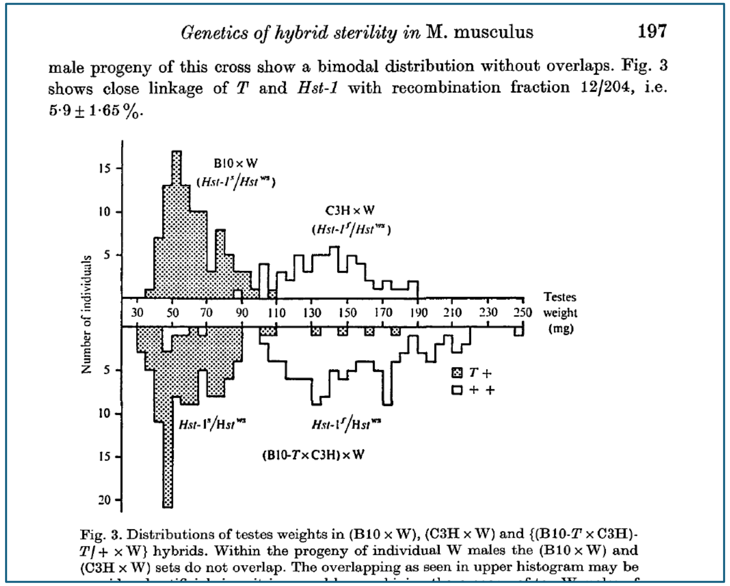
Fifty Years of Hybrid Sterility 1

1974 - 2024

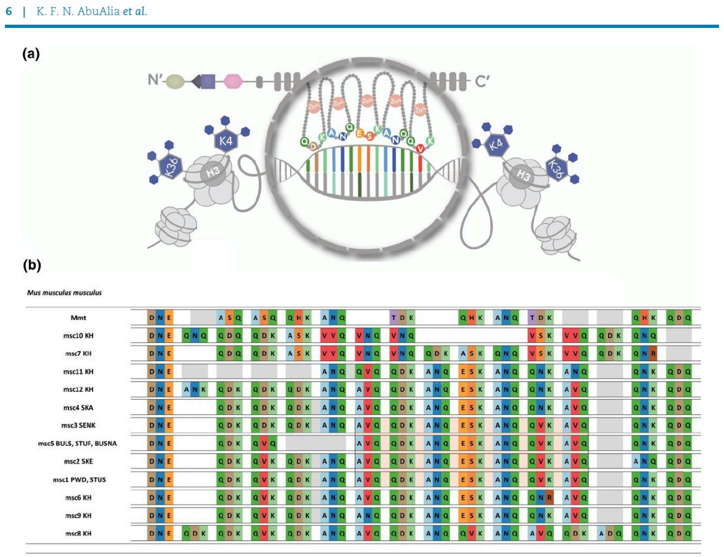
Genetical Research 1974


Genetics of hybrid sterility in M. musculus 197

male progeny of this cross show a bimodal distribution without overlaps. Fig. 3 shows close linkage of *T* and *Hst-1* with recombination fraction 12/204, i.e. 5·9 ± 1·65 %.

Fig. 3. Distributions of testes weights in (B10 × W), (C3H × W) and {(B10-*T* × C3H)-*T*/+ × W} hybrids. Within the progeny of individual W males the (B10 × W) and (C3H × W) sets do not overlap. The overlapping as seen in upper histogram may be

Genetics 2024



Outline

- Hybrid Sterility and Speciation
- Milestones of the Genetics of Hybrid Sterility in Mus musculus
- Ongoing Projects

Genetics of hybrid sterility

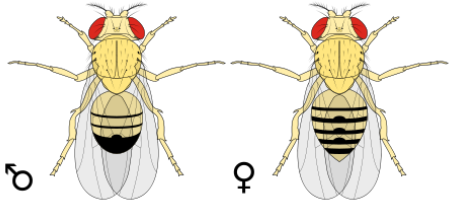
Hybrid sterility refers to a situation when two fertile parental taxa produce a hybrid that is sterile

Horse x donkey

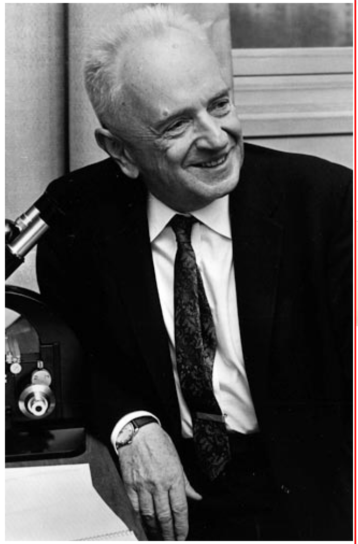
Mule (infertile)

Genetics of Drosophila hybridization

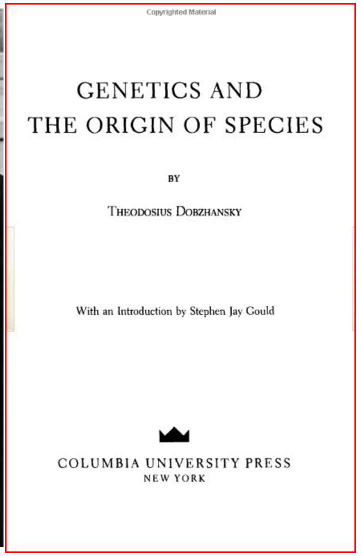
Dobzhansky, Muller, Haldane, Coyne, Barbash, Presgraves



1930 → 2020



Theodosius Dobzhansky
(1900 – 1975)



GENETICS AND
THE ORIGIN OF SPECIES

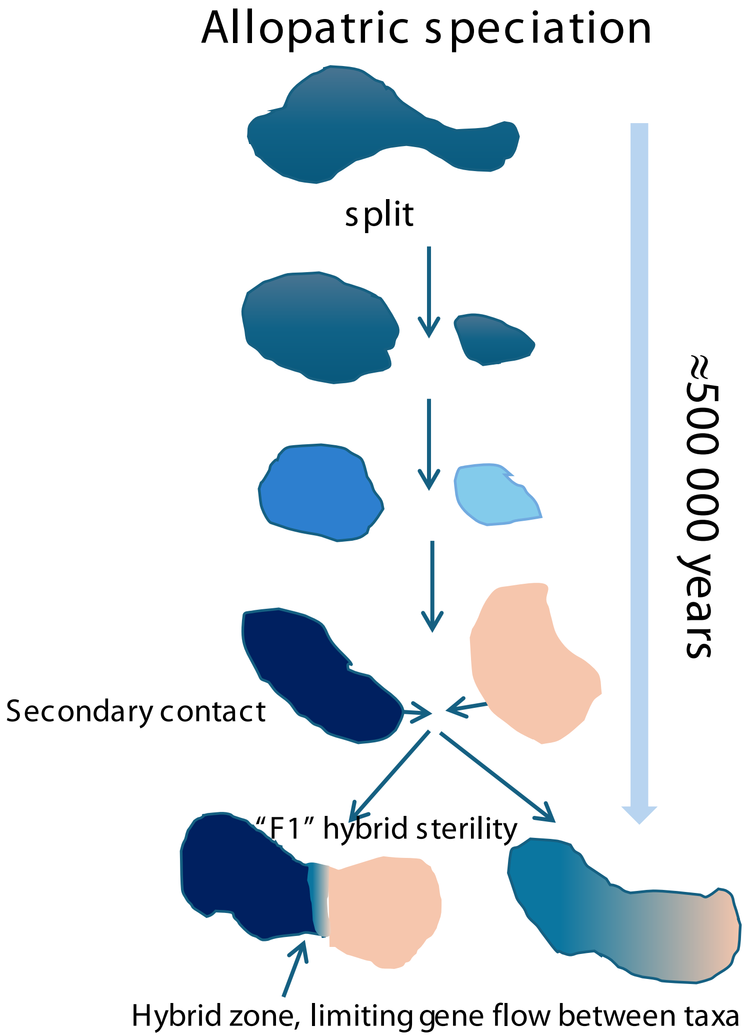
BY

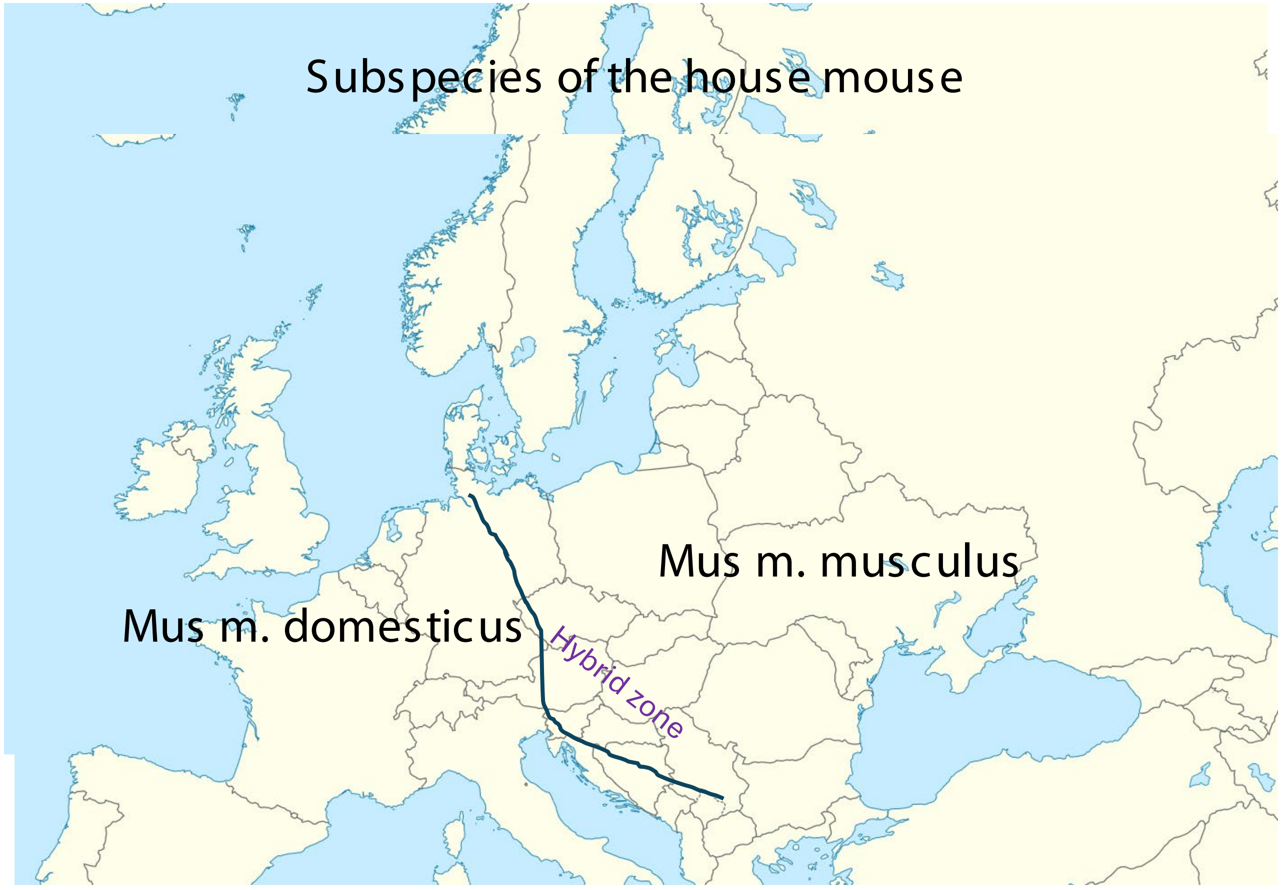
THEODOSIUS DOBZHANSKY

With an Introduction by Stephen Jay Gould

COLUMBIA UNIVERSITY PRESS
NEW YORK

Hybrid sterility and origin of species

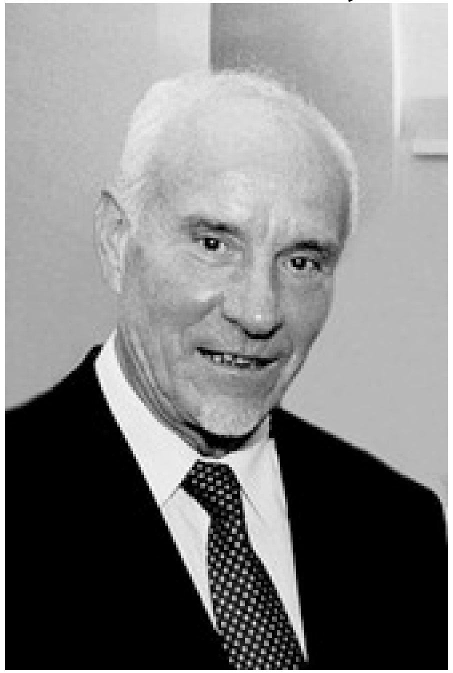




Milestone 1:

Hybrid sterility 1 – Hst1 genetic locus

Pavol Iványi



(1930–2005)

Genet. Res., Camb. (1975), 24, *pp.* 189-206 **189**
With 1 *plate and* 4 *text-figures*
Printed in Great Britain

Genetic studies on male sterility of hybrids between laboratory and wild mice *(Mus musculus* L.)

BY J. FOREJT AND P. IVANYI
Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences, 142 20 Praha 4, Krc, Czechoslovakia
{Received 24 *June* 1974)

Genetic studies on male sterility of hybrids between laboratory and wild mice *(Mus musculus* L.) BY J. FOREJT AND P. IVANYI

B10 x W-I	sterile	C3H x WI fertile
B10 x W-II	fertile	
B10 x WIII	sterile and fertile	

Genetics of hybrid sterility in M. musculus 197

male progeny of this cross show a bimodal distribution without overlaps. Fig. 3 shows close linkage of *T* and *Hst-1* with recombination fraction 12/204, i.e. $5 \cdot 9 \pm 1 \cdot 65\,\%$.

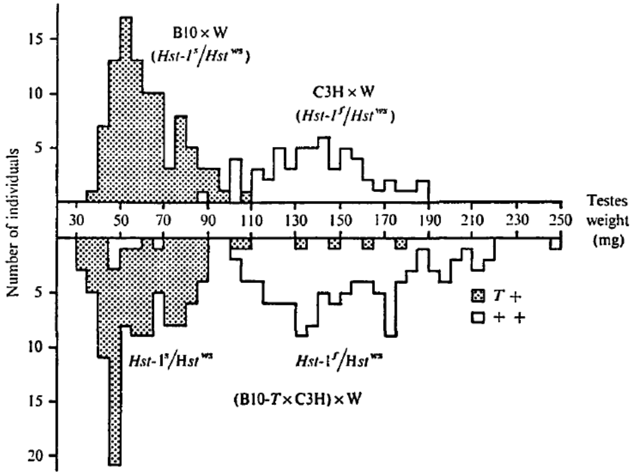


Fig. 3. Distributions of testes weights in (B10 × W), (C3H × W) and {(B10-*T* × C3H)-*T*/+ × W} hybrids. Within the progeny of individual W males the (B10 × W) and (C3H × W) sets do not overlap. The overlapping as seen in upper histogram may be

	Recombinations	%	Mb
T – Hst-1	4/89	4·5	8.65
Hst-1 – H-2	12/89	13·5	18
T – H-2	16/89	18·0	26.65

> Nature. 1976 Mar 11;260(5547):143-5. doi: 10.1038/260143a0.

Spermatogenic failure of translocation heterozygotes affected by H-2-linked gene in mouse

J Forejt

PMID: 1256555 DOI: 10.1038/260143a0

Hybrid sterility 1 – Hst1 genetic locus

Mus m. musculus inbred and consomic strains

PWD/Ph
PWK/Ph

C57BL/10J (B10)
C57BL/6J (B6)
C3H/J

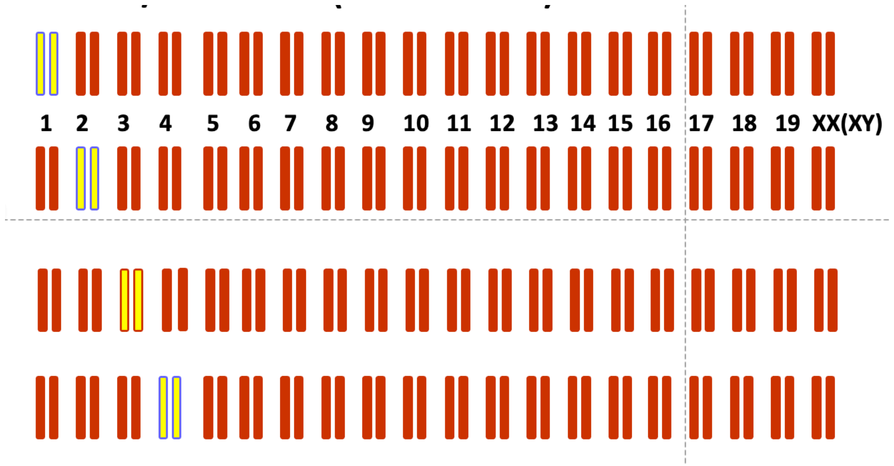
PWD/Ph and PWK/Ph inbred mouse strains of Mus m. musculus subspecies--a valuable resource of phenotypic variations and genomic polymorphisms. Gregorová S, Forejt J. Folia Biol (Praha). 2000;46(1):31-41.

GENOME RESEARCH

Mouse consomic strains: Exploiting genetic divergence between Mus m. musculus and Mus m. domesticus subspecies

Sona Gregorová, Petr Divina, Radka Storchova, Zdenek Trachtulec, Vladana Fotopulosova, Karen L. Svenson, Leah Rae Donahue, Beverly Paigen and Jiri Forejt

Genome Res. 2008 18: 509-515; originally published online Feb 6, 2008;



B6-Chr1^{PWD}

B6-Chr2^{PWD}

Soňa Gregorová

Bev and Ken Paigen's

The Jackson Laboratory, Bar Harbor, ME

GENOME RESEARCH

Mouse consomic strains: Exploiting genetic divergence between Mus m. musculus and Mus m. domesticus subspecies

Sona Gregorová, Petr Divina, Radka Storchova, Zdenek Trachtulec, Vladana Fotopulosova, Karen L. Svenson, Leah Rae Donahue, Beverly Paigen and Jiri Forejt

Genome Res. 2008 18: 509-515; originally published online Feb 6, 2008;
Access the most recent version at doi:10.1101/gr.7160508

Milestone 2:

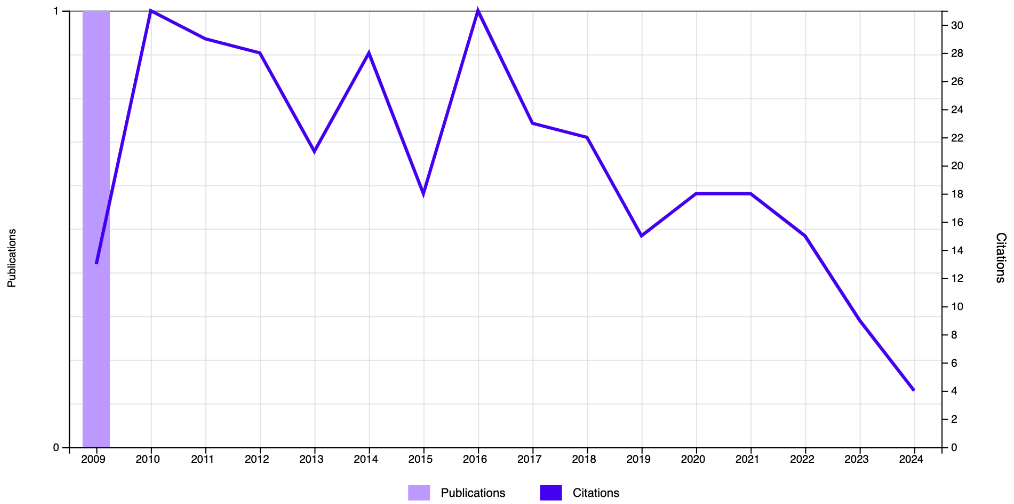
Hybrid sterility 1 locus identified with *Prdm9* gene

A Mouse Speciation Gene Encodes a Meiotic Histone H3 Methyltransferase

ONDREJ MIHOLA, ZDENEK TRACHTULEC, CESTMIR VLCEK, JOHN C. SCHIMENTI, AND JIRI FOREJT

SCIENCE 16 Jan 2009 Vol 323, Issue 5912 pp. 373-375
DOI: 10.1126/science.1163601

Ondra Mihola

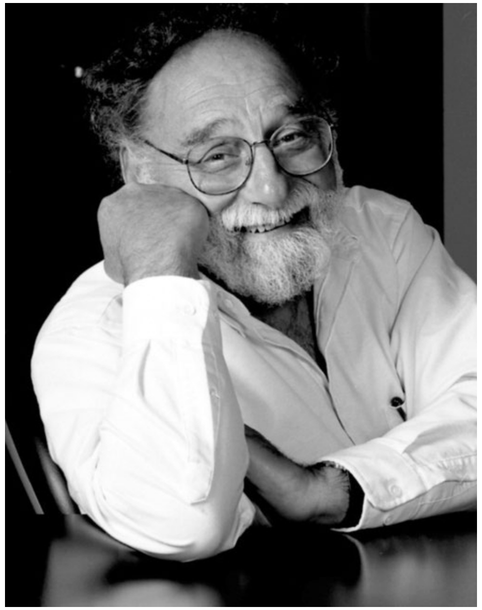


Zdeněk Trachtulec

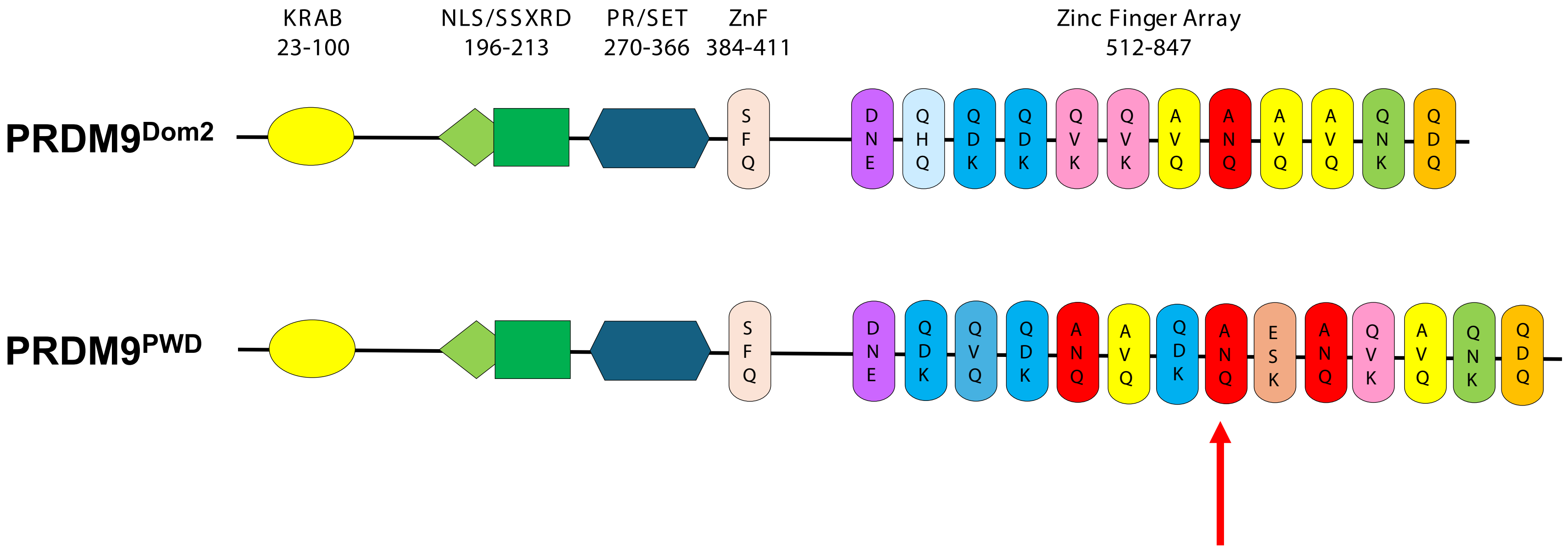
Milestone 2:

Hybrid sterility 1 locus identified with *Prdm9* gene

PRDM9 histone methyltransferase determines positions of recombination hotspots



Ken Paigen 1927-2020



Sequence-specific DNA-binding zinc-finger domain

Baudat et al. Science 2010
Parvanov et al. Science 2010
Myers et al. Science 2010

2019

Od Ken Paigen <ken.paigen@jax.org>

Komu Professor Jiri Forejt 11.10.2019 18:38

Kopie Natalie Powers <Natalie.Powers@jax.org>, Petko Petkov <Petko.Petkov@jax.org>

Předmět **Re: manuscript**

Dear Jiri,

I am sorry not to reply sooner. I fell last weekend and suffered a concussion. I had time to read your comments, which are very helpful, but not to draft a reply. I am recovering, but am not allowed screen time (I am stealing this). Natalie has indeed been workin on a reply. Will get back to you as soon as we can

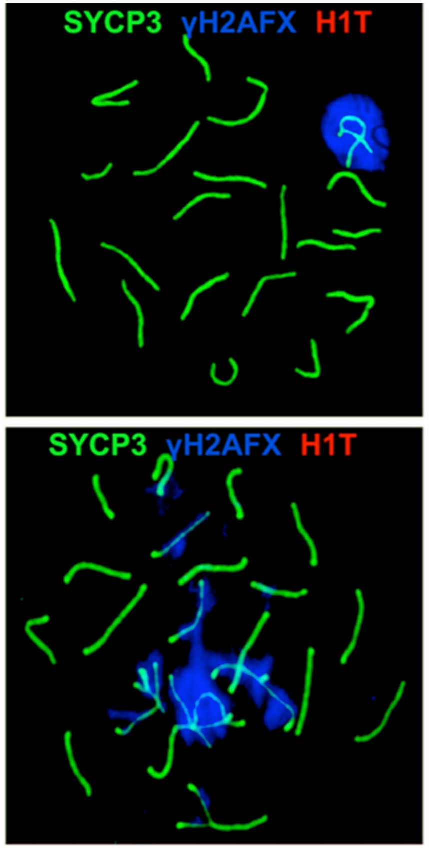
On 10/11/19, 10:25 AM, "Jiri Forejt" <jiri.forejt@img.cas.cz> wrote:

> Dear Ken,
>
> I feel that your silence means either that you feel offended or that you
> asked Tanmoy and Nathalie to prepare a response showing that I am

† Feb, 2020

Milestone 3:

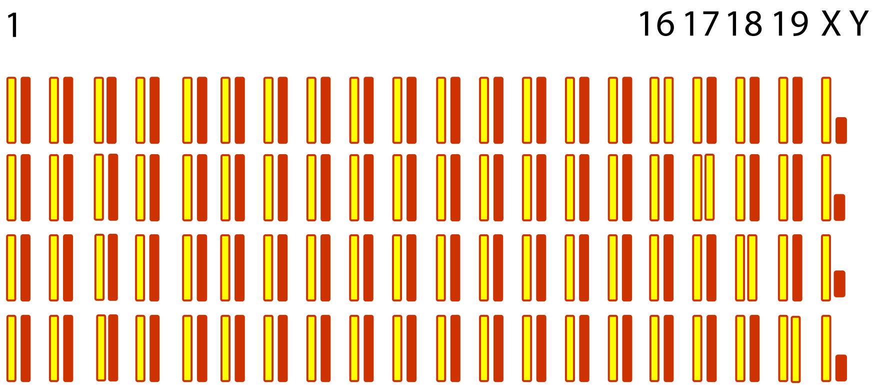
Recognition of homologous chromosomes is impaired in sterile hybrids

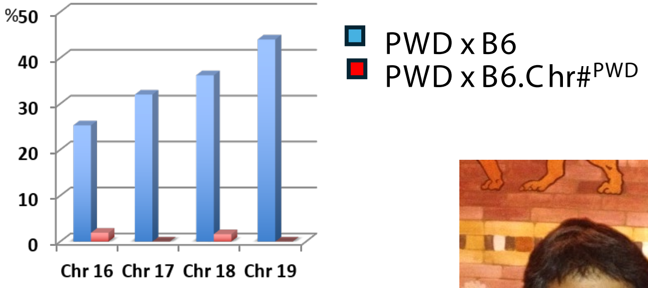


B6

(PWD x B6)

Mechanistic basis of infertility of mouse intersubspecific hybrids.
Bhattacharyya T, Gregorova S, Mihola O, Anger M, Sebestova J, Denny P, Simecek P, Forejt J.
Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):E468-77. doi: 10.1073/pnas.1219126110.





Modulation of *Prdm9*-controlled meiotic chromosome asynapsis overrides hybrid sterility in mice. Gregorova S, Gergelits V, Chvatalova I, Bhattacharyya T, Valiskova B, Fotopulosova V, Jansa P, Wiatrowska D, Forejt J
Elife. 2018 Mar 14;7:e34282. doi: 10.7554/eLife.34282.

Soňa Gregorová

Tanmoy Bhattacharyy

Milestone 4:

Asymmetric erasure of PRDM9 binding sites in sterile hybrids

Nature
2016 Feb 11;530(7589):171-176.
doi: 10.1038/nature16931. Epub 2016 Feb 3.

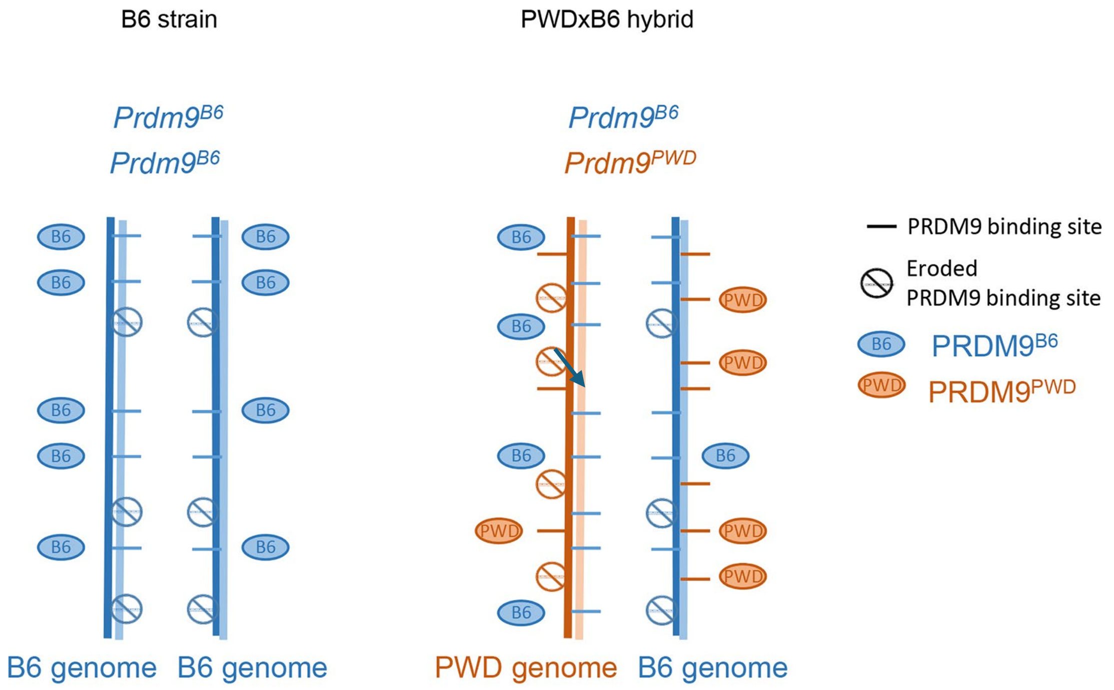
Re- engineering the zinc fingers of PRDM9 reverses hybrid sterility in mice

Benjamin Davies[#,1], Edouard Hatton[#,1], Nicolas Altemose[1,2], Julie G Hussin[1], Florencia Pratto[3], Gang Zhang[1], Anjali Gupta Hinch[1], Daniela Moralli[1], Daniel Biggs[1], Rebeca Diaz[1], Chris Preece[1], Ran Li[1,2], Emmanuelle Bitoun[1], Kevin Brick[3], Catherine M Green[1], R Daniel Camerini-Otero[3], Simon R Myers[1,2], Peter Donnelly

Genetics: Asymmetric breaks in DNA cause sterility.
Forejt J. **Nature**. 2016 Feb 11;530(7589):167-8. doi: 10.1038/nature16870. Epub 2016 Feb 3.PMID: 26840487 No abstract available.

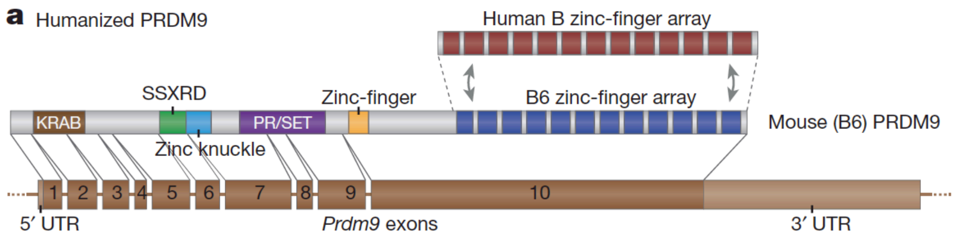
Milestone 4:

Asymmetric erasure of PRDM9 binding sites in sterile hybrids



PWDxB6^{Hu}

“Humanized” PRDM9



Milestone 4:

Hstx2 locus determines asymmetric fertility of reciprocal hybrids

X chromosome control of meiotic chromosome synapsis in mouse inter-subspecific hybrids.
Bhattacharyya T, Reifova R, Gregorova S, Simecek P, Gergelits V, Mistrik M, Martincova I, Pialek J, Forejt J. PLoS Genet. 2014 Feb 6;10(2):e1004088. doi: 10.1371/journal.pgen.1004088

Genomic Structure of Hstx2 Modifier of Prdm9-Dependent Hybrid Male Sterility in Mice.
Lustyk D, Kinský S, Ullrich KK, Yancoskie M, Kašíková L, Gergelits V, Sedlacek R, Chan YF, Odenthal-Hesse L, Forejt J, Jansa P. Genetics. 2019 Nov;213(3):1047-1063. doi: 10.1534/genetics.

Tanmoy Bhattacharyya

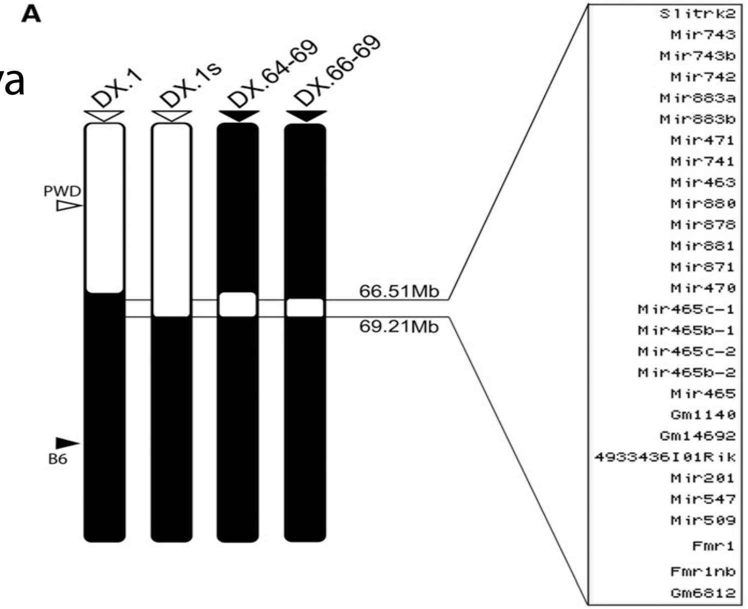
Diana Lustyk

PWD♀ x B6♂
$Hstx2^{PWD}$ $Prdm9^{PWD/B6}$

Sterile

B6♀ x PWD♂
$Hstx2^{B6}$ $Prdm9^{PWD/B6}$

Fertile



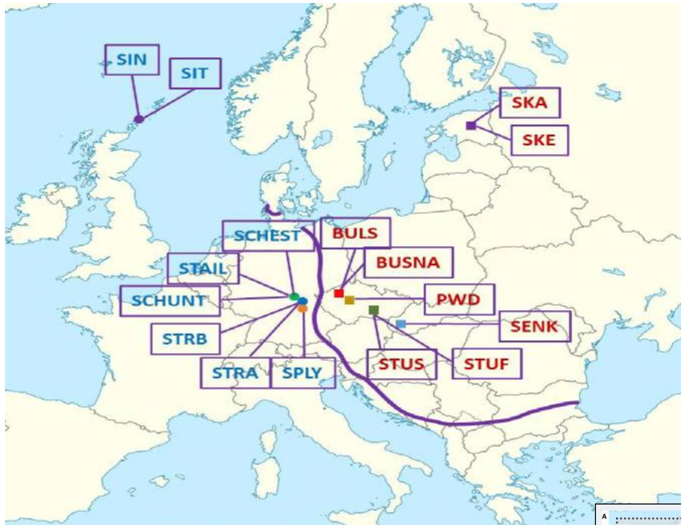
Petr Jansa

Milestone 5:

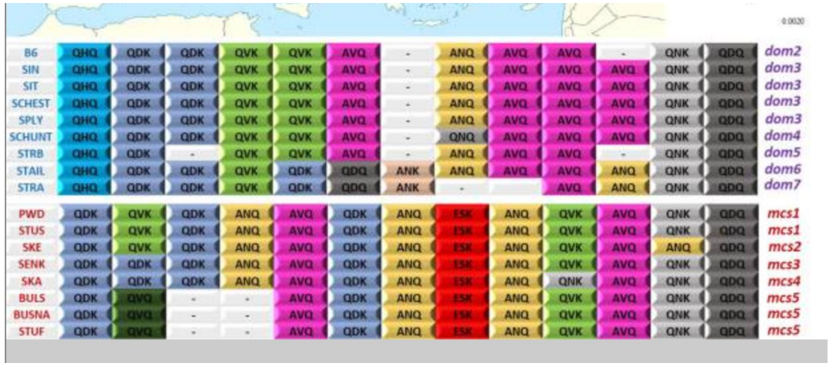
PRDM9 acts as a hybrid sterility gene in wild mice from different European populations

Prdm9 Intersubspecific Interactions in Hybrid Male Sterility of House Mouse. Mukaj A, Piálek J, Fotopulosova V, Morgan AP, Odenthal-Hesse L, Parvanov ED, Forejt J. Mol Biol Evol. 2020 Dec 16;37(12):3423-3438. doi: 10.1093/molbev/msaa167.

Natural variation in the zinc-finger-encoding exon of Prdm9 affects hybrid sterility phenotypes in mice. AbuAlia KFN, Damm E, Ullrich KK, Mukaj A, Parvanov E, Forejt J, Odenthal-Hesse L. Genetics. 2024 Mar 6;226(3):iyae004. doi: 10.1093/genetics/iyae004



Linda Odenthal-Hesse


0.0020

B6	QHQ	QDK	QDK	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	-	QNK	QDQ	dom2
SIN	QHQ	QDK	QDK	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	AVQ	QNK	QDQ	dom3
SIT	QHQ	QDK	QDK	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	AVQ	QNK	QDQ	dom3
SCHEST	QHQ	QDK	QDK	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	AVQ	QNK	QDQ	dom3
SPLY	QHQ	QDK	QDK	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	AVQ	QNK	QDQ	dom3
SCHUNT	QHQ	QDK	QDK	QVK	QVK	AVQ	-	QNQ	AVQ	AVQ	AVQ	QNK	QDQ	dom4
STRB	QHQ	QDK	-	QVK	QVK	AVQ	-	ANQ	AVQ	AVQ	-	QNK	QDQ	dom5
STAIL	QHQ	QDK	QDK	QVK	QDK	QDQ	ANK	ANQ	AVQ	AVQ	ANQ	QNK	QDQ	dom6
STRA	QHQ	QDK	QDK	QVK	QDK	QDQ	ANK	-		AVQ	ANQ	QNK	QDQ	dom7
PWD	QDK	QVK	QDK	ANQ	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs1
STUS	QDK	QVK	QDK	ANQ	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs1
SKE	QDK	QVK	QDK	ANQ	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	ANQ	QDQ	mcs2
SENK	QDK	QDK	QDK	ANQ	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs3
SKA	QDK	QDK	QDK	ANQ	AVQ	QDK	ANQ	ESK	ANQ	QNK	AVQ	QNK	QDQ	mcs4
BULS	QDK	QVQ	-	-	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs5
BUSNA	QDK	QVQ	-	-	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs5
STUF	QDK	QVQ	-	-	AVQ	QDK	ANQ	ESK	ANQ	QVK	AVQ	QNK	QDQ	mcs5

Emil Parvanov Amisa Mukaj Jaroslav Piálek

Ongoing projects: Synthetic hybrid sterility

A minimal hybrid sterility genome assembled by chromosome swapping between mouse subspecies (Mus musculus)

Vladana Fotopulosova*[1], Giordano Tanieli*, Petr Jansa and Jiri Forejt^

Molecular Biology and Evolution, in revision 2024

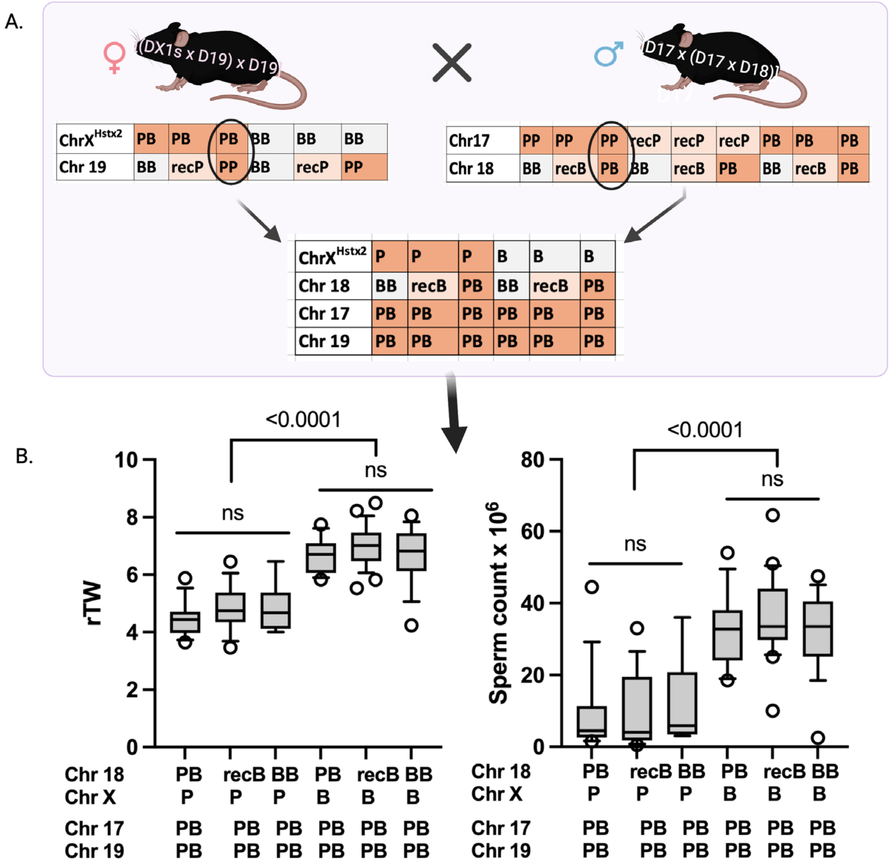


Fig. 3

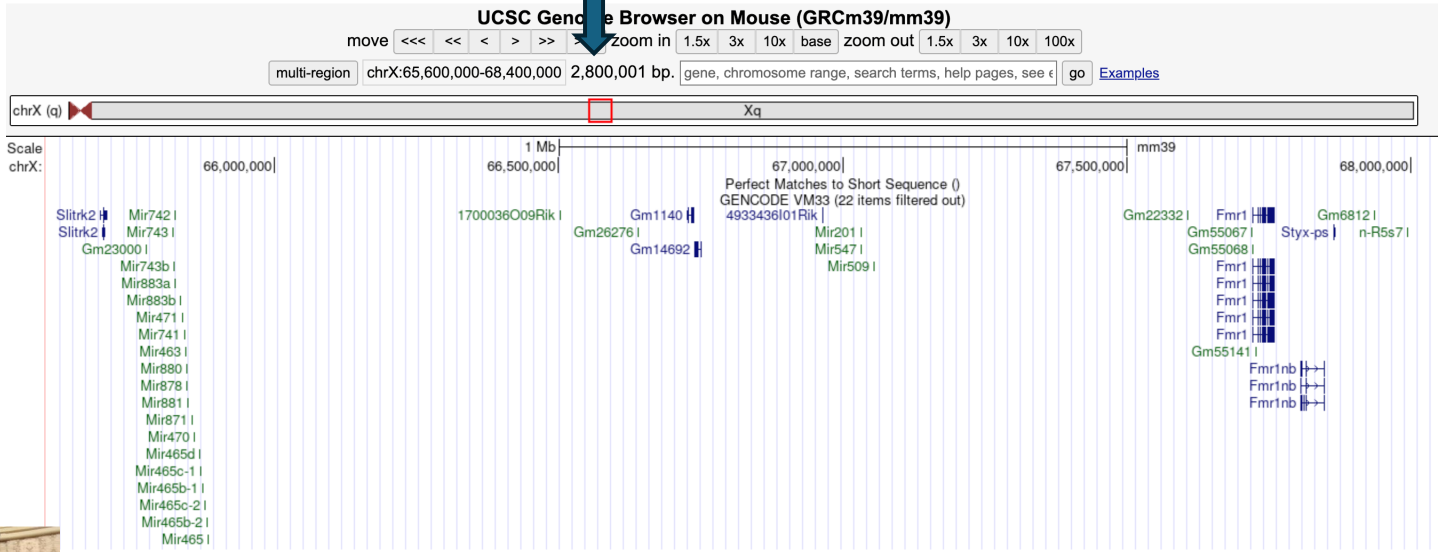
Conclusions: Musculus/domesticus heterozygosity of the three smallest chromosomes is sufficient to reconstitute a major part of Prdm9-driven hybrid male sterility.

Vladana Fotopulosová

Giordano Tanieli

Ongoing projects: Mir465 cluster

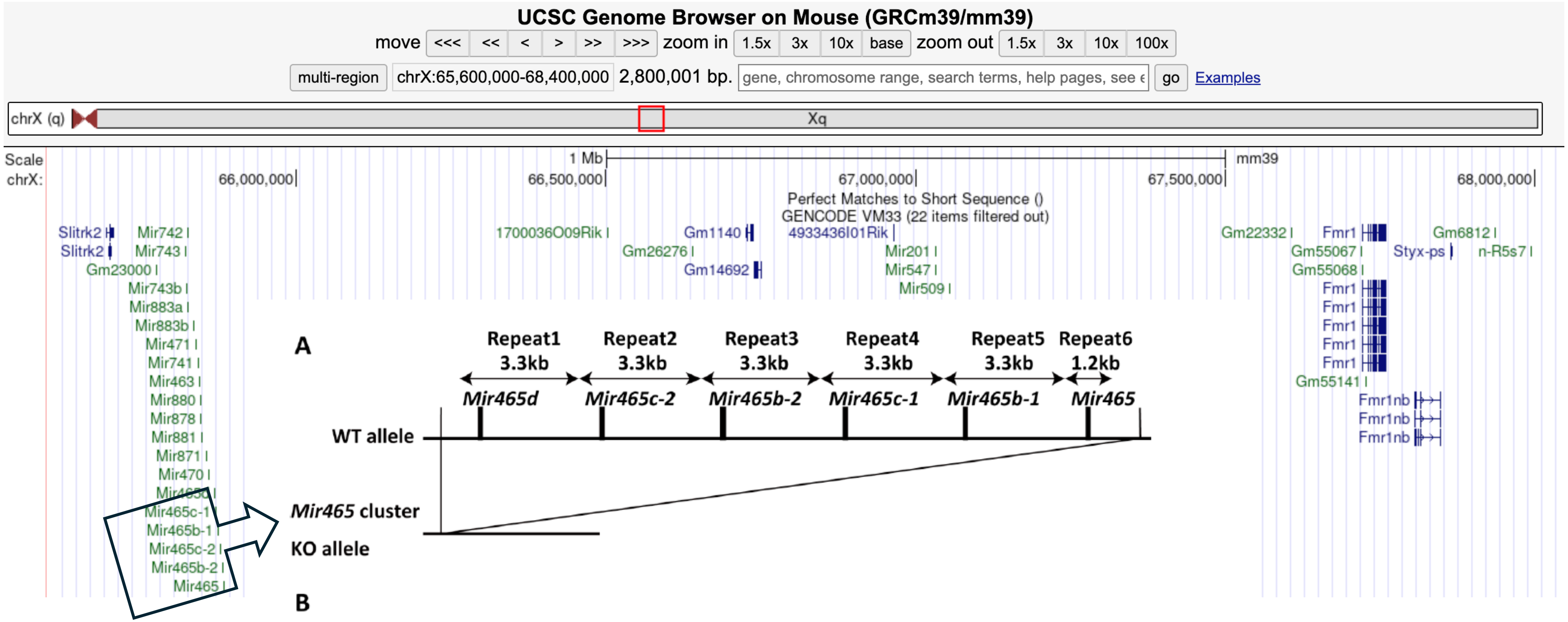
- B6. Hstx1
- B6.Hstx2
- B6.Meir1



Radek Sedláček
Jaroslav Pialek
Kento Morimoto
Linda-Odenthal-Hesse

Petr Jansa Karel Fusek Giordano Tanieli

Mir465 cluster is the Hstx2 candidate



identity	Repeat1	Repeat2	Repeat3	Repeat4	Repeat5	Repeat6
Repeat1	-	88.9%	94.4%	88.9%	87.9%	85.2%
Repeat2	-	-	91.3%	100.0%	89.3%	85.2%
Repeat3	-	-	-	91.3%	93.3%	87.8%
Repeat4	-	-	-	-	89.3%	85.2%
Repeat5	-	-	-	-	-	87.8%
Repeat6	-	-	-	-	-	-

B6 – 1 Mir465 cluster

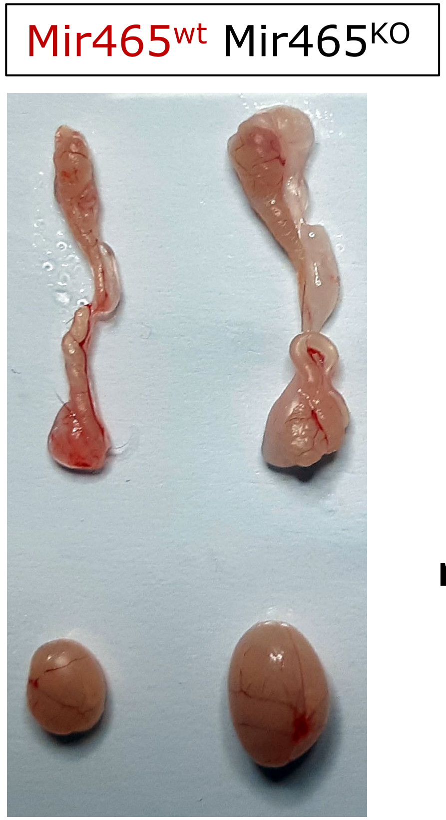
PWD -2 Mir465 clusters

The Mir465 microRNA cluster is responsible for the phenotypes of Hstx1, Hstx2 and Meir1 (and much more ☺)

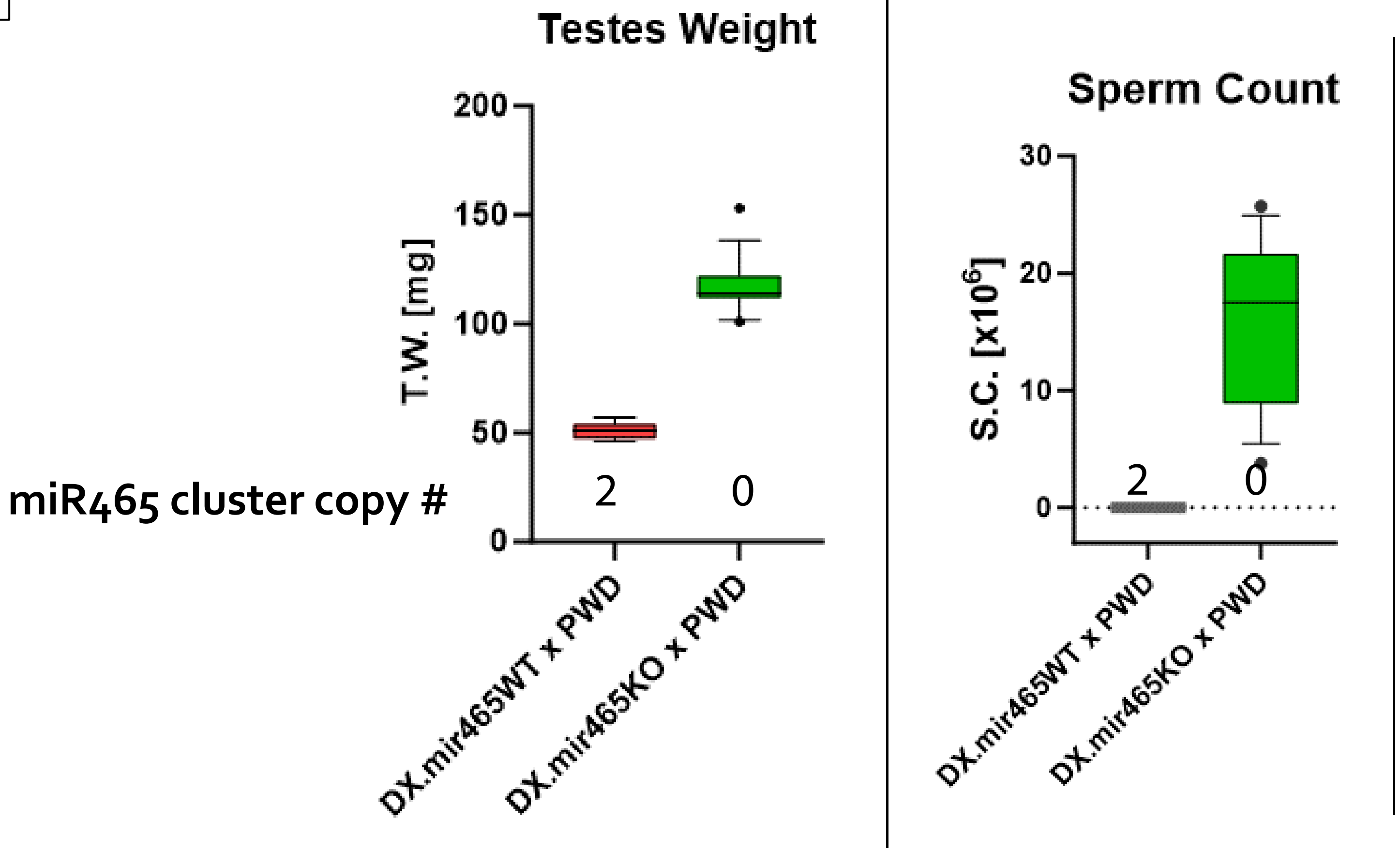
Genetic tools – Mir465 cluster copy number variants:

➢ B6 male	- 1 copy
➢ PWD male	- 2 copies
➢ Mir465 cluster transgene (BAC clone) on B6 background	1 → 2 copies
➢ Mir465 cluster deletion on B6 background	1 → 0 copy
➢ Mir465 cluster deletion on B6.$X^{64\text{-}69PWD}$ background	2 → 0 copy

The male fertility of (B6.$X^{64\text{-}69PWD}$/$X^{64\text{-}69PWD\text{-}DEL}$ x PWD)F1 hybrids differs according to Mir465 copy number



(B6.Hstx2^{PWD} x PWD)F1



Fertility of hybrids differs according to the number of Mir465 copies

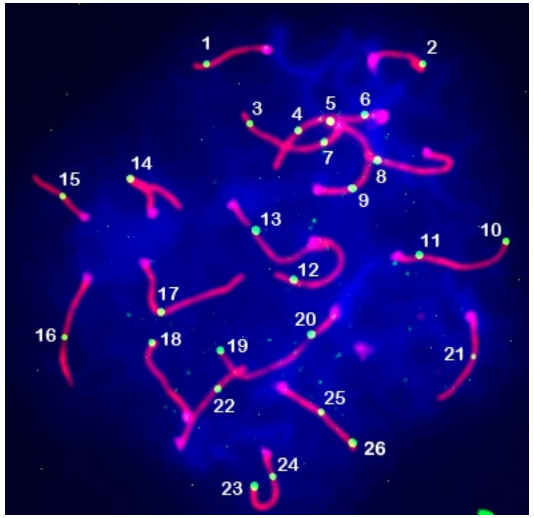
F1 Hybrid	Mir465 copy No.	Testes weight (mg)	Sperm count (*10^6)	Asynapsis (%)	No. offsprings sired by one male/ B6 female/1 month
B6 x PWD $^{\#}$	1	95,6	2,75	58,7	4,1
B6-Tg(Mir465) x PWD	2	54,0	0	74,6	not tested
DX1s-Tg(Mir465) x PWD	3	58,0	0	91,1	not tested
B6.*Mir465*^{Del} x PWD $^{\#}$	0	125,3	8,64	46,8	13,3
B6.*Hstx2*^{PWD} x PWD $^{\$}$	2	50,1	0	67,8	0
B6.*Hstx2*^{PWD}-*Mir465*^{Del} x PWD $^{\$}$	0	113,5	12,52	26,5	13,0
PWD x B6	2	65,2*	0	85,3*	0

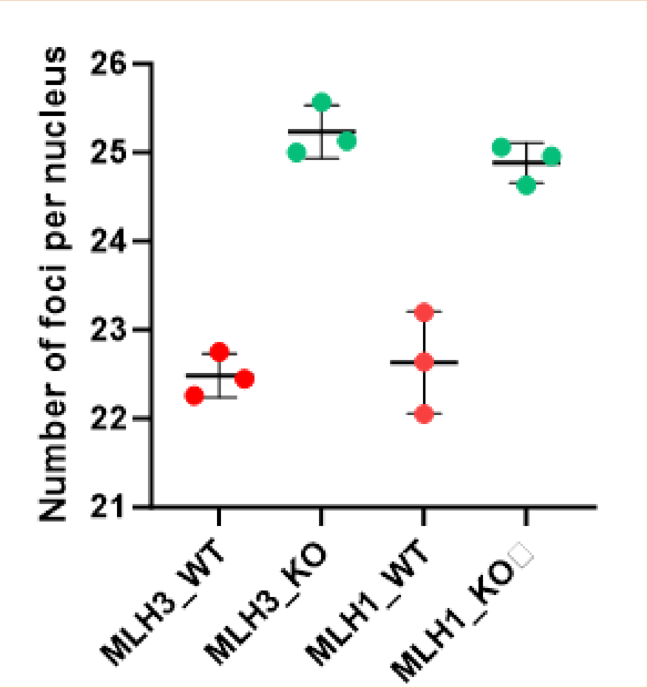
Mir465 controls homologous DNA recombination

Congenic strains: B6-X.PWD$^{64-69}$ versus B6-X.PWD$^{64-69}$-Mir465$^{\underline{KO}}$

Mir465 copy No. 2 0 2 0

Hybrid Sterility Locus on Chromosome X Controls Meiotic Recombination Rate in Mouse. Balcova M, Faltusova B, Gergelits V, Bhattacharyya T, Mihola O, Trachtulec Z, Knopf C, Fotopulosova V, Chvatalova I, Gregorova S, Forejt J. PLoS Genet. 2016 Apr 22;12(4):e1005906. doi: 10.1371/journal.pgen.1005906. eCollection 2016





Hstx2 (hybrid sterility) and Meir1 (crossover rate) are identical. They are controlled by the Mir465 cluster.

Mir465 cluster deletion rescues T43H chromosomal male ste

Segmental trisomy of chromosome 17: A mouse model of human aneuploidy syndromes

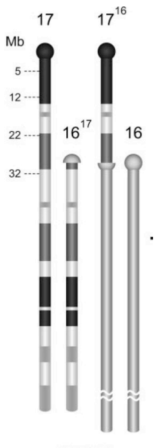
Tomáš Vacík*, Michael Ort†‡, Soňa Gregorová*, Petr Strnad*, Radek Blatný*, Nathalie Conte§, Allan Bradley§, Jan Bureš†, and Jiří Forejt*¶

Institutes of *Molecular Genetics and †Physiology, Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic; ‡Department of Psychiatry, First School of Medicine, Charles University, 11636 Prague, Czech Republic; and §The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

Chromosomal rearrangement interferes with meiotic X chromosome inactivation

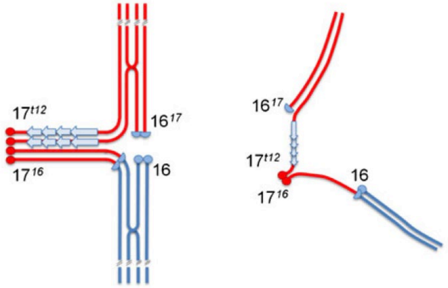
David Homolka, Robert Ivanek, Jana Capkova, Petr Jansa and Jiri Forejt

Genome Res. published online Aug 23, 2007;
Access the most recent version at doi:10.1101/gr.6520107

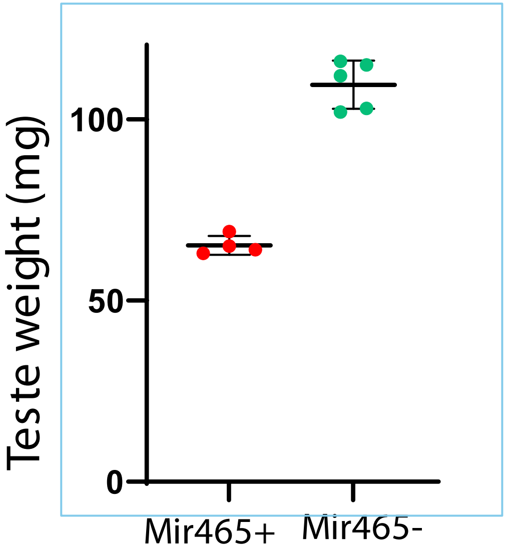


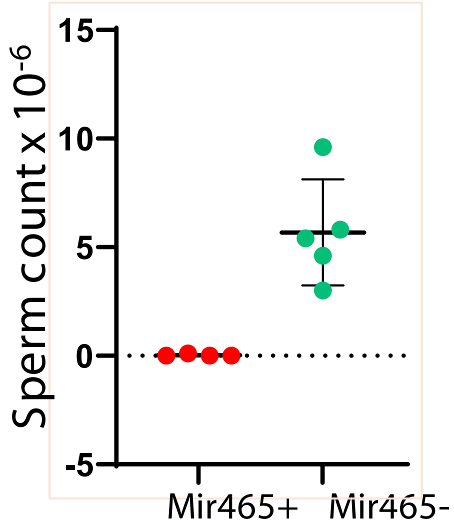
T43/+
♂sterile

Translocation T16:17)43H



B6.Mir465+/- x B6.T43H/T43H





Mir465 (Hstx2) acts as a pachytene checkpoint in chromosomally induced male sterility on inbred domesticus background

Molecular mechanisms of Mir465 action

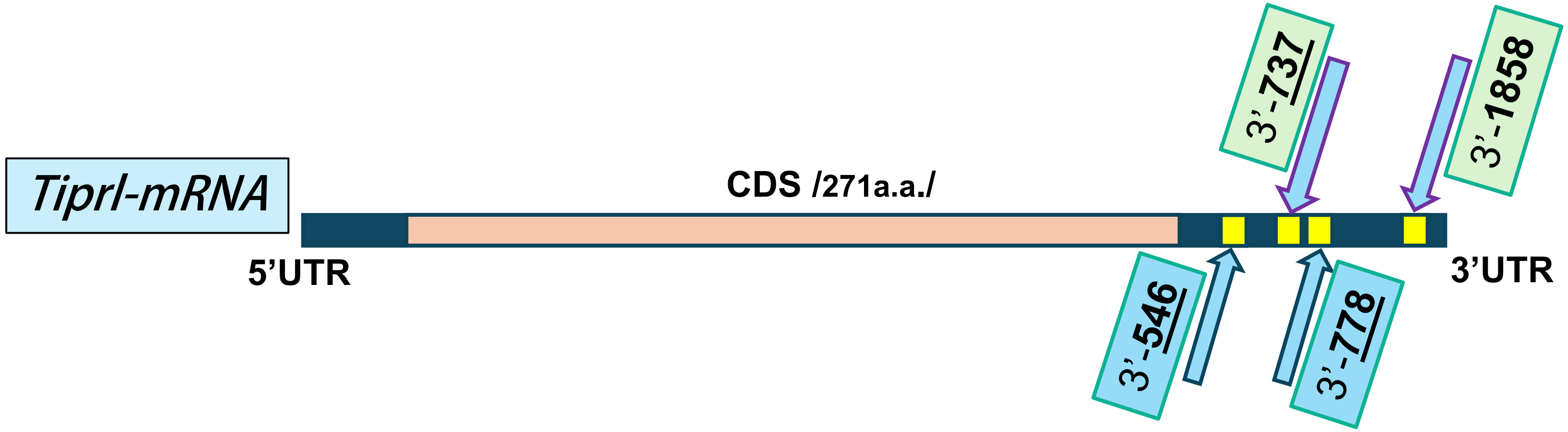
Pilot experiments

- RNAseq
- Mass Spectrometry on machine ORBITRAP

Differentiall Expressed Genes - **F.C. =** B6-X.PWD$^{64-69}$ versus B6-X.PWD$^{64-69}$-Mir465^{KO}

Protein	F.C.	Full Name
Fbp1	**16,6**	Fructose-1,6-bisphosphatase 1
Ldhc	**11,3**	L-lactate dehydrogenase C chain
Piwil1	**11,1**	Piwi-like protein 1
Slc2a3	**9,99**	Solute carrier family 2, facilitated glucose transporter member 3
Shcbp1l	**7,59**	Testicular spindle-associated protein SHCBP1L
Ybx2	**4,02**	Y-box-binding protein 2
Clgn	**3,75**	Calmegin
Sumo2	**3,35**	Small ubiquitin-related modifier 2
Rimbp3	**3,23**	RIMS-binding protein 3
Dnaaf1	**3,16**	Dynein axonemal assembly factor 1
Mertk	**2,66**	Tyrosine-protein kinase Mer
Ybx3	**2,59**	Y-box-binding protein 3
Ub2a	**1,97**	Ubiquitin-conjugating enzyme E2 A
Cfap36	**1,77**	Cilia- and flagella-associated protein 36
Tiprl	**1,71**	TIP41-like protein
Hspa2	**1,7**	Heat shock-related 70 kDa protein 2
Nsrp1	**1,7**	Nuclear speckle splicing regulatory protein 1

Mir465 and Mir743 target sites in Tiprl 3'-UTR



miRNA SEED seq 465a-5p-binding to Tiprl-3'UTR

```
                      987654321
miR-465a-5p    AGUGUAGUCACGGUAAGAUUUAU-5'
                          |||||||||
Tiprl 3' UTR             ccatTTCTAAATAtgacat-3'
```

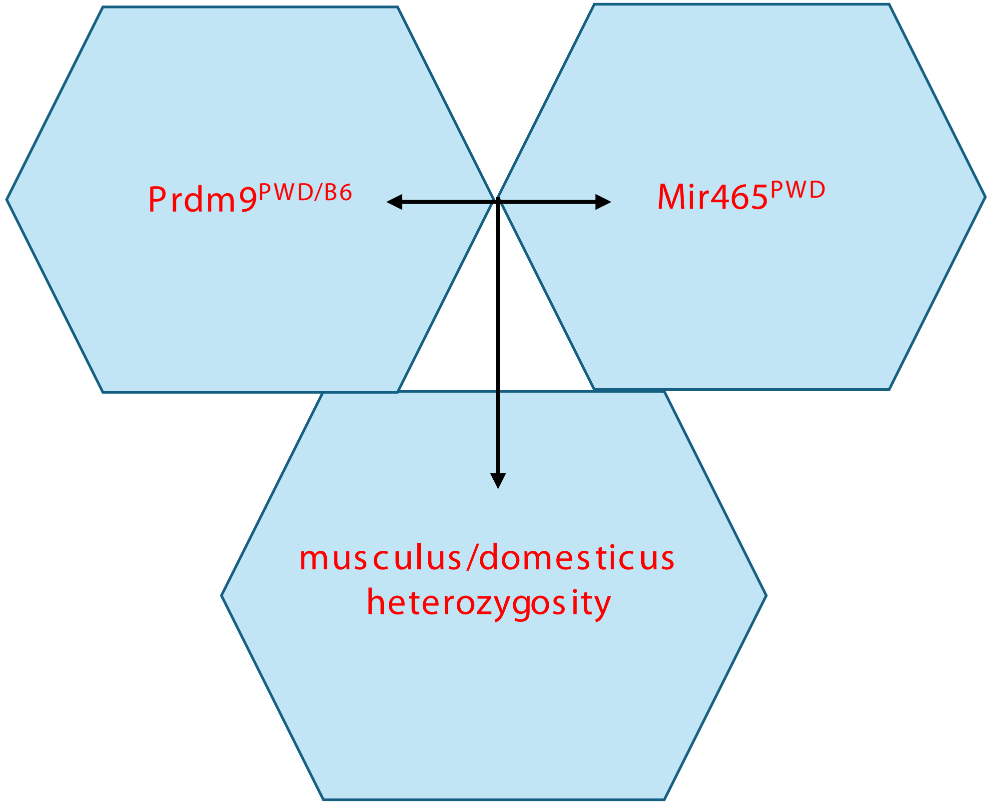
miRNA SEED seq 743a-3p-binding to Tiprl-3'UTR

```
               .......................987654321
miR-743a-3p    AGAUGAGUCGAACCACAGAAAG-5'
                            |||||||||
TiprL 3' UTR   NNNNNNNGTCCAAGTGTCTTTTGTAAG-3'
```

The target sites of Mir465-5p and miR743a-3p are very close to each other, so they may act synergistically to increase the silencing effect.
TIPRL is proposed to function in DNA damage response by promoting H2AX phosphorylation on C-terminal Ser-140 (gH2AX). Furthermore it may play an important role in the regulation of ATM/ATR signaling pathway controlling DNA REPAIR and DNA replication.

Conclusion:
Genetic architecture of hybrid sterility in mice is composed from three main components

(PWD x B6)F1 ♂



Mouse Molecular Genetics 2012

My group 1974 -2023

S. Gregorová
M. Dzur-Gejdošová-Balcová
P. Šimeček
P. Jansa
P. Flachs
T. Bhattacharya
B. Faltusová-Vališková
I. Chvátalová
V. Gergelits
D. Wiatrowska-Lustyk
L. Wang
L. Kašíková
J. Čapková
H. Pavljuková
K. Dohnal
M. Loudová
B. Mosinger
J. Králová

Z. Trachtulec
M. Mňuková-Fajdelová
J. Hatina
S. Dimitrov
M. Brennerová
T. Vacík
P. Divina
O. Mihola
R. Storchová-Reifová
R. Blatný
D. Homolka
R. Ivánek
V. Fotopulosová
A. Mukaj
D. Parvanov
K. Fusek
G. Tanieli