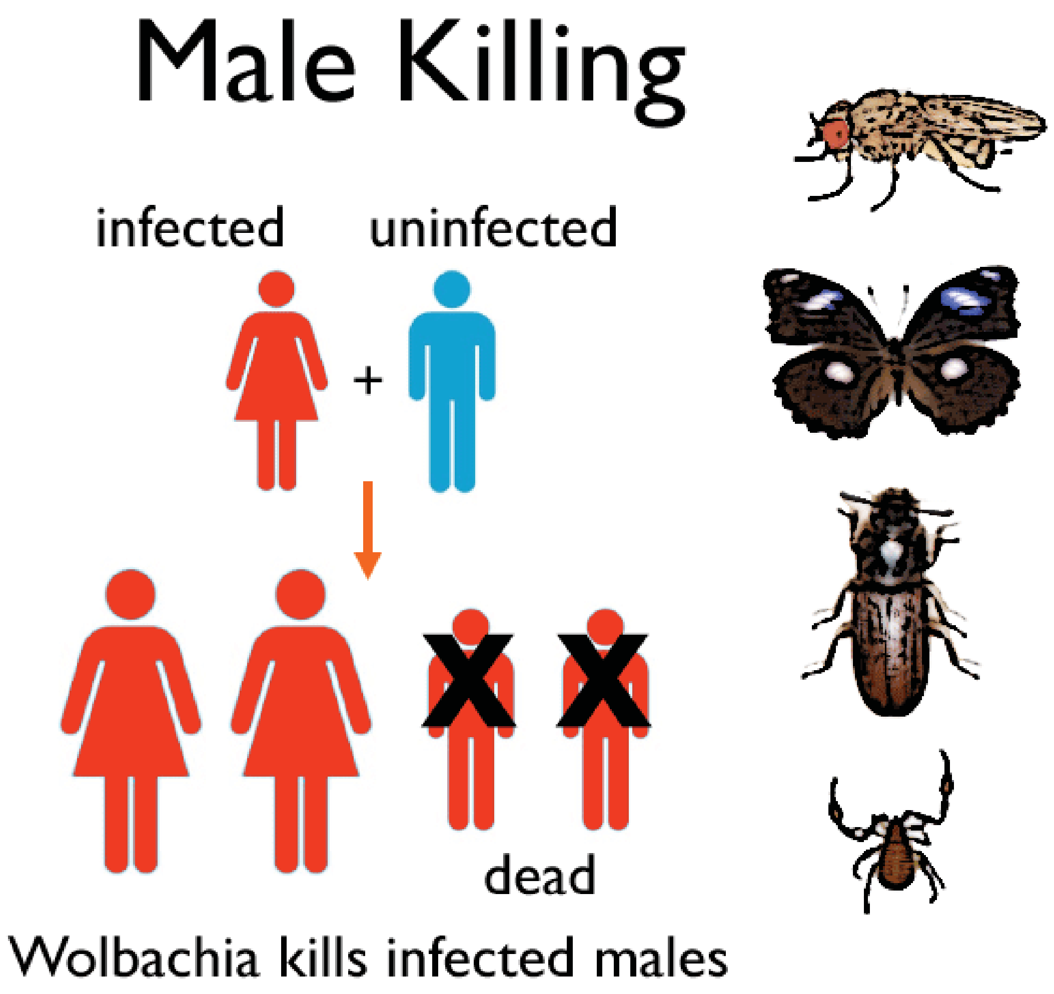
http://www.rochester.edu/college/bio/labs/WerrenLab/WerrenLab-WolbachiaBiology.html



Feminization
Feminization is perhaps the most obviously beneficial strategy for a maternally inherited bacterium such as Wolbachia. With males being dead-ends for the inheritance of most cytoplasmic factors (such as Wolbachia and mitochondria), conversion of infected genetic male offspring into females doubles the potential Wolbachia transmission to the following generation. To date however, Wolbachia-induced feminization is the most infrequently described of the four main Wolbachia-induced phenotypes, reported in only three Arthropod orders. Wolbachia-induced feminization has been documented most commonly in several species of terrestrial isopod within the order Oniscidae. Among insects, Wolbachia-induced feminization is only currently known to occur in Lepidoptera and Hemiptera.

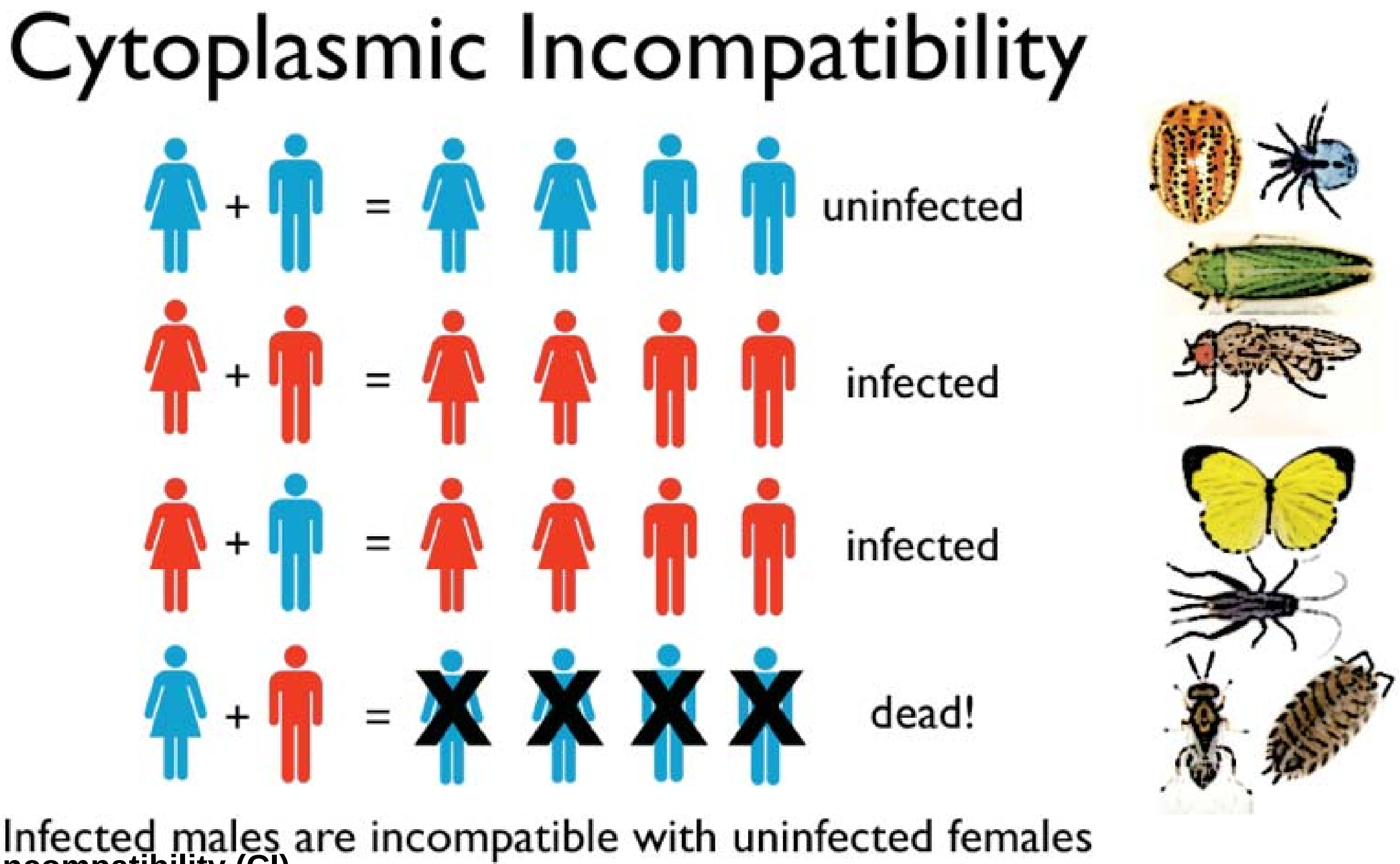


Parthenogenesis
With males being an evolutionary dead end for Wolbachia inheritance, another obvious strategy of host manipulation by a maternally inherited endosymbiont is to induce parthenogenesis, the production of female offspring without fertilization by sperm. As with Wolbachia-induced feminization, parthenogenesis induction doubles the potential transmission of Wolbachia to the next generation, because all the progeny are female. Currently, Wolbachia-induced parthenogenesis is known from three different Arthropod orders; Thysanoptera (Thrips), Acari (mites) and Hymenoptera (wasps).



Male **Killing**

The third phenotype caused by Wolbachia is the killing of genetic males. Wolbachia should only evolve this phenotype when the killing of infected males benefits the surviving infected female siblings. Consequently, only hosts with high sibling competition for resources are those in which male-killing Wolbachia should persist. To date, male-killing Wolbachia infections have been described in four different Arthropod orders. Within insects, these include Diptera, Coleoptera and Lepidoptera. Outside of Insecta, male killing has been reported in pseudoscorpiones (class Arachnida).



Cytoplasmic Incompatibility (CI)

The most commonly described, and phylogenetically diverse Wolbachia-induced phenotype is CI, currently known from at least eight different arthropod orders: Acari, Coleoptera, Diptera, Isopoda, Lepidoptera, Hymenoptera, Homoptera and Orthoptera. CI is manifest when a Wolbachia-infected male mates with a female lacking the same Wolbachia type (either uninfected or infected with a different Wolbachia type). All other combinations of crosses are compatible. In diploid organisms, the result of an incompatible cross is increased embryonic mortality. At its most extreme, when a Wolbachia-infected male mates with an uninfected female, all offspring die (incompatible cross). The same infected male mated to a similarly infected female (compatible cross) sees no increase in offspring mortality. The spread of such Wolbachia through a population is easily explained theoretically. Briefly, in a mixed population (with both infected and uninfected individuals), the presence of Wolbachia-infected males increases the relative fitness of infected females by reducing the fitness of uninfected females.

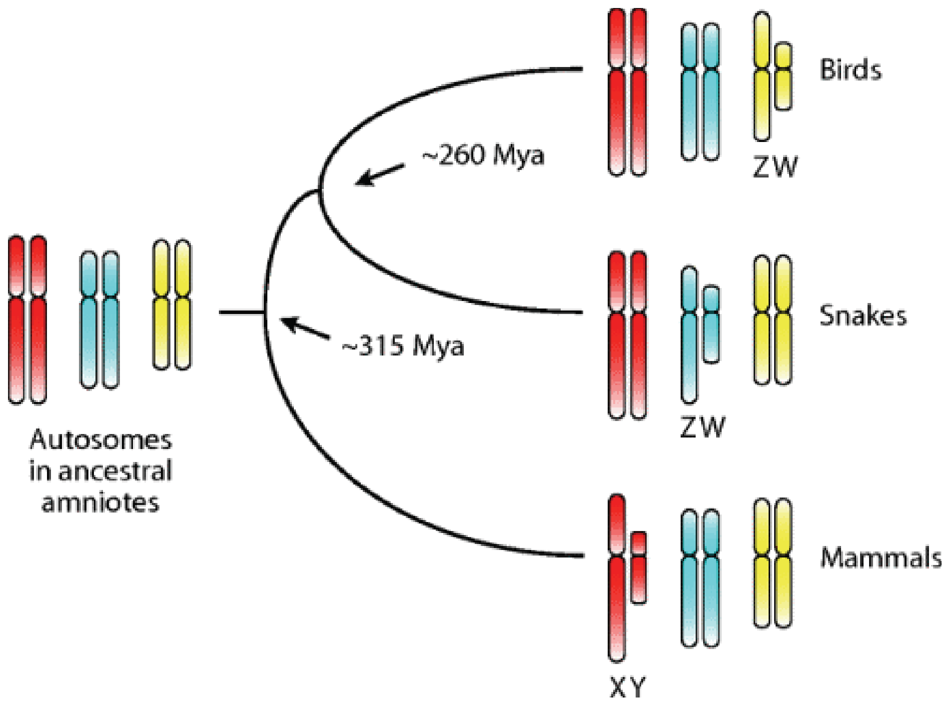
Human Health

- Wolbachia confers immunity to flies against viruses such as flockhouse, Dengue, Malaria. Strategy being tested is to infect mosquitos with wolbachia, and give mosquitoes immunity against malaria, dengue etc. and avoid expensive and harmful methods such as fumigation.
- Nematodes cause elephantiasis, but nematodes need wolbachia to survive, for iron metabolism, here wolbachia acts as an endo-symbiont. So easy to treat patients for wolbachia using cheap antibiotics, which indirectly kills worms, instead of directly trying to kill worms.

Elephantiasis

Haldane's rule

When in the offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous.



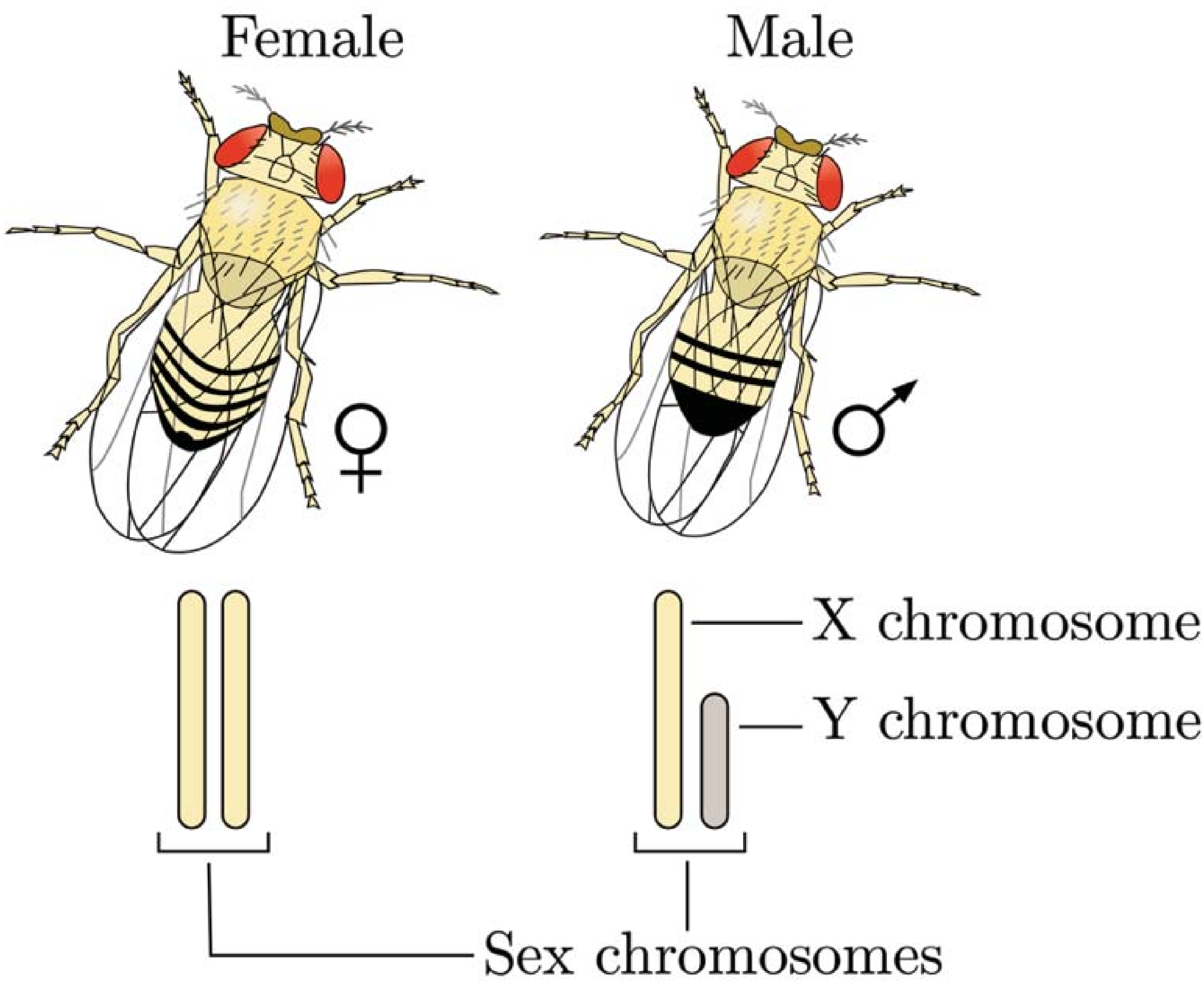
Multiple Independent Origins of Sex Chromosomes in Amniotes

The general consensus in the scientific community is that amniotes were sexually determined by environmental factors originally, and chromosomal sex determination systems appeared late on the scene.

Birds evolved the ZZ/ZW sex determination system, and snakes also evolved this system independently. On the other hand, mammals evolved the XX/XY system independently. The split of the mammals from the rest of the amniotes occurred around 315 million years ago, whereas the archosaurs (birds, crocs, dinosaurs, possibly turtles) diverged from the lepidosaurs (snakes, lizards) around 260 million years ago.

There are 2 ways to explain the presence of the ZZ/ZW system in both birds and snakes. The first model is that the ZZ/ZW system appeared before archosaurs and lepidosaurs diverged, and some of the archosaurs/ lepidosaurs reversed to temperature-dependent sex determination later. This model has serious problems, as the regaining of a feature is considered extremely unlikely in the Theory of Evolution.

The second model predicts that snakes and birds developed the ZZ:ZW system independently. This system fits the Theory of Evolution nicely, and has been proven genetically. After all, the autosome being converted to sex chromosomes in birds is different from that in snakes.



In this system, females have XX chromosomes, and are known as the **homogametic** sex; males have XY chromosomes, and are called the **heterogametic** sex. This system is found in most mammals (including humans), some insects of the genus ***Drosophila***, and some plants of the genus ***Gingko***.

XX/X0 System

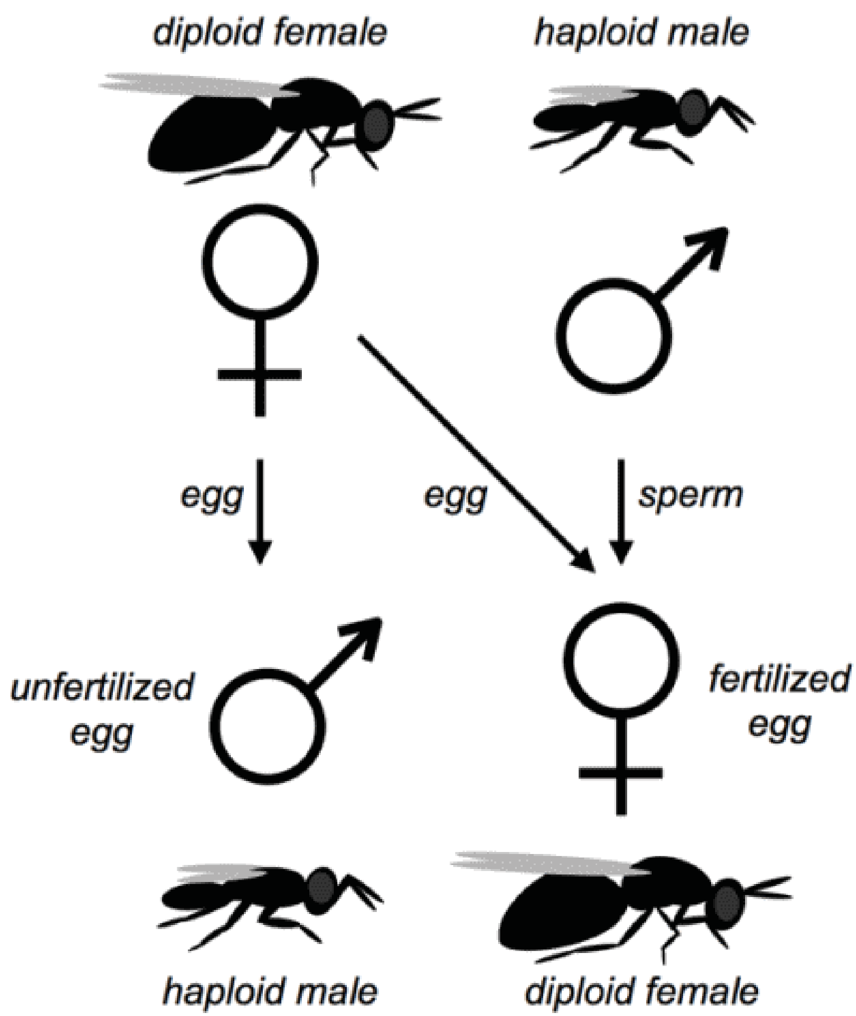
This system is similar to the XX/XY system above. Females have XX chromosomes, while males have X0 chromosomes (the 0 indicates none). This system is observed in a number of insects, including the grasshoppers and crickets of order ***Orthoptera*** and in cockroaches (order ***Blattodea***).

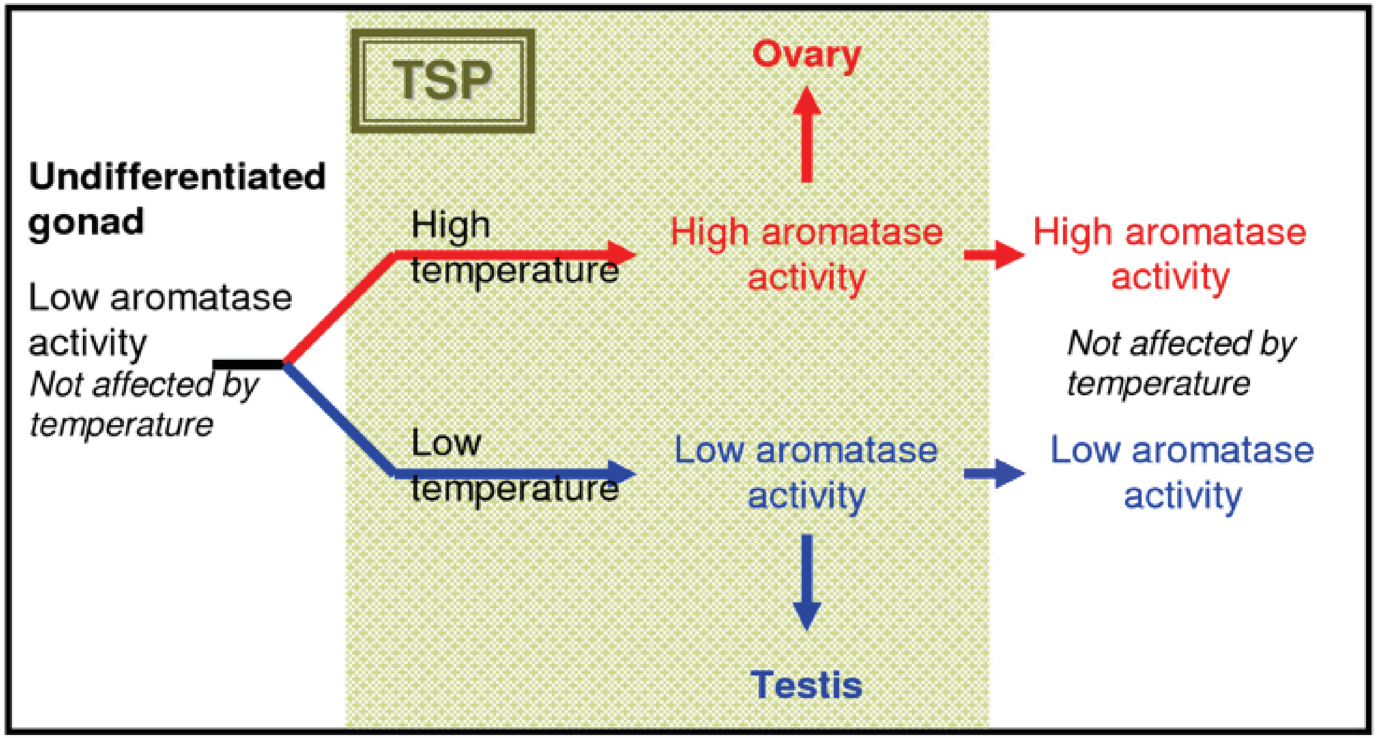
ZZ/ZW System

In this system, it is the ovum that determines the sex of the offspring, instead of the sperm as the XX/XY and the XX/X0 system. In this system, males are the homogametic sex with ZZ chromosomes; while females are heterogametic with ZW chromosomes. This is the system used in birds, some fish, and some insects (including butterflies and moths), and some reptiles, including Komodo dragons.

Haplodiploid System

This system is special in the sense that an offspring that is formed from the union of a sperm and an egg (fertilised) becomes a female; an unfertilised offspring becomes a male. Males have have only half the chromosome count of females, and are haploid; females are diploids. This system determines the sex of the offspring of many **hymenopterans** (bees, ants, and wasps), spider mites, coleopterans (bark beetles) and rotifers.





Temperature-dependent sex determination

The sex of the offspring of this system is determined by the temperature of the eggs. Instead of chromosomal sex determination systems, this is a environmental sex determination system. The eggs are affected by the temperature at which they are incubated during the middle one-third of embryonic development. This critical period of incubation is known as the thermo-sensitive period (TSP).

Polyphenic System

A sex-determining polyphenism allows a species to reproduce normally while permitting different sex ratios. In tropical clown fish, the dominant individual in a group becomes female while the other ones are male, and blue wrasse fish are the reverse. If the dominant individual dies, another individuals will change its sex and replace it. This system ensures that there will always be a mating couple when two individuals of the same species are present.

Other Systems

Some species, such as some snails, practice sex change: adults start out male, then become female. In ***Bonellia viridis***, larvae become males if they make physical contact with the female, and females if they end up on soil. In some **arthropods**, sex is determined by infection, as when bacteria of the genus ***Wolbachia*** alter their sexuality; some species consist entirely of ZZ individuals, with sex determined by the presence of *Wolbachia*.

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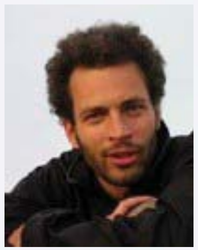
Colin Malone

Ian Weisberger

Bruce Gelb

Bob Desnick

Valentina Di pierro



Julius Brennecke

Rozy Rampersaud