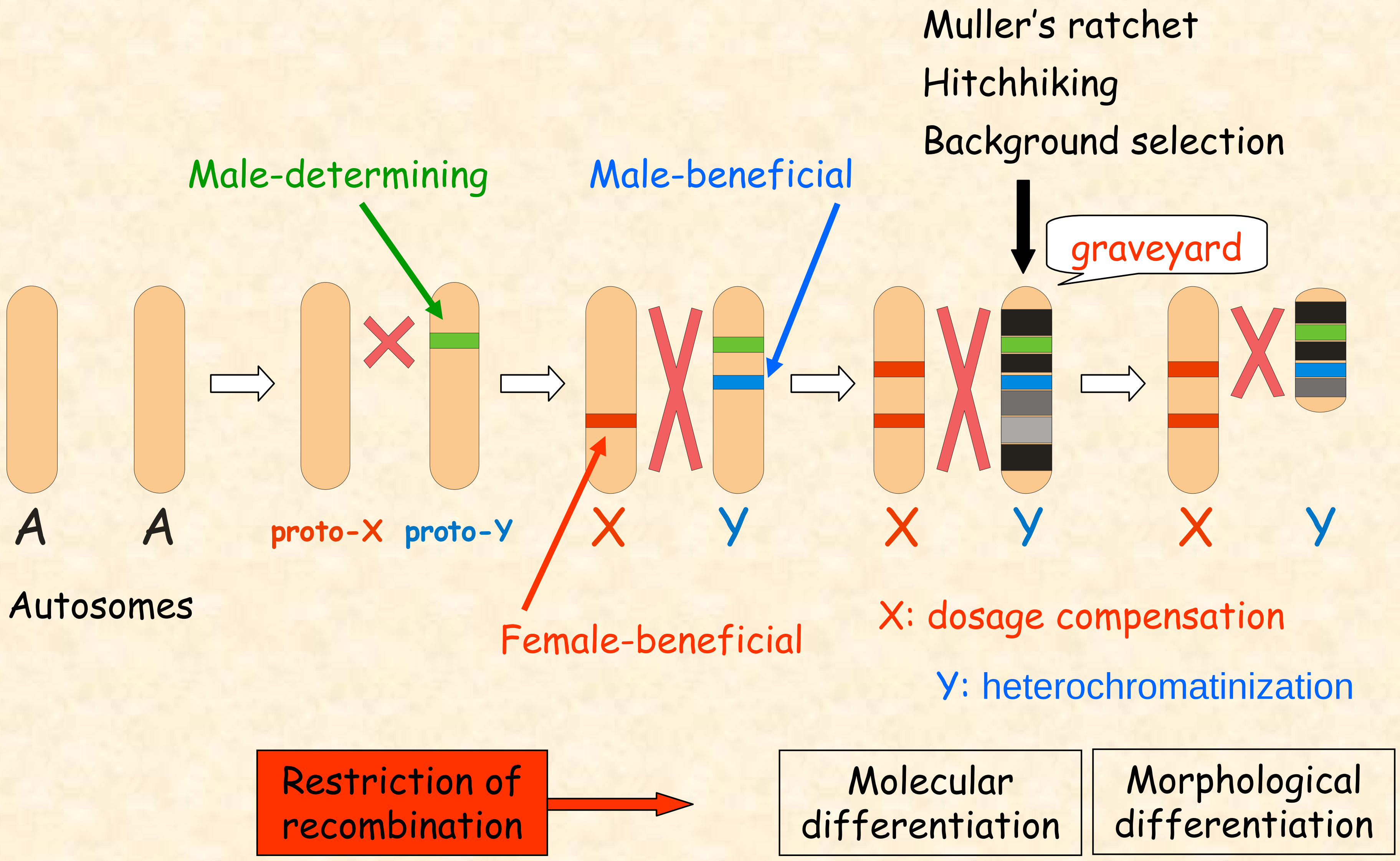
Sex chromosome evolution in organisms with female heterogamety and holokinetic structure of chromosomes: moths and butterflies

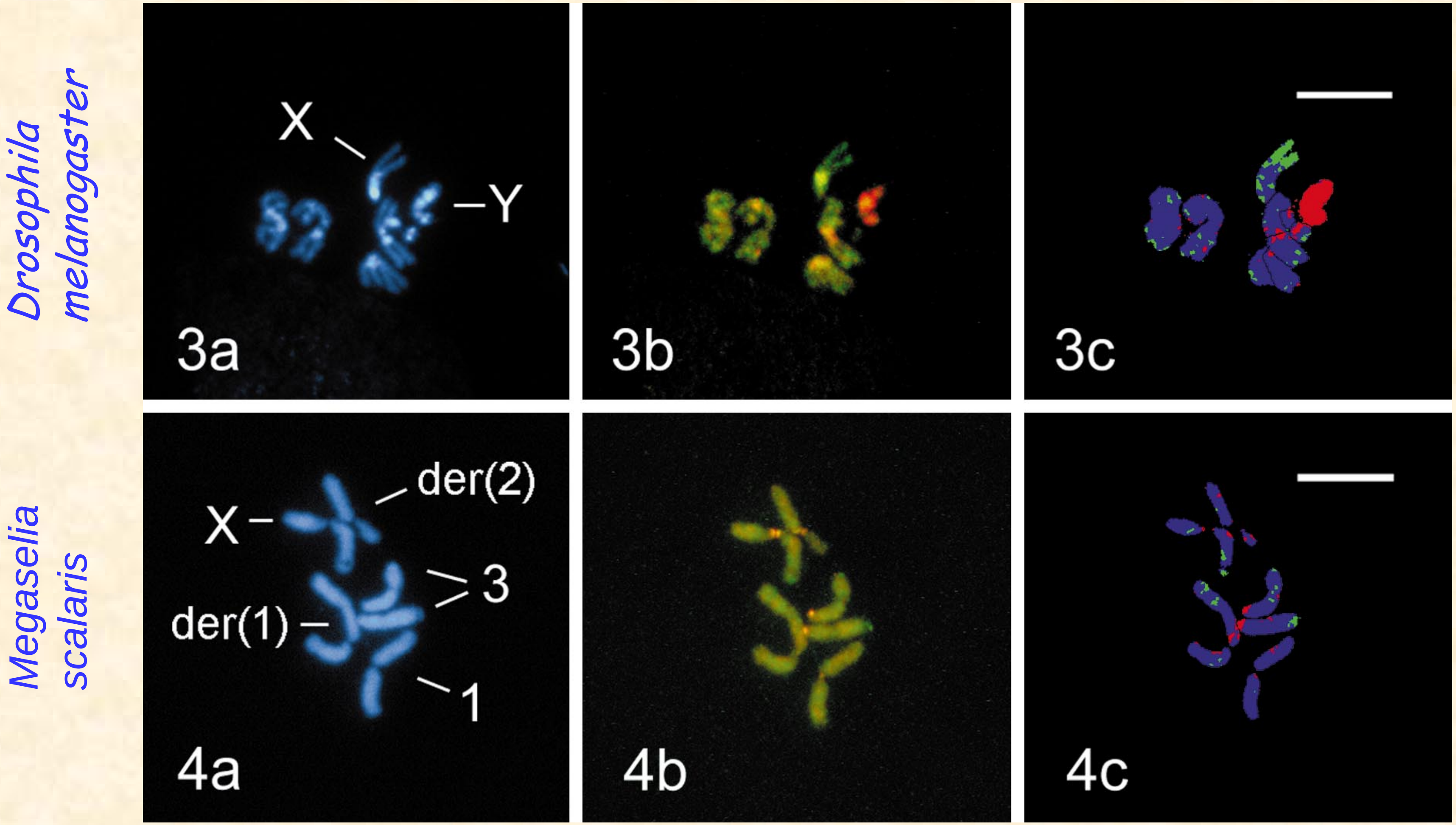
Frantisek Marec[1,2]

[1]Biology Centre ASCR, Institute of Entomology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Rep.

Theory: evolution of sex chromosomes



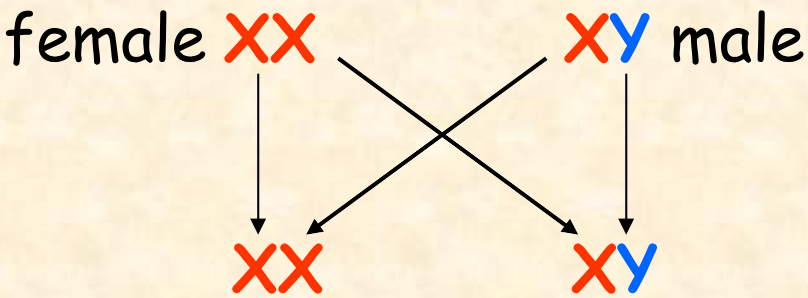
Molecular differentiation of sex chromosomes by CGH



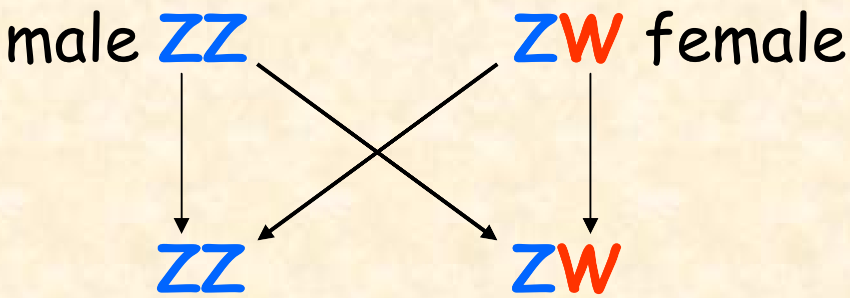
Traut et al. (1999) *Chromosoma* 108: 173-180.

Two basic sex-chromosome systems had evolved:

Male heterogamety
(mammals, *Drosophila* ...)



Female heterogamety
(birds, butterflies ...)



The conception of Y as a "graveyard" revised

- Y chromosomes of primates: palindrome-driven sister chromatid and intrachromatid recombination prevents degradation and loss of genes => continuing evolution

  Lange et al. (2009) *Cell* 138: 855–69.

  Hughes et al. (2010) *Nature* 463: 536–39.

- *Drosophila* Y chromosome: an important role in male fitness – it contributes to adaptive phenotypic variation through a regulatory role of Y-linked polymorphic elements in gene expression

  Lemos et al. (2008) Science 319: 91–93.

WZ/ZZ system (female/male)

Heterogametic female sex inferred from sex-linked inheritance in *Abraxas* 100 ya
- sex chromosomes never seen.

Abraxas glossulariata
(Geometridae)

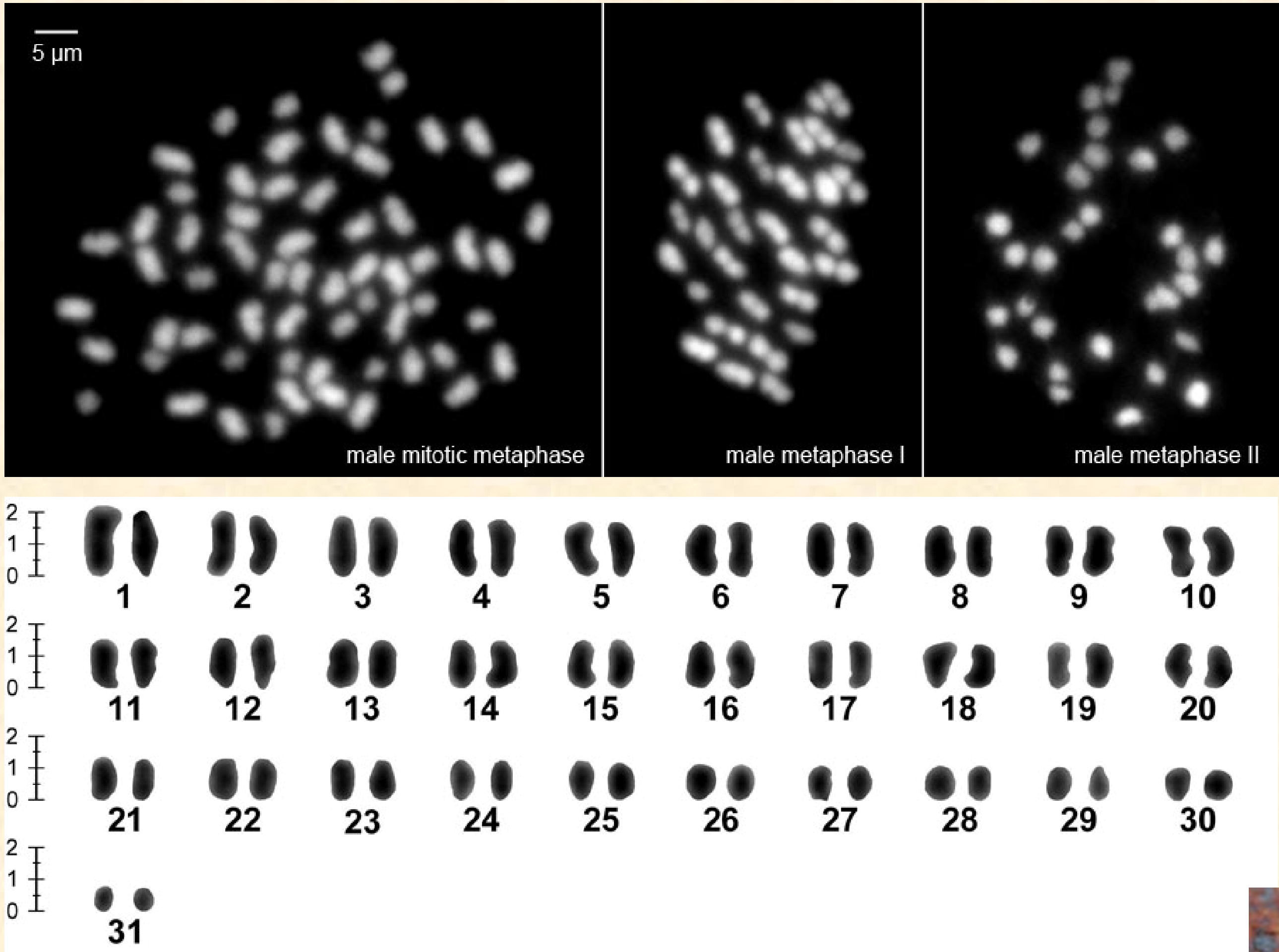
- **Lepidoptera**
- Trichoptera (Z0)
- snakes (Reptilia: Serpentes)
- birds (Aves)

- some fishes (*Xiphophorus maculatus*)
- number of amphibians (*Xenopus laevis*)
- some lizards (*Lacerta vivipara*)
- some parasitic worms (*Schistosoma*)
- several plants (*Fragaria elatior*) ???

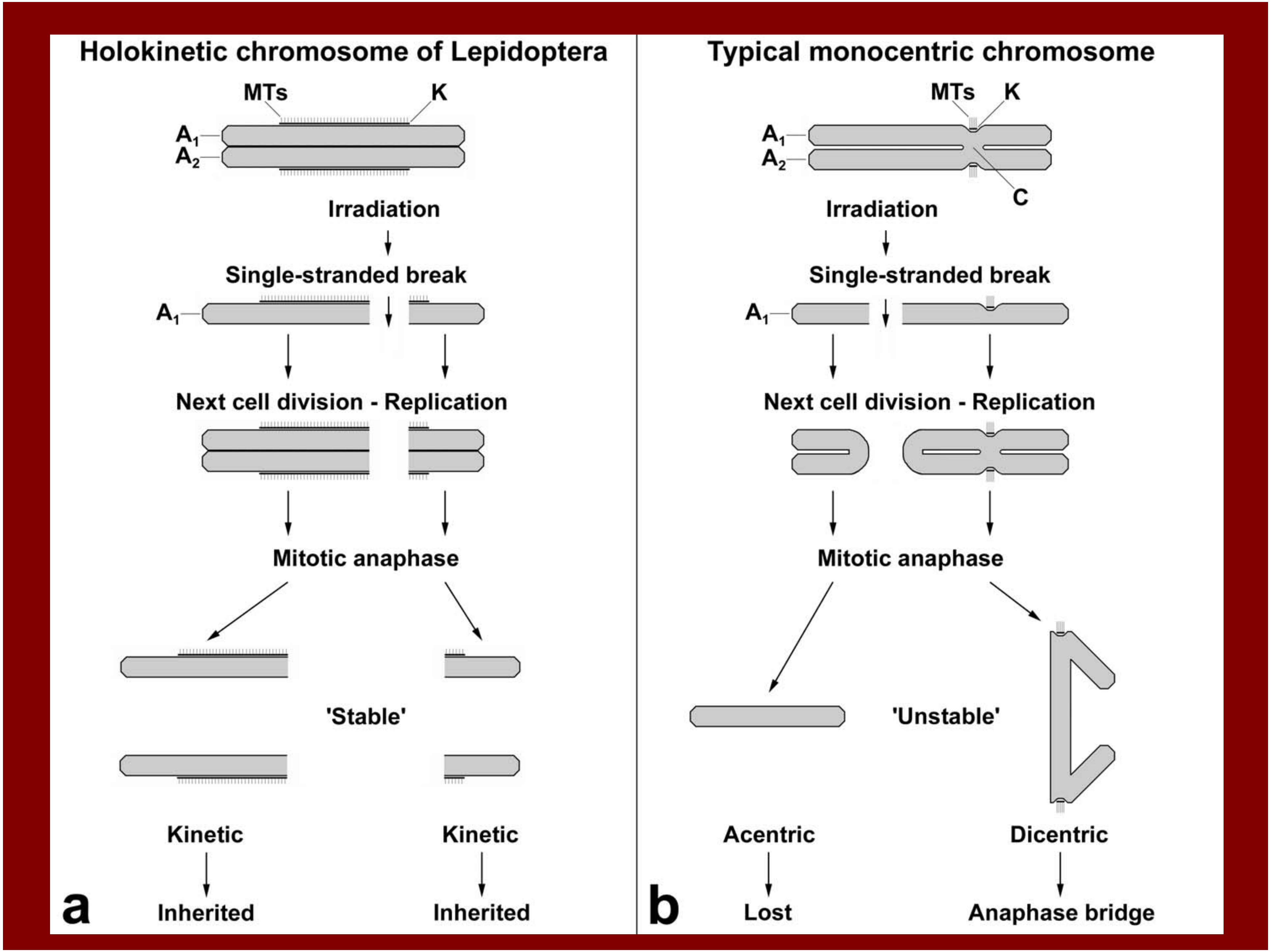
Lepidoptera
moths and butterflies

- Largest taxon with female heterogamety
  - more than 150.000 species described
- The only taxon with female heterogamety having holokinetic chromosomes
- Achiasmatic female meiosis => no cross-over
- Sex chromatin in somatic cells of females

Holokinetic chromosomes of Lepidoptera



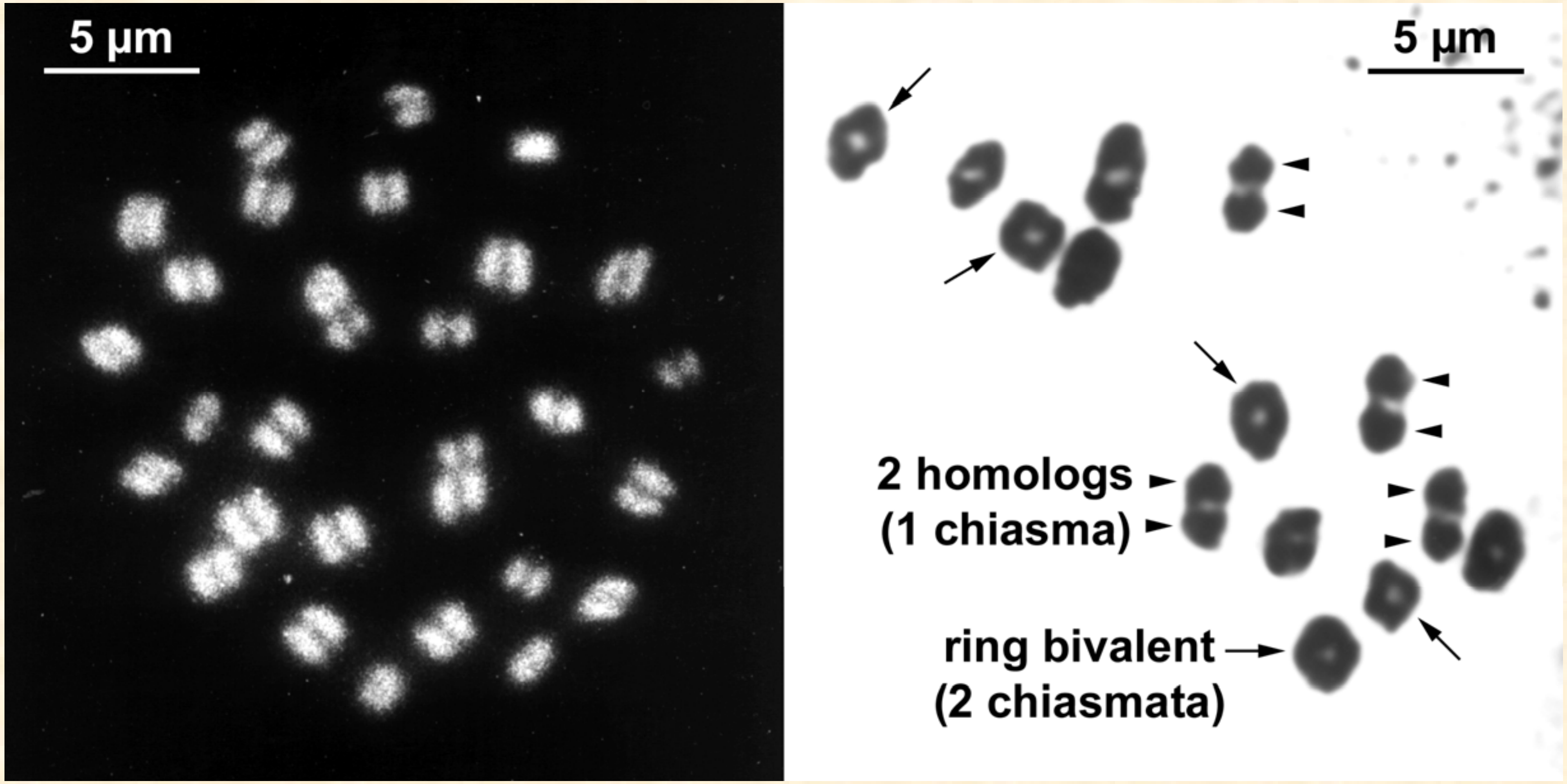
Carob moth, *Ectomyelois ceratoniae* (Pyralidae)
n = 31 ; 2n = 62


Holokinetic chromosome of Lepidoptera
MTs
K
A1
A2
Irradiation
Single-stranded break
A1
Next cell division - Replication
Mitotic anaphase
'Stable'
Kinetic
Kinetic
a
Inherited
Inherited
Typical monocentric chromosome
MTs
K
A1
A2
C
Irradiation
Single-stranded break
A1
Next cell division - Replication
Mitotic anaphase
'Unstable'
Acentric
Dicentric
b
Lost
Anaphase bridge

Holokinetic chromosomes of Lepidoptera

- contribute to resistance to clastogenic compounds and ionizing radiation
  - chromosome breaks do not lead to break-fussion-bridge cycle
  - chromosome fragments inherited
  - reduced risk of dicentric chromosomes
- facilitate karyotype evolution by rearrangements
  - fusion => reduced chromosome numbers
  - fission => increased chromosome numbers
- **BUT surprising stability of genomes/karyotypes**
  - common /ancestral chromosome number n = 31 (exceptions occur)
  - widely conserved synteny of genes between species

Achiasmatic versus chiasmatic meiosis in Lepidoptera

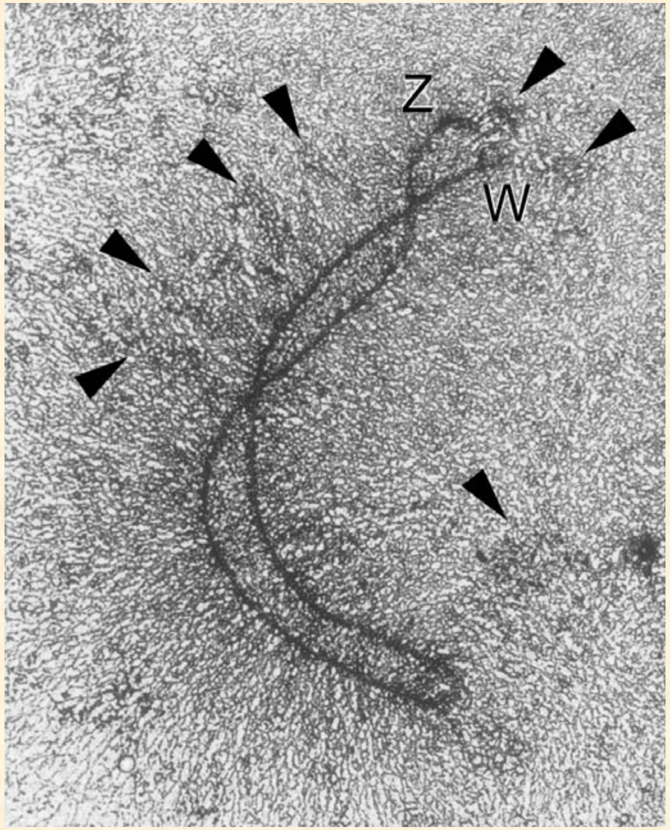


Metaphase I bivalents of the flour moth, *Ephestia kuehniella* (Pyralidae) - female (n=30)

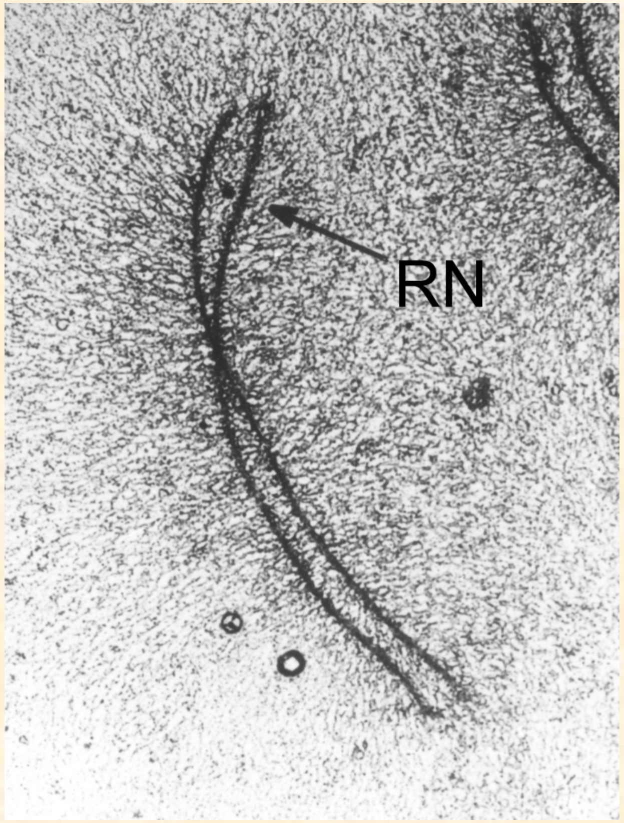
Metaphase I bivalents of the vapourer moth, *Orgyia antiqua* (Lymantriidae) - male (n=14)

⇒ no recombination between W-Z, not even between autosomes

The flour moth, *Ephestia kuehniella* (Pyralidae)



female SC

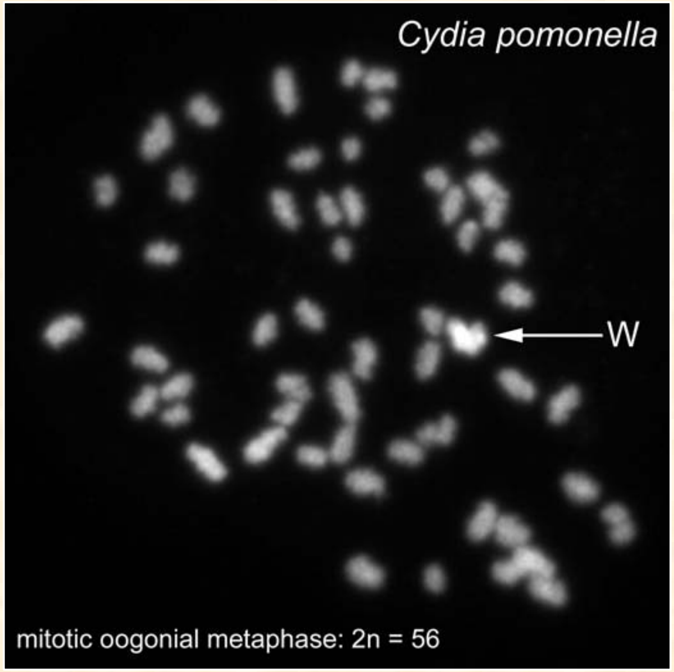


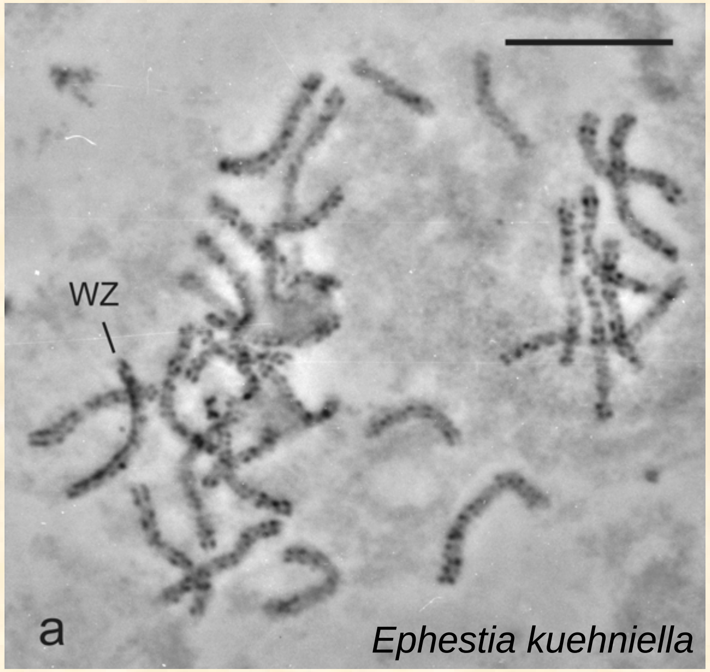
male SC

Marec and Traut (1993) *Heredity* 71: 394-404.

W chromosome in Lepidoptera

- consists of heterochromatin => mainly composed of repetitive DNA
- small holokinetic chromosomes => resist identification in metaphase
- distinguishable in pachytene; WZ bivalent
- in contrast to gene-rich Z, W carries only a few genes (*Fem* factor)

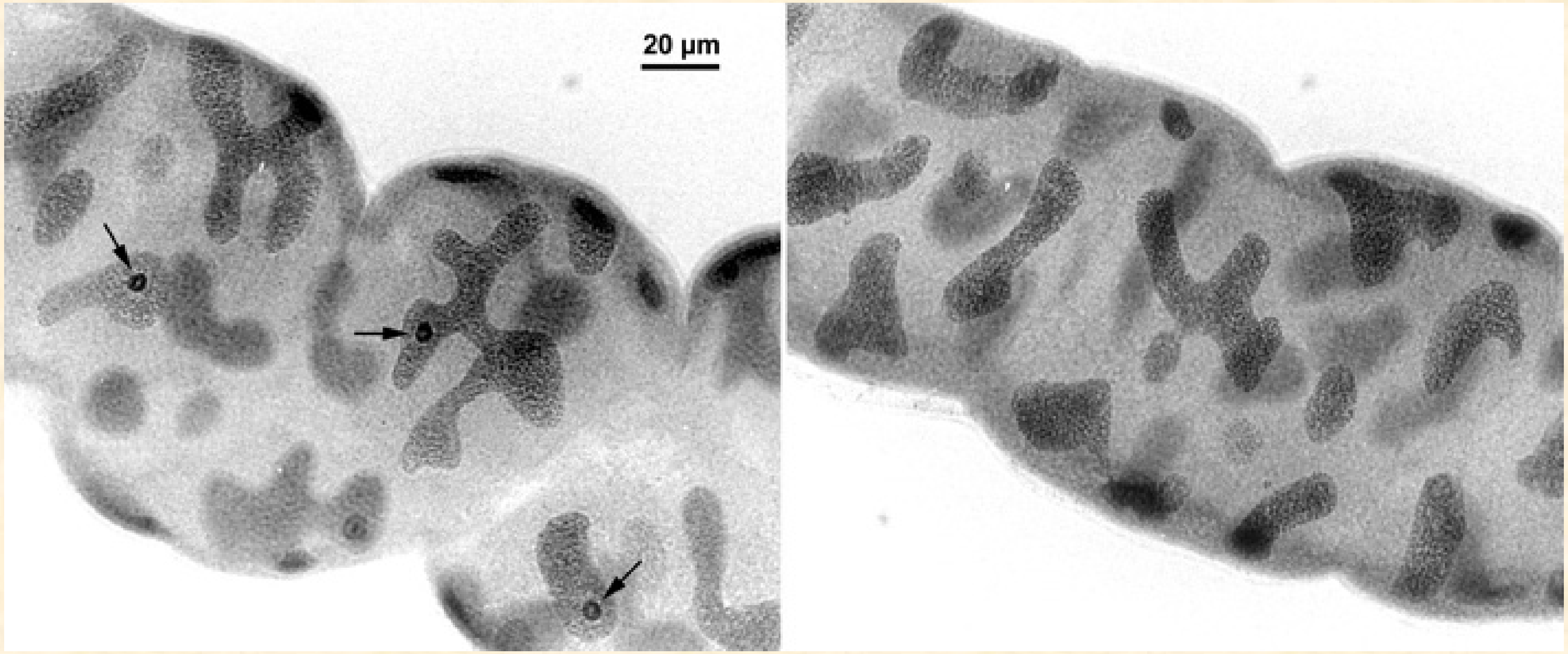




Sex chromatin in Lepidoptera

Ephestia kuehniella - Malpighian tubules

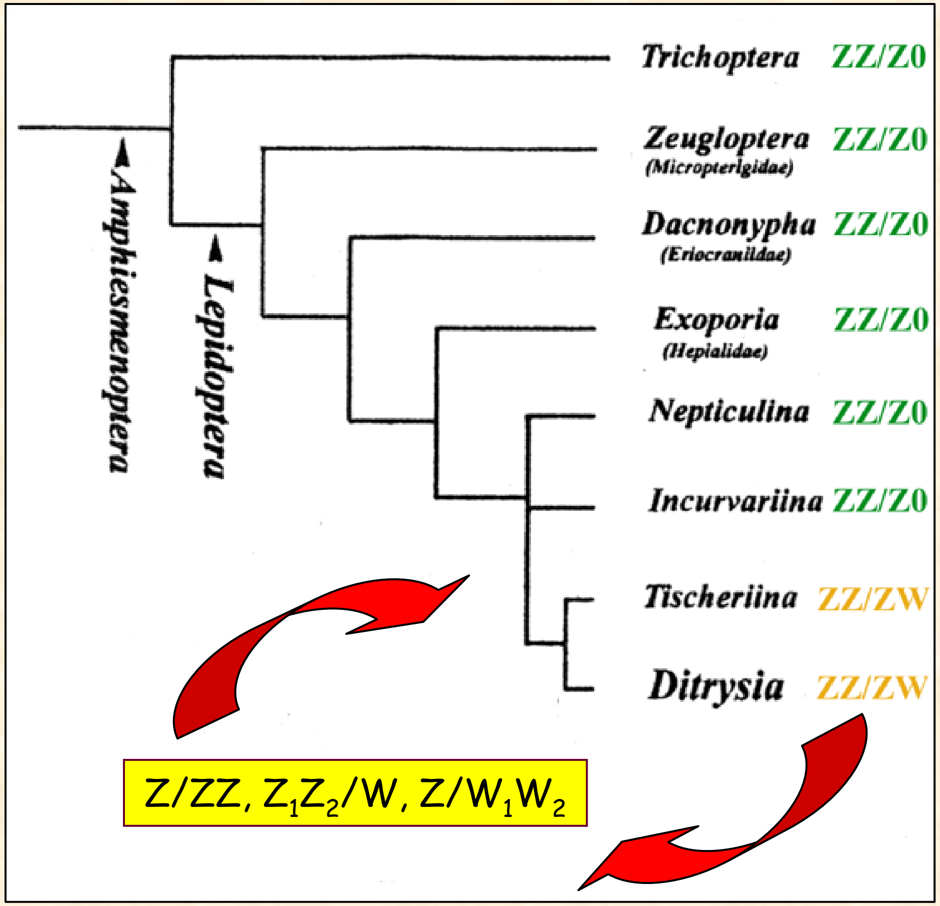
female male



Characteristic trait of females in 80% of Ditrysia, derived from W

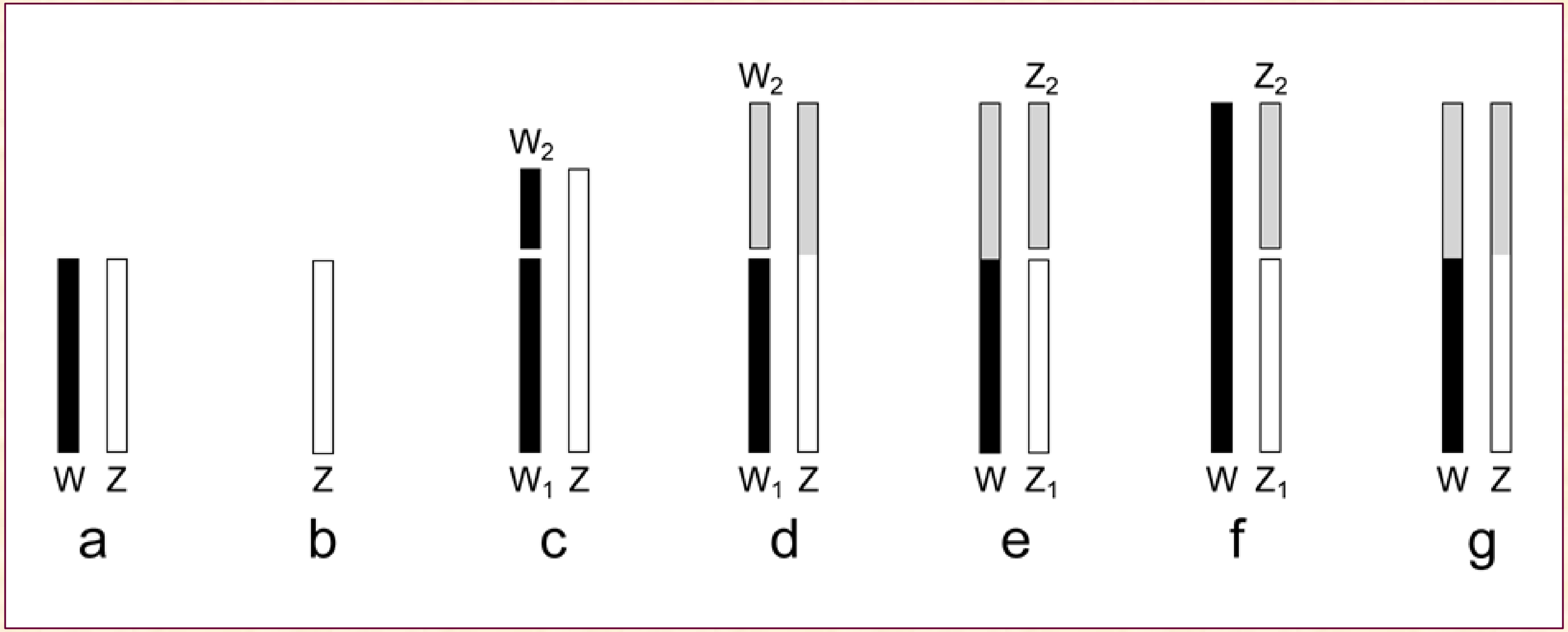
reviewed by Traut and Marec (1996) *Q. Rev. Biol.* 71: 239-256.

Phylogeny of sex chromosomes in caddis-flies, moths and butterflies



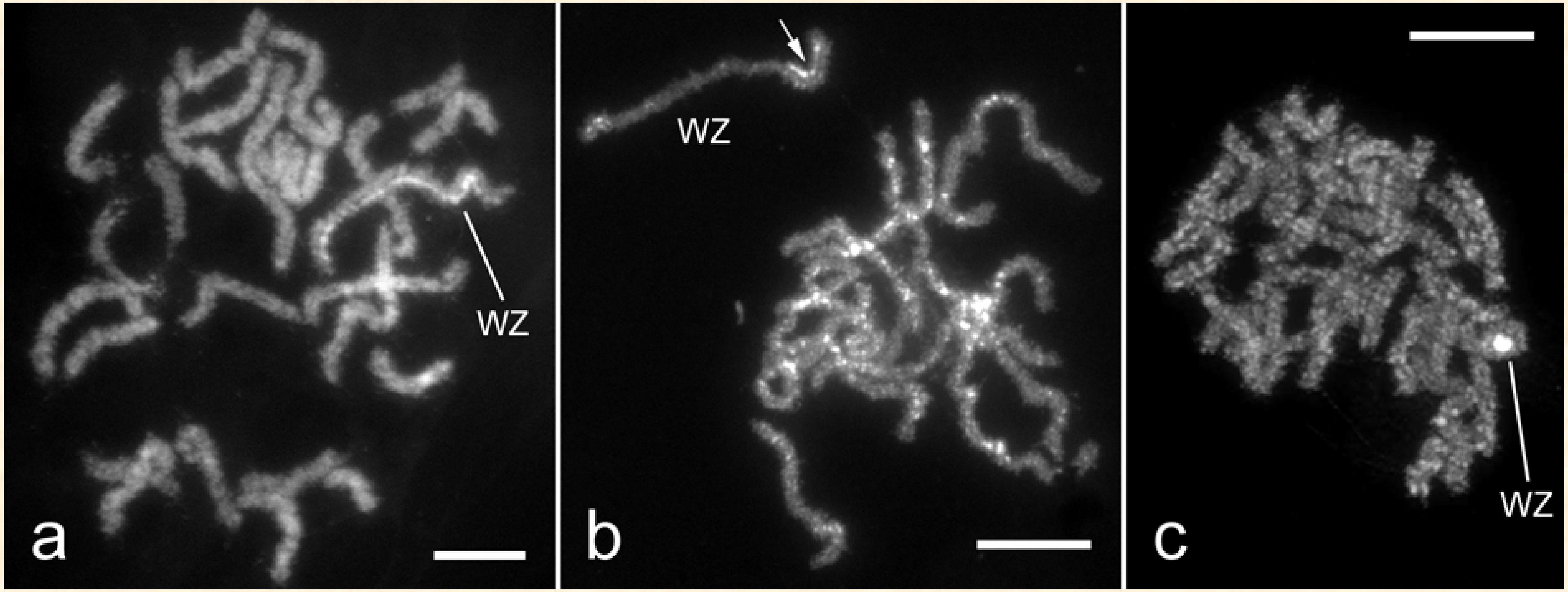
Compiled from: Traut and Marec (1996, 1997)
Marec and Novak (1998)
Lukhtanov (2000)

Variations of sex chromosomes in Lepidoptera



Marec et al. (2010) in: *Molecular Biology and Genetics of the Lepidoptera*. CRC Press.

W-chromosome forms



Cydia pomonella *Orgyia antiqua* *Bicyclus anynana*

Marec et al. (2010) in: Molecular Biology and Genetics of the Lepidoptera. CRC Press.

Tools of molecular cytogenetics for W-chromosome identification

Our project:

Evolutionary history of
sex chromosomes in Lepidoptera

Origin of the W chromosome

W-chromosome origin
Hypotheses

1) **Translocation/Fusion of an autosome onto the Z chromosome:**
   - T(Z;A) => neo-Z chromosome
   - autosome homologue => neo-W chromosome

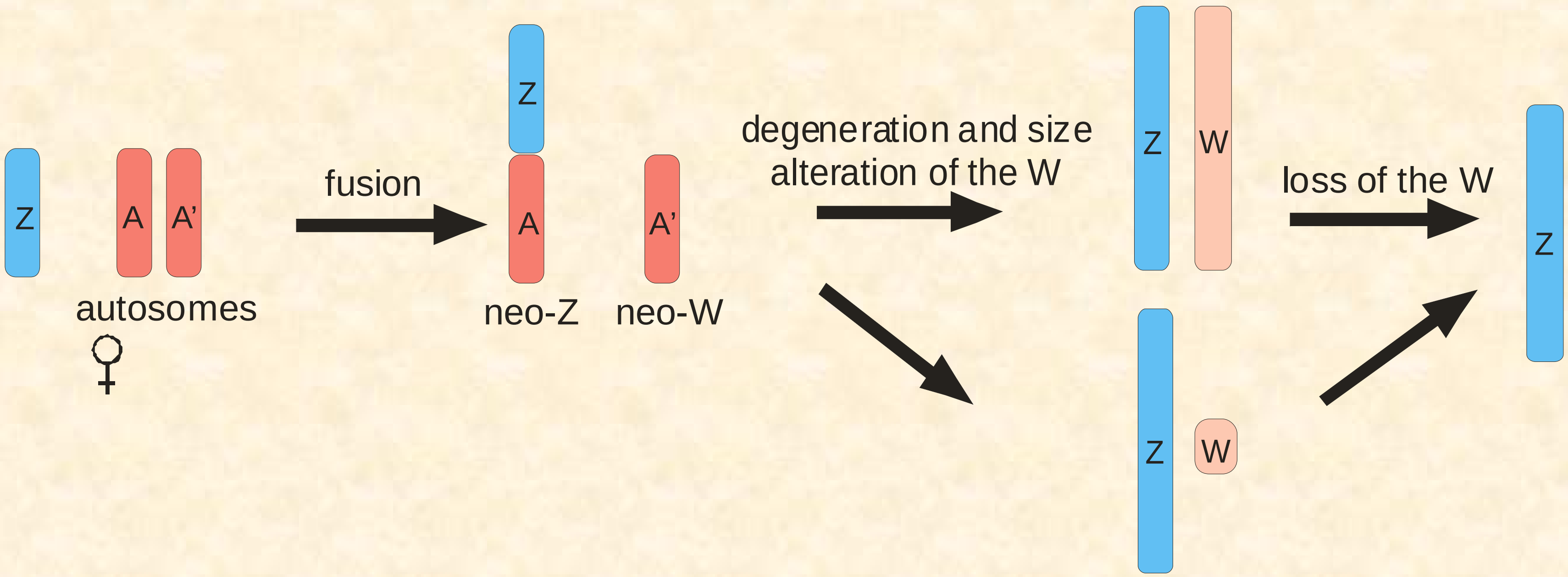
[Traut and Marec 1996]

2) **B (supernumerary) chromosome => neo-W**

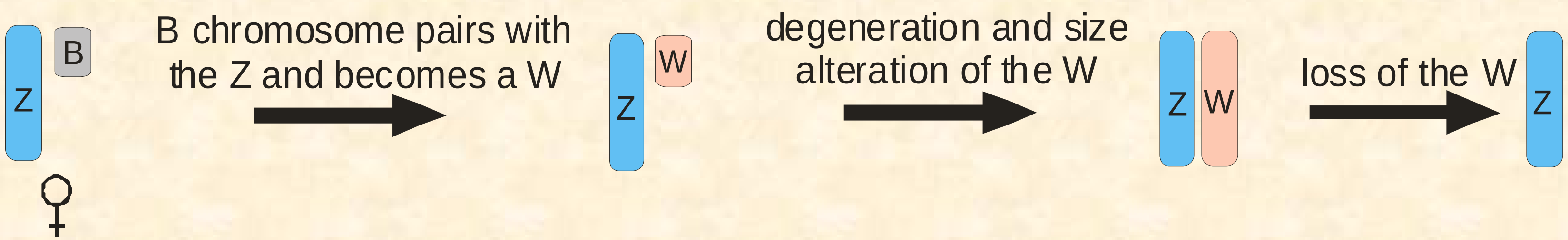
[Lukhtanov 2000]

Rise of the W chromosome

Hypothesis 1: fusion of the Z chromosome with an autosome



Hypothesis 2: rise of the W chromosome from a B chromosome

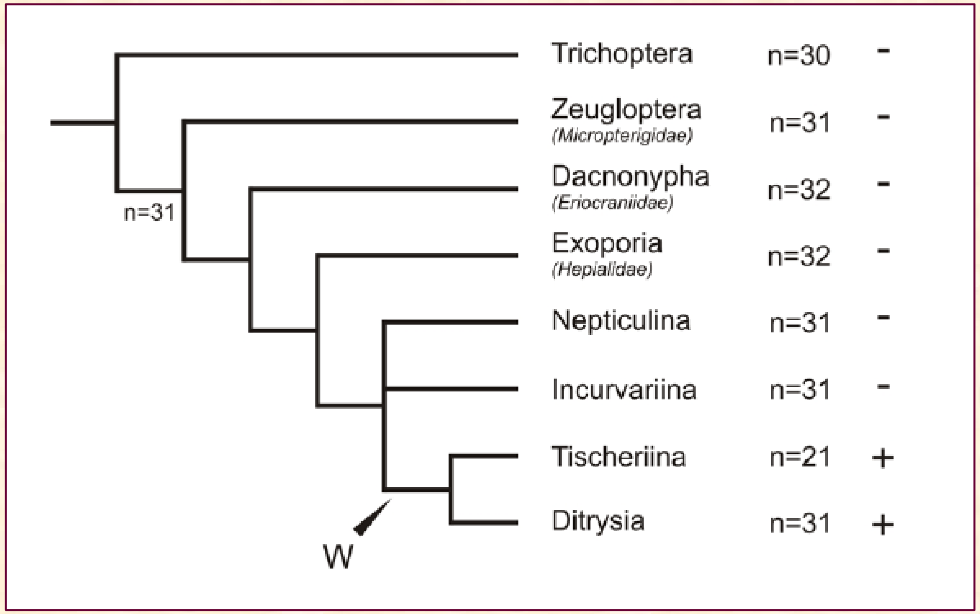


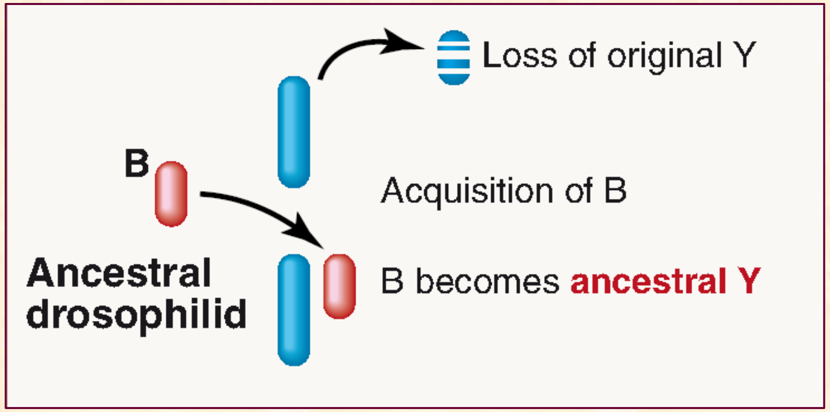
1) Autosomal origin of W – supported by:

- WZ pairs in a bivalent and form a regular SC
- neo-W repeatedly arose by T(W;A) in Ditrysia
- W and Z often belong to largest chromosomes

2) B-chromosome origin of W – preferred due to:

- autosomal origin lacks support in modal/ancestral chromosome number (n=31)
- similar origin predicted for Y in drosophilids



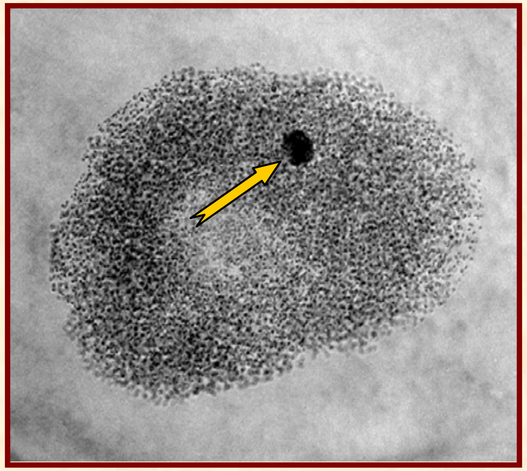


To answer the question of the W-chromosome origin

1) Does the W chromosome contain remnants of homology to an autosome or “autosomal part“ of the Z chromosome? Or to a B chromosome???

2) Does the Z chromosome in Ditrysia contain an autosomal part or is it fully orthologous to the ancestral Z of primitive Lepidoptera and Trichoptera?

Probing the W chromosome for evolutionary studies in Lepidoptera



Our first approach:

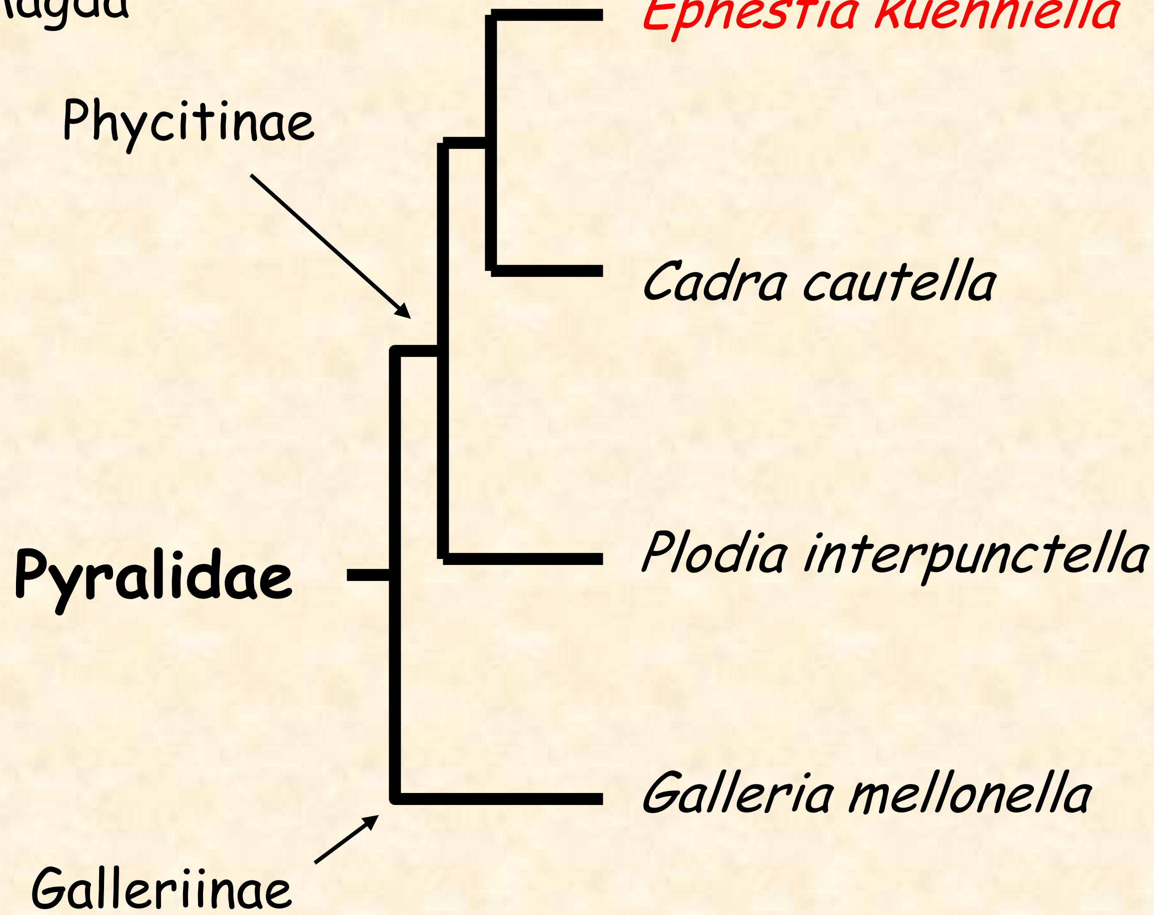
- preparation of W-chromosome painting probes by laser microdissection of W-chromatin bodies

- comparative chromosome painting (Zoo-FISH) of W chromosomes in related species


OLYMPUS

Zoo-FISH

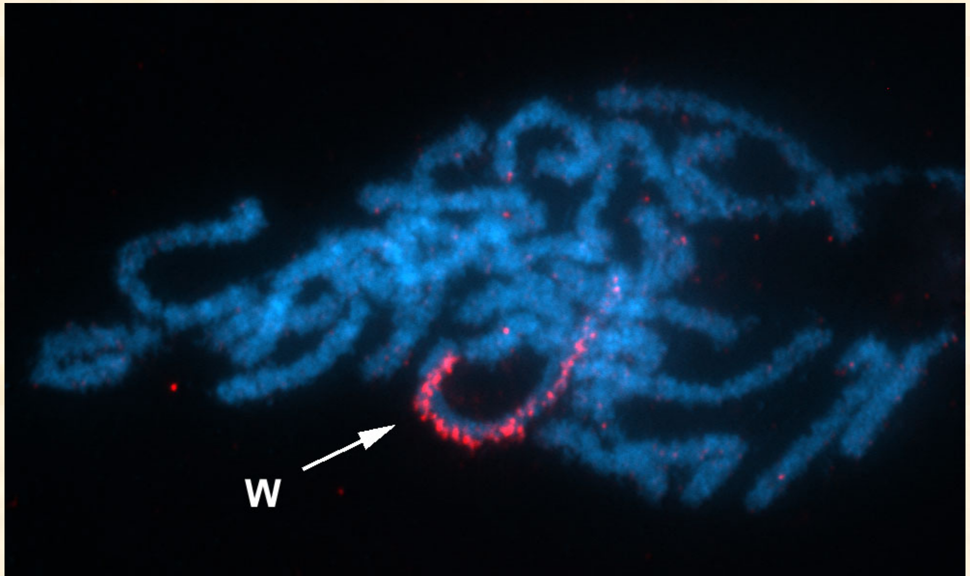
Magda



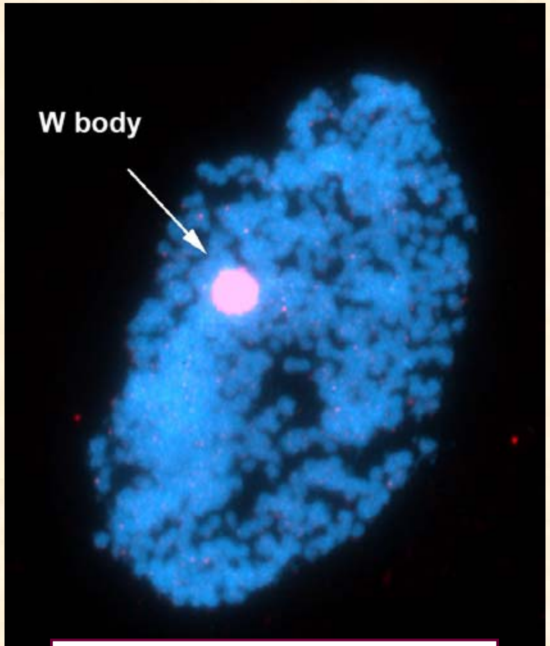
Vitkova et al. (2007) *Chromosome Res.* 15: 917-930.

Zoo-FISH with W-specific probe, derived from microdissected sex chromatin

- DOP-PCR amplification and labelling of sex chromatin sample
- Cross-hybridization of *Ephestia* W-probe to other pyralids



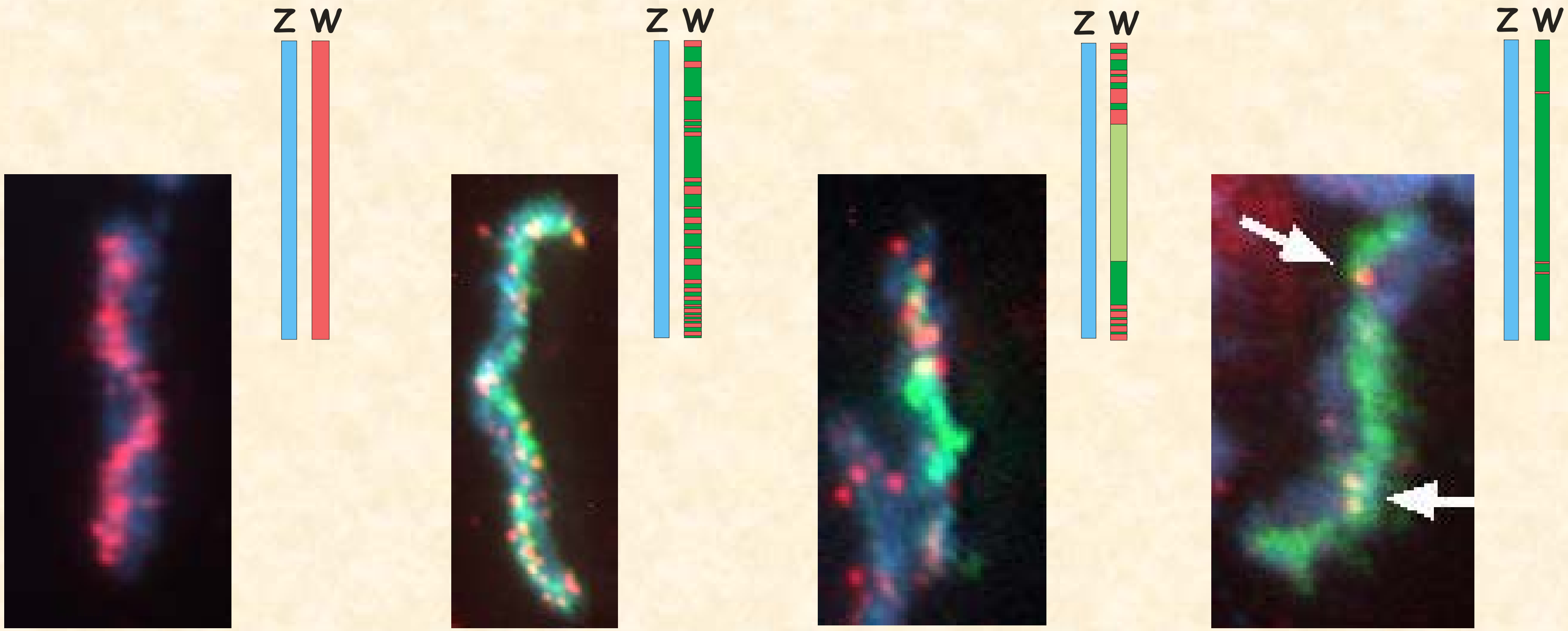
Ephestia kuehniella – pachytene oocyte



female interphase

Zoo-FISH with the W-specific probe from *Ephestia*

Red signal = W-probe from *Ephestia*
Green signal = W-probe from the same species as chromosomes
Blue = DAPI counterstaining



Ephestia *Cadra* *Plodia* *Galleria*

Conclusions

Results of Zoo-FISH in pyralids suggest rapid molecular evolution of the W chromosome.

W chromosome cannot be used for comparative evolutionary studies between higher taxa.

Z chromosomes for tracking sex-chromosome evolution in Lepidoptera

Two different approaches:

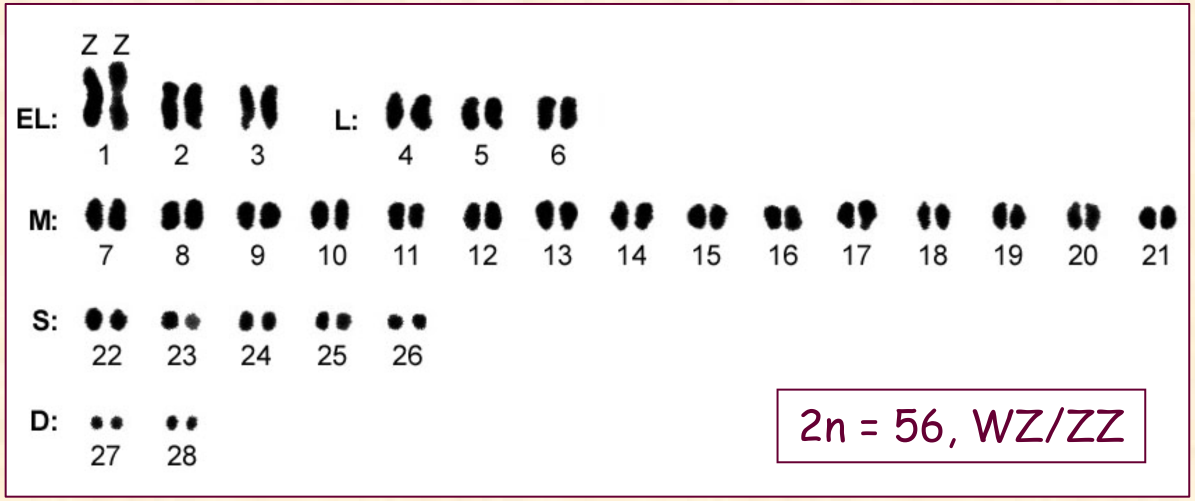
(i) preparation of Z-chromosome painting probes by laser microdissection and their use for
- creation of Z-DNA libraries and search for molecular markers of Z chromosomes
- comparative chromosome painting (Zoo-FISH)

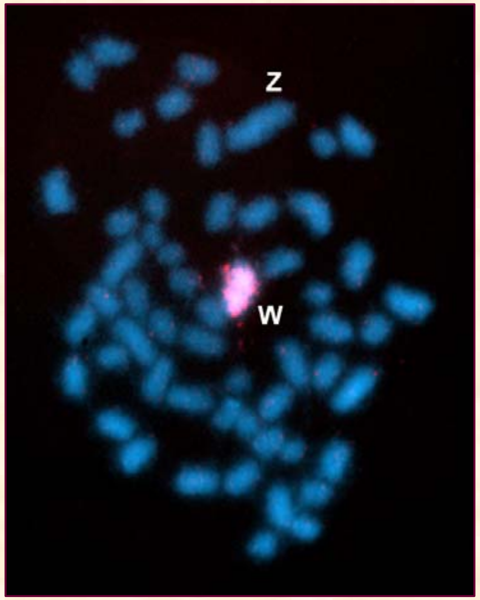
(ii) conserved synteny mapping of orthologs of Z-linked genes known from *Bombyx* or other sp.
- by BAC-FISH, if BAC library available
- by direct gene mapping, if BAC library not available

Codling moth (*Cydia pomonella*)
F: Tortricidae

Why codling moth?

- Karyotype and sex chromosome analysis done; Z largest element; tools of molecular cytogenetics established **(Fukova et al. 2005, 2007)**, W/Z molecular markers developed **(Fukova et al. 2009)**
- Bacterial Artificial Chromosome (BAC) library available
- <u>Most basal species of Lepidoptera phylogeny with some genetics</u>
- Another motivation: development of genetic sexing strains for SIT control of this pest **(Marec et al. 2005, 2007)**

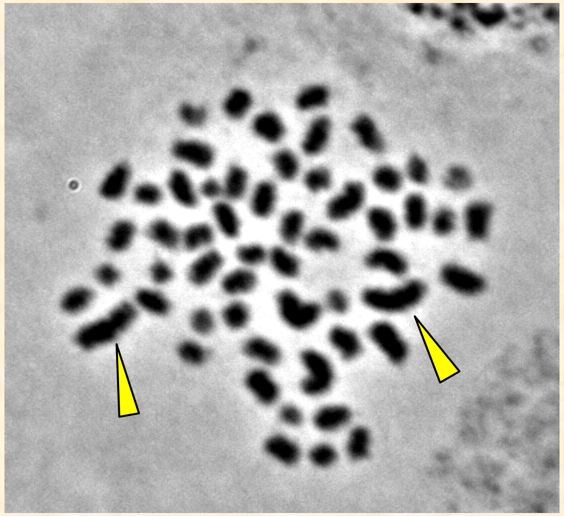




Creation of Z-chromosome library

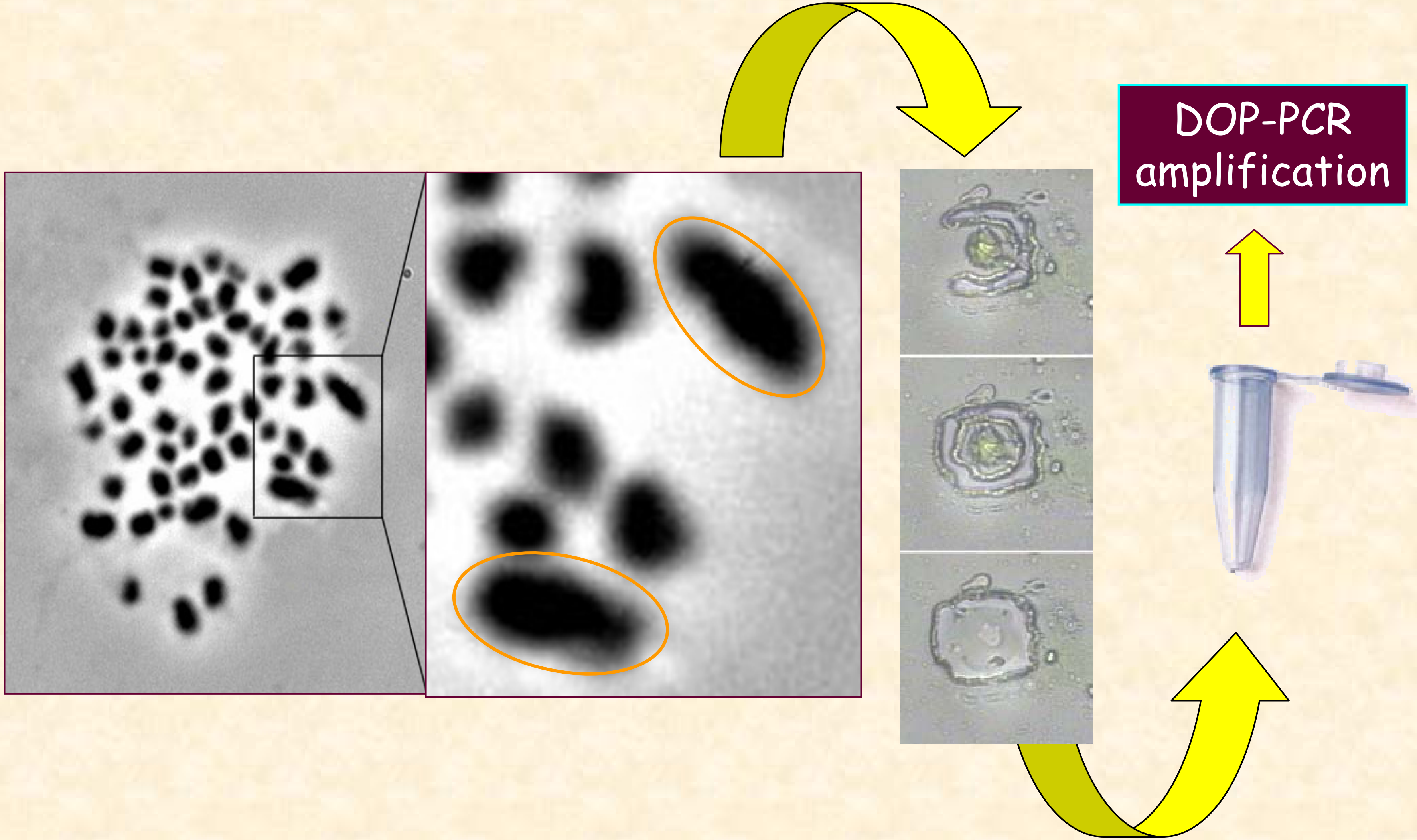
- laser microdissection of Z chromosomes from spermatogonial metaphase sets
- DOP-PCR amplified samples tested as Z-probe by FISH
- samples cloned, sequenced and analysed

Jindra

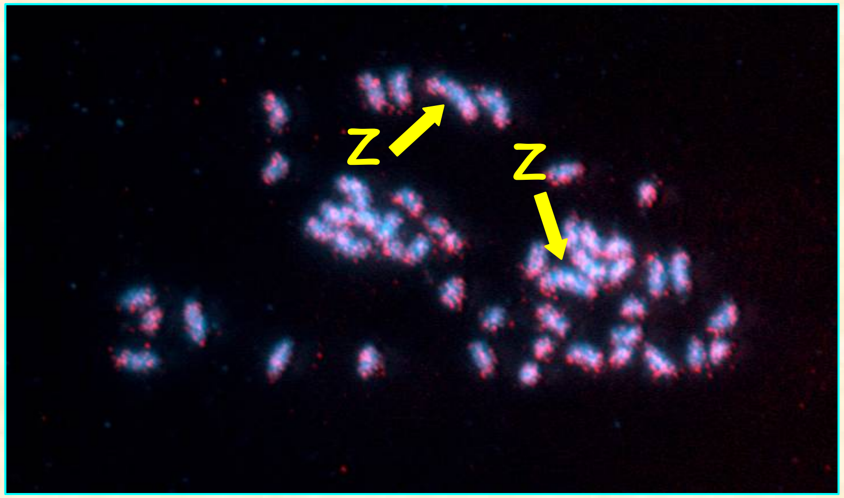


Magda

Laser microdissection of Z chromosomes



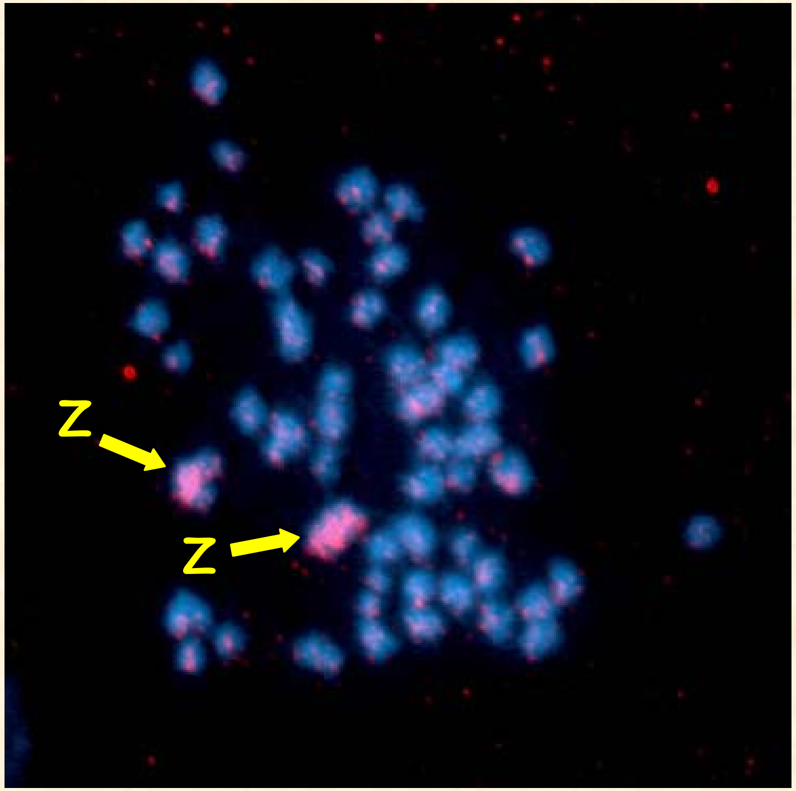
A low specificity of FISH with Z-chromosome-derived probes in codling moth males



FISH with Z-chromosome-derived probe highlighted W in females

Alternative system for balanced amplification of laser-microdissected Z-chromosome samples

- amplification using WGA4 kit (Sigma-Aldrich)
  - even a single chromosome can serve as a template
- re-amplification using WGA3 kit



Laser microdissection of Z chromosomes

Summary

- Low specificity of Z probes and W-painting: DOP-PCR amplified mainly repetitive DNA, enriched in W, as confirmed by cloning, sequencing, and Southern hybridization
- Balanced amplification using WGA4 / WGA3 system => sufficient specificity for Z chromosomes
- Z-painting probes will be used to determine similarity of Z chromosomes between related species of the family Tortricidae:
  - Plum fruit moth (*Cydia funebrana*)
  - Oriental fruit moth (*Grapholita molesta*)
  - European grapevine moths (*Lobesia botrana* and *Eupoecilia ambiguella*)

Synteny mapping of *Bombyx mori* Z-linked genes in codling moth by BAC-FISH

Mapping codling moth orthologs of Z-linked genes by BAC-FISH

First step: search for orthologs of *Bombyx* Z-linked genes

Petr

Candidate genes:

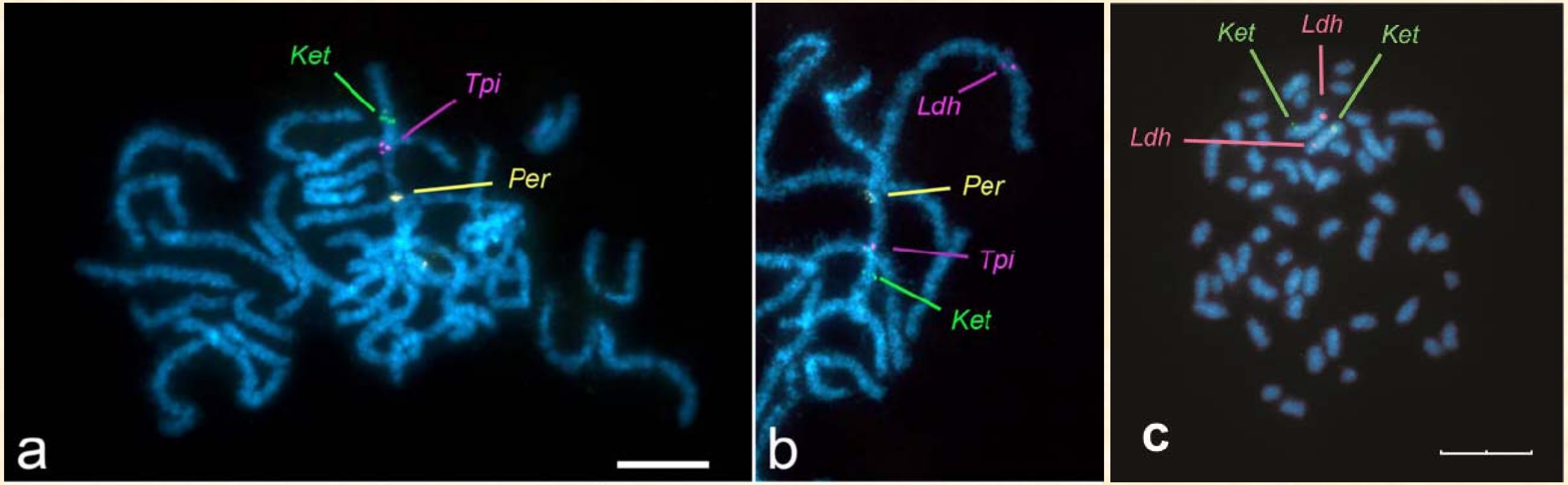
- *period* => *Cpper* (Fukova et al. 2009)
- *Bmkettin*
- *apterous*
- *Triose-phosphate isomerase* (*Tpi*)
- *Lactate dehydrogenase* (*Ldh*)
- 6-*Phosphogluconate dehydrogenase* (6-*pgd*)

Martina

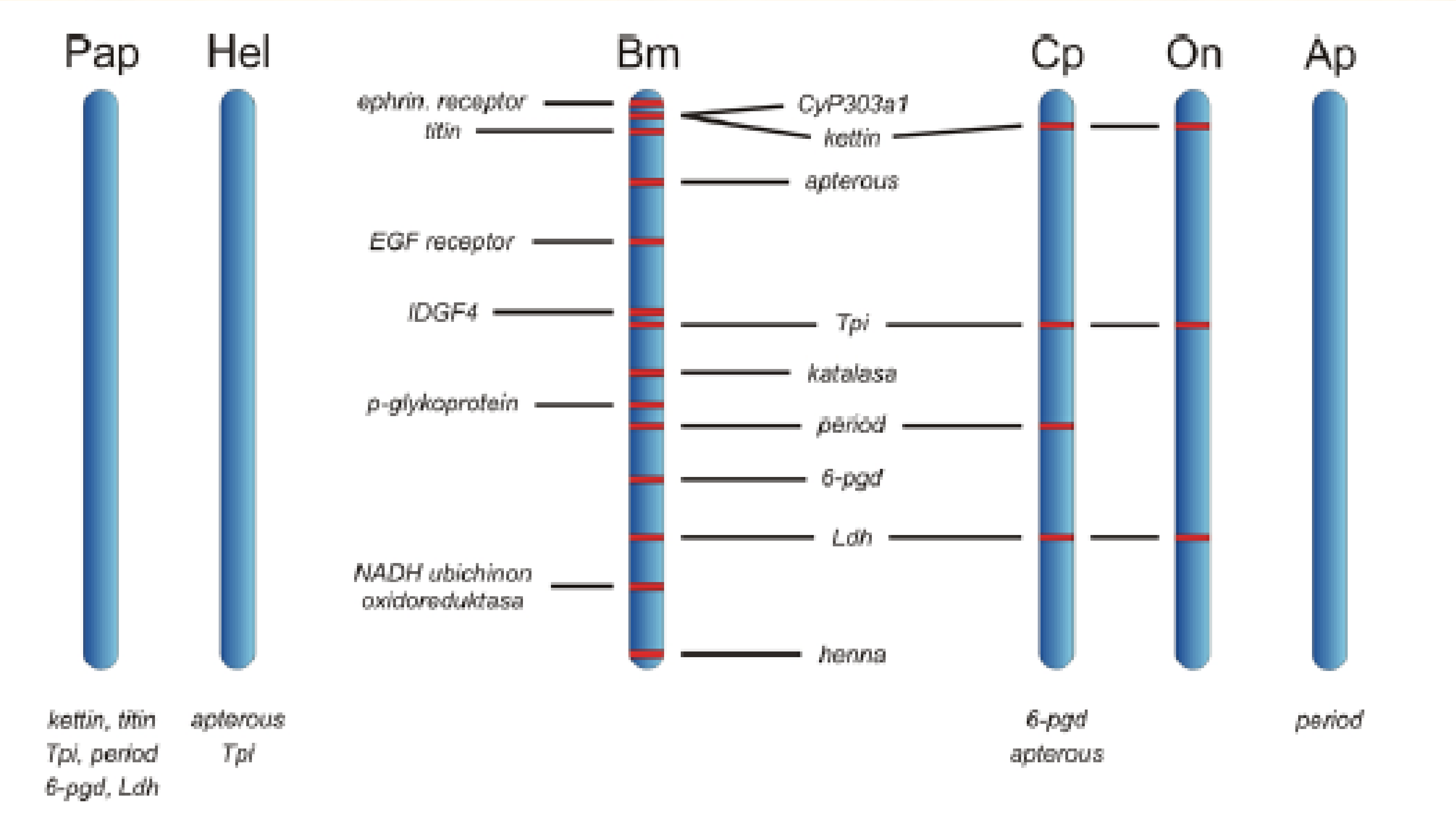
Second step: screening BAC library with isolated orthologs

Third step: BAC-FISH mapping

BAC-FISH mapping of codling moth orthologs of Z-linked genes

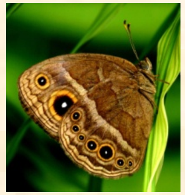


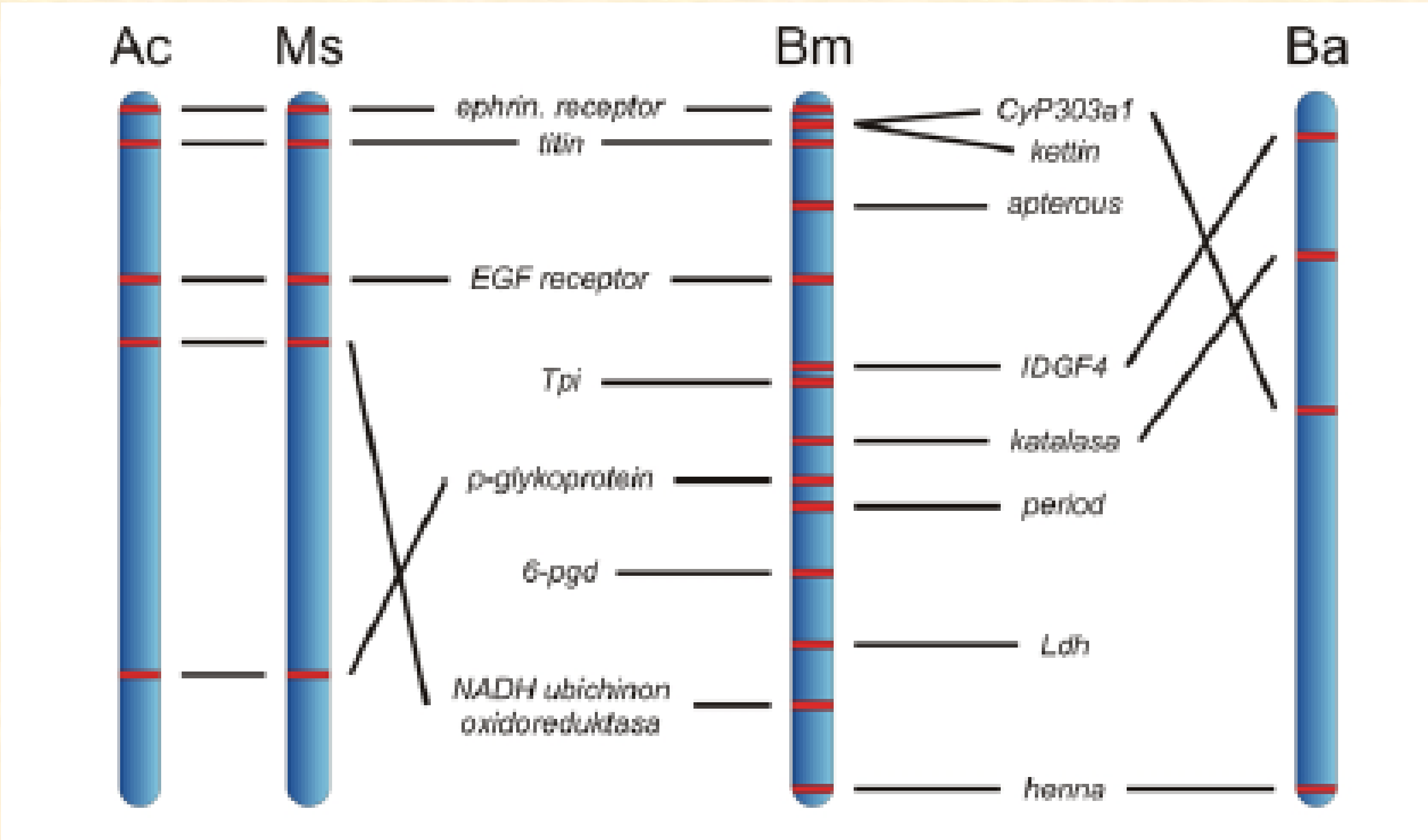
Comparison of Z-chromosome maps



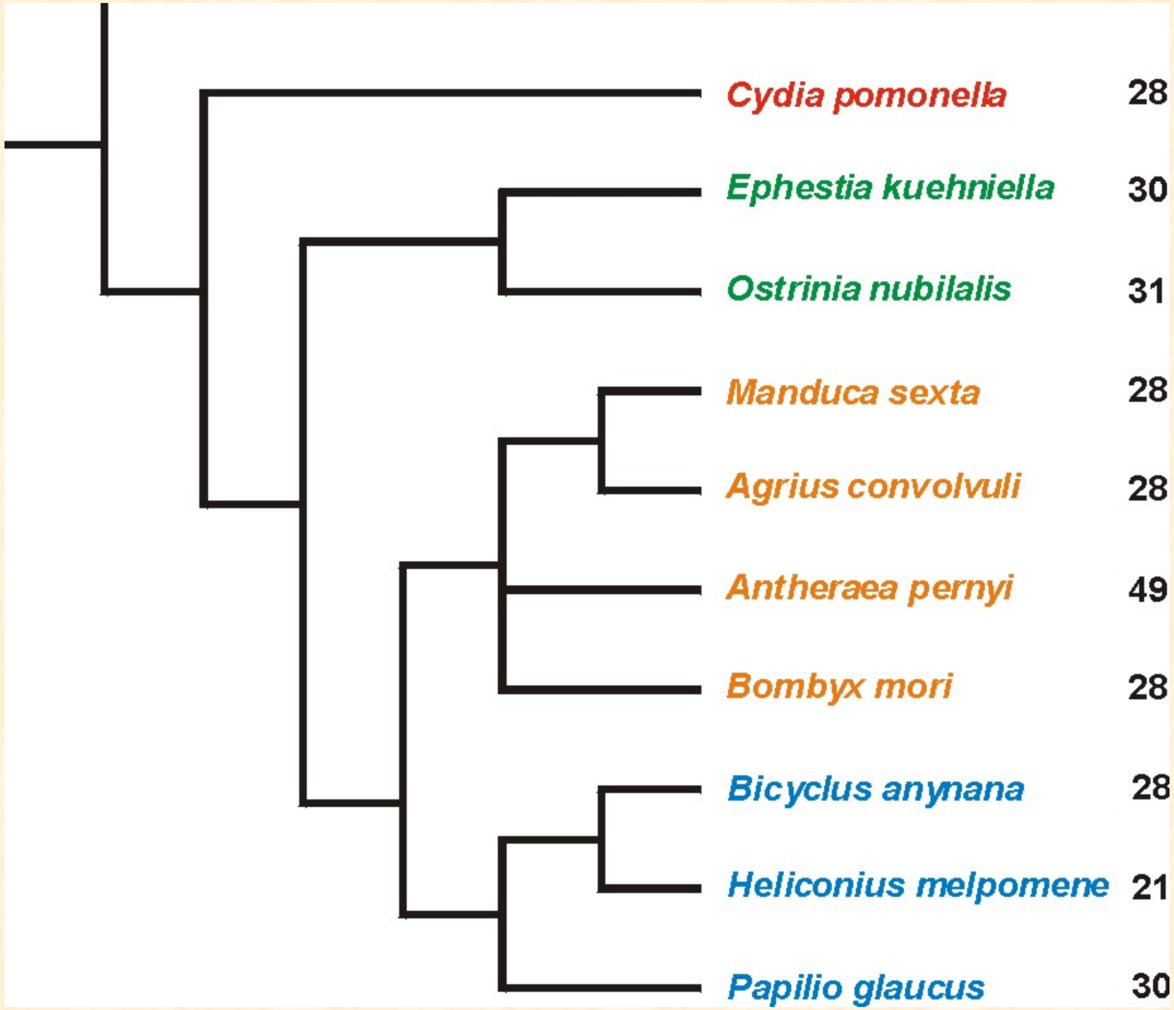
Comparison of Z-chromosome maps





Conserved synteny of Z-linked genes



BAC-FISH mapping of codling moth genes

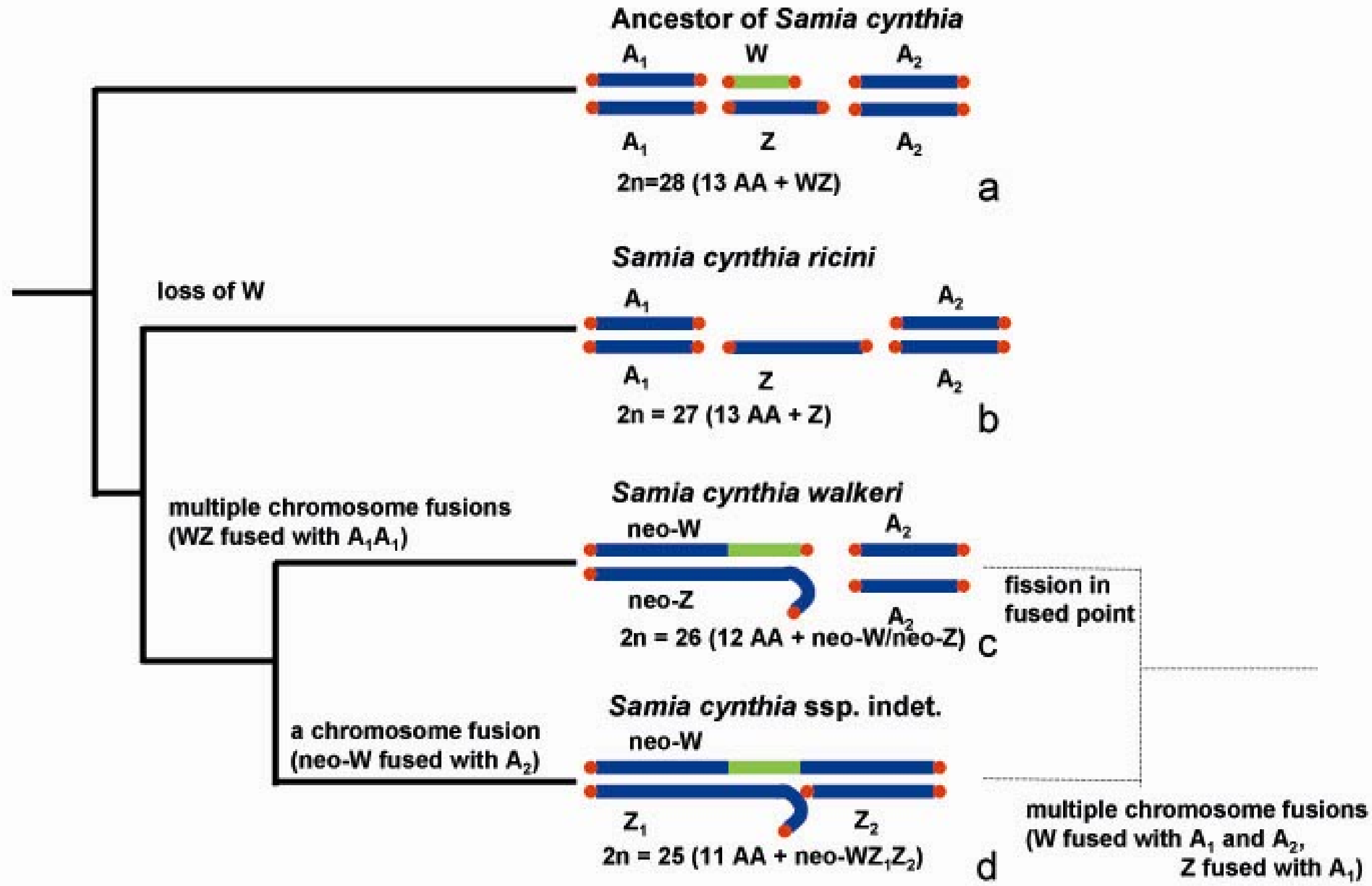
Summary and Conclusions

- Study confirmed conserved synteny of genes and conserved gene order between Z chromosomes of *B. mori* and *C. pomonella*
- Conserved synteny of Z-linked genes between all species studied including codling moth as representative of a basal ditrysian clade suggests the common origin of Z chromosomes in Ditrysia
- Results add further evidence to stability of lepidopteran genomes, in spite of holokinetic nature of their chromosomes

A case study

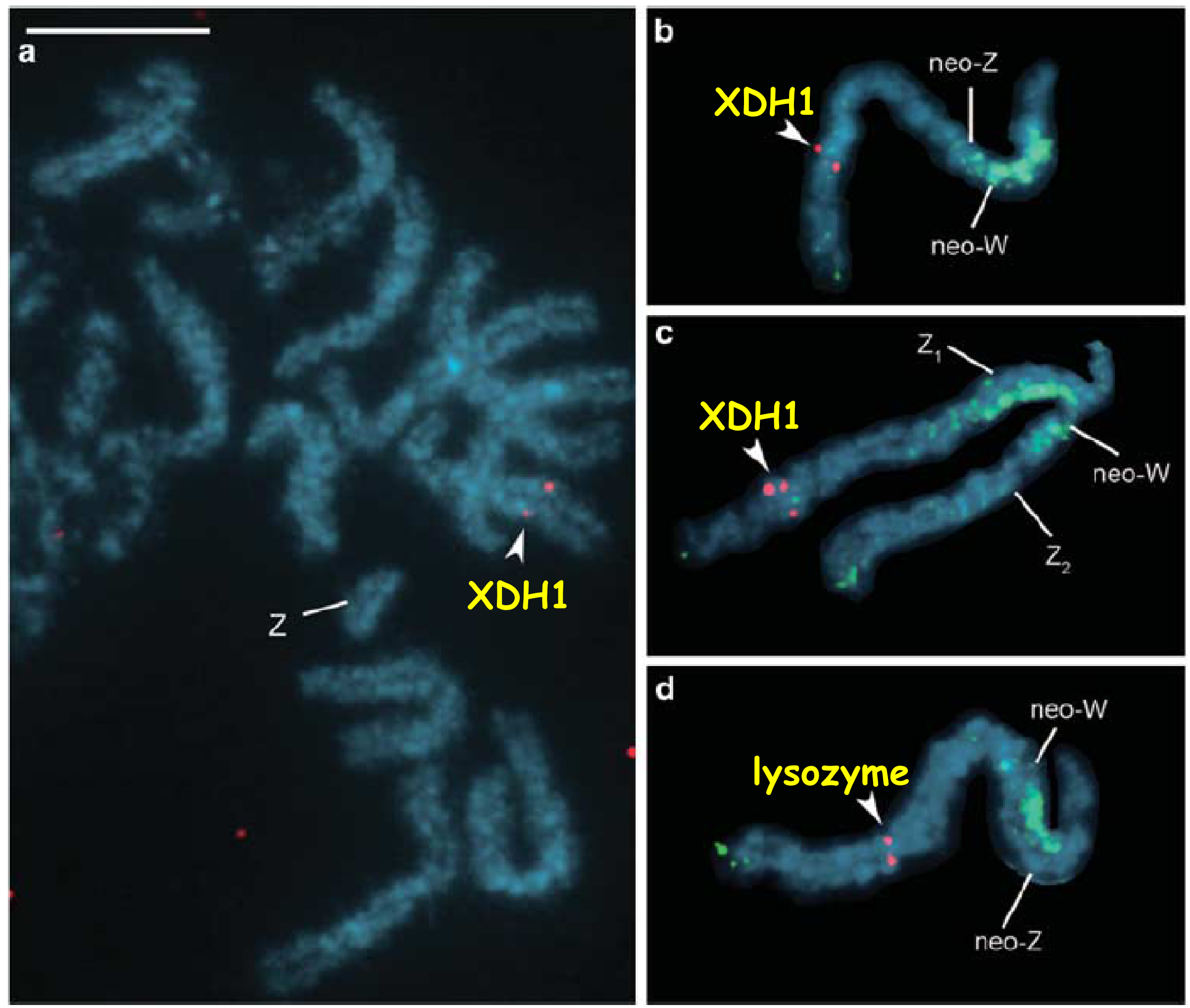
Wild silkmoth (Samia *cynthia*)
F: Saturniidae

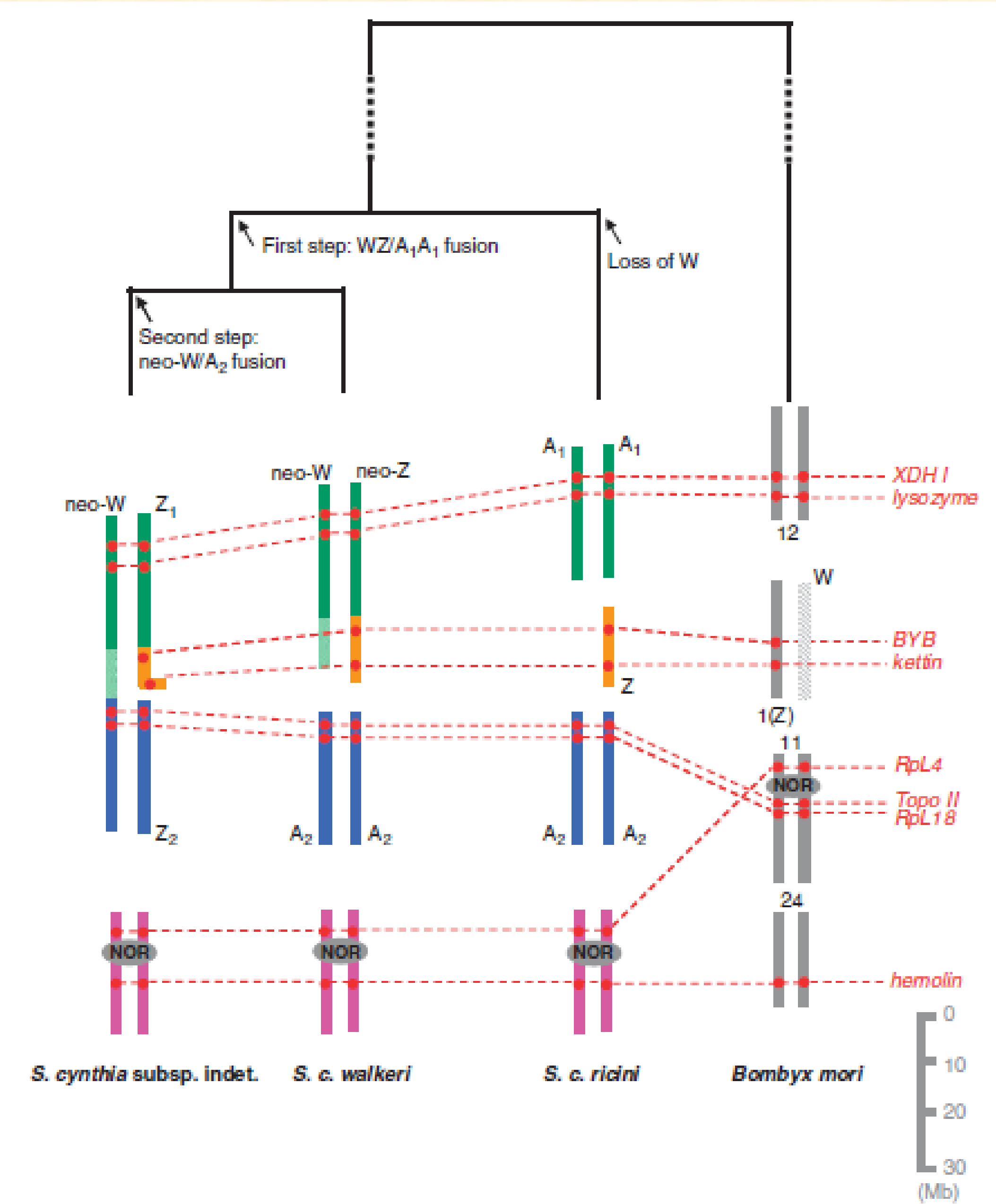
Sex-chromosome evolution in geographical subspecies



Yoshido, Marec, Sahara (2005) *Chromosoma* 114: 193-202.

Direct FISH-mapping of *Bombyx mori* orthologous genes on chromosomes of *Samia cynthia* ssp.





Yoshido, Sahara, Marec, Matsuda (2010) *Heredity*. Online 28 July 2010.

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