Testing models of microsatellite mutation:

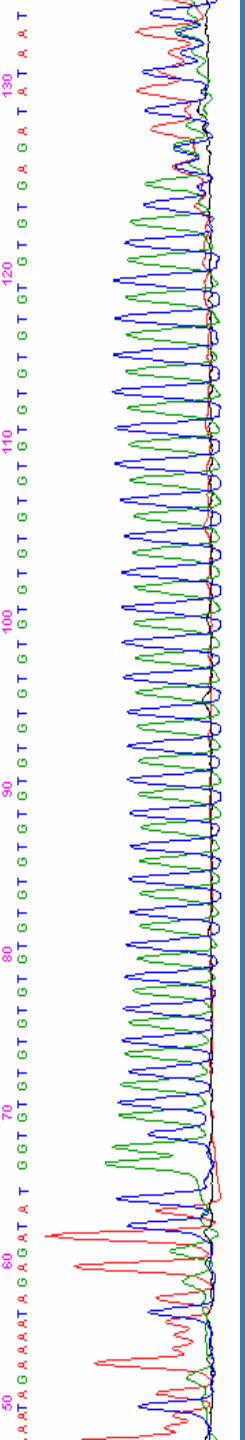
a cross-chromosomal comparison in the tammar wallaby

Anna MacDonald

UNIVERSITY OF CANBERRA
Institute for Applied Ecology
Ecological Solutions for a Healthy Environment

Overview

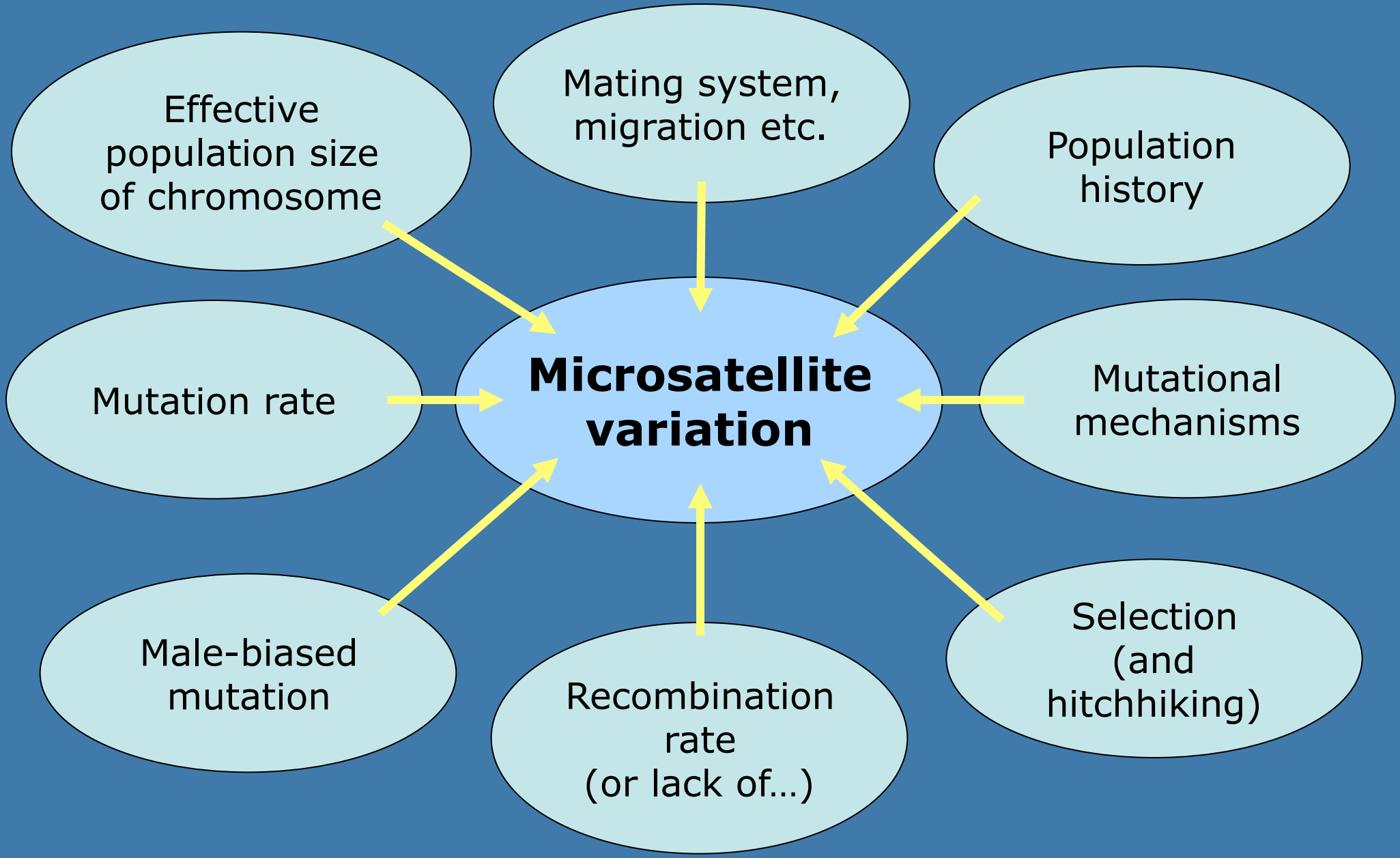
- Allelic richness and microsatellite repeat length
- Allelic richness varies with chromosome
- Reconstruction experiments: testing ability to detect mutations in sperm cells



Common assumptions about microsatellite mutations

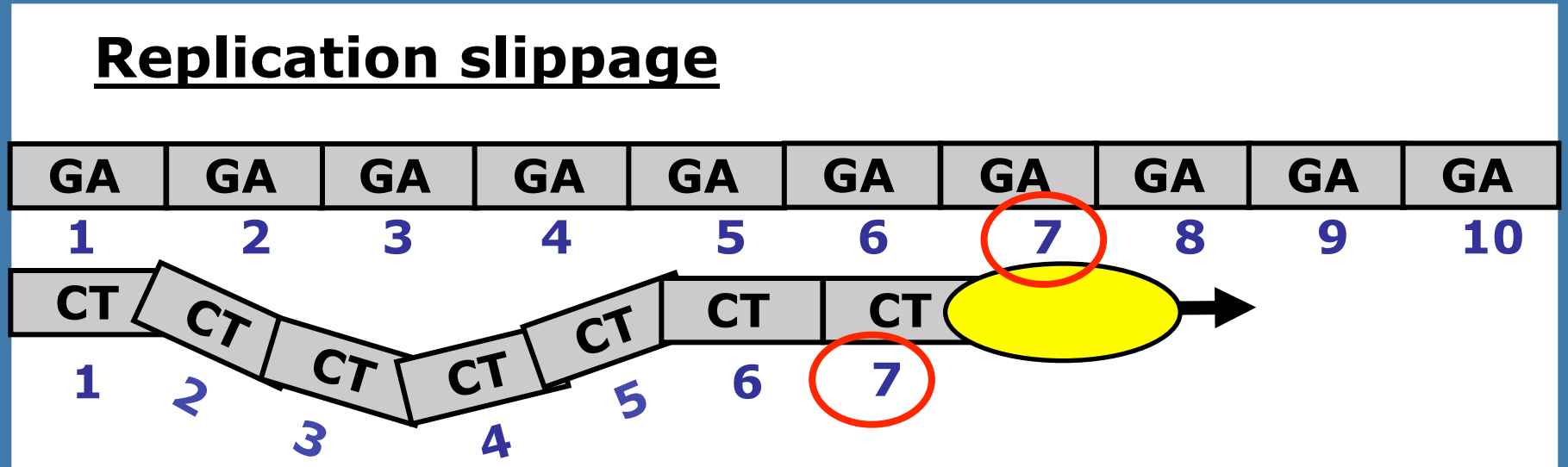
- mutation rate
- single-step vs. multi-step
- size homoplasy
- constraints on allele size
- directionality of mutations

Microsatellite variation may be affected by many factors:



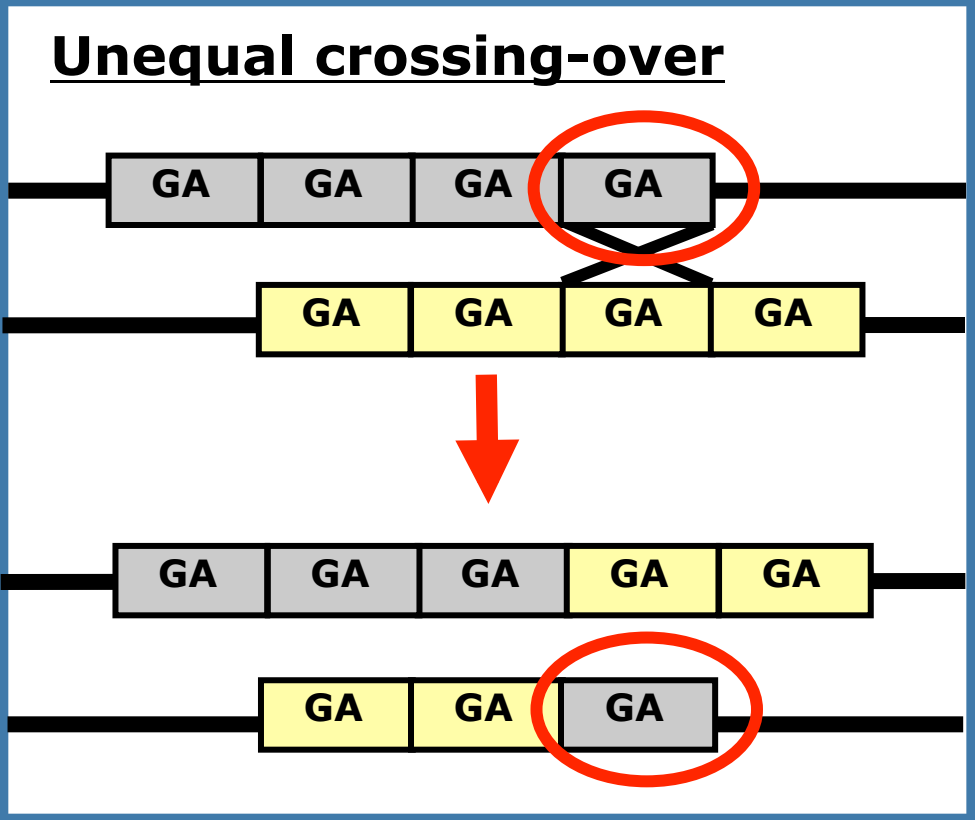
Most microsatellite mutations are thought to occur during DNA replication

Polymerase misaligns due to repeats, creating a loop

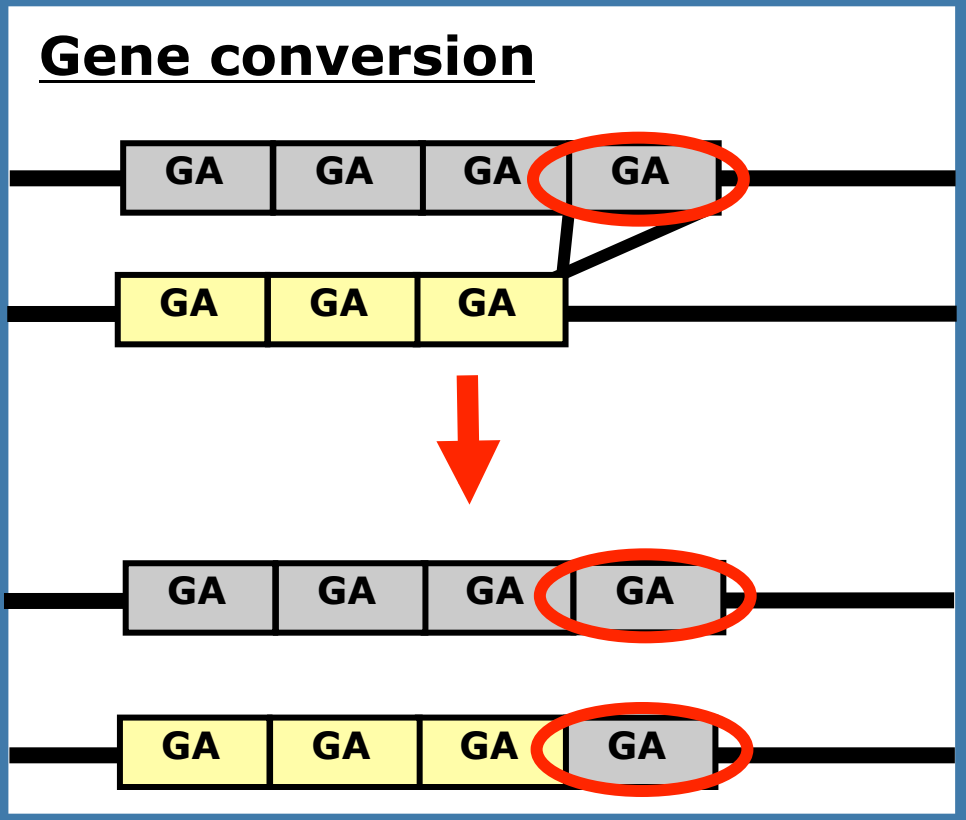


A repeat is added (or lost) – a mutation!

Mutations may also occur during DNA recombination?



Homologous chromosomes exchange sections of DNA



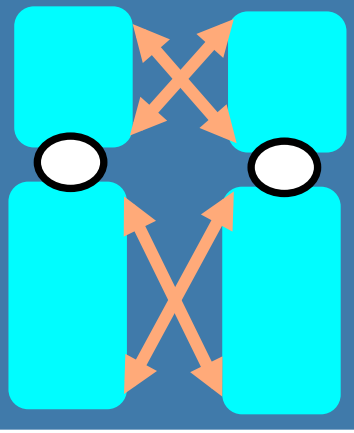
DNA is transferred from one chromosome to the other – no exchange

Microsatellite variation in a model marsupial

Tammar wallaby
(*Macropus eugenii)*

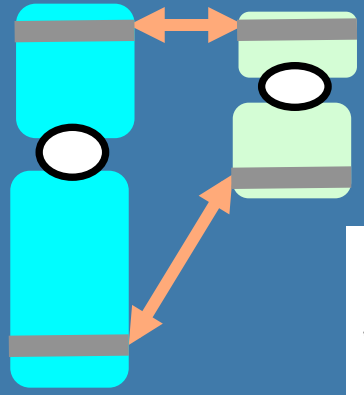
- Kangaroo genome project
- DNA sequence from X and Y chromosome BAC clones

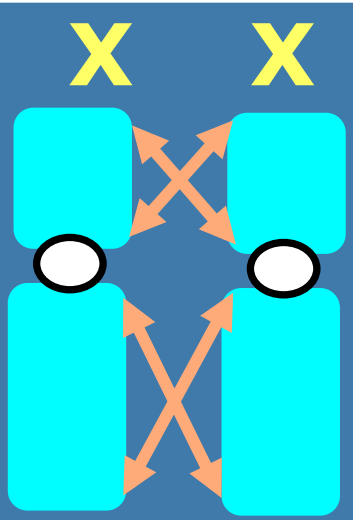
Compare microsatellite variation on different chromosomes



Eutherian Mammals

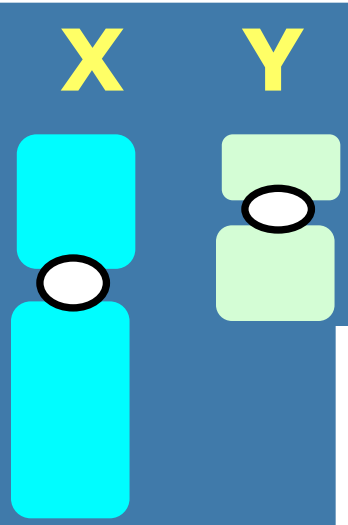
Pseudoautosomal Regions:
some recombination between X and Y



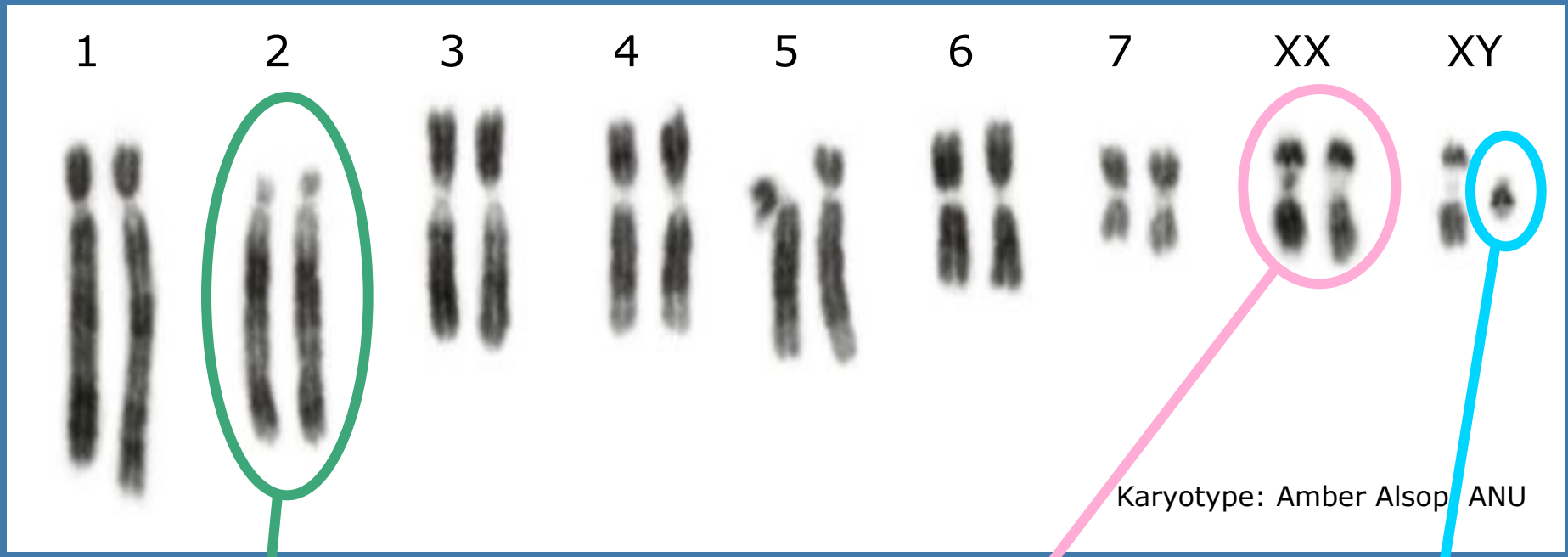


Marsupial Mammals

No Pseudoautosomal Regions:
Y does not recombine



Chromosome-specific microsatellites developed from tammar wallaby



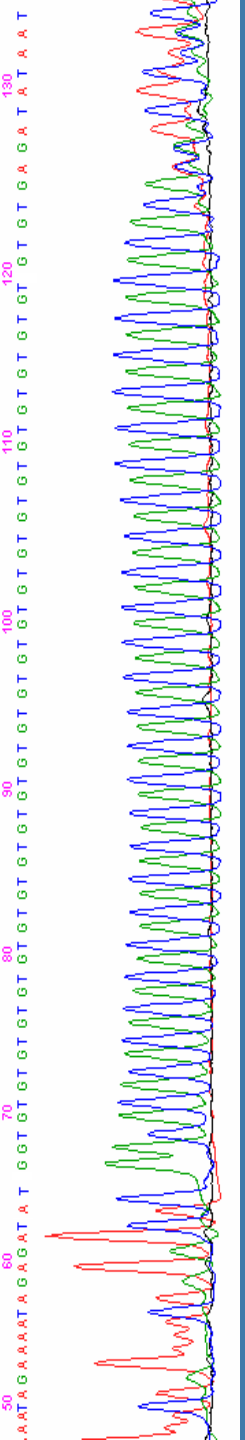
11 microsatellites from chromosome 2

9 X chromosome microsatellites

9 Y chromosome microsatellites

Y primers: MacDonald *et al.* (2006) Molecular Ecology Notes **6:** 1202-1204

X and 2 primers: MacDonald *et al.* (2007) Molecular Ecology Notes (Online Early)

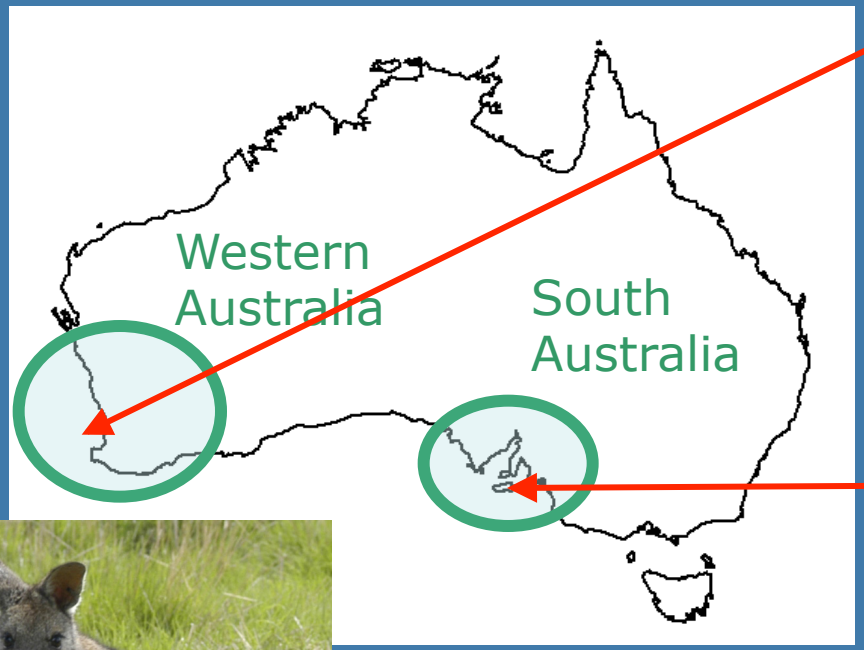


Microsatellite development

- from chromosome-specific BAC sequence
- di- and tri-nucleotide repeats
- repeats mostly AC or AT
- ≥5 consecutive repeat units

Microsatellite variation in tammar wallaby

Historical range:



Garden Island

- isolated, inbred population
- samples collected from naval base by Brian Chambers
- 78 males + 34 females

Kangaroo Island

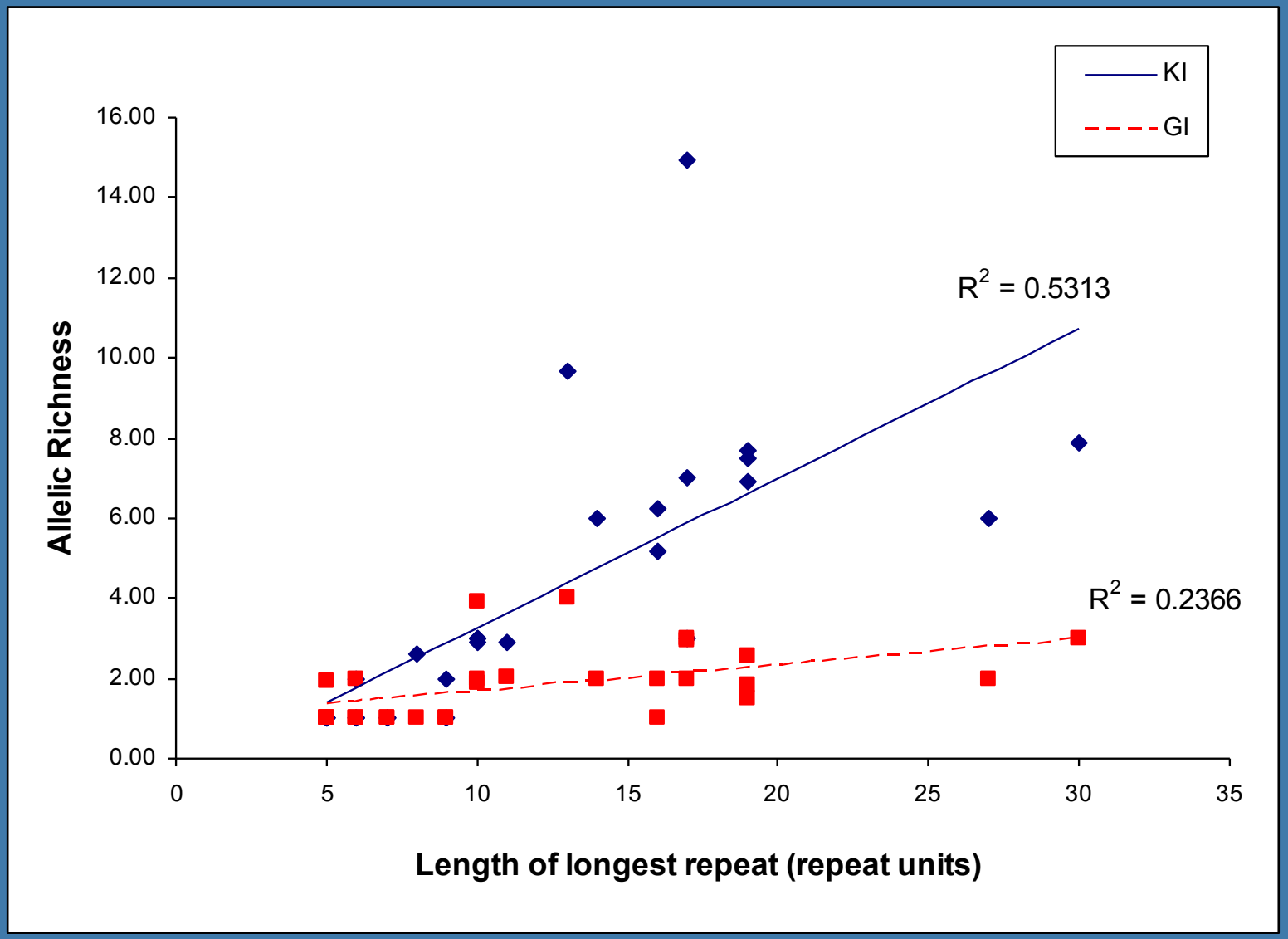
- largest remnant population
- samples collected by Marilyn Renfree's group
- 97 males + 102 females

Microsatellite variation in tammar wallaby

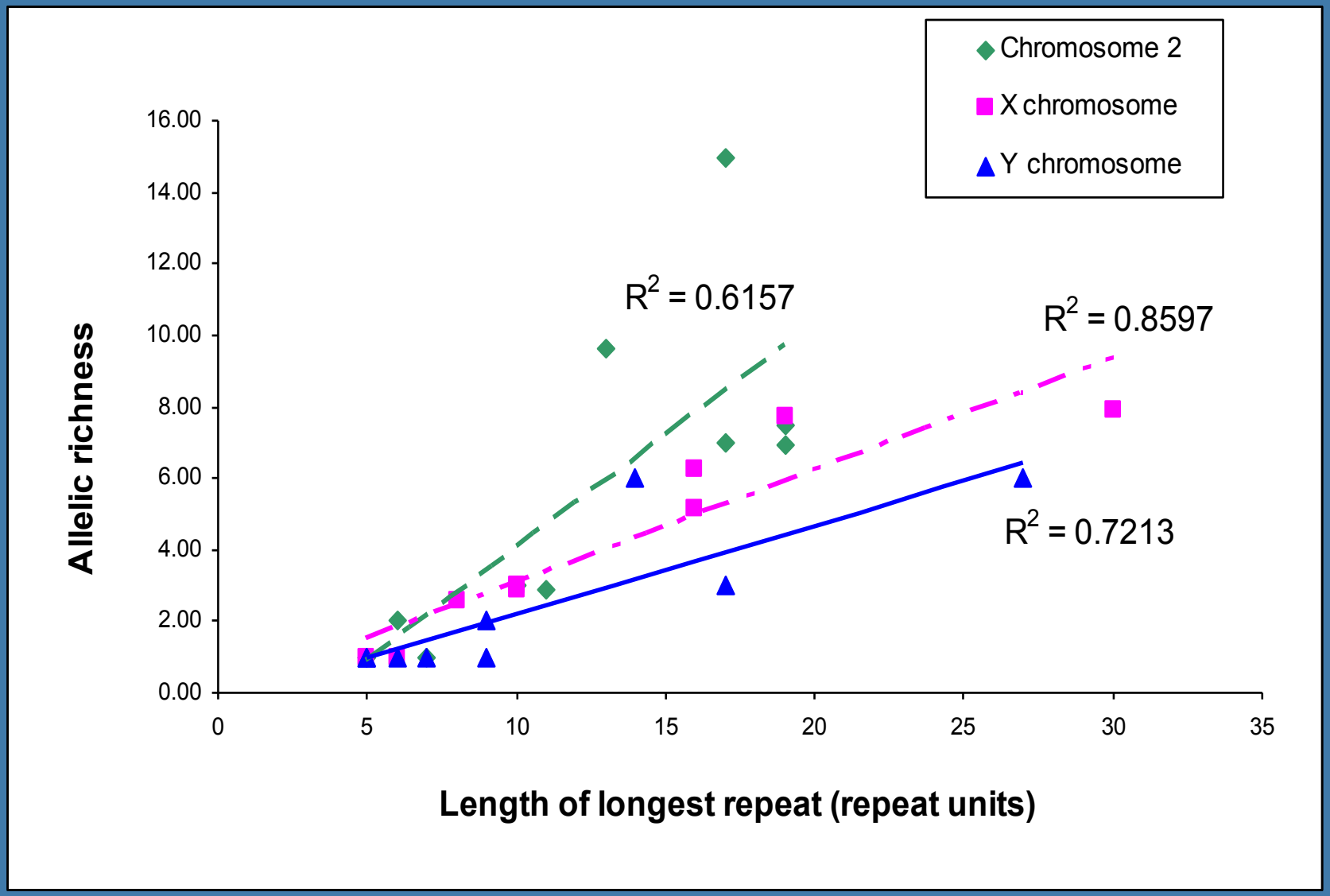
	Kangaroo Island			Garden Island		
	Chr 2	X	Y	Chr 2	X	Y
# loci	11	9	9	11	9	9
# polymorphic	8	7	4	8	5	4
# alleles	1-19	1-8	1-6	1-4	1-4	1-3
mean Hexp	0.441	0.373	-	0.093	0.175	-
mean allelic richness	*5.2	4.2	*2.4	2.0	1.9	1.5

*(P=0.03)

Allelic richness increases with repeat length (P<0.01)

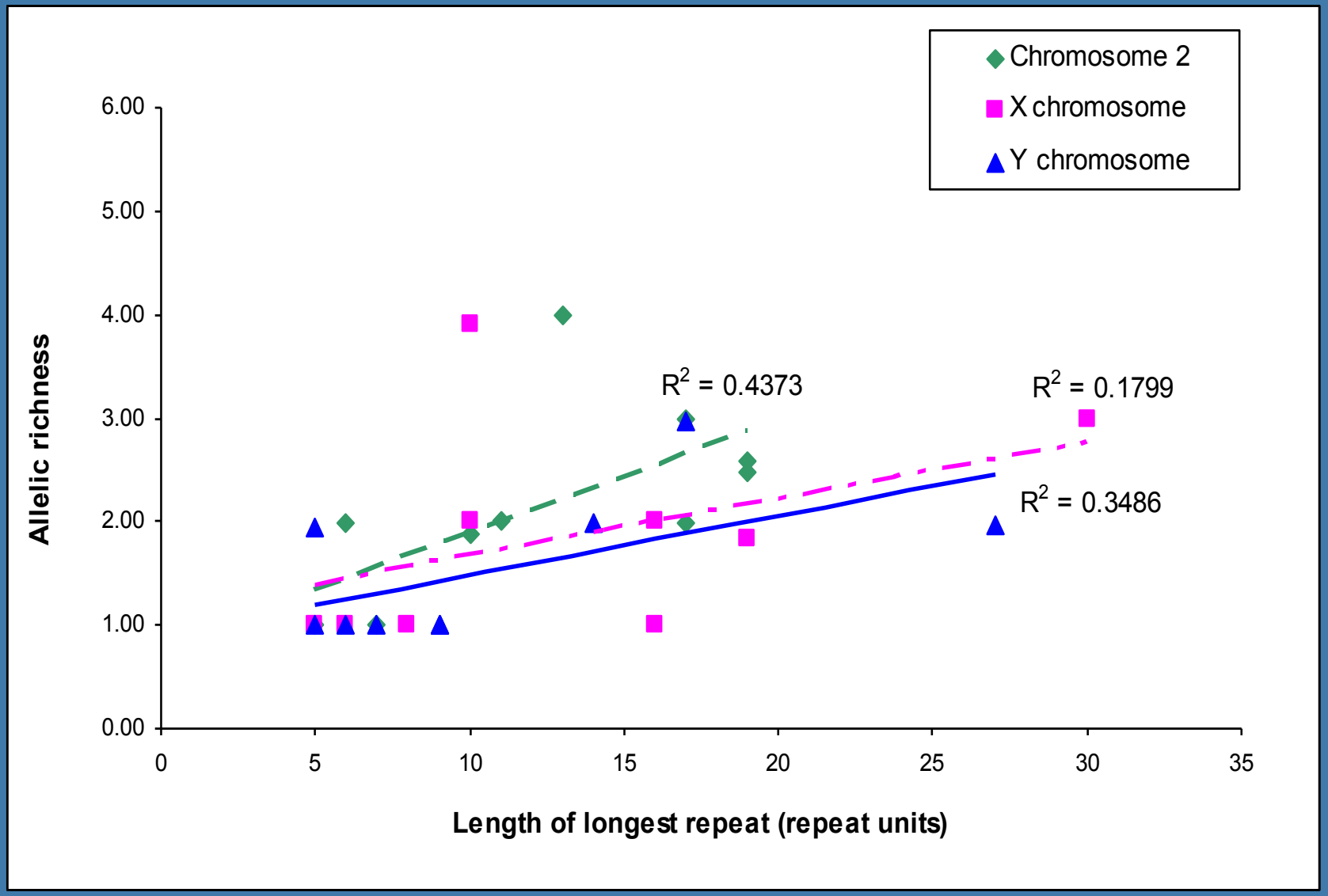


Allelic richness varies with chromosome



Kangaroo Island: 2* > X > Y* (P=0.03)

Allelic richness varies with chromosome



Garden Island: 2 > X > Y

Reasons for reduced Y chromosome variation?

- Lack of recombination?
- May also result from:

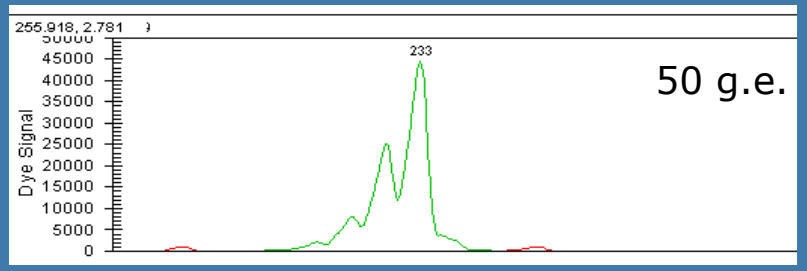
  - Lower effective population size

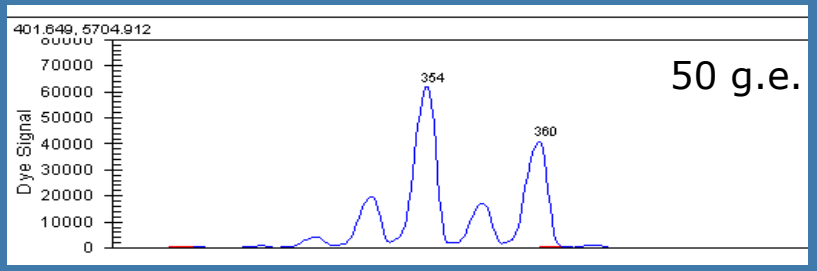
  - Demographic factors

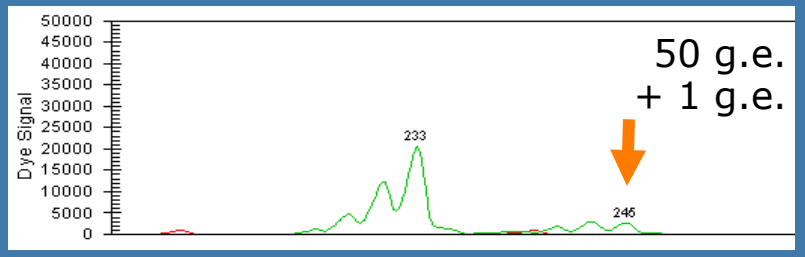
Need to observe mutations to understand mutational mechanisms

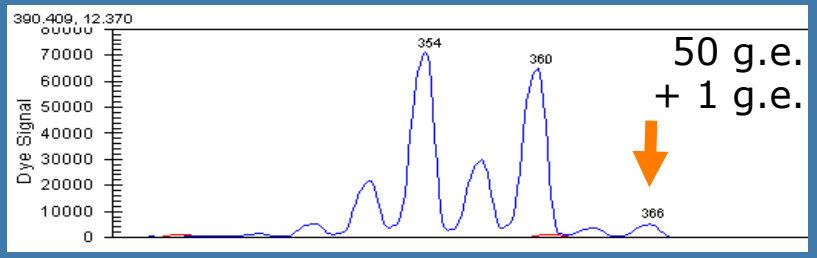
Identifying mutations in wallaby sperm

- Small-pool PCR from sperm DNA
- 1 haploid genome equivalent ≈3.5 pg
- Reconstruction experiments
  Test ability to detect mutant DNA at low concentrations: ‘spike’ with different allele

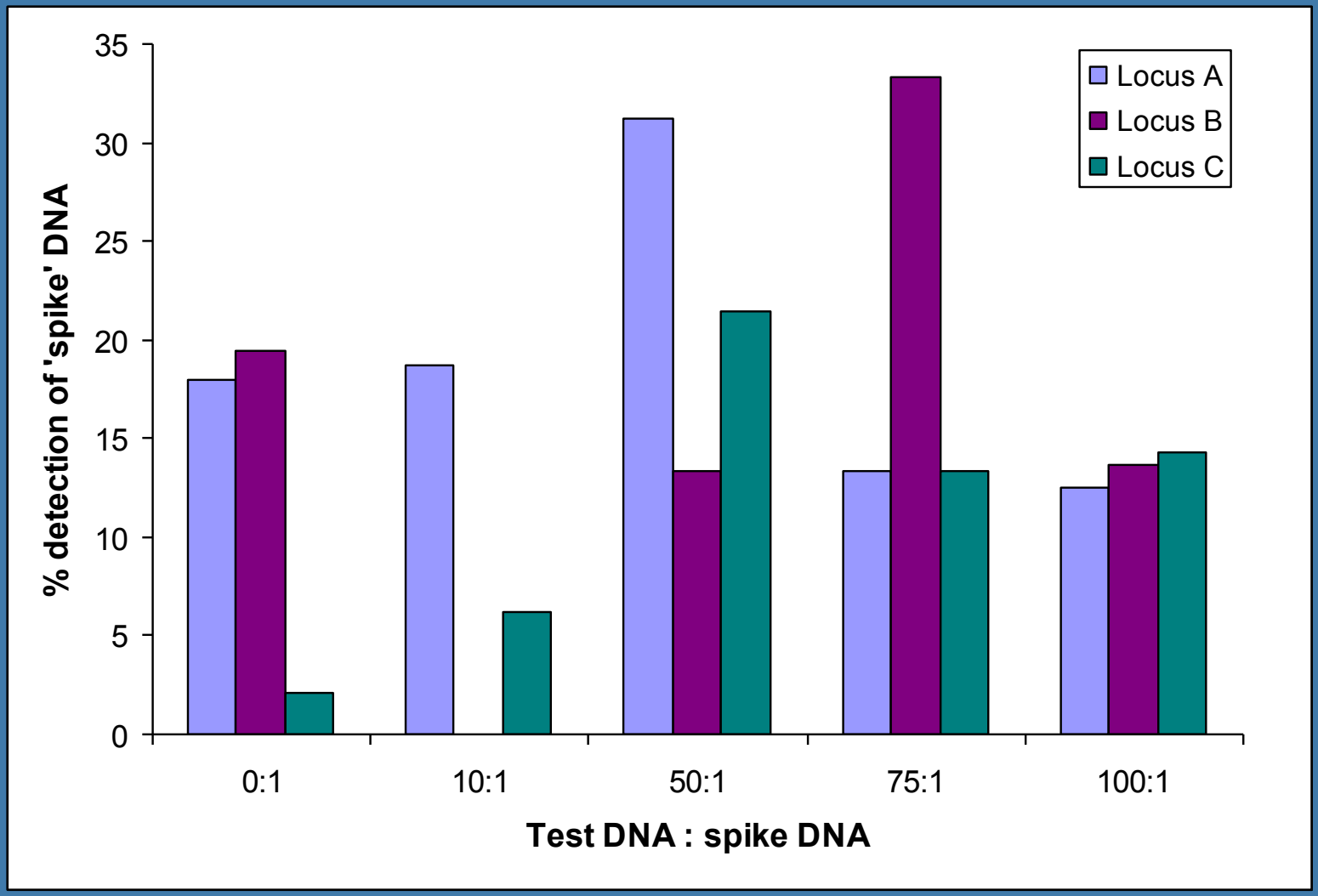








PCR efficiency from low copy number DNA:



Summary

- Allelic richness increases with length of longest repeat.
- Allelic richness is reduced on the Y chromosome. Experimental testing is required to confirm the role of recombination in generating mutations.
- Experiments established to observe mutations occurring in sperm cells.

Acknowledgements

University of Canberra

Stephen Sarre

Nancy FitzSimmons

Niccy Aitken

Erika Alacs

Oliver Berry

Arthur Georges

Lachlan Farrington

Marion Höhn

Will Osborne

David Pederson

Alex Quinn

Rachel Walsh

ARC Centre for Kangaroo Genomics

Amber Alsop

Terry Fletcher

Jenny Graves

Barbara Harriss

Danielle Hickford

Edda Koina

Marilyn Renfree

Natasha Sankovic

Geoff Shaw

Matthew Wakefield

Lee Webley

Kejun Wei

Kyall Zenger

This project is funded by the Australian Research Council

Australian Government

Australian Research Council

CSIRO

Lyn Hinds

University of Western Australia

Brian Chambers

University of Newcastle

Merrilee Harris

Contact me: macdonald@aerg.canberra.edu.au