Development of Single Nucleotide Polymorphisms (SNPs) and Sex-Specific Markers for Genetic Monitoring of Razorback Sucker (*Xyrauchen texanus*)

Photo: T. Kennedy

Funded by:

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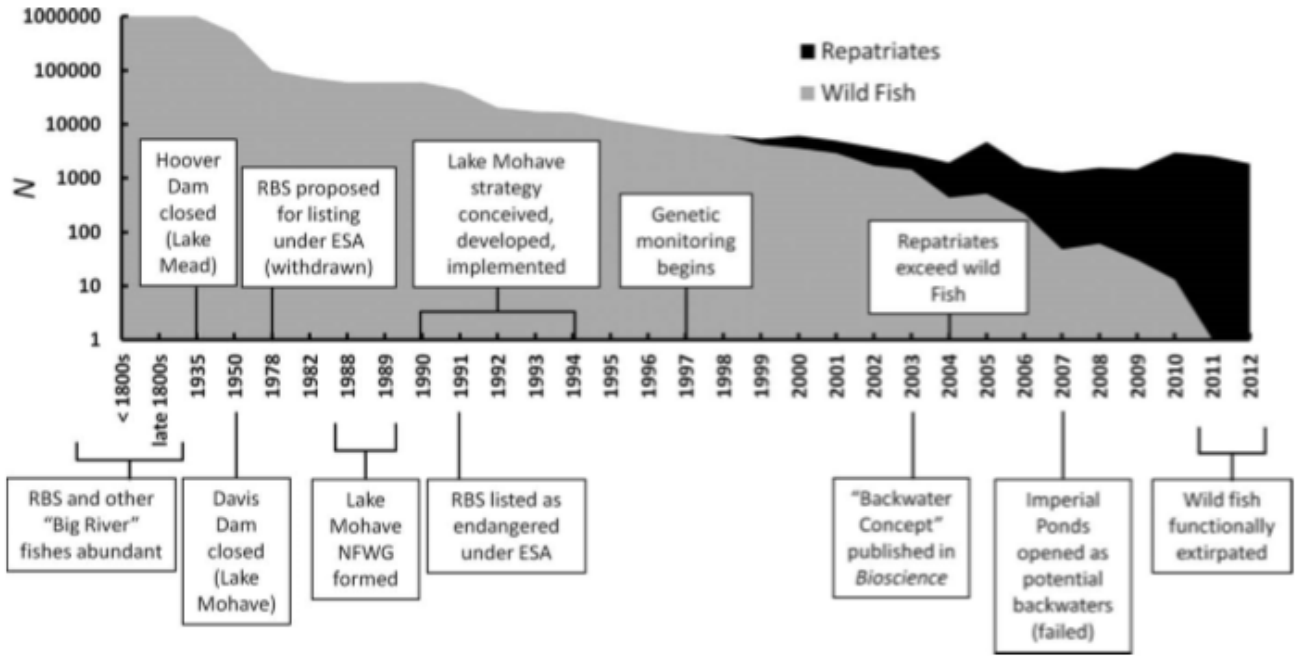
Summary of events

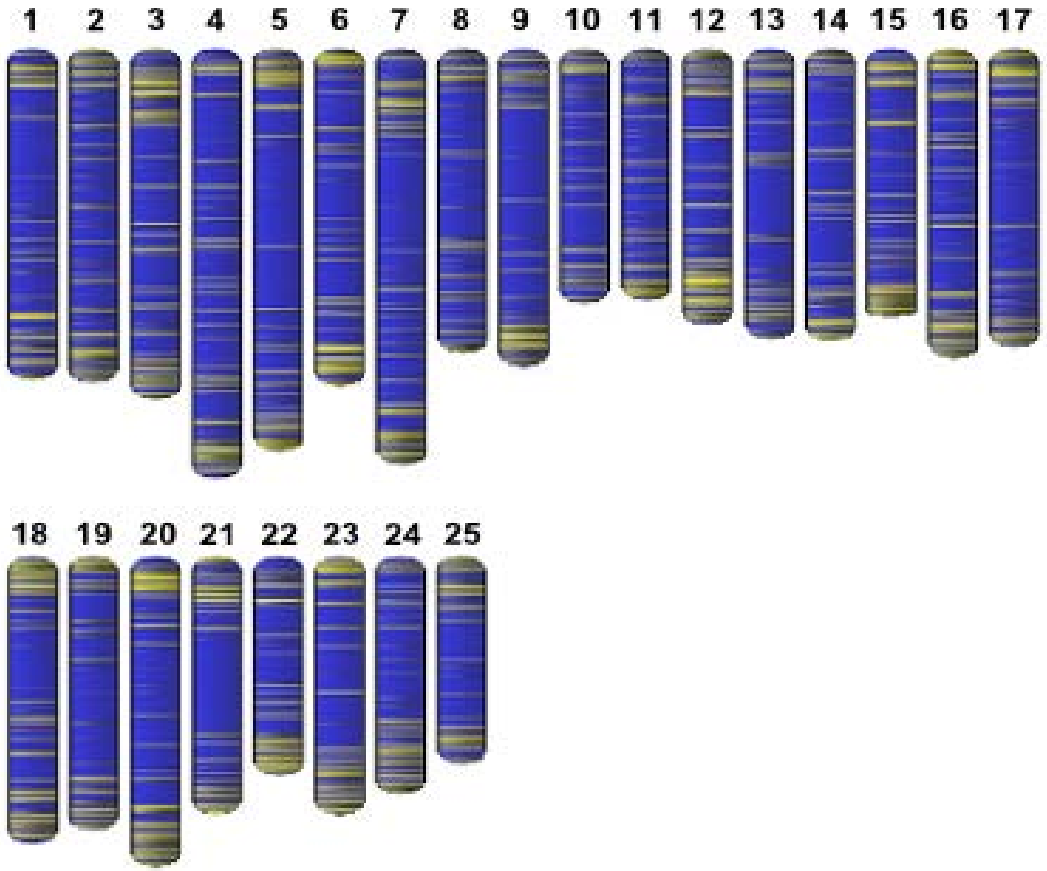
- Highlights – genetic studies
  - 1997 – Genetic monitoring initiated
  - 2005 – Studies of mtDNA shows genetic variation maintained
  - 2007 – First estimate of the effective number of breeders
  - 2010 – Introduction microsatellites
  - 2010 – Initiate backwater studies
  - 2014 – Comprehensive genetic and demographic study published
  - **FY2017 – Development of single nucleotide polymorphism (SNP) panel initiated**



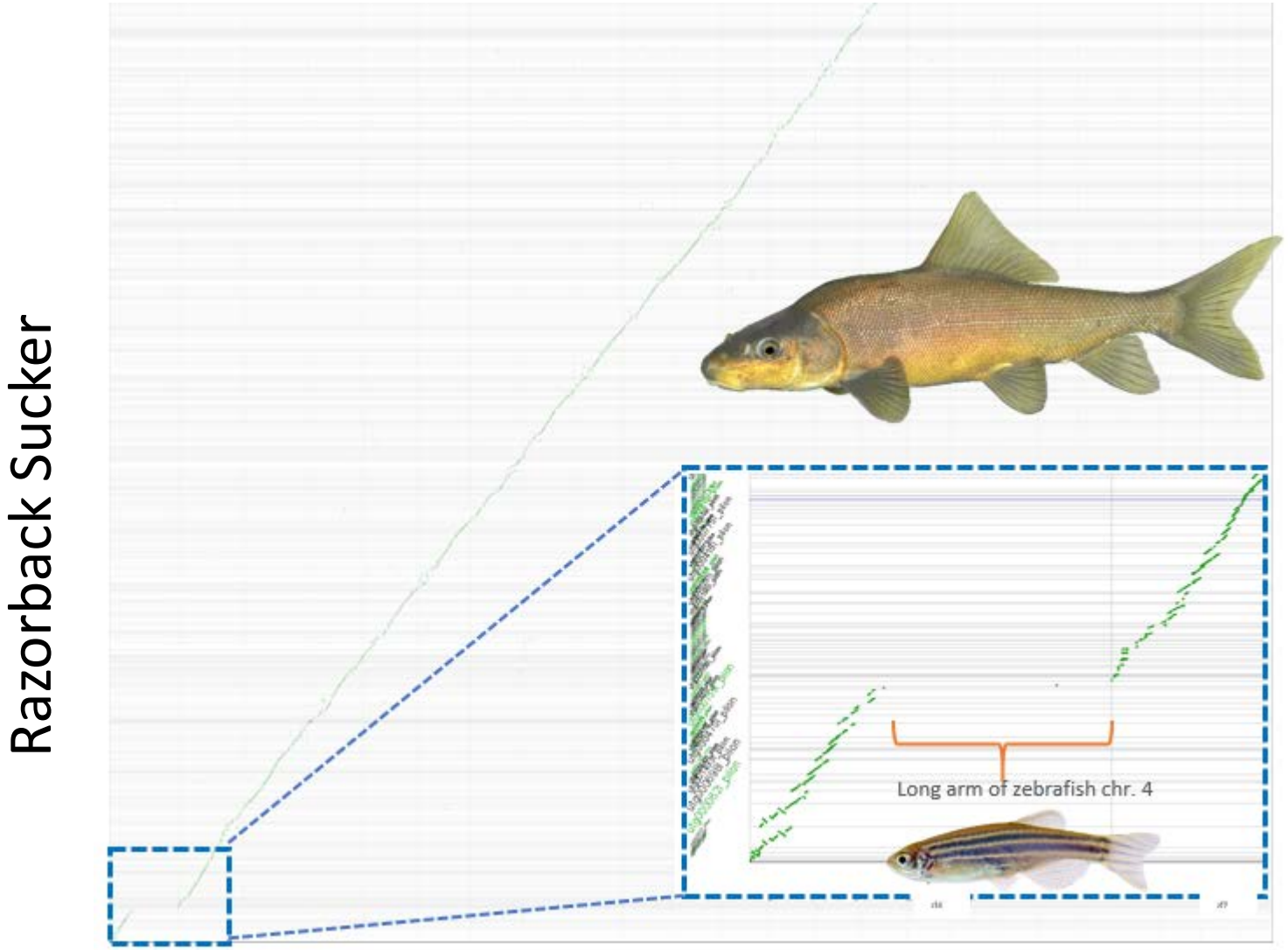
From Marsh et al. 2015

Complete genome

- Needed to generate a map of the razorback sucker genome
  - Map sites of markers to insure independence
  - Identify region of sex determination
- Full genome 2.4 gigabases (GB)
  - Human genome = 2.9 GB
- 60X coverage (copies of each sequence) with multiple methods
- All 50 chromosomes assembled
  - Tetraploid – 2x as many chromosomes as zebrafish



Zebrafish genome



Aligns very well with zebrafish

LETTER

nature

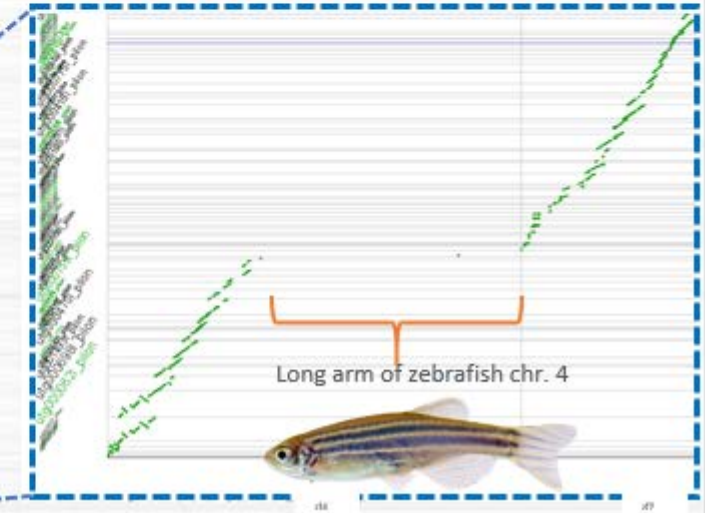
doi:10.1038/nature12111

The zebrafish reference genome sequence and its relationship to the human genome

Kerstin Howe[1]*, Matthew D. Clark[1,2]*, Carlos F. Torroja[1,3], James Torrance[1], Camille Berthelot[4,5,6], Matthieu Muffato[7],

The long arm of chromosome 4 also has a special structure with respect to gene orthology and synteny. Approximately 80% of the genes present have no identifiable orthologues in human. In fact, 110 genes (out of 663) have no identifiable orthologues in any other sequenced teleost genome and indeed seem to be zebrafish-specific genes. The genes in this region are highly duplicated, with 31 ancestral

the subtelomeric region of the long arm of chromosome 4 has been found to contain a major sex determinant with alleles that are 100% predictive of male development and 85% predictive of female development, suggesting that this chromosome may be, might have been, or may be becoming, a sex chromosome in this particular population[20].

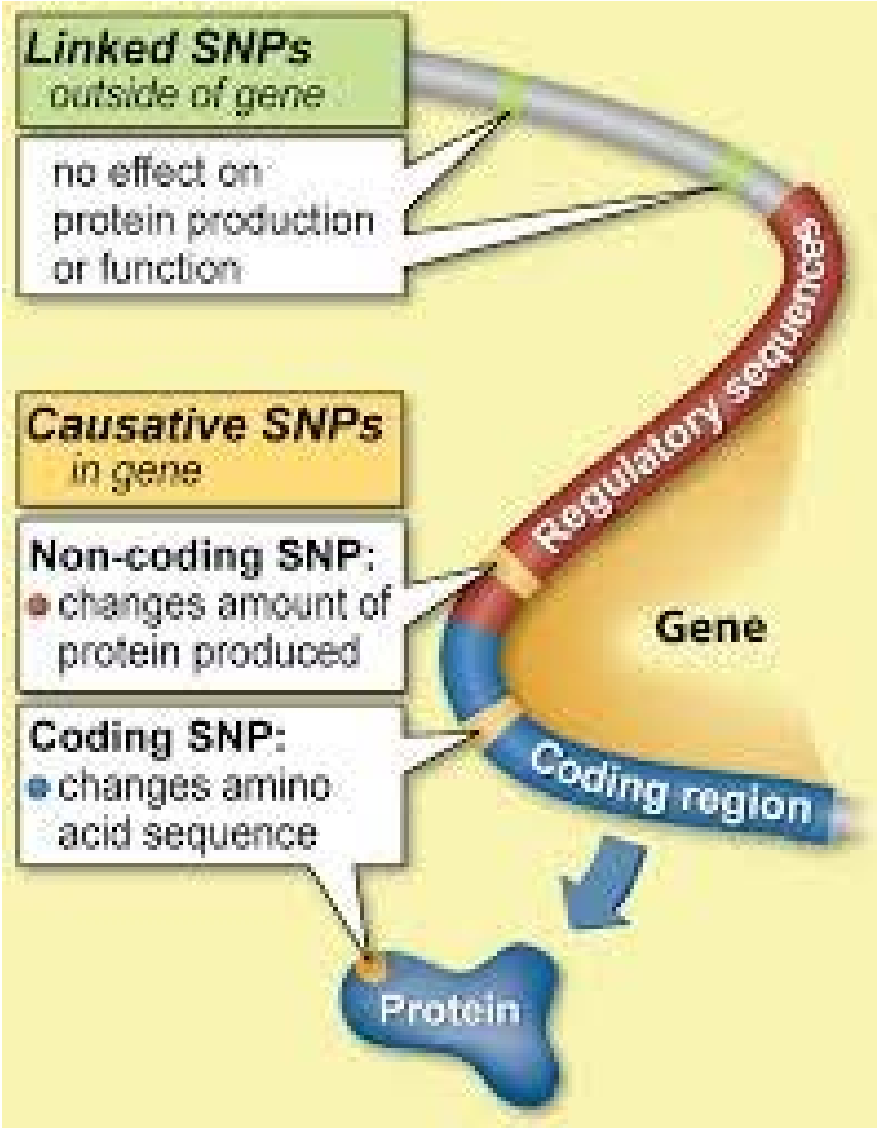


Zebrafish

Aligns very well with zebrafish

What are SNPs?

- Single Nucleotide Polymorphisms – changes in the DNA sequence
- 1000s of SNP markers available
  - Much increased statistical power
  - Independence of markers
  - Some functional others neutral

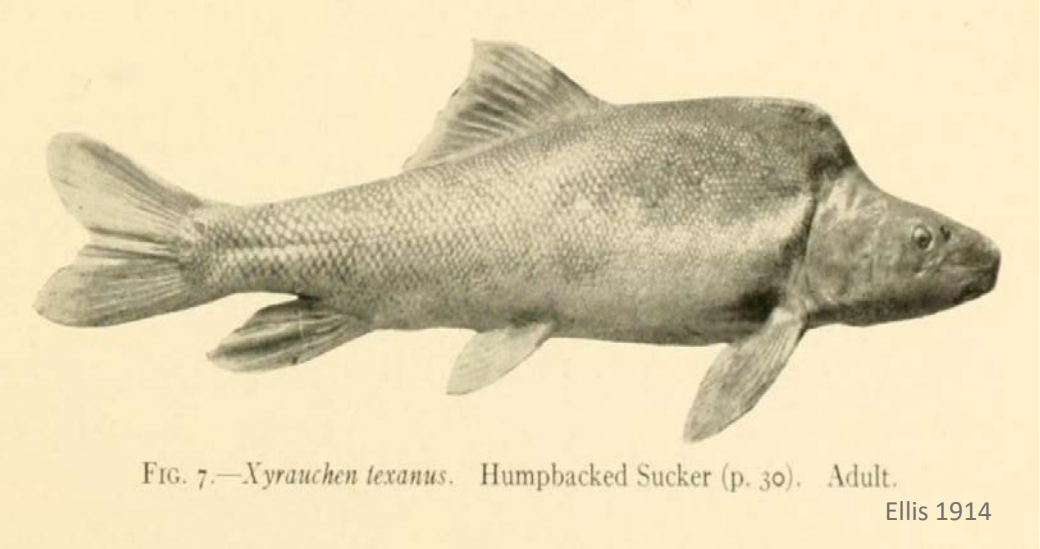


Why develop a SNP panel for genetic monitoring?

- Genetic diversity
- Inbreeding
- Sex specific markers
  - Variation in sex ratios
- Parentage analyses
  - Variation in individual reproductive success in backwaters
- Identify hybrids between razorback and flannelmouth suckers

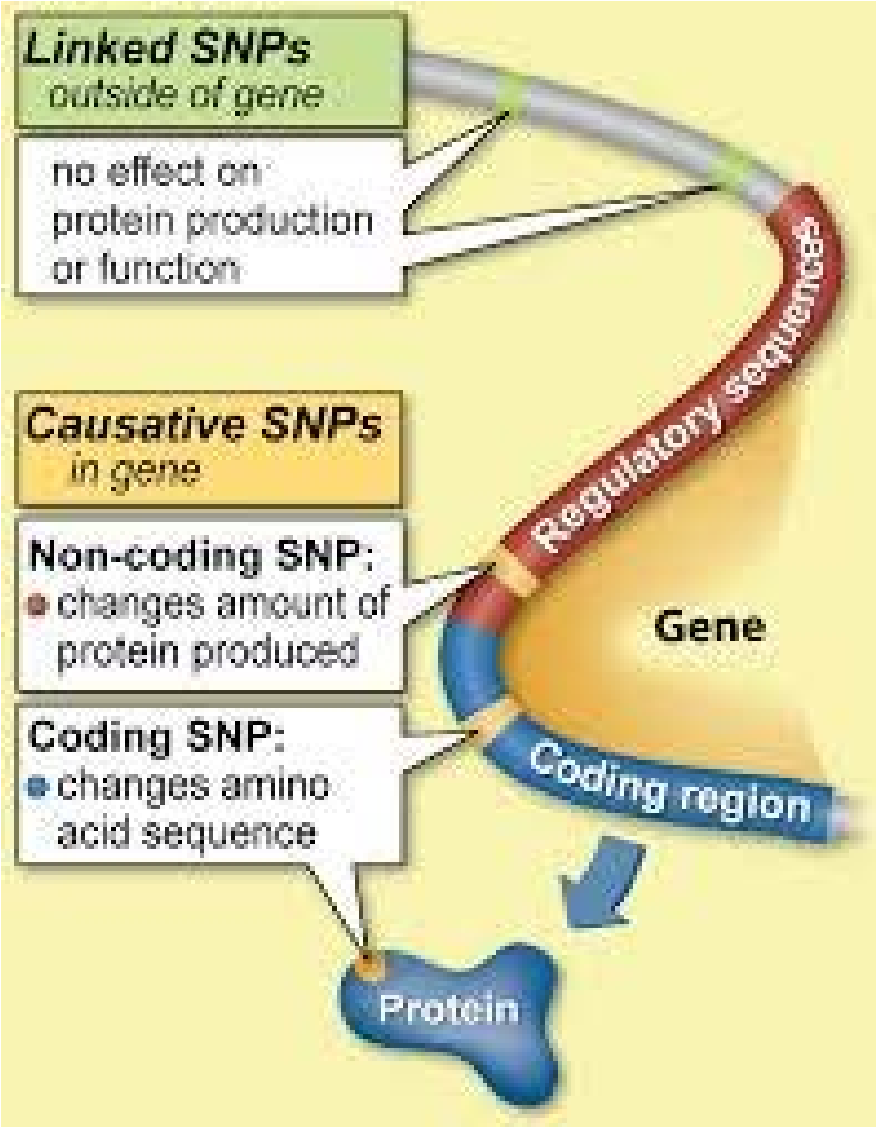
Restriction site-associated DNA sequencing (RAD-seq)

- 64 adult males
- 64 adult females
  - Sex determined phenotypically and by known reproduction
  - All contributed offspring (microsatellite parentage analysis)
- 40 larvae (10 larvae x 4 families)
- 24 flannelmouth sucker



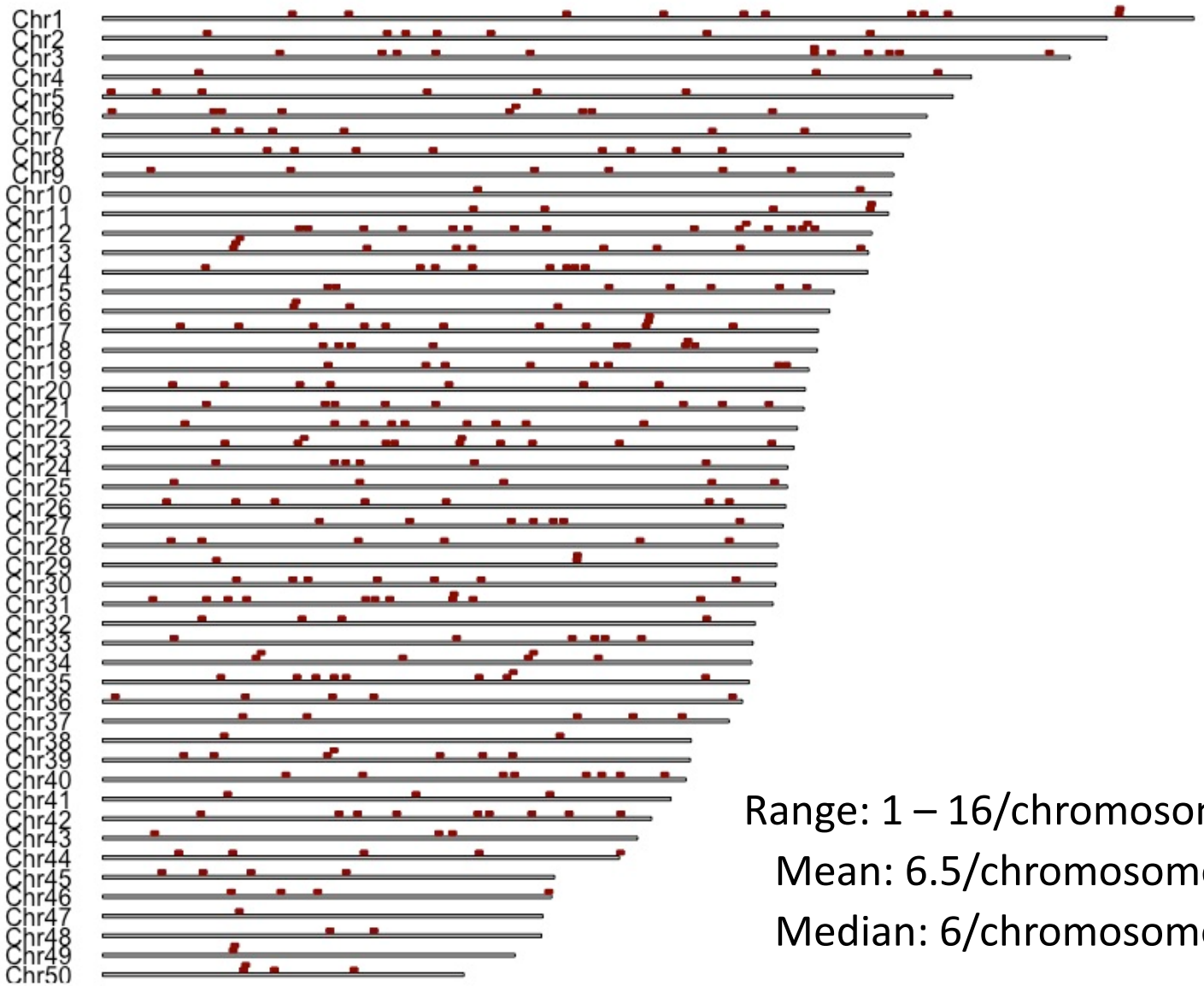
SNP panel development

- 394 loci tested to date
  - 205 loci optimized
    - Filtered
    - Amplify well with each other
    - Average coverage >5x
    - All in Hardy-Weinberg equilibrium
- Parentage analyses
  - Developing computational methods to use all SNPs in the fragment
  - More than two alleles per locus = greater statistical power



What about SNPs and the genome?

- Selected SNPs scattered throughout the genome

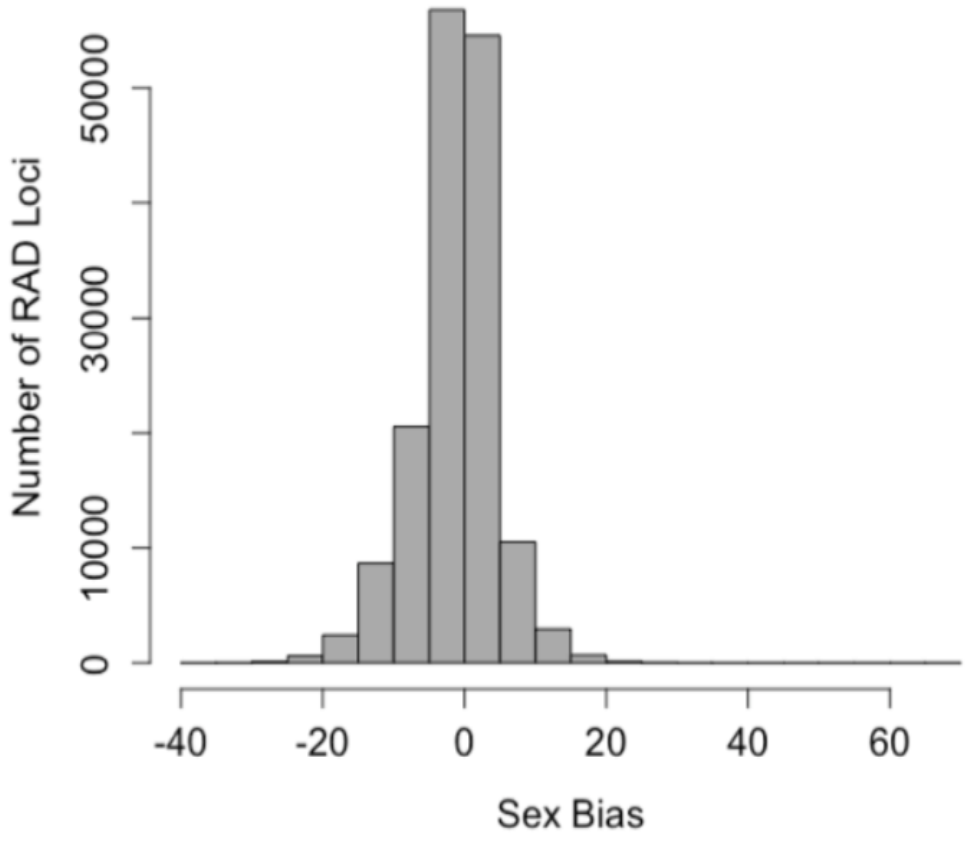


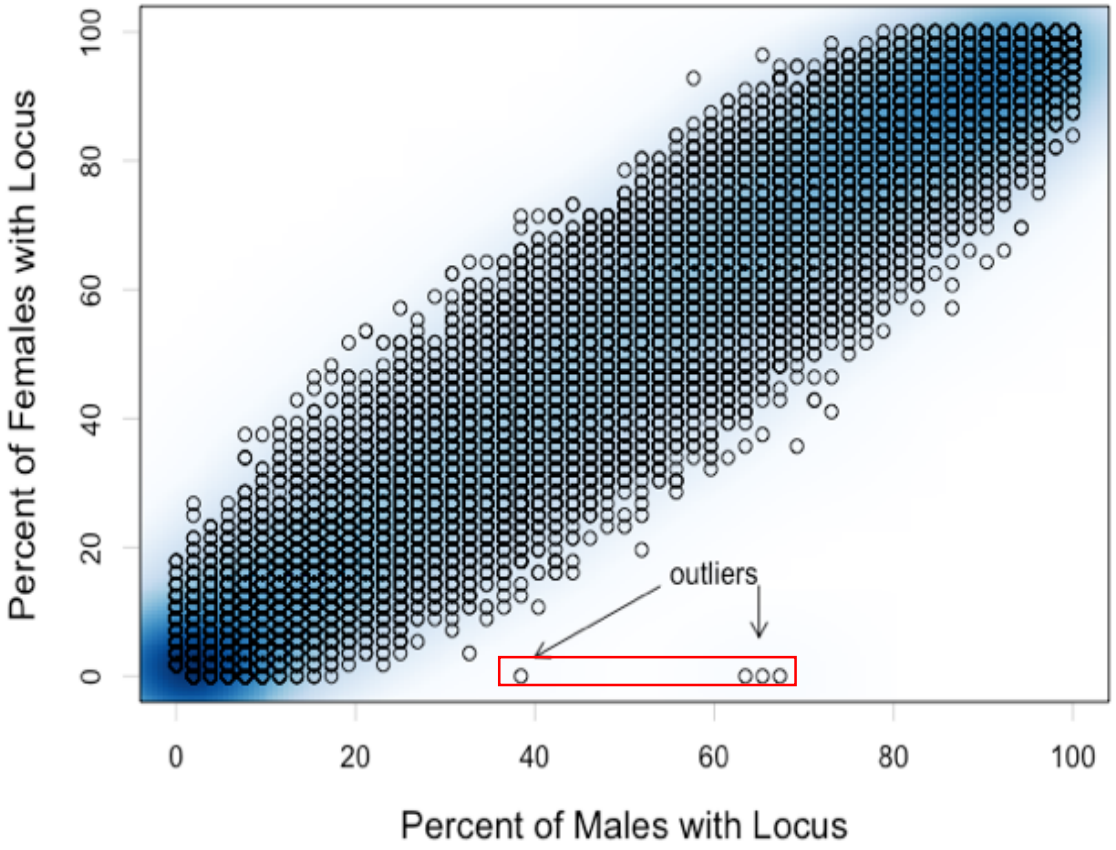
Range: 1 – 16/chromosome

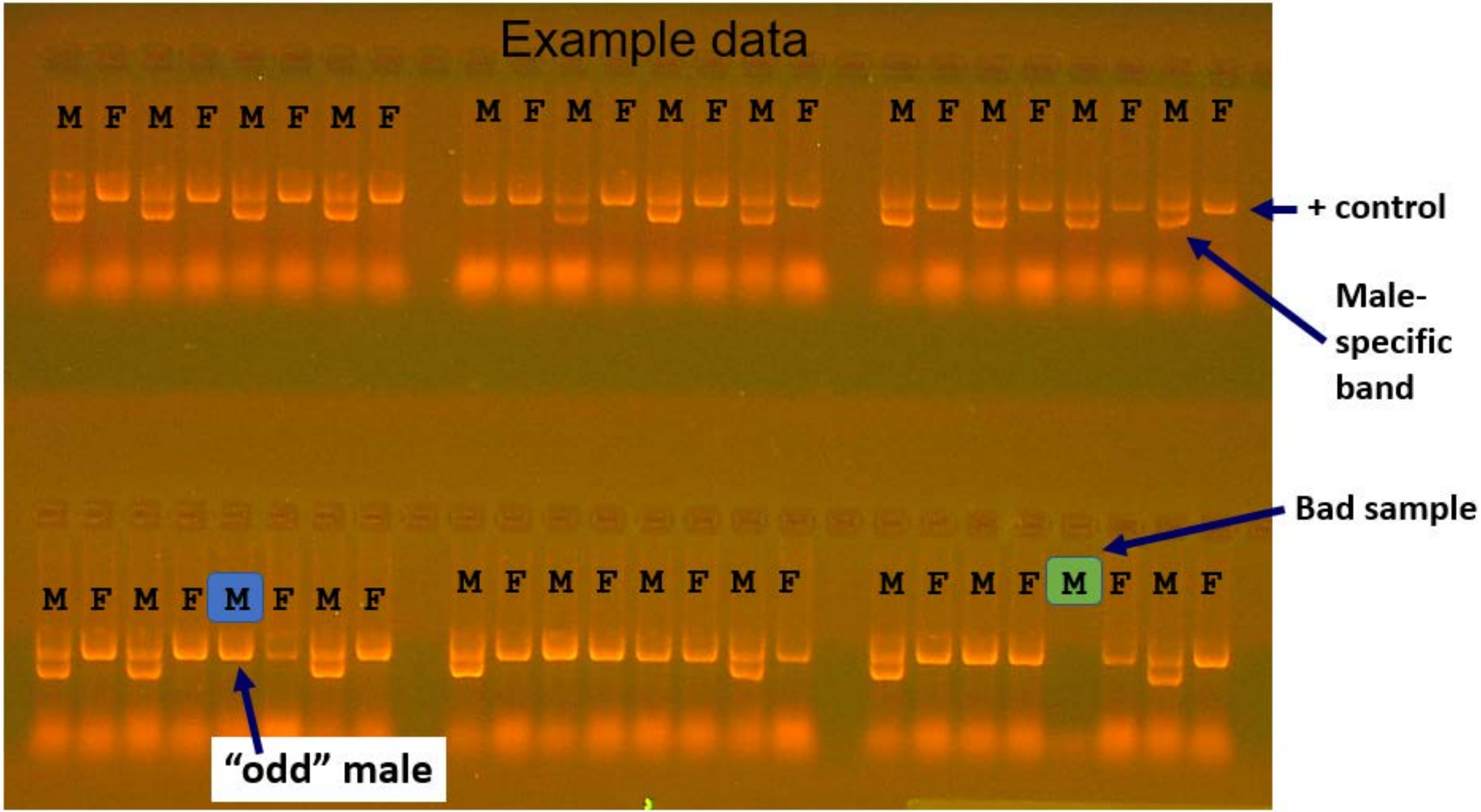
Mean: 6.5/chromosome

Median: 6/chromosome

Sex-specific presence-absence markers






Example data
M F M F M F M F
M F M F M F M F
M F M F M F M F
+ control
Male-specific band
Bad sample
M F M F M F M F
M F M F M F M F
M F M F M F M F
"odd" male

Male-Specific Markers: PCR Validation (RAD samples)

Group	Marker 1	Marker 2	Marker 3	Overall
Adult females	0/63 0%	0/64 0%	0/64 0%	**0/64** **0%**
Adult males	50/62 80.1%	52/64 81.3%	51/63 81.0%	**52/64** **81.3%**

Razorback sucker: 90.6% correctly sexed
(116 of 128 known adults)
12 phenotypic males without the male-specific markers

Male-Specific Markers: PCR Validation (Lake Mohave samples: 1997-2017)

Phenotypic sex	N	Marker 1	Marker 2	Marker 3	Overall
Adult females	71	2/65 3.0%	2/66 3.0%	2/68 2.9%	**2/68** **2.9%**
Adult males	68	55/64 85.9%	57/66 86.4%	57/66 86.4%	**58/67** **86.6%**

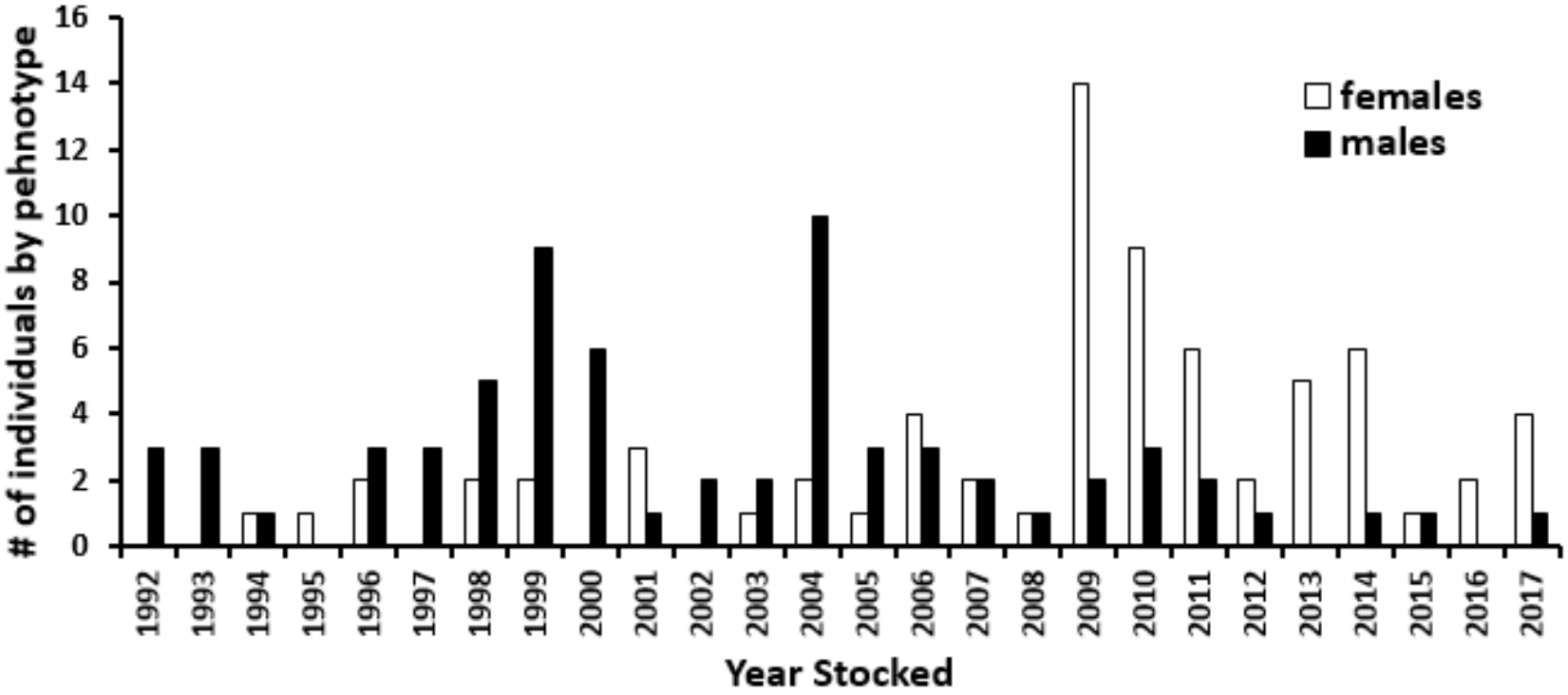
91.9% (124 of 135 known adults) correctly sexed

- Two unusual females were fish that had been re-tagged
- Nothing unusual about the nine odd males

- **Why do some males lack the male-specific markers?**

Temporal variation

- Unusual males and females (mismatched between phenotype and genetic identification) scattered across time
  - Sampled in 1993, 1998, 1999(3), 2004, 2007(2), 2009, 2015, 2017
- Distribution of males and females structured over time
  - 68 males, 71 females



Test pre-2008 vs post 2007
G-test
G = 41.0
Df = 1
P < 0.0001

Male-Specific Markers: Unknowns (Lake Mohave larvae: 2018)

48 larvae from six locations, January - April

Group	Marker 1	Marker 2	Marker 3	Overall
Larval females	24	24	24	**24**
Larval males	23	23	23	**23**

1:1 sex ratio in larvae

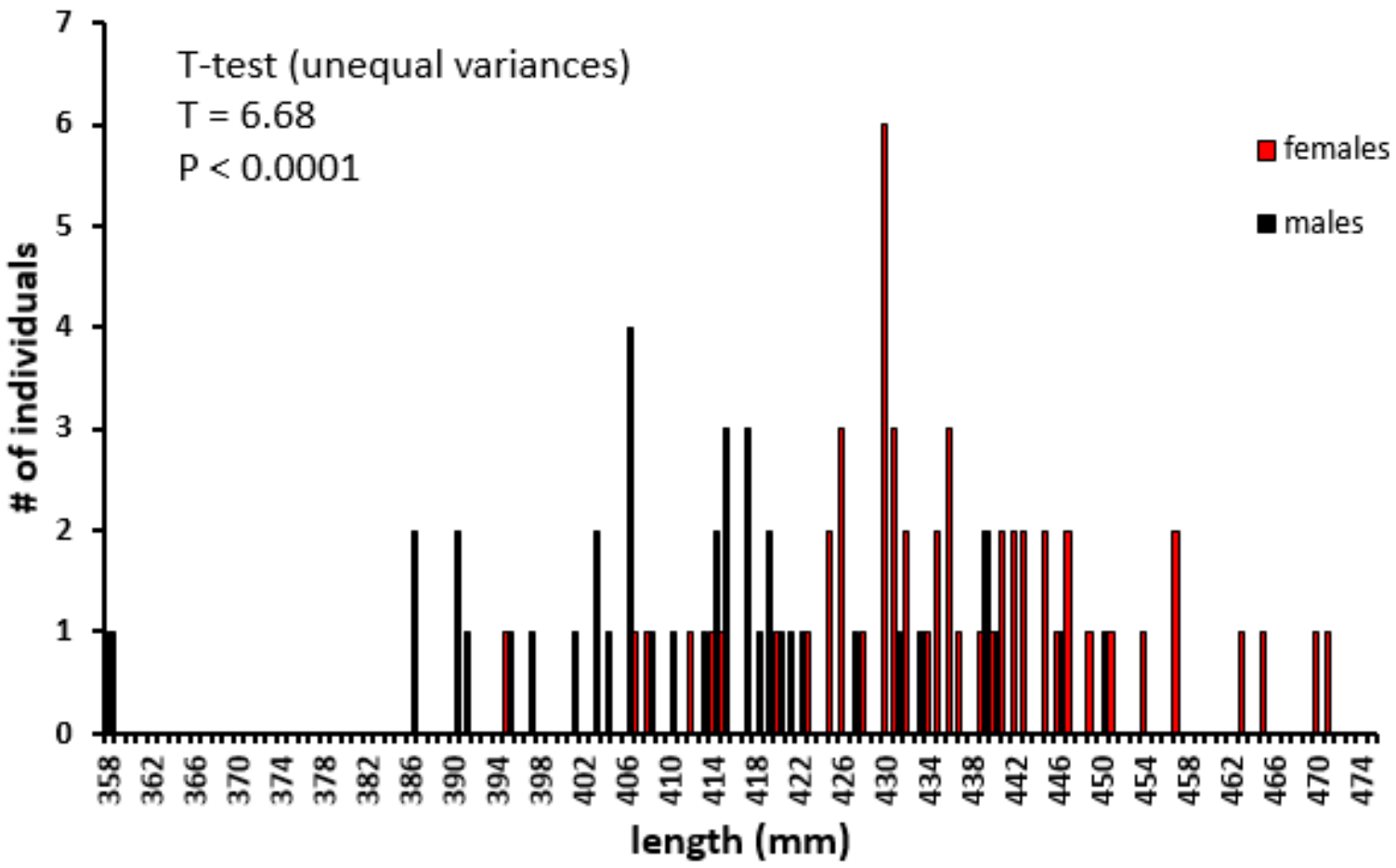
Odd results for adults reflect hatchery operations?

Male-Specific Markers: Unknowns (Achii-Hanyo samples: 2016)

Group	Number	Ave. length
Females	**55**	**436**
Males	**41**	**412**

Stocking more females than males

Females significantly larger than males

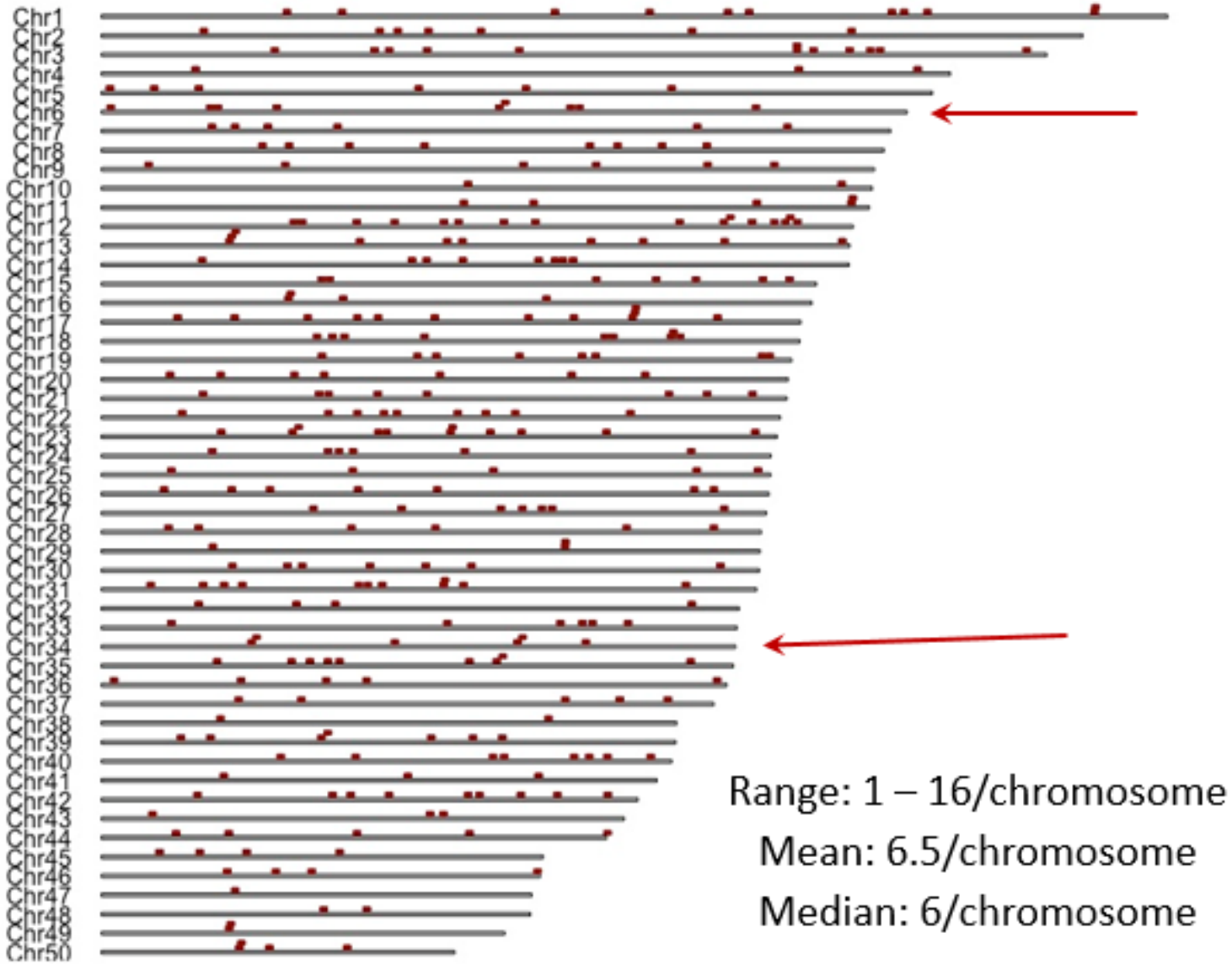


Summary – SNP results

- Many SNPs optimized
  - Starting application in parentage analyses
- Male-specific markers consistent with phenotypes in >92% of all individuals tested
- Sex appears to be polygenic (loci on several chromosomes) and environmentally determined
  - Environmental sex determination similar to what we know from work with goldfish and other fishes
  - For goldfish, sex sensitive to environment up to 22 days with females becoming males at higher temperatures (> 23 C)
- Why is this important?

What about sex specific markers and the genome?

- Difficult to place because of their short length and structure
  - MM1 = chr 34
  - MM2 = chr 6
  - MM3 = chr 1 or 11?
- Sex is polygenic as well as environmentally determined?



Why are sex ratios important?

ANIMAL BEHAVIOUR, 1999, **58**, 287–294
Article No. anbe.1999.1149, available online at http://www.idealibrary.com on IDEAL

Operational sex ratio influences female preference and male–male competition in guppies

MULLICA JIROTKUL

Animal Conservation (2002) **5**, 13–20 © 2002 The Zoological Society of London
DOI:10.1017/S1367943002001026 Printed in the United Kingdom

Manipulating sex ratios for conservation: short-term risks and long-term benefits

C. Wedekind
Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

Oecologia (Berl) (1982) 53:146–151
Oecologia
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THE
GENETICAL THEORY OF
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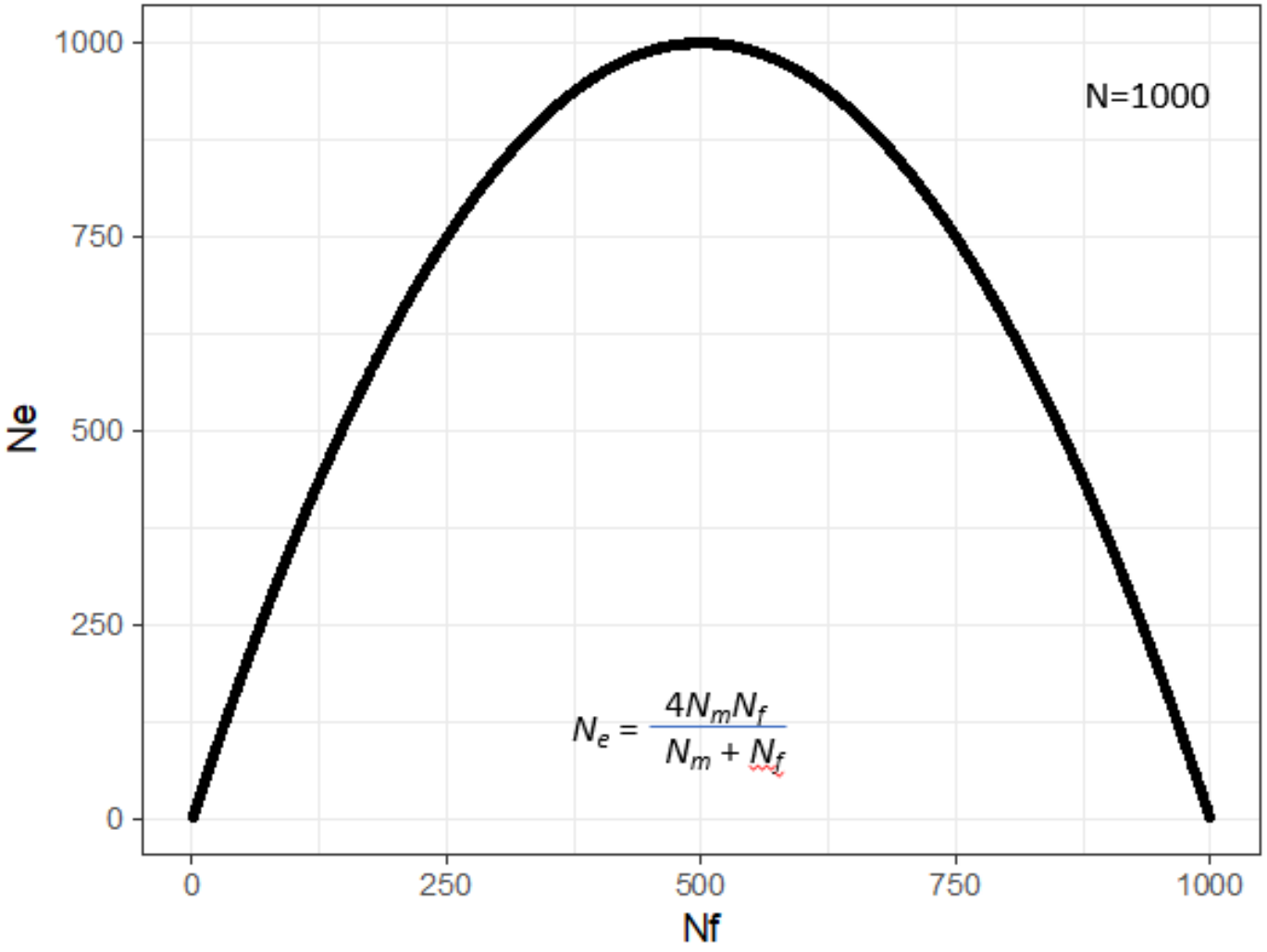
By
R. A. FISHER

Size Specific Predation by Herons and its Effect on the Sex-Ratio of Natural Populations of the Mosquito Fish *Gambusia affinis* Baird and Girard

Robert H. Britton[1] and Michael E. Moser[1,2]

Sex ratios and effective population size



- Any deviation from a 50:50 sex ratio reduces effective number of breeders
- Reduces levels of genetic variation
- Therefore, important to consider such effects as thinking about conservation

Acknowledgements

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Photo: T. Kennedy