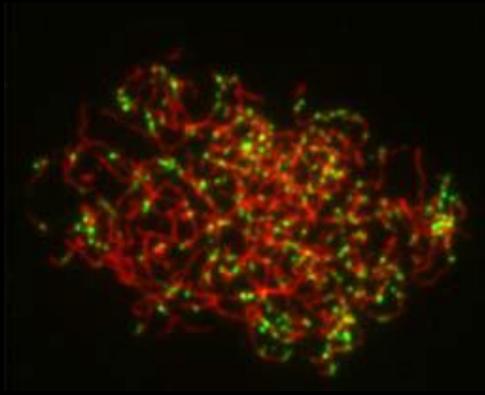# Controlling meiotic recombination:
To mix or not to mix

Raphael Mercier, IJPB INRA Versailles

Meiotic Crossovers

→ Crossovers generate <u>genetic diversity</u> among siblings

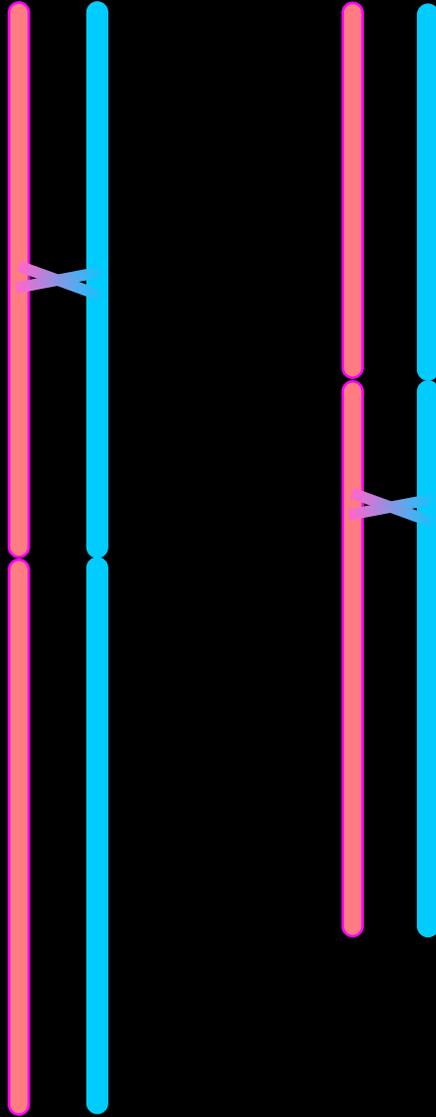→ Crossovers are <u>essential</u> for accurate distribution of chromosomes at meiosis

Crossover distribution is not random

Crossover distribution is not random
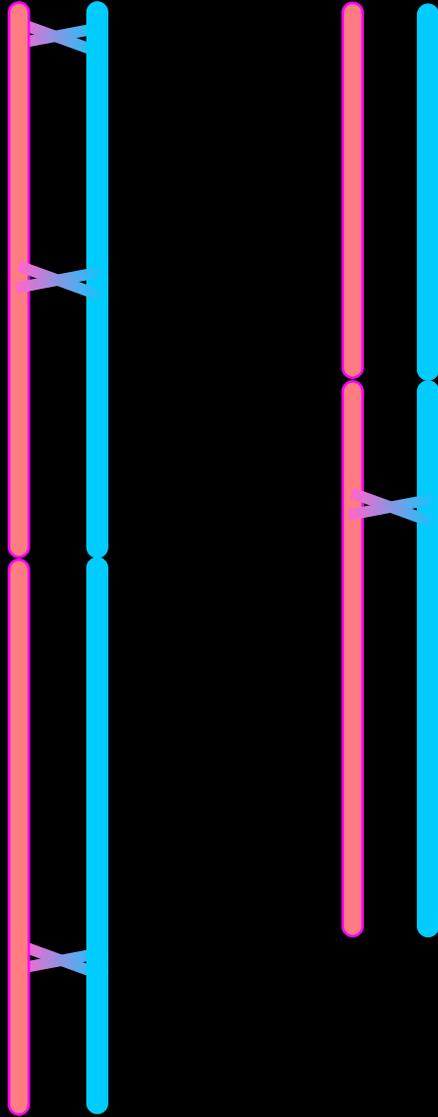
1) Obligate crossover:

At least one per chromosome pair
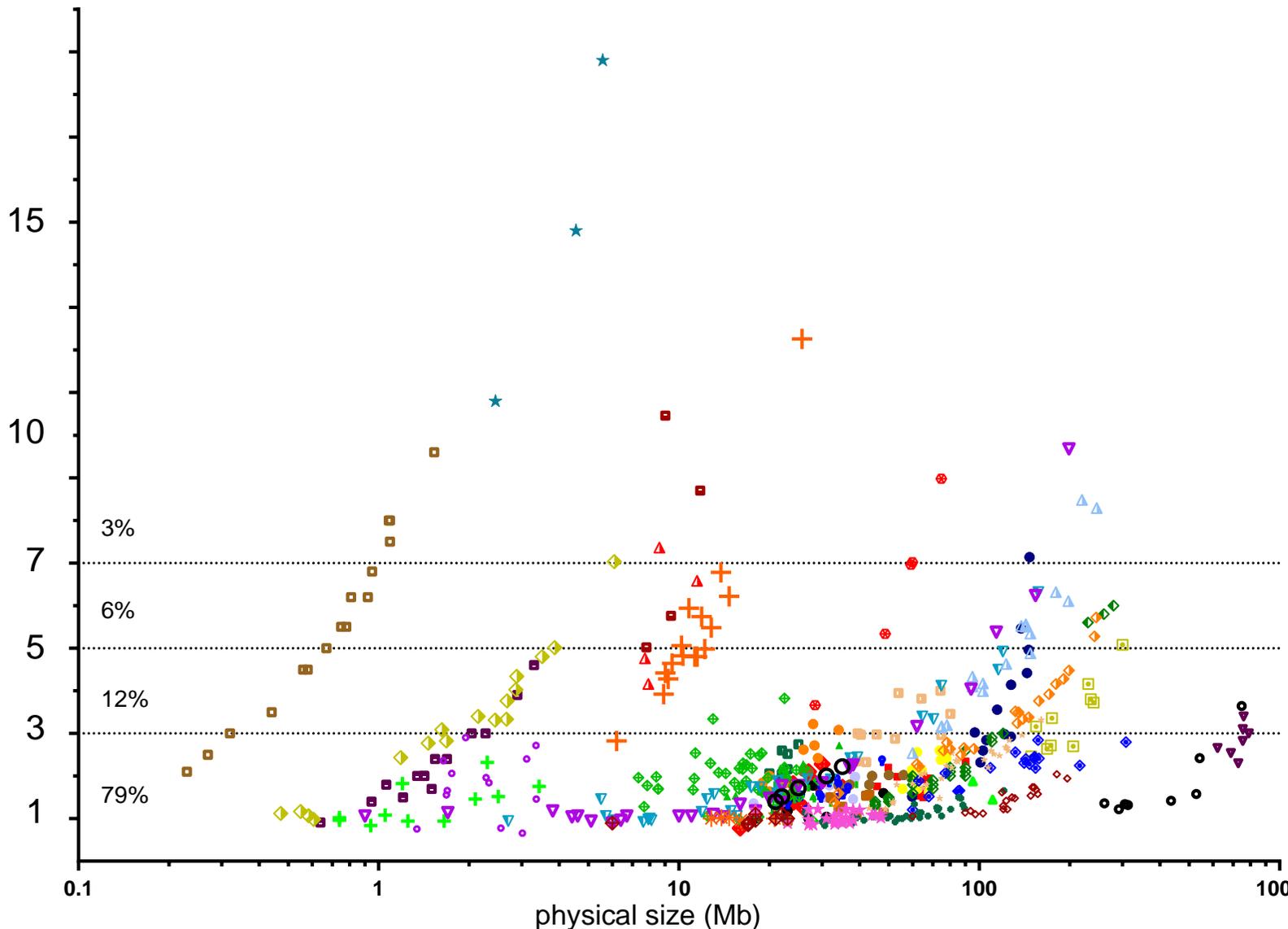
Crossover distribution is not random

2) Not numerous:

Typically 1 to 3 per chromosome pair

Genetic (cM) *vs* physical size (Mb)
of chromosomes from a range of species
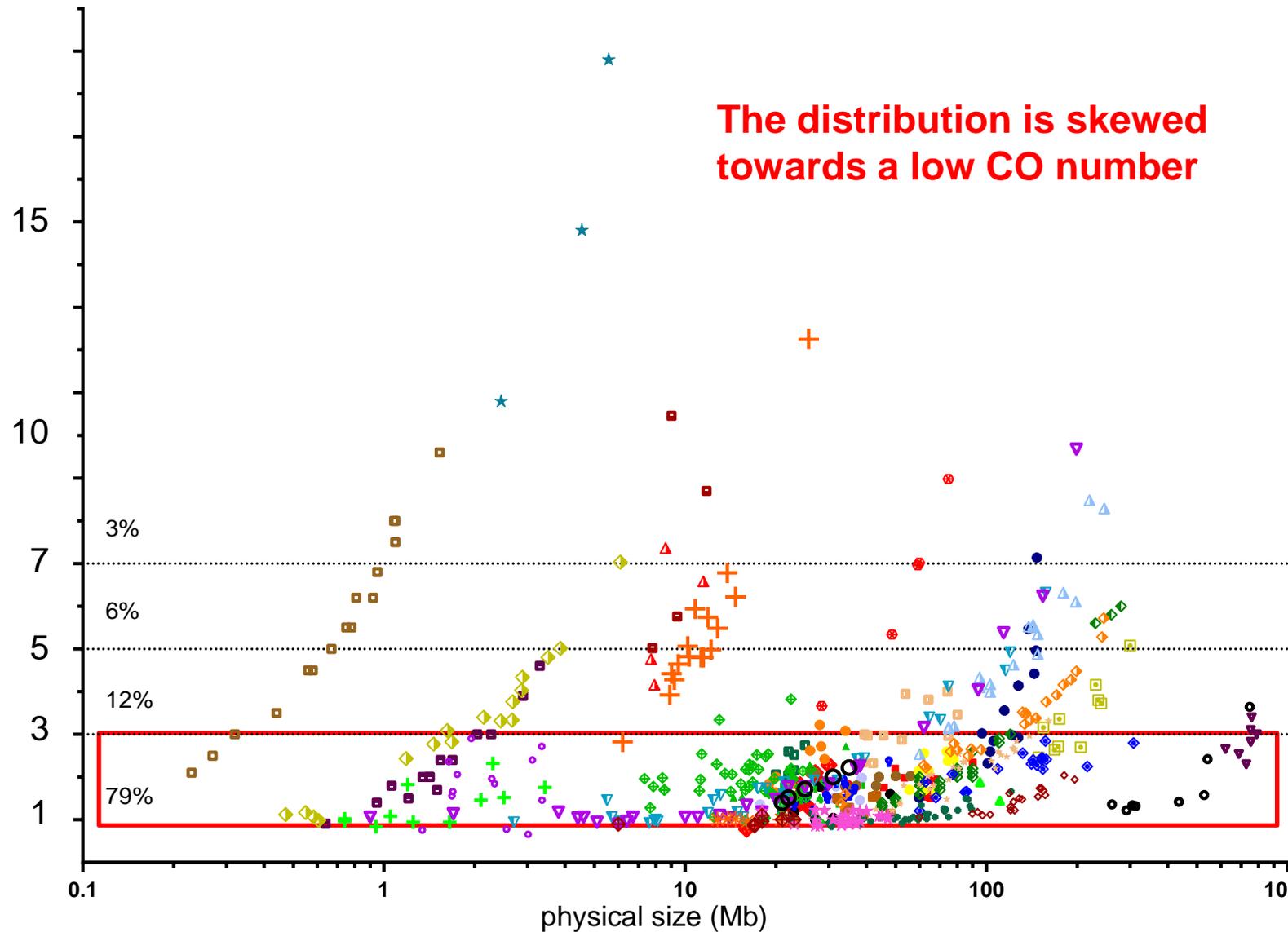
Crossovers per meiosis

physical size (Mb)

Legend:

- ○ *Arabidopsis thaliana*
- *Sorghum bicolor*
- *Eucalyptus grandis*
- *Medicago truncatula*
- *Brachypodium distachyon*
- *Cardamine hirsuta*
- Corn *Zea mays*
- Apple *Malus domestica*
- wild strawberry *Fragaria vesca*
- Sugar Beet *Beta vulgaris*
- Chinese cabbage *Brassica rapa*
- Coton *Gossypium arboreum*
- Peach *Prunus persica*
- *Capasella rubella*
- Cacao *Theobroma cacao*
- Melon *Cucumis melo*
- Tomato *Solanum lycopersicum*
- Cucumber *Cucumis sativus*
- Barley *Hordeum vulgare*
- Fission yeast *Schizosaccharomyces Pombe*
- Budding yeast *Saccharomyces cerevisiae*
- Button mushroom *Agaricus bisporus*
- *Fusarium gramineanum*
- Fusarium *Gibberella zeae*
- *Zymoseptoria tritici*
- chicken *Gallus gallus domesticus*
- Human *Homo sapiens*
- Oposum *Monodelphis domestica*
- Mouse *Mus Musculus*
- Dog *Canis familiaris*
- Pig *Sus scrofa*
- Cattle *Bos taurus*
- Cat *Felis catus*
- Soay sheep *Ovis aries*
- Flycatcher
- Carp *Cyprinus carpio*
- mangrove killifish *Kryptolebias mammoratus*
- *Caenorhabditis elegans*
- Honey bee *Apis mellifera*
- Anopheles gambiae
- *Trypanosoma brucei*
- Plasmodium falciparum

Genetic (cM) *vs* physical size (Mb)
of chromosomes from a range of species

Crossovers per meiosis

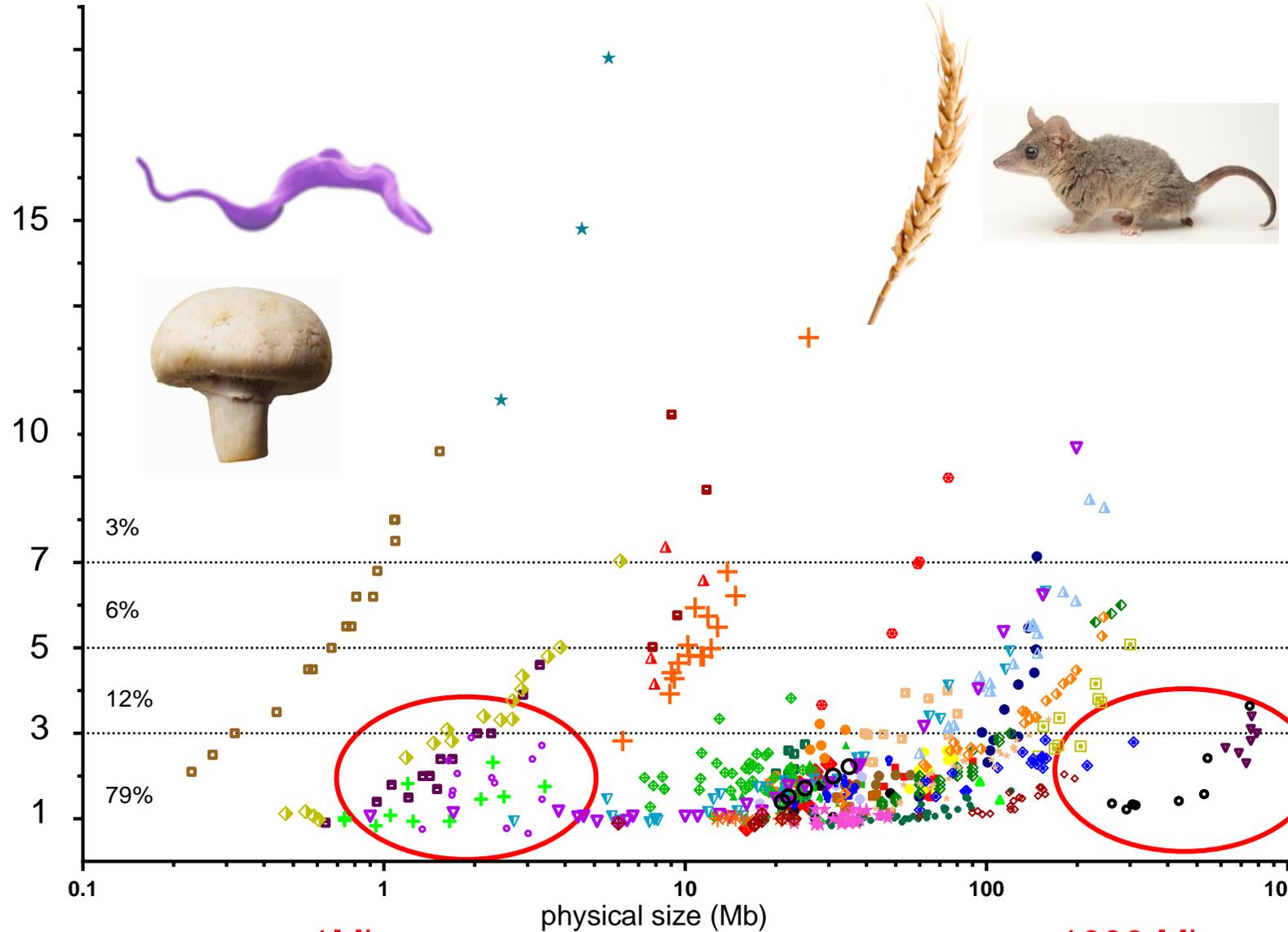**The distribution is skewed towards a low CO number**

physical size (Mb)

Legend:
- Arabidopsis thaliana
- Sorghum bicolor
- Eucalyptus grandis
- Medicago truncatula
- Brachypodium distachyon
- Cardamine hirsuta
- Corn Zea mays
- Apple Malus domestica
- wild strawberry Fragaria vesca
- Sugar Beet Beta vulgaris
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- Peach Prunus persica
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- Melon Cucumis melo
- Tomato Solanum lycopersicum
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- Budding yeast Saccharomyces cerevisiae
- Button mushroom Agaricus bisporus
- Fusarium gramineanum
- Fusarium Gibberella zeae
- Zymoseptoria tritici
- chicken Gallus gallus domesticus
- Human Homo sapiens
- Oposum Monodelphis domestica
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- Dog Canis familiaris
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- Honey bee Apis mellifera
- Anopheles gambiae
- Trypanosoma brucei
- Plasmodium falciparum

Genetic (cM) *vs* physical size (Mb)
of chromosomes from a range of species

Crossovers
per meiosis

physical size (Mb)

~1Mbase

~1000 Mbases

Legend:
- ○ *Arabidopsis thaliana*
- *Sorghum bicolor*
- *Eucalyptus grandis*
- *Medicago truncatula*
- *Brachypodium distachyon*
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- Carp *Cyprinus carpio*
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- *Caenorhabditis elegans*
- Honey bee *Apis mellifera*
- Anopheles gambiae
- *Trypanosoma brucei*
- Plasmodium falciparum

Genetic (cM) *vs* physical size (Mb)
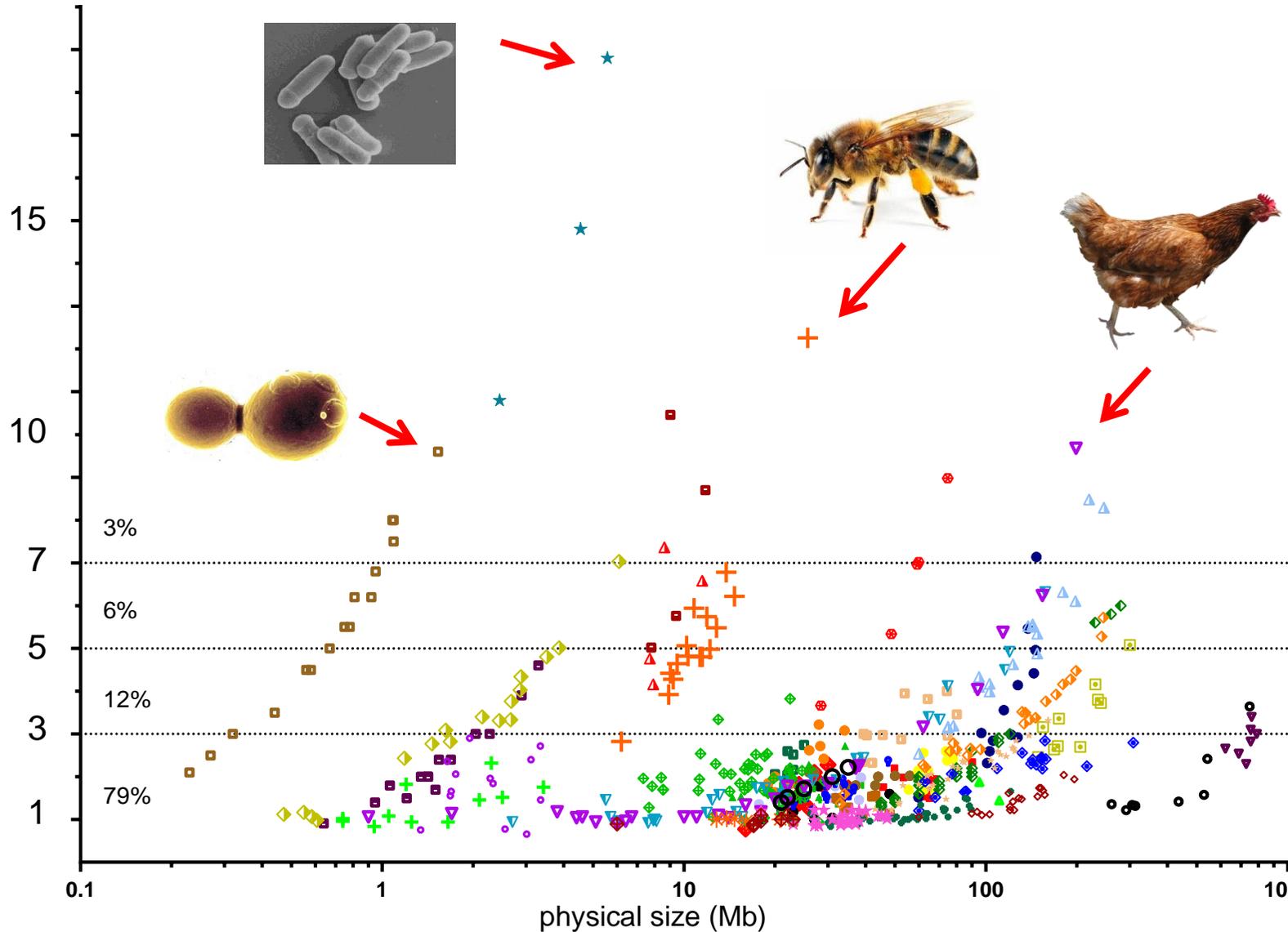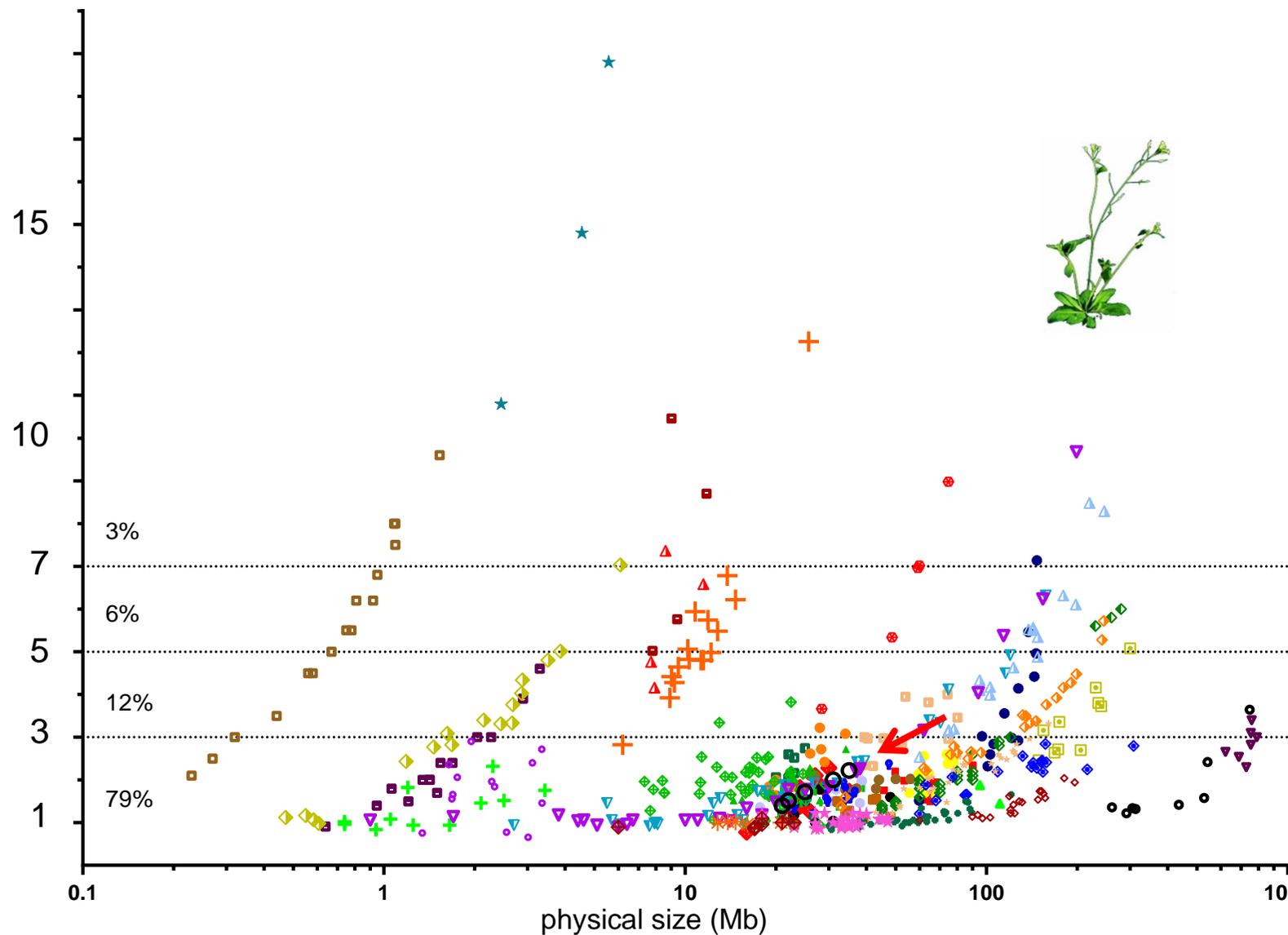of chromosomes from a range of species

Crossovers per meiosis

15

10

7 — 3%

6%

5

12%

3

79%

1

physical size (Mb)
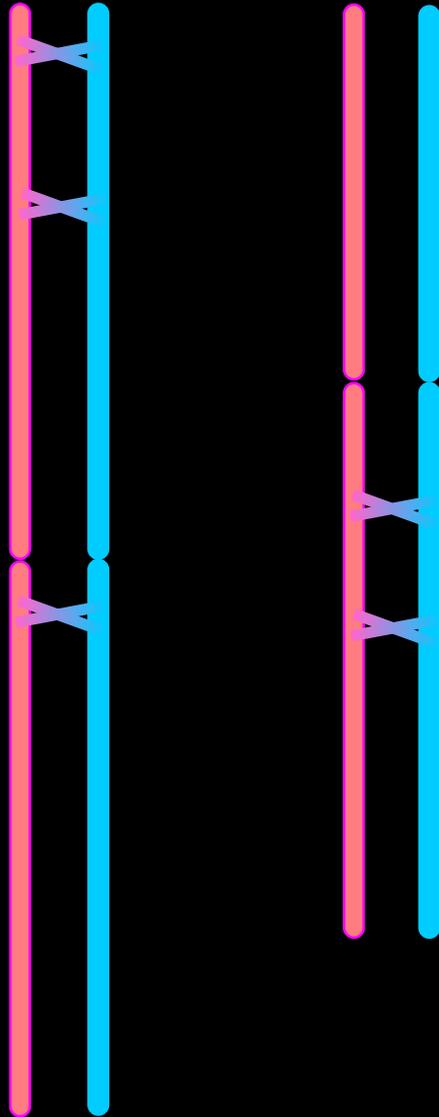
0.1   1   10   100   1000

○ *Arabidopsis thaliana*

● *Sorghum bicolor*
□ *Eucalyptus grandis*
■ *Medicago truncatula*
✶ *Brachypodium distachyon*
■ *Cardamine hirsuta*
□ Corn *Zea mays*
● Apple *Malus domestica*
· wild strawberry *Fragaria vesca*
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● Chinese cabbage *Brassica rapa*
● Coton *Gossypium arboreum*
● Peach *Prunus persica*
✚ *Capasella rubella*
● Cacao *Theobroma cacao*
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▲ Fusarium *Gibberella zeae*
◇ *Zymoseptoria tritici*
▽ chicken *Gallus gallus domesticus*
◇ Human *Homo sapiens*

● Oposum *Monodelphis domestica*
◇ Mouse *Mus Musculus*
● Dog *Canis familiaris*
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· Cattle *Bos taurus*
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✶ mangrove killifish *Kryptolebias mammoratus*
✳ *Caenorhabditis elegans*
✚ Honey bee *Apis mellifera*
▲ Anopheles gambiae
✚ *Trypanosoma brucei*
■ Plasmodium falciparum

Genetic (cM) *vs* physical size (Mb)
of chromosomes from a range of species

Crossovers
per meiosis

physical size (Mb)

3%
6%
12%
79%

○ *Arabidopsis thaliana*

● *Sorghum bicolor*
□ *Eucalyptus grandis*
■ *Medicago truncatula*
✳ *Brachypodium distachyon*
■ *Cardamine hirsuta*
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✚ *Trypanosoma brucei*
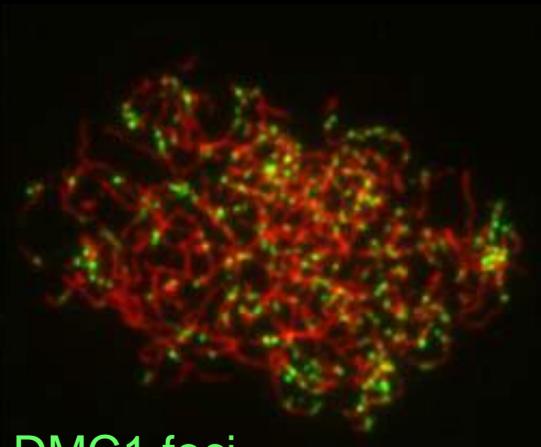■ Plasmodium falciparum

Crossover distribution is not random

3) crossover interference:

Crossovers tend to be away from each others

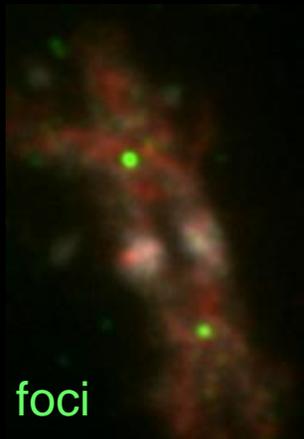# Precursors largely excess crossovers

DMC1 foci

MLH1 foci

Double Strand Breaks
(~200)

Non-Crossover repair

Class I COs
(~9)
(Interfering)

Class II COs
(~1)
(Non interfering)

- Crossover number is very low in most species
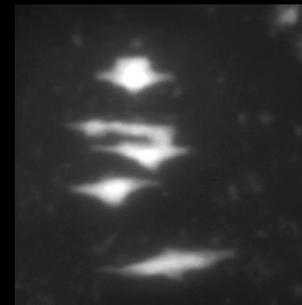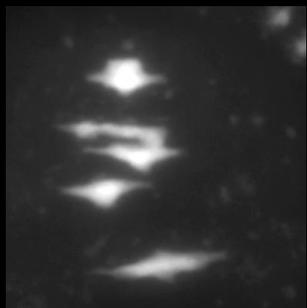- molecular precursors largely outnumber crossovers

What limits crossover formation?

Looking for anti-crossover factors

Wild-type

zmm mutant

Supressors ?

zmm-/- (T-DNA)

gene?-/- (EMS)

Anti-crossover screen summary

- 7000 lines screened
- 8 genes characterized so far
- 3 pathways

FANCM
MHF1
MHF2

RECQ4/BLM
TOP3α
RMI1

FIGL1
FLIP

The FANCONI pathway
Crismani et al, Science 2012
Girard et al, NAR 2014

The BTR pathway
Séguéla et al, PNAS 2015
Séguéla et al, NAR 2016

The FIDGETIN pathway
Girard et al, PLoS Genetics 2015
Fernandes et al, unpublished

Class II crossovers are specifically increased in all these mutants

Wild type

Double Strand Breaks

Non-Crossover repair

ZMM   MUS81

Class I COs   **Class II COs**

FANCM MHF1 MHF2

RECQ4 TOP3α RMI1

FIGL1 FLIP

Double Strand Breaks

Non-Crossover repair

ZMM   MUS81

Class I COs   **Class II COs**

Crismani et al, 2012. Girard et al, 2014, 2015. Seguéla et al 2015, 2016

Genome wide analysis of recombination in single and multi-mutants

Col/Ler F1 Hybrid

↓ selfing

>200 F2 plants
Genotyping for segregating markers

♂♀

Genome wide analysis of recombination in single and multi-mutants

Genome wide analysis of recombination in single and multi-mutants

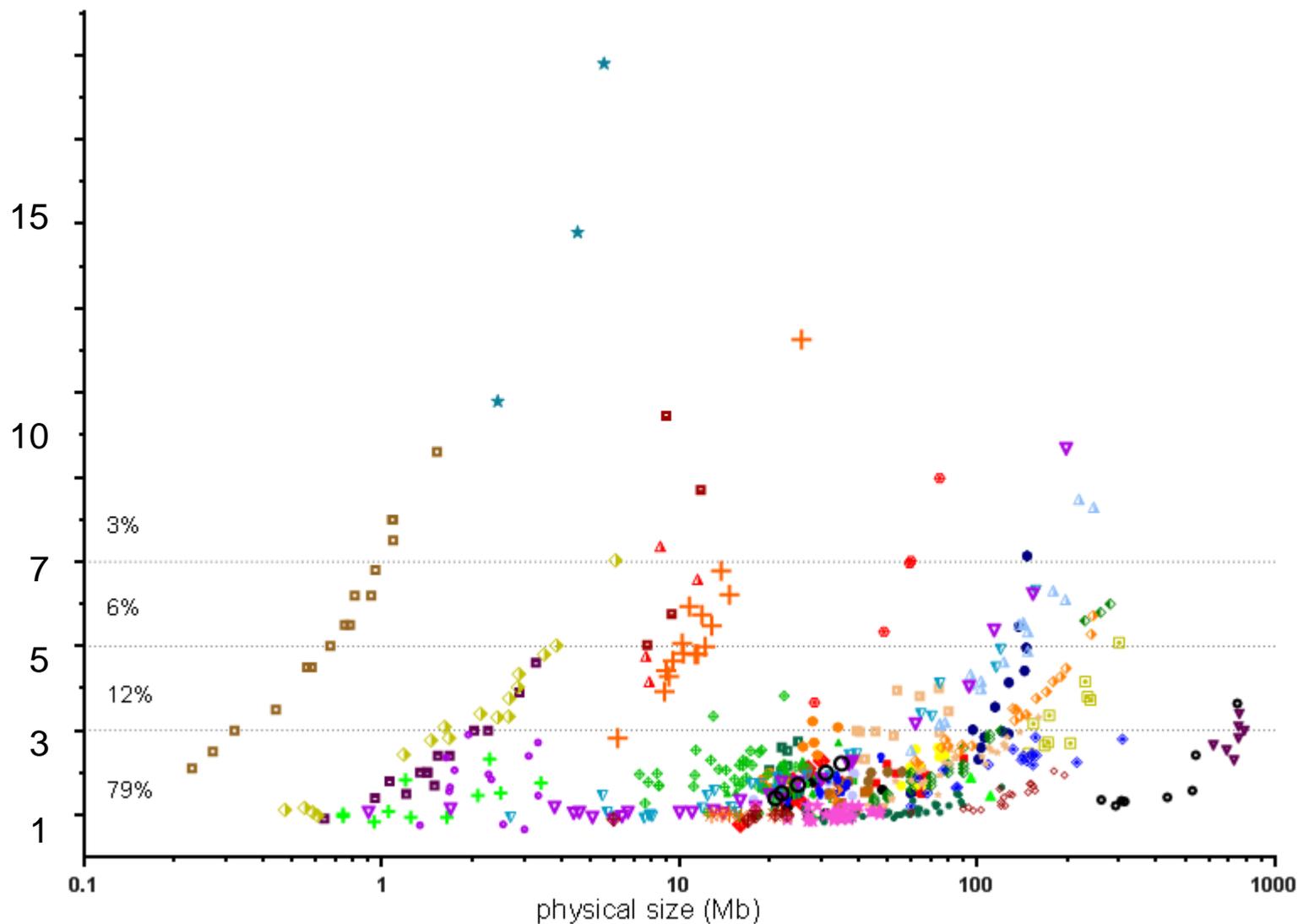# Genome wide analysis of recombination in single and multi-mutants

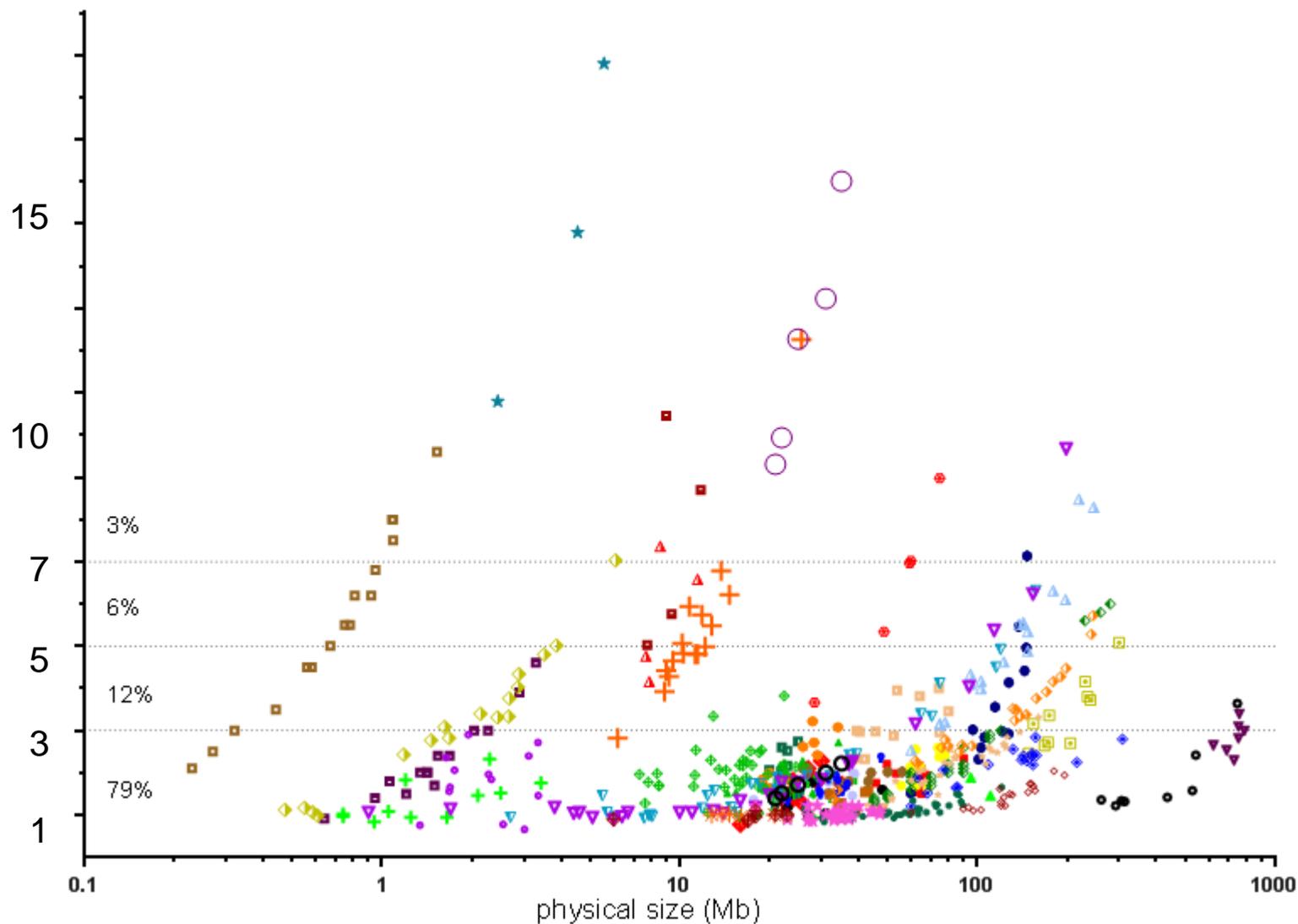Genetic (cM) *vs* physical size (Mb) of chromosomes from in a range of species

Crossovers per meiosis

physical size (Mb)

○	*Arabidopsis thaliana*
●	*Sorghum bicolor*
□	*Eucalyptus grandis*
■	*Medicago truncatula*
✶	*Brachypodium distachyon*
■	*Cardamine hirsuta*
□	Corn *Zea mays*
●	Apple *Malus domestica*
·	wild strawberry *Fragaria vesca*
●	Sugar Beet *Beta vulgaris*
●	Chinese cabbage *Brassica rapa*
●	Coton *Gossypium arboreum*
●	Peach *Prunus persica*
⊕	*Capasella rubella*
●	Cacao *Theobroma cacao*
◆	Melon *Cucumis melo*
■	Tomato *Solanum lycopersicum*
▲	Cucumber *Cucumis sativus*
▼	Barley *Hordeum vulgare*
★	Fission yeast *Schizosaccharomyces Pombe*
▣	Budding yeast *Saccharomyces cerevisiae*
●	Button mushroom *Agaricus bisporus*
▣	*Fusarium gramineanum*
▲	Fusarium *Gibberella zeae*
◇	*Zymoseptoria tritici*
▽	chicken *Gallus gallus domesticus*
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●	Cattle *Bos taurus*
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◆	Soay sheep *Ovis aries*
▽	Flycatcher
✦	Carp *Cyprinus carpio*
★	mangrove killifish *Kryptolebias mammoratus*
✳	*Caenorhabditis elegans*
✚	Honey bee *Apis mellifera*
▲	Anopheles gambiae
✚	*Trypanosoma brucei*
■	Plasmodium falciparum

Genetic (cM) *vs* physical size (Mb)
of chromosomes from in a range of species

Crossovers per meiosis

Legend:
- Arabidopsis thaliana
- Arabidopsis thaliana recq4 figl1
- Sorghum bicolor
- Eucalyptus grandis
- Medicago truncatula
- Brachypodium distachyon
- Cardamine hirsuta
- Corn Zea mays
- Apple Malus domestica
- wild strawberry Fragaria vesca
- Sugar Beet Beta vulgaris
- Chinese cabbage Brassica rapa
- Coton Gossypium arboreum
- Peach Prunus persica
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- Oposum Monodelphis domestica
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- mangrove killifish Kryptolebias mammoratus
- Caenorhabditis elegans
- Honey bee Apis mellifera
- Anopheles gambiae
- Trypanosoma brucei
- Plasmodium falciparum

physical size (Mb)

Fertility in these Hyper-recombinant Mutants ?

Wild type

recq4 figl1

Number of seeds per fruit
Col/Ler Hybrid

ns

n=6 | n=3 | n=3 | n=3 | n=3 | n=3 | n=3

Wild type | figl1 | recq4 | figl1 fancm | recq4 fancm | recq4 figl1 | recq4 figl1 fancm

Number of pollen grains per anther
Col/Ler hybrid

ns | *** | *** | *** | *** | *** | ***

■ dead
alive

n=6 | n=3 | n=3 | n=5 | n=3 | n=3 | n=3 | n=3

Wild type | fancm | figl1 | recq4 | figl1 fancm | recq4 fancm | recq4 figl1 | recq4 figl1 fancm

Recombination frequency along chromosomes

recombination
(cM/mb +/- 95% CI)

recq4
figl1

Wild type

Coordinates (Mb)

Transfer to crops

Transfer to crops

recq4 compared to wild type rice: x 3.3

CONCLUSIONS for « to mix »

- Meiotic crossovers can be largely increased (x7.8 genome wide)

  ➢ Potential great tool for plant breeding

  ➢ But no effect in peri-centromeres

  ➢ Why having so few crossovers in most species?

not To Mix:
towards apomixis

Apomixis
(clonal reproduction through seeds)

- Relatively common in the plant kingdom

Erigeon   *Taraxacum*   *Heracium*   *Tripsacum*   *Boechera*   Ranunculus

- Absent in crops
- Its introduction in crops has potential revolutionary application

P1
(inbred line #1)

X

P2
(inbred line #2)

SEX

APOMIXIS

F1

F2

F2, F3, F4, ...

T. Dresselhaus

Hybrid parent

Clonal embryon

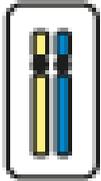Hybrid parent

Clonal diploid
gamete

Clonal embryon

The combination of three mutations turn meiosis into mitosis

- Recombination
- Two rounds of chromosome segregation
- Co-segregation of sister chromatids at division I

*MiMe
(spo11/rec8/osd1)*

male

Female

d'Erfurth et al, Plos Biol 2009.
d'erfurth, Cromer et al, Plos genet 2010

100% clonal gametes

Doubling of ploidy at each generation in the *MiMe* line

2n (10 chr)

4n (20 chr)

8n (40 chr)

meiosis

Hybrid parent

MiMe

Clonal diploid
gamete

Clonal embryon

Hybrid parent

MiMe
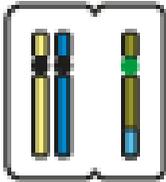
Clonal diploid gamete
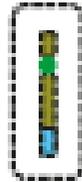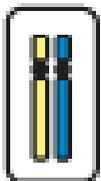
GEM Gamete

Fertilization

Clonal embryon

Haploid plants produced by centromere-mediated genome elimination

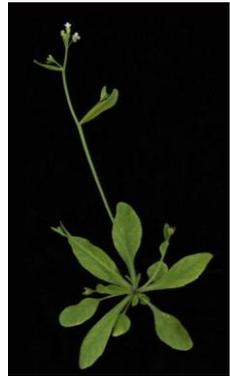Maruthachalam Ravi[1] & Simon W. L. Chan[1]

Simon Chan

Ravi et al, Nature, 2010

cross (♀ x ♂)	Seeds per siliqua	Germination rate (%)	Total plants analysed	Hybrid diploid* (%)	Triploid (%)	Aneuploid (%)	Clones* (%)
MiMe x GEM	15	92	156	0.6	13	53	34
GEM x MiMe	23	0.5	12	0	25	33	42
cloned *MiMe* x GEM	14	91	79	1.3	20	54	24

MiMe♀ x GEM♂

locus	OSD1	REC8	SPO11	CENH3	a	b	c	d	e	f	g	h	i	j	k	l	m
chromosome	3	5	3	1	1	1	1	2	2	2	3	3	4	4	4	5	5

♀ parent

♂ parent

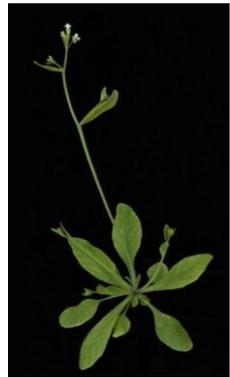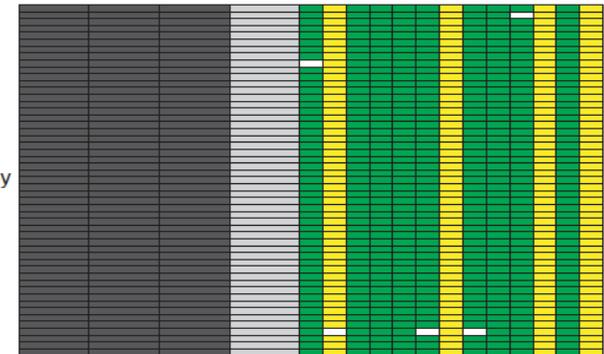Marimuthu, Jolivet et al. Science 2011

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Marimuthu, Jolivet et al. Science 2011

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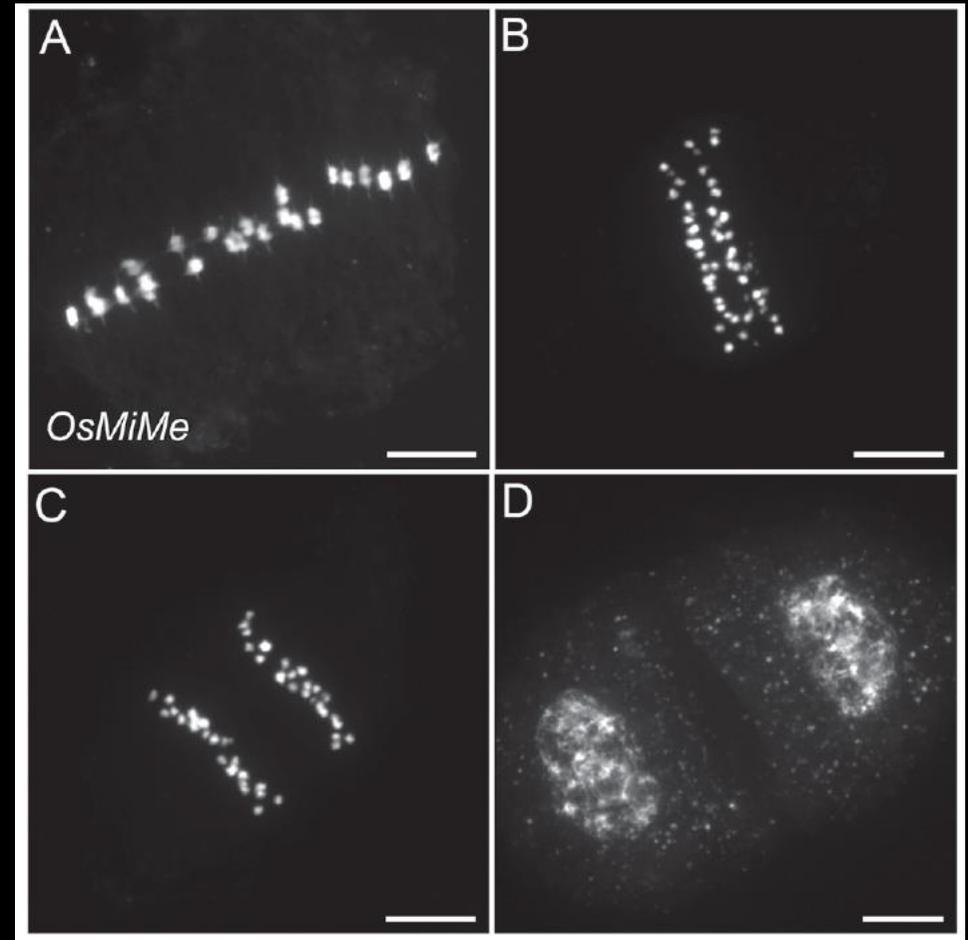MiMe♀ x GEM♂

cloned *MiMe*♀ x GEM♂

Mitosis instead of Meiosis

\+ Cross with the genome elimination line
_____

\= Clonal seeds !

The combination of the same three mutations turn meiosis into mitosis in rice

OsMiMe

CONCLUSIONS for « not to mix »

➢ It is possible to engineer clonal reproduction through seeds

➢ Some limitations remains

  - Frequency of clonal seeds
  - Induction of embryogenesis in crops

THANKS!

INRA Versailles
Meiotic Mechanism Team

To mix

Not to mix

Mathilde Séguéla-Arnaud
Joiselle Fernandes
Chloé Girard
Wayne Crismani
et al

Isabelle d'Erfurth
Sylvie Jolivet
Laurence Cromer
et al

CIRAD Montpellier
UMR AGAP

Delphine Mieulet
Emmanuel Guiderdoni