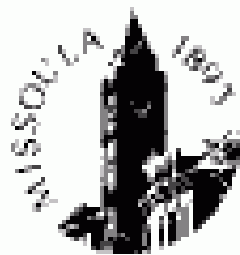


*Use of genomics to detect and understand
spread of introgression from introduced
rainbow trout into native westslope
cutthroat trout*

Fred Allendorf
&
Gordon Luikart



University of Montana

Victoria

UNIVERSITY OF WELLINGTON

*Te Whare Wānanga
o te Ūpoko o te Ika a Māui*



TRENDS in Ecology & Evolution

Conservation genetics

Cryptic northern refugia

Carnivorous plants

Evolutionary constraints on digit identity

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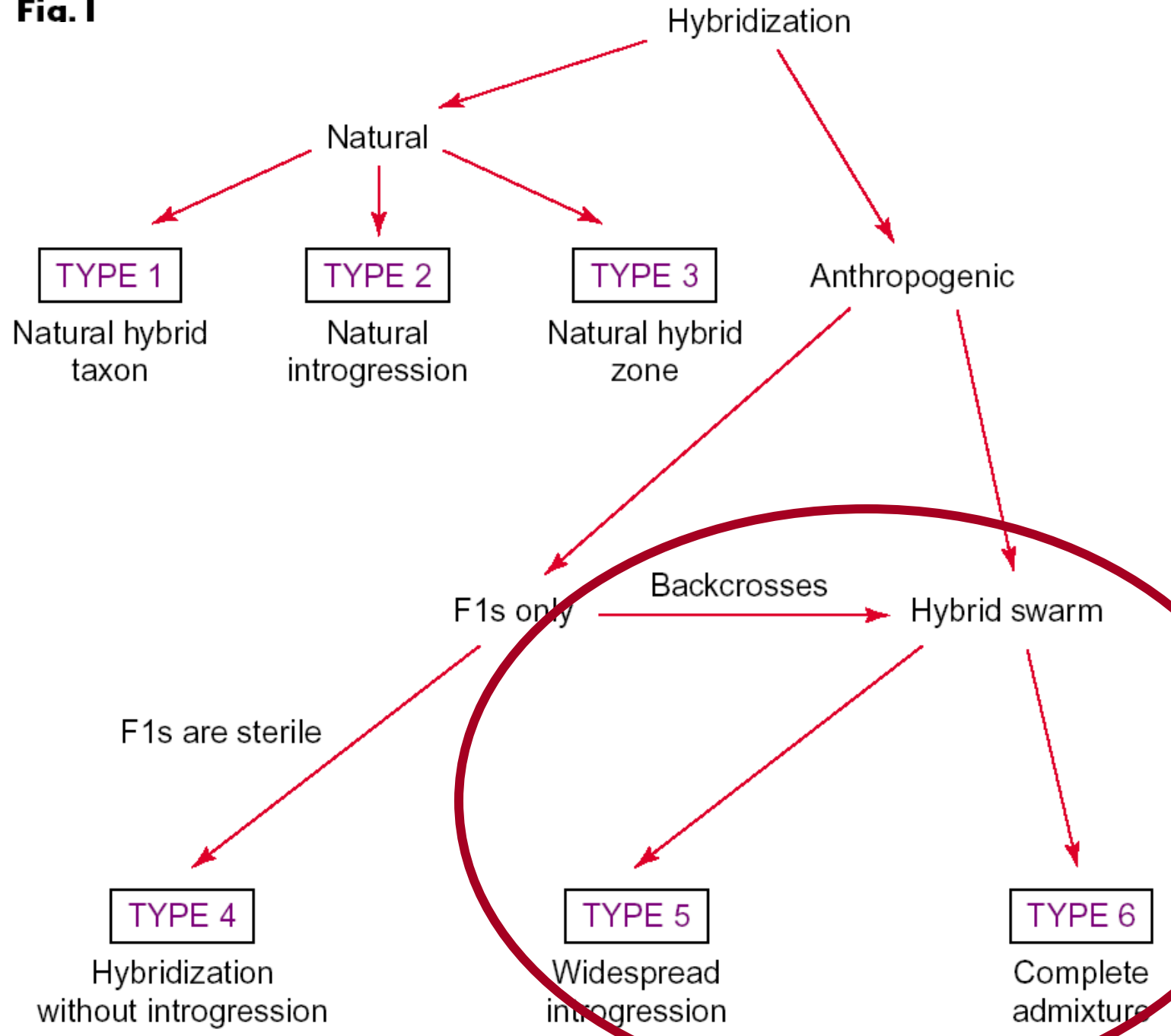
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The problems with hybrids: setting conservation guidelines

Fred W. Allendorf, Robb F. Leary, Paul Spruell
and John K. Wenburg

2001. Trends in
Ecology & Evolution
16:613-622.

Fig. 1



Genomic extinction of New Zealand grey duck, *Anas superciliosa*



Hybridize with introduced mallard ducks.
Few (none?) pure populations remain (Murray
Williams, Victoria University of Wellington).

Genomic extinction: Irretrievable loss by hybridization of genome-wide combination of genotypes that have evolved over long periods of evolutionary time.

What has been lost?

Not the genes; they still are present in the admixed hybrid swarm.

The grey duck genome has been lost through admixture with mallard genes.

What is the effect of this?

Fitness

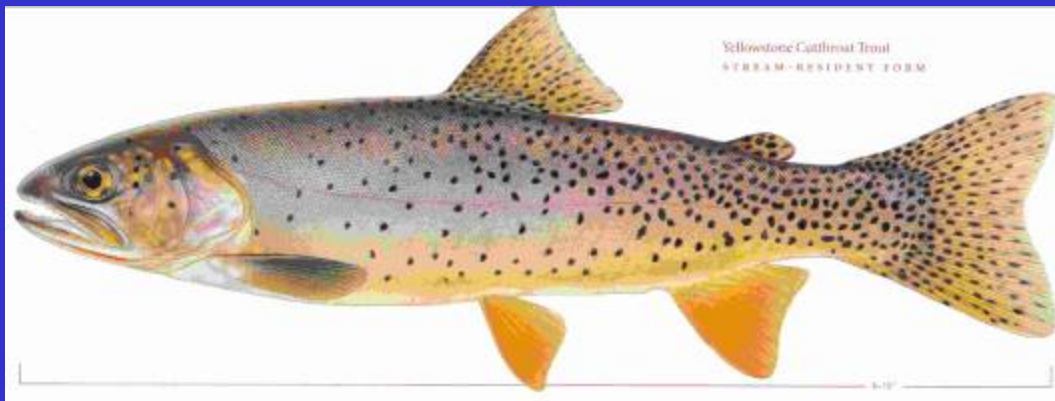
Alleles that enhance fitness may reduce fitness in the novel genetic background produced by hybridization. Such interactions between alleles are known as Bateson-Dobzhansky-Muller incompatibilities.

Loss of the grey duck genotype is expected to bring about the loss of adaptations due to multiple locus interactions.



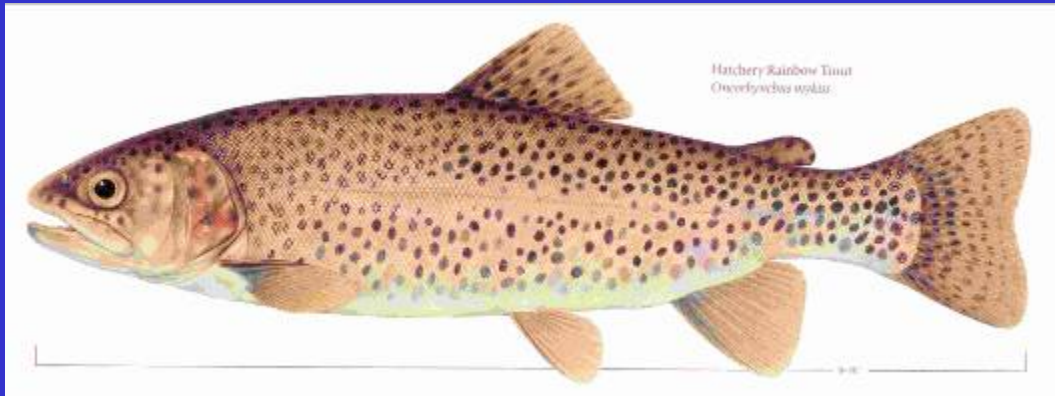
Westslope cutthroat trout
(WCT)

Oncorhynchus clarkii lewisi



Yellowstone cutthroat trout
(YCT)

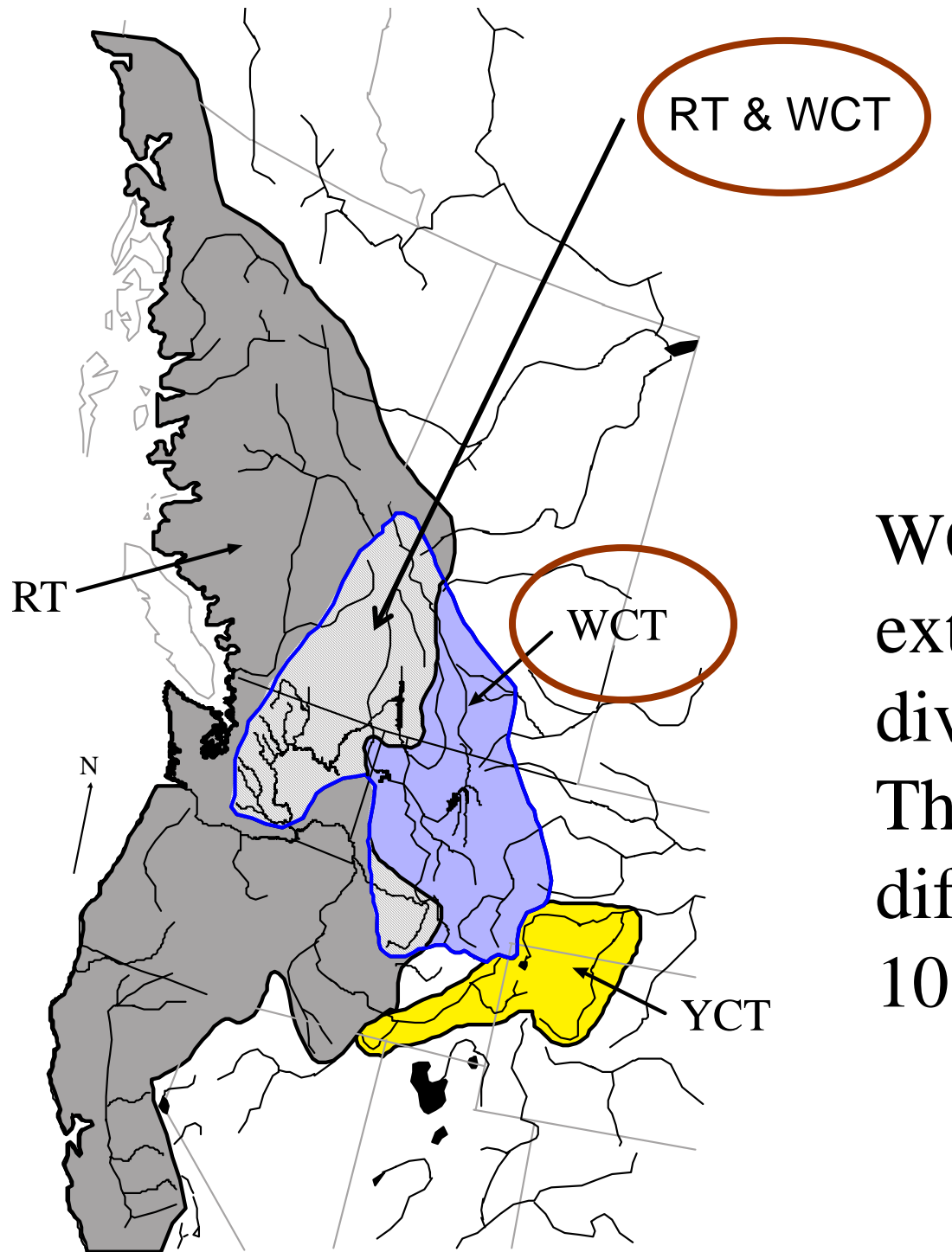
O. c. bouvieri



Rainbow trout
(RT)

O. mykiss

(Illustrations by Joseph R. Tomelleri)



WCT and YCT are extremely genetically divergent subspecies. They are fixed for different alleles at 10 of 52 allozyme loci.

A history of markers to study hybridization between westslope cutthroat and rainbow trout:

1975	Allozymes
1980	mtDNA
1990	PCR using interspersed nuclear elements
2000	Microsatellites In/Dels SNPs
2010	RADs (500-1,000 diagnostic SNPs) ESTs (candidate loci)

ASSOCIATIONS BETWEEN MITOCHONDRIAL AND NUCLEAR GENOTYPES IN CUTTHROAT TROUT HYBRID SWARMS

S. H. FORBES AND F. W. ALLENDORF

Division of Biological Sciences, University of Montana, Missoula, MT 59812 USA

Hybrid swarm in Forest Lake, Montana

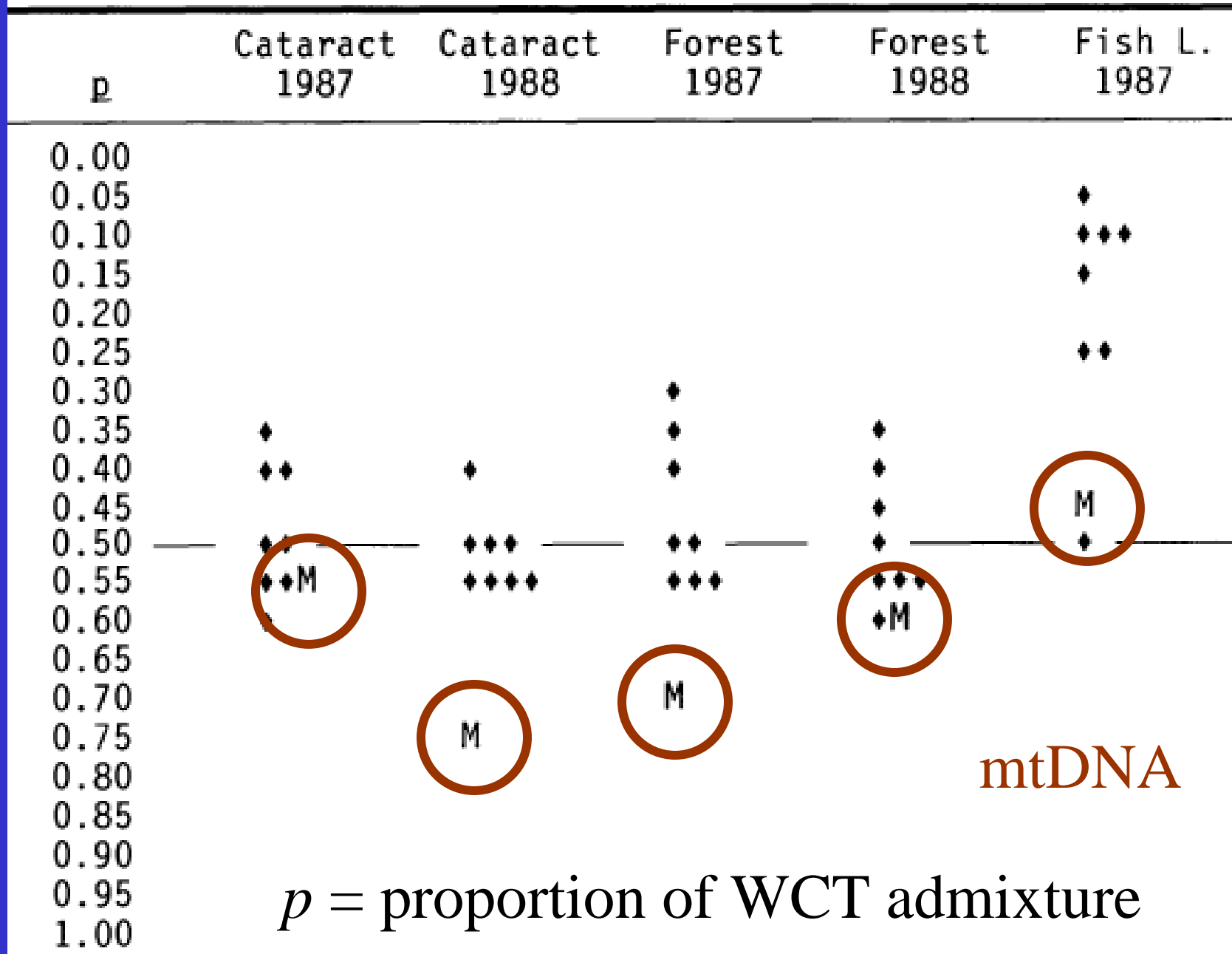


Table 3. Allozyme genotypes at 8 nuclear loci and mtDNA genotypes in a hybrid swarm of westslope and Yellowstone cutthroat trout in Forest Lake, Montana.

No.	mtDNA	<i>Nuclear encoded loci</i>								
		<i>Aat1</i>	<i>Gpi3</i>	<i>Idb1</i>	<i>Lgg</i>	<i>Me1</i>	<i>Me3</i>	<i>Me4</i>	<i>Sdb</i>	
1	YS	W	W	WY	W	W	W	W	W	Y
2	YS	W	WY	WY	WY	Y	W	WY	WY	Y
3	WS	WY	Y	Y	W	Y	WY	Y	WY	WY
4	WS	Y	W	WY	WY	W	Y	W	WY	WY
5	YS	Y	Y	Y	WY	WY	WY	Y	Y	Y
6	YS	WY	Y	W	WY	W	W	W	W	Y
7	WS	WY	WY	Y	W	WY	W	W	W	W
8	WS	WY	Y	WY	WY	Y	W	Y	Y	Y
9	WS	Y	Y	WY	WY	W	WY	WY	WY	W
10	WS	WY	Y	WY	WY	WY	Y	W	Y	Y
11	YS	Y	W	W	WY	W	Y	W	Y	Y
12	WS	W	WY	Y	WY	W	WY	WY	WY	Y
13	YS	W	Y	W	Y	W	WY	W	W	W
14	YS	Y	Y	WY	WY	WY	WY	WY	WY	W
15	WS	WY	Y	WY	Y	W	Y	WY	WY	W

W= homozygous WCT; WY=heterozygous; Y= homozygous YCT

Hybrid swarm: all individuals are hybrids by varying numbers of generations of backcrossing with parental types and mating among hybrids.



Conclusions

(1) All 8 nuclear loci show similar amounts of admixture.

No evidence for differential rates of introgression because of natural selection.

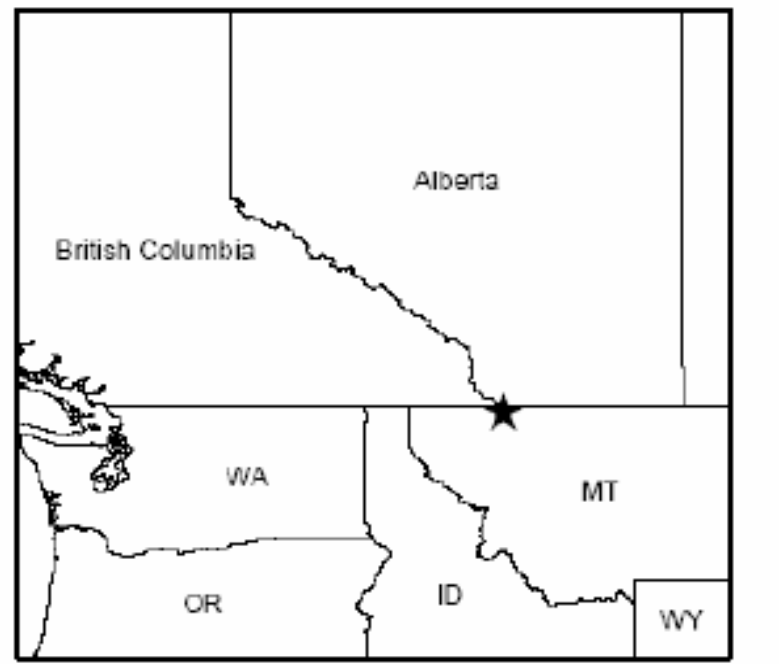
(2) Frequency of mtDNA genotype of native fish almost always greater than introduced fish.

Native females appear to have relatively greater fitness than native males.

Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*

Nathaniel P. Hitt, Christopher A. Frissell, Clint C. Muhlfeld, and Fred W. Allendorf

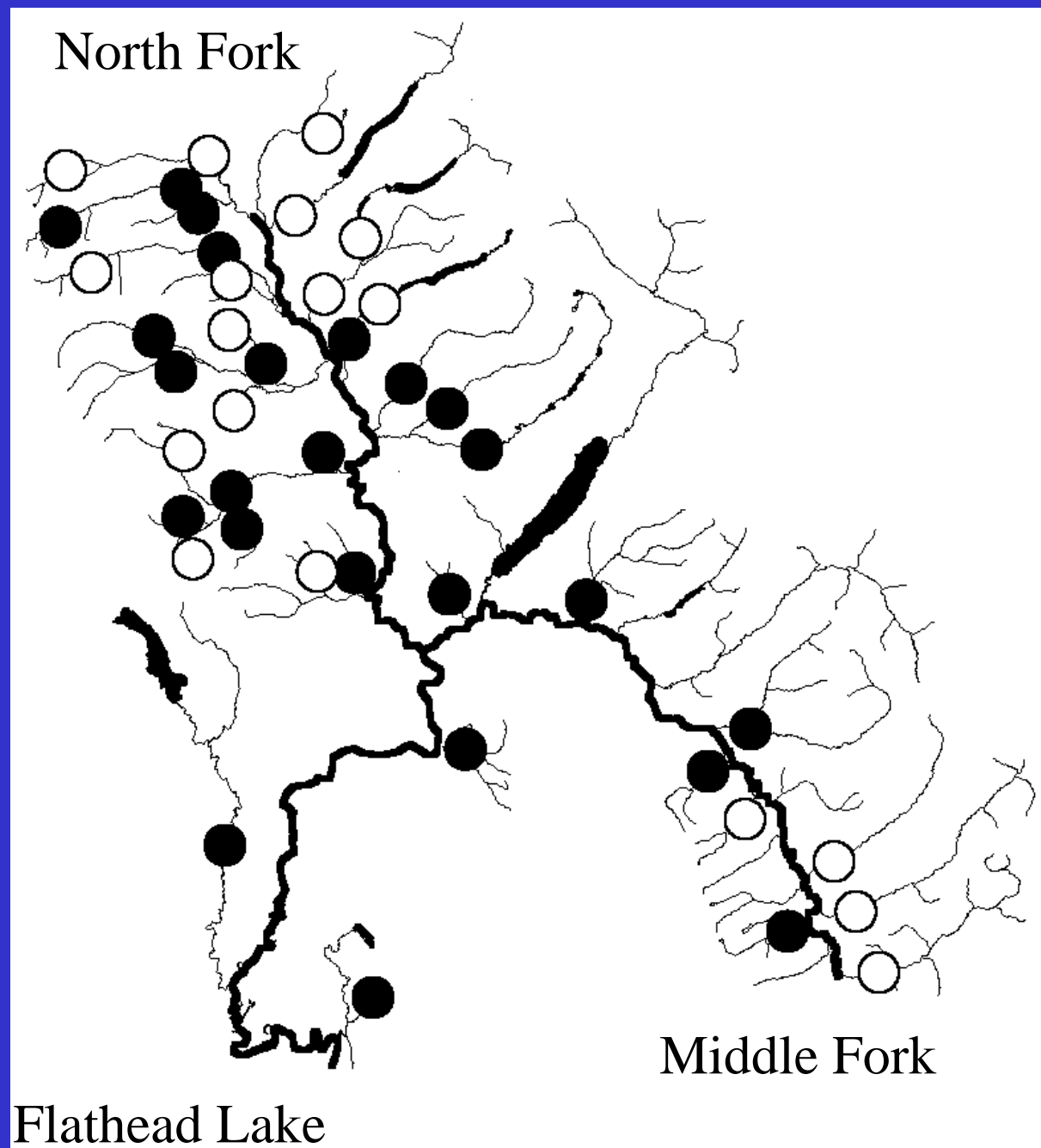
Can. J. Fish. Aquat. Sci. 60: 1440–1451 (2003)



Examined hybridization in 42 putative WCT samples from Flathead River.

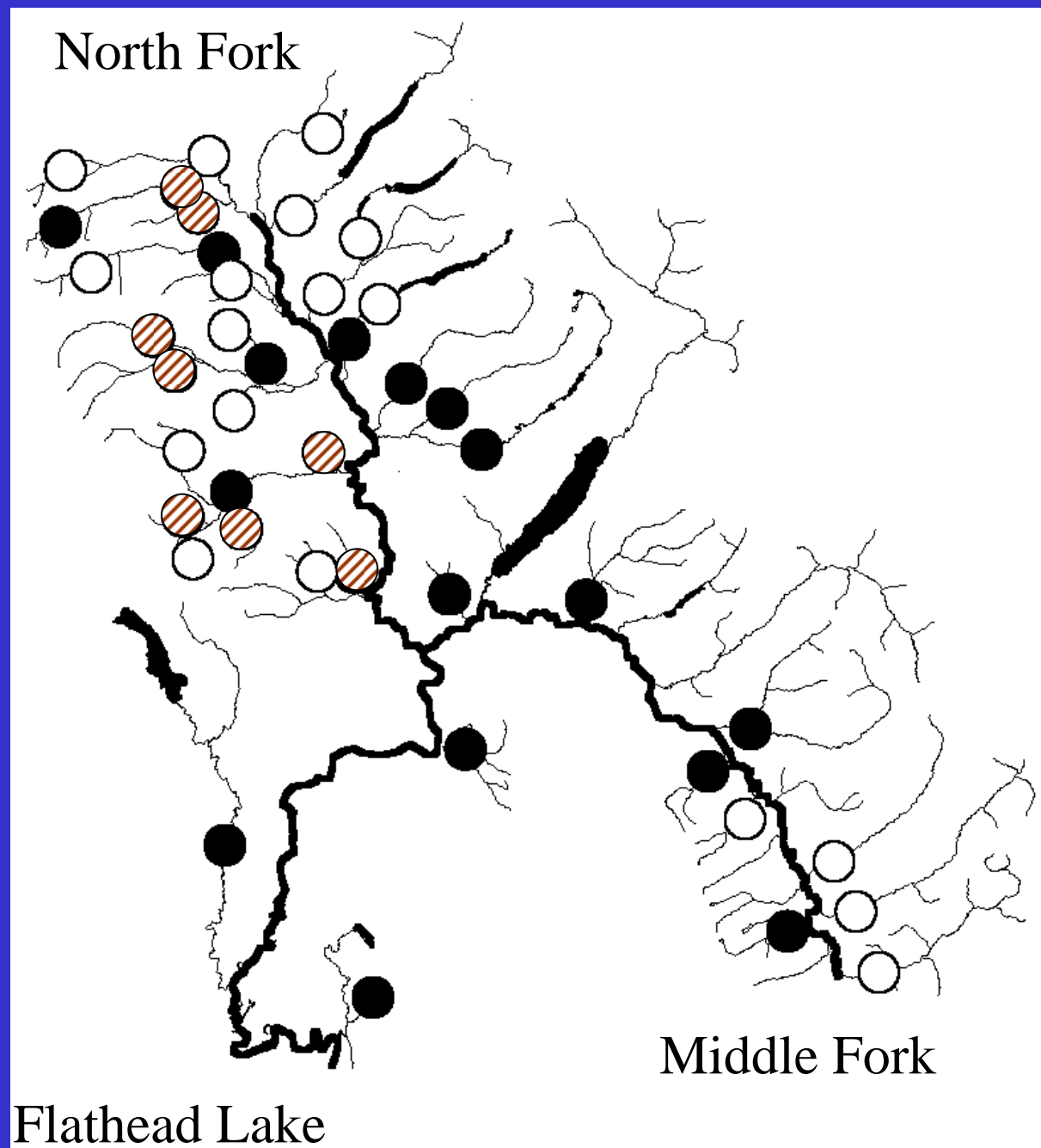
Hybridization widespread

- WCT ($n=17$)
- Hybridized ($n=25$)



Hybridization spreading

- WCT ($n=17$)
- Hybridized ($n=17$)
- ◐ Hybridized post-1985 ($n=8$)

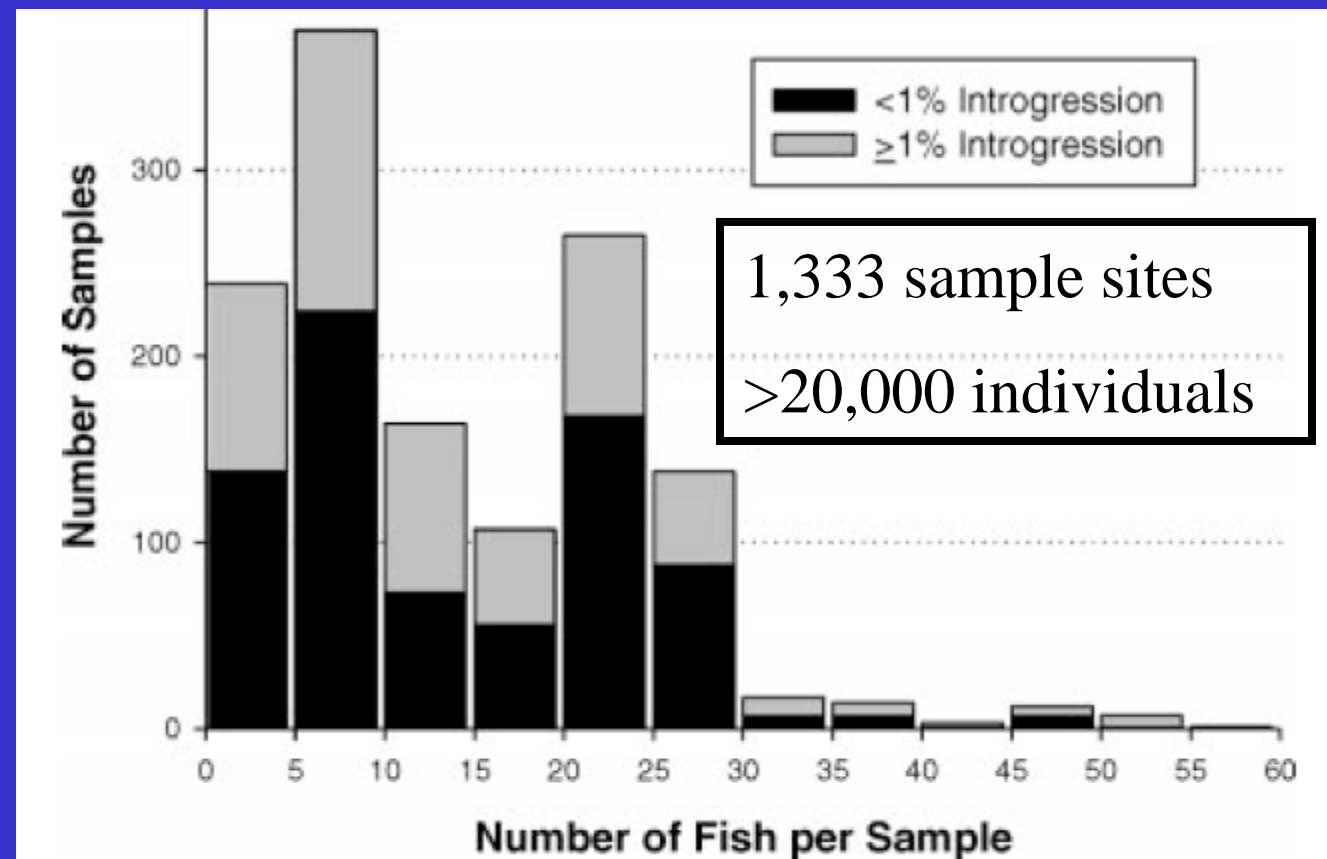


Status and Conservation of Westslope Cutthroat Trout within the Western United States

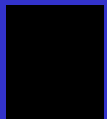
BRADLEY B. SHEPARD*

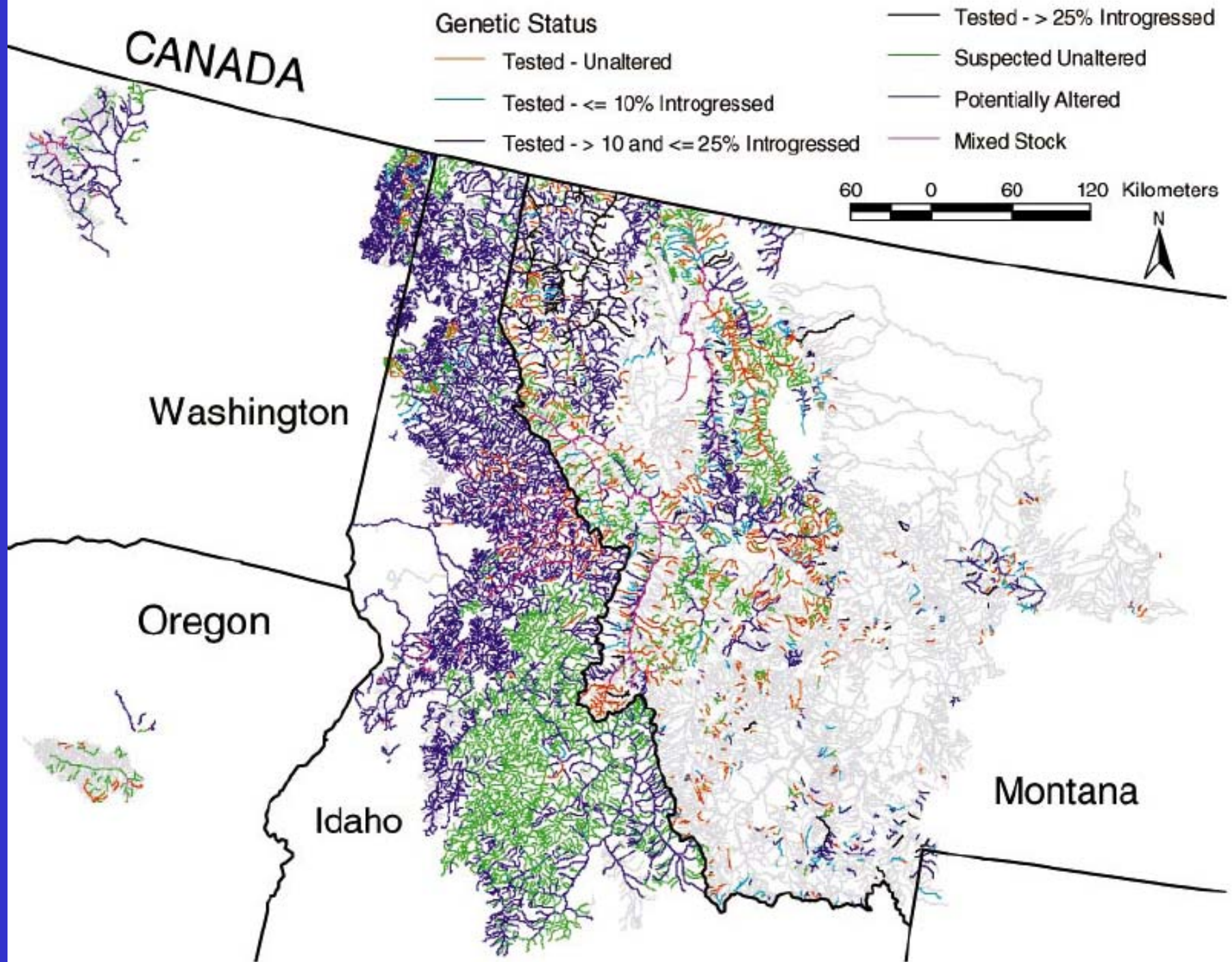
*Montana Fish, Wildlife, and Parks; and
Ecology Department, Montana State University,
1400 South 19th Avenue, Bozeman, Montana 59718, USA*

BRUCE E. MAY AND WENDI URIE



<1%
admixture





Westslope cutthroat trout (WCT) and the ESA

- Formal petition in 1997 to list.
- USFWS (1999) found not warranted because of widespread distribution and current status of the overall WCT population.
- Lawsuit argued that this was incorrect because it included hybridized populations as WCT.
- Court (2002) ruled that this was not based on best available science and ordered USFWS to reconsider whether to list WCT as after taking into account prevalence of hybridization.

Intercrosses and the U.S. Endangered Species Act: Should Hybridized Populations be Included as Westslope Cutthroat Trout?

FRED W. ALLENDORF,* ROBB F. LEARY, NATHANIEL P. HITT,† KATHY L. KNUDSEN,
LAURA L. LUNDQUIST, AND PAUL SPRUELL

Conservation Biology, Pages 1203–1213
Volume 18, No. 5, October 2004

Alternative 1: Include only non-hybridized WCT.

Alternative 2: Include WCT populations with less than 10% admixture.

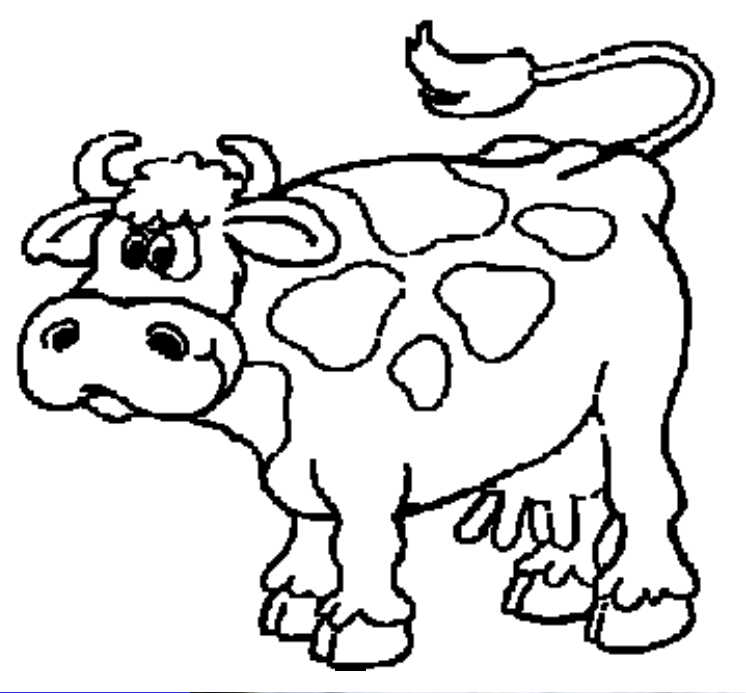
Alternative 3: Include all populations that retain the phenotypic attributes of WCT.

Our Recommendation: WCT are WCT.

Alternative 1: Only non-hybridized (<1% admixture) populations should be included as WCT in the unit to be considered for listing.

- WCT are a monophyletic lineage that has been evolutionary isolated for 1-2 million years.
- Inclusion of hybrid swarms in listing unit will protect source of ongoing hybridization and accelerate genomic extinction.

Hybrids sometimes should be part of the listing unit under the ESA



Use genomic screen to remove individuals with cattle genes from breeding stock.

USFWS Finding, August 2003

Alternative 3: Include all populations that retain the phenotypic attributes of WCT.

“all measured individuals in the population have morphological characters that are within the scientifically-recognized ranges of those characters for the WCT subspecies”.

Morphological identification primary: If there are no morphological data available, then a population will be considered WCT if it has less than 20% of its gene from taxa other than WCT.

The USFWS Finding appears driven by desire to justify preferred policy outcome to not list WCT under the ESA:

- (1) There are no morphological data.
- (2) Morphological detection of hybrids doesn't work.

Westslope Cutthroat Trout, Hybridization, and the U.S. Endangered Species Act

DONALD E. CAMPTON* AND LYNN R. KAEDING†

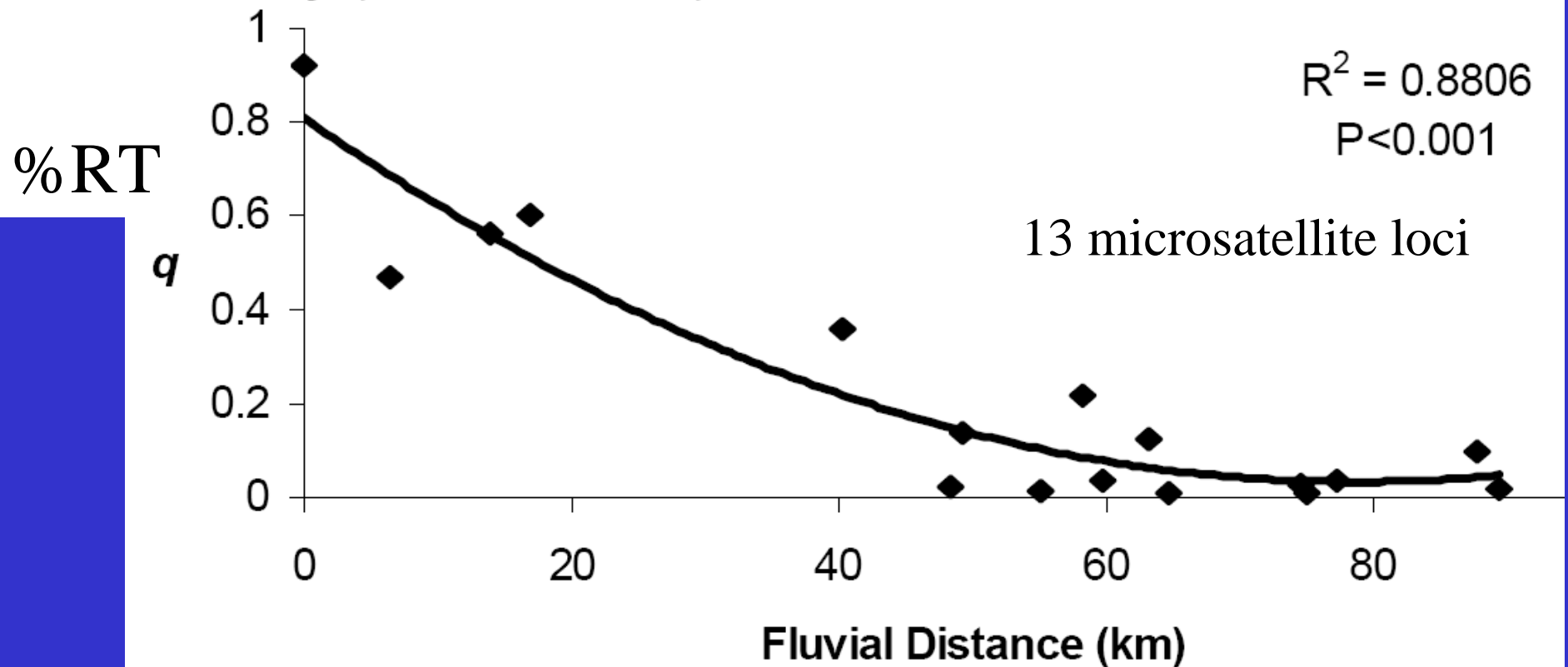
2005. *Con. Biol.* 19:1323-1325

USFWS further argued that there is no empirical evidence for our contentions that -

- (1) Admixed populations source of spread.
- (2) Reduced fitness of the hybrids in the wild.

Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)

Matthew C. Boyer, Clint C. Muhlfeld, and Fred W. Allendorf 2008. CJFAS



RT invasion facilitated by increased dispersal from hybrid swarms.

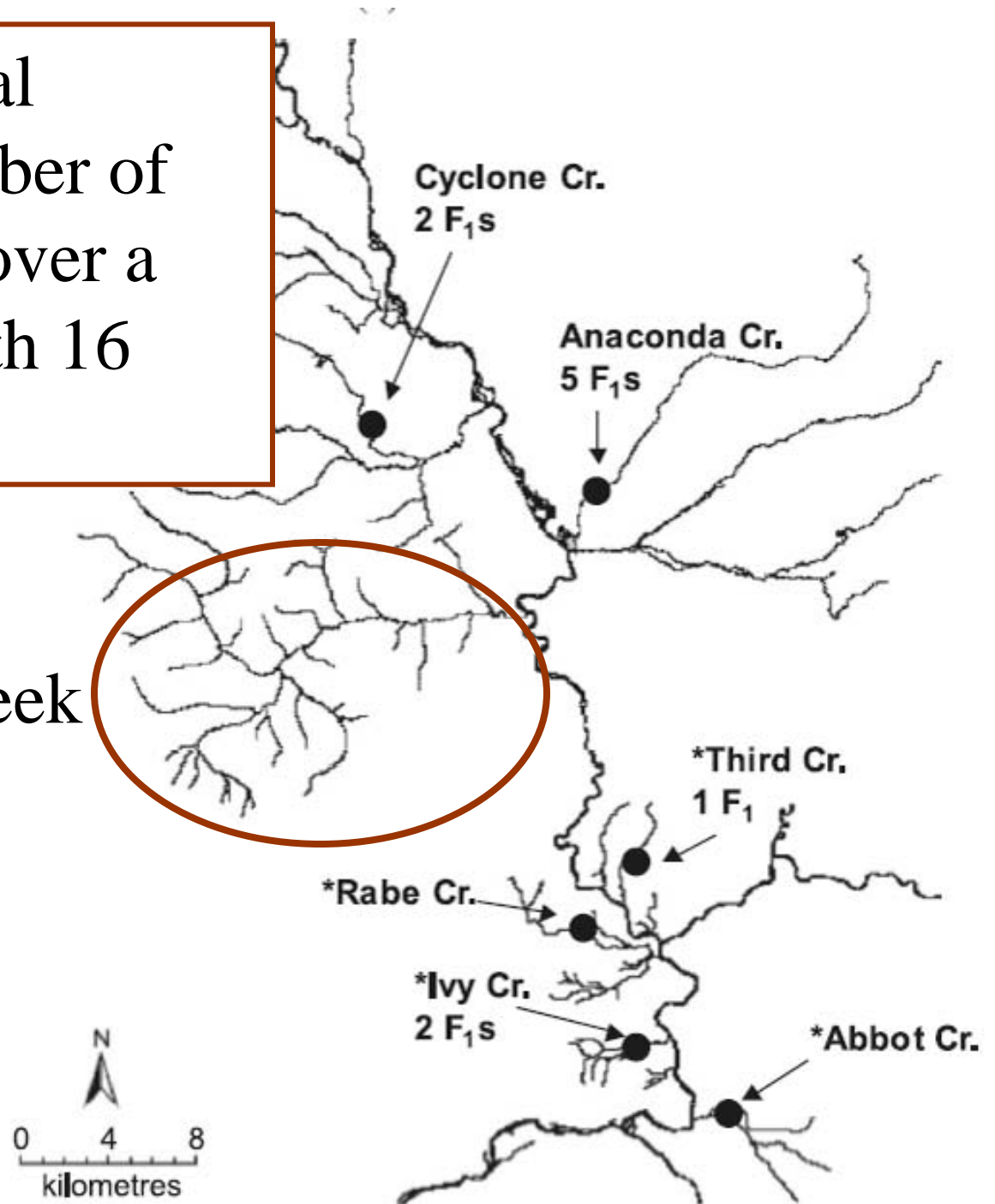
Hybridization rapidly reduces fitness of a native trout in the wild

Clint C. Muhlfeld^{1,2,*}, Steven T. Kalinowski²,
Thomas E. McMahon², Mark L. Taper²,
Sally Painter³, Robb F. Leary⁴
and Fred W. Allendorf³

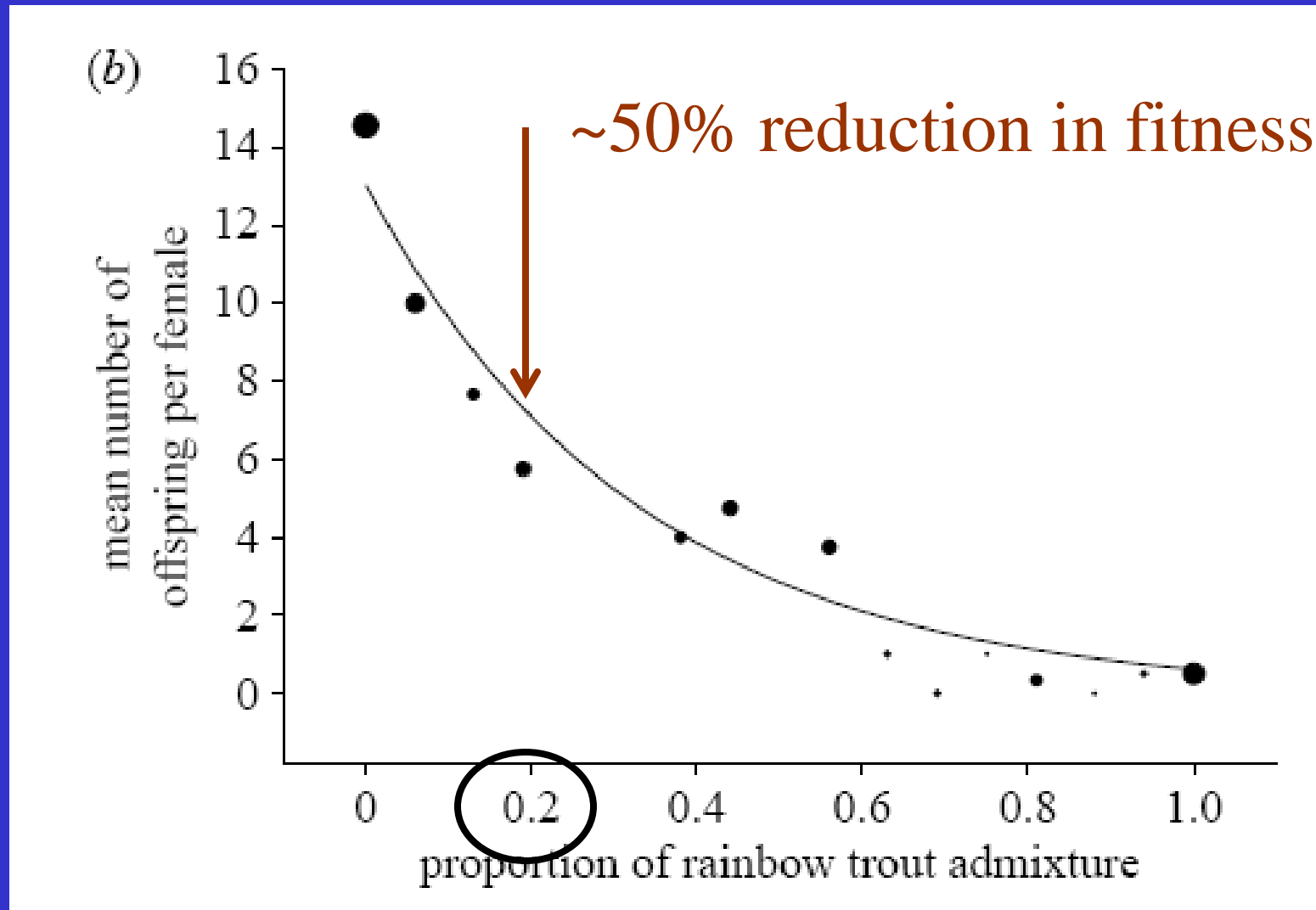
Hybridized fish have greatly reduced fitness in the wild.

Estimated individual admixture and number of progeny produced over a five year period with 16 microsatellite loci.

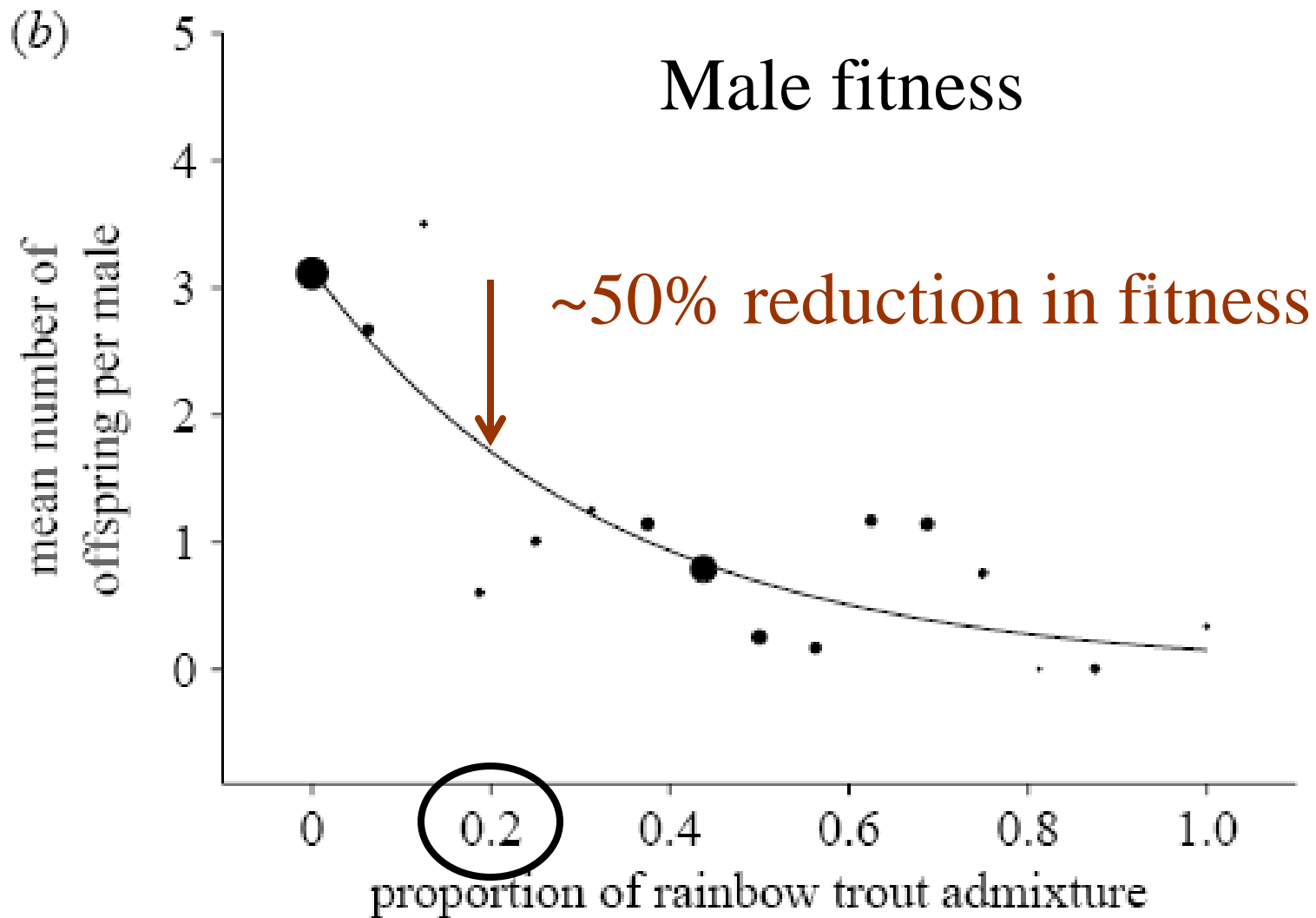
Langford Creek



Female fitness



WCT females produced ~14X progeny than RT

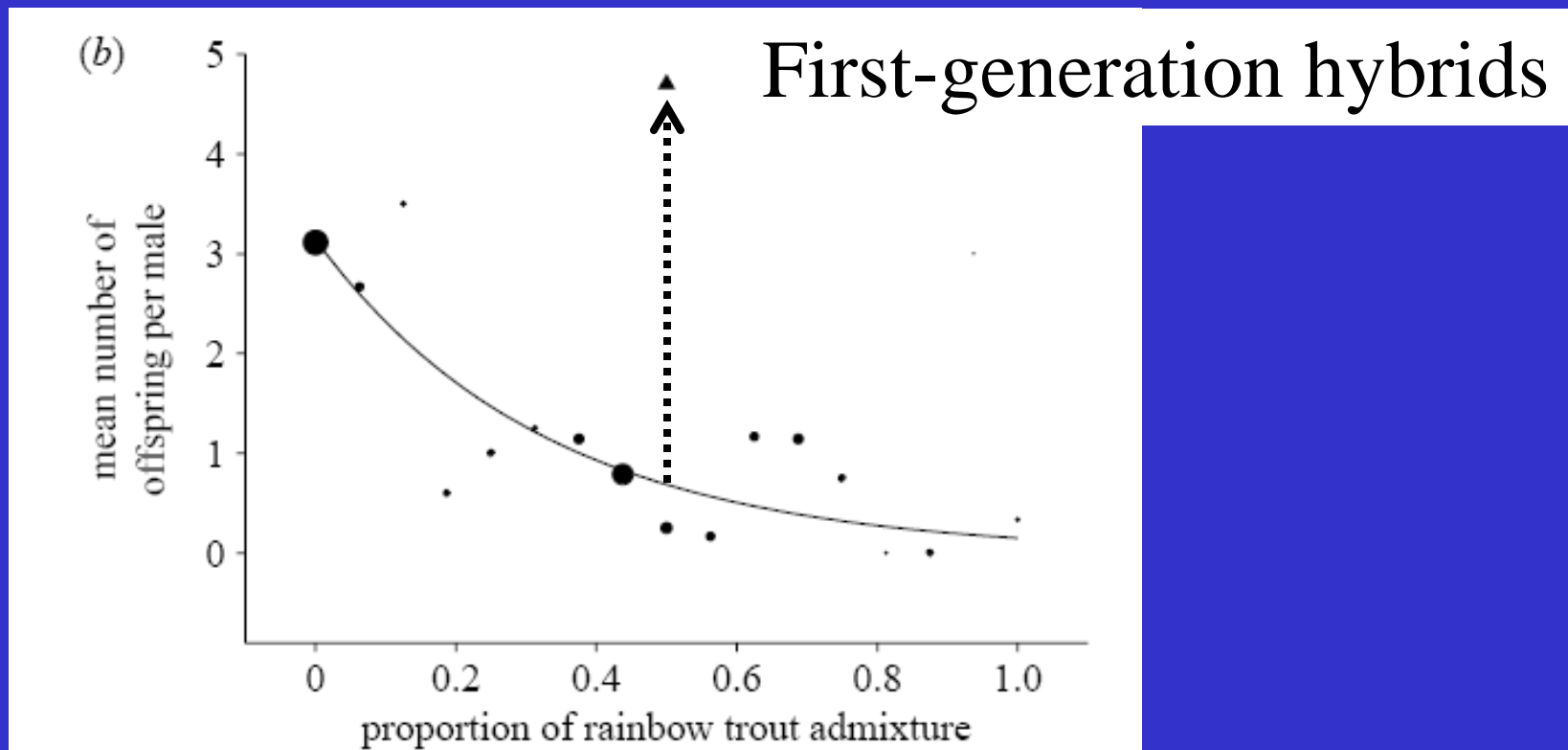


Why is hybridization spreading so rapidly if the hybrids have such reduced fitness?

Heterosis increases the effective migration rate

Pär K. Ingvarsson^{1*} and Michael C. Whitlock

Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4



Sheltering of deleterious recessive alleles in first-generation hybrids can increase effective rate of gene flow and cause loss of local adaptations.

“Genomic Ratchet”

... parental taxa will trend toward extinction as introgression proceeds in spite of even a heavy fitness penalty for the hybrids.

Epifanio and Philipp (2001)

- All progeny of hybrid will be hybrids.
- Frequency of hybrids within a population may increase even when most of the hybrid progeny do not survive.

Consider a population of grey duck hybridizing with mallards. Assume that we start with 90% grey and 10% mallards that mate at random, and the fitness of the hybrids is reduced by t .

That is, the fitness of the grey ducks is 1 and the fitness of the hybrids is $1 - t$.

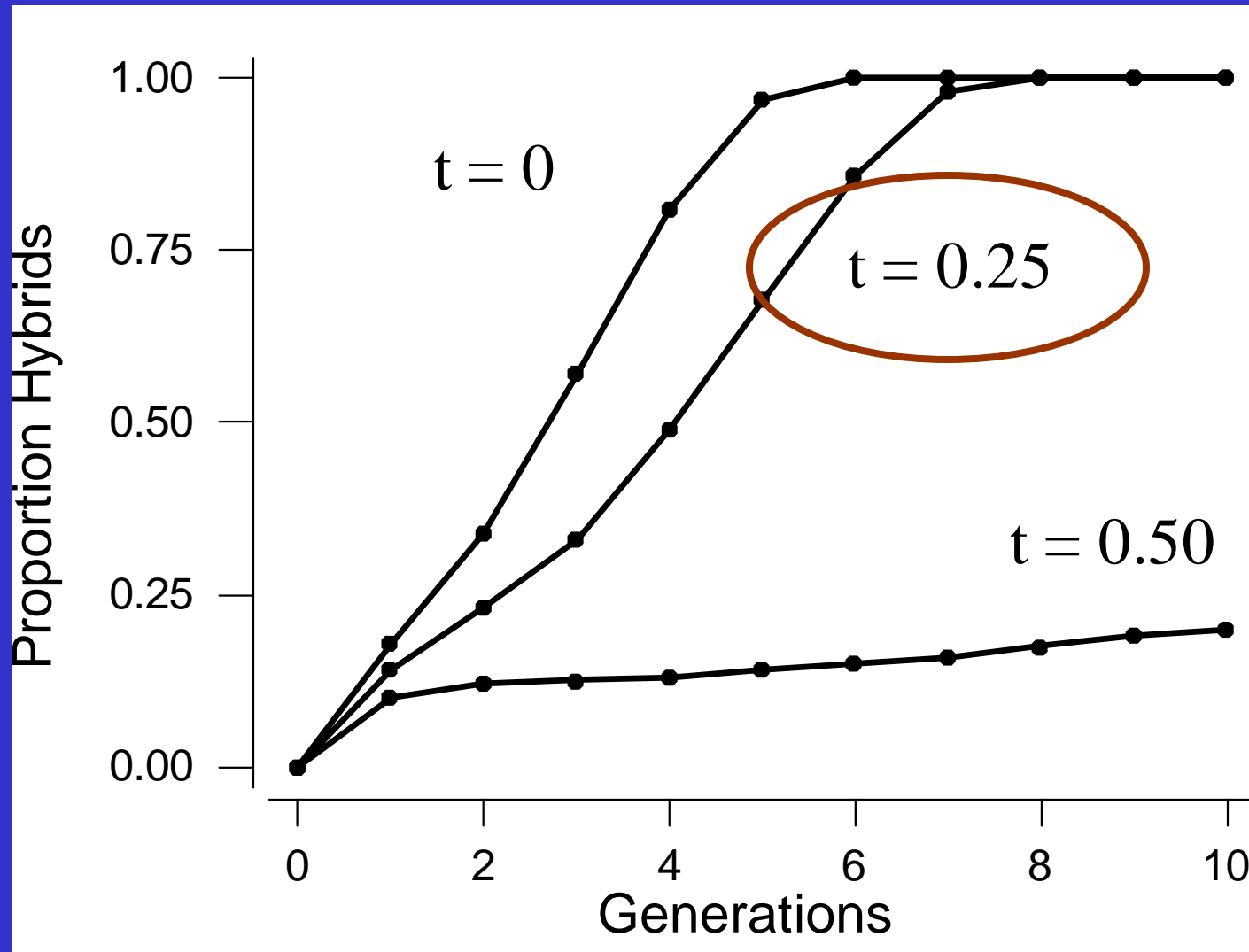
(Fitness of the mallards is irrelevant since they are so rare; 1% after panmixia.)



Hybrid fitness = 0.75 ($t = 0.25$)

Gen	G	G x M	M
0	0.90	0.00	0.10
	0.81	0.18	0.01
	0.81	0.14	0.01
1	0.85	0.14	0.01
	0.72	0.28	0.00
	0.72	0.21	0.00
2	0.77	0.23	0.00
	0.60	0.40	0.00
	0.60	0.30	0.00
3	0.67	0.33	0.00

Genomic ratchet

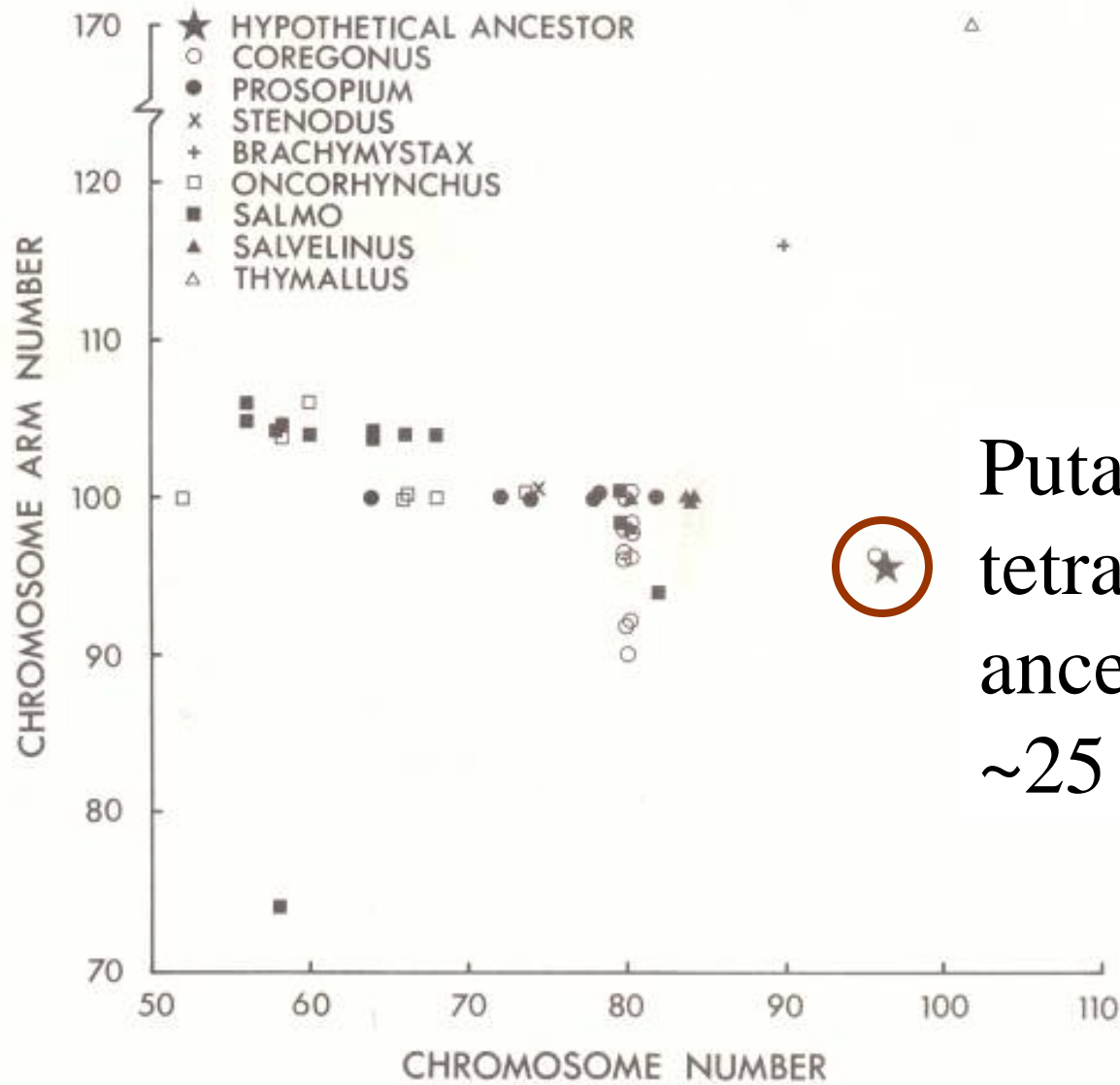


t = reduction
in fitness of
hybrids

The proportion of hybrids increases rapidly even if they have greatly reduced fitness (25%).

Tetraploidy and the Evolution of Salmonid Fishes

FRED W. ALLENDORF and GARY H. THORGAARD



Putative tetraploid ancestor ~25 MYA

Present Status of 33 Ancestral Protein Loci in the Rainbow Trout

Locus	Duplicated		Single locus
	Isoloci	Diverged	
<i>Aat-A</i>	—	Yes	—
<i>Aat-B</i>	Yes	—	—
<i>Aat-m</i>	—	Yes	—
<i>Adh</i>	—	—	Yes
<i>Ada</i>	—	—	Yes
<i>Ck-A</i>	—	Yes	—
<i>Ck-B</i>	—	—	Yes
<i>Ck-C</i>	—	Yes	—
<i>Dia</i>	—	—	Yes
<i>Fdp</i>	—	—	Yes
<i>Gap-A</i>	—	Yes	—
<i>Gap-B</i>	—	Yes	—
<i>Gpi-A</i>	—	Yes	—
<i>Gpi-B</i>	—	—	Yes
<i>Idh-A</i>	Yes	—	—
<i>Idh-m</i>	—	Yes	—
<i>Ldh-A</i>	—	Yes	—
<i>Ldh-B</i>	—	Yes	—
<i>Ldh-C</i>	—	—	Yes
<i>Mdh-A</i>	Yes	—	—
<i>Mdh-B</i>	Yes	—	—
<i>Mdh-m</i>	—	Yes	—
<i>Me-A</i>	Yes	—	—
<i>Me-m</i>	Yes	—	—
<i>Mpi</i>	—	—	Yes
<i>Pal</i>	Yes	—	—
<i>Pgd</i>	—	—	Yes
<i>Pgk</i>	—	—	Yes
<i>Pgm-A</i>	—	Yes	—
<i>Pgm-B</i>	Yes	—	—
<i>Sdh</i>	—	Yes	—
<i>Sod</i>	—	—	Yes
<i>Tfn</i>	—	—	Yes
Total	8	13	12

33 enzyme loci

Single loci 12

Duplicated 21

Isoloci* 8

Diverged 13

*Pairs of homeologous loci that share alleles

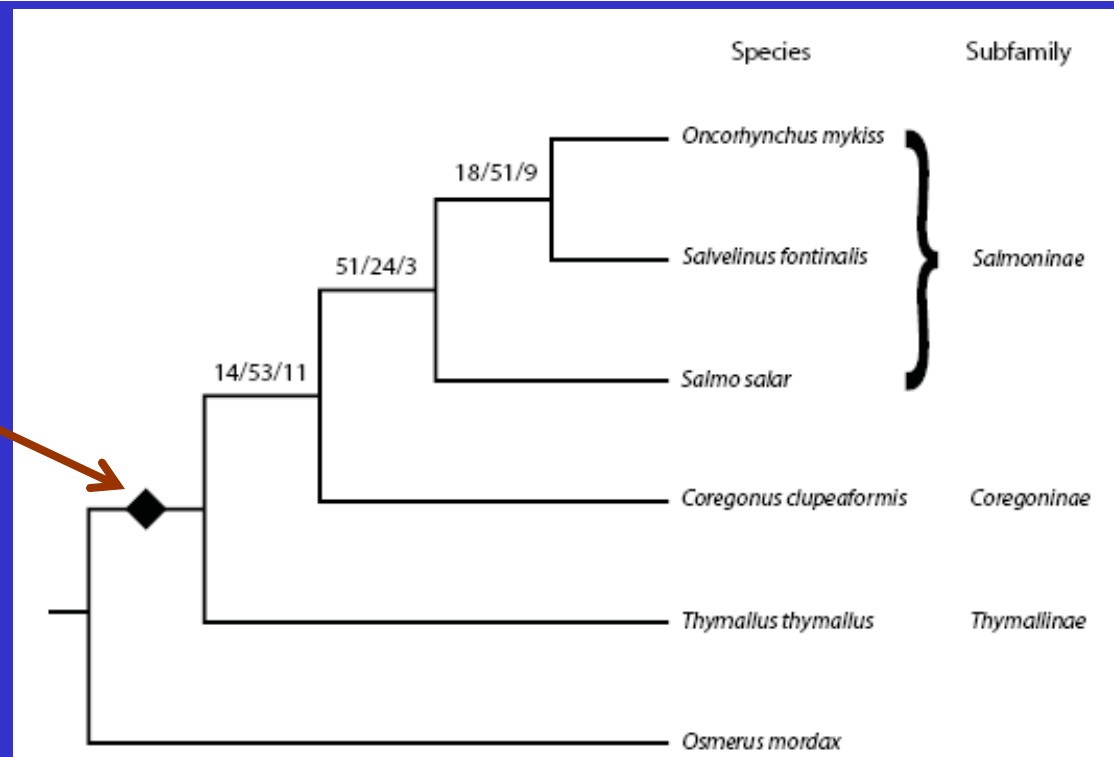
Research article

Open Access

A salmonid EST genomic study: genes, duplications, phylogeny and microarrays

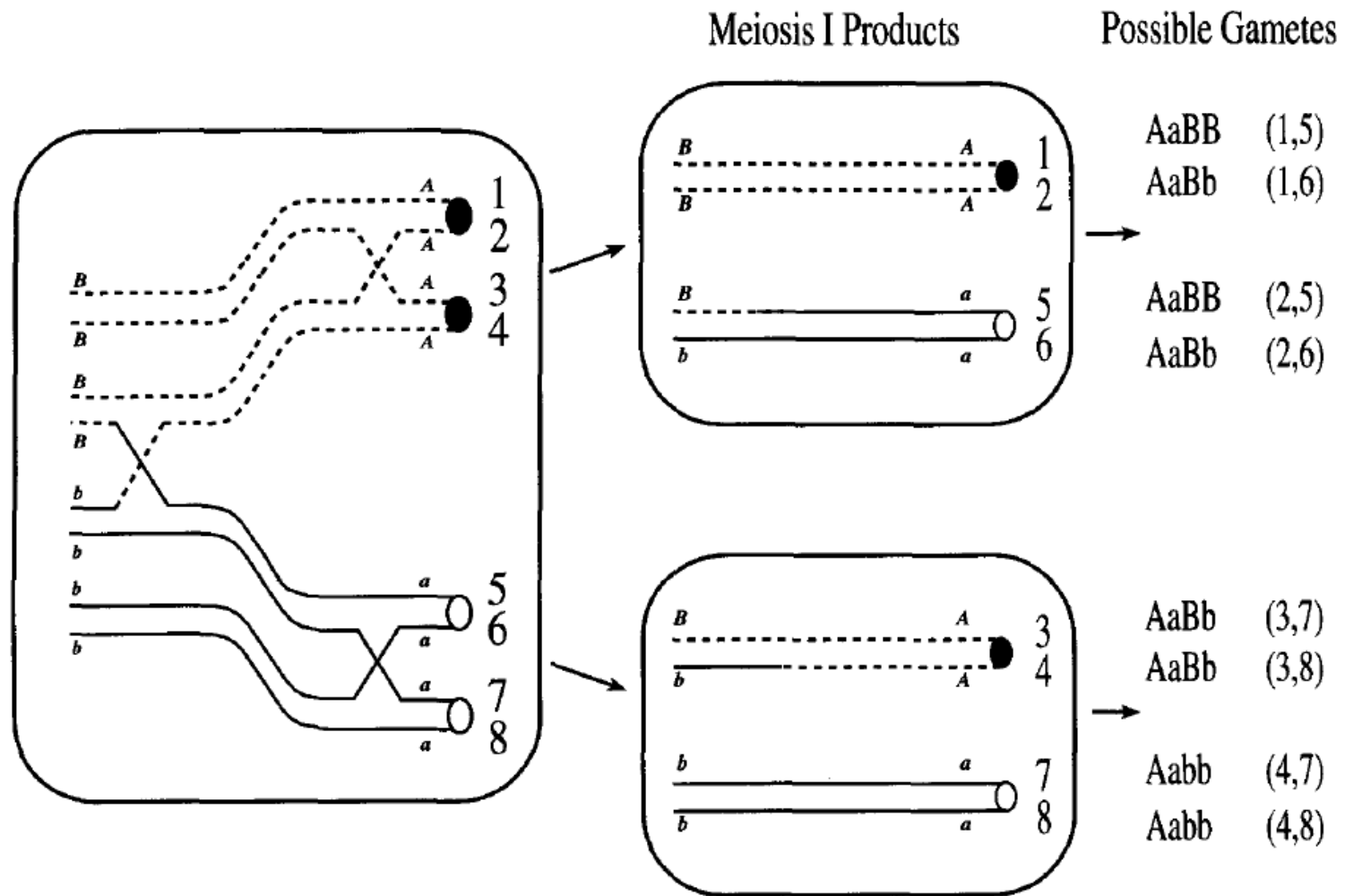
Ben F Koop^{*1,6}, Kristian R von Schalburg¹, Jong Leong¹, Neil Walker¹, Ryan Lieph¹, Glenn A Cooper¹, Adrienne Robb¹, Marianne Beetz-Sargent¹, Robert A Holt², Richard Moore², Sonal Brahmhatt³, Jamie Rosner³, Caird E Rexroad III⁴, Colin R McGowan⁵ and William S Davidson⁵

Gene duplications appear



Secondary Tetrasomic Segregation of *MDH-B* and Preferential Pairing of Homeologues in Rainbow Trout

Fred W. Allendorf and Roy G. Danzmann*



Recombination between homeologues in male rainbow trout for *MDH-B*

TABLE 5

Frequency of progeny phenotypes in families from nulliplex females and apparently homozygous duplex males

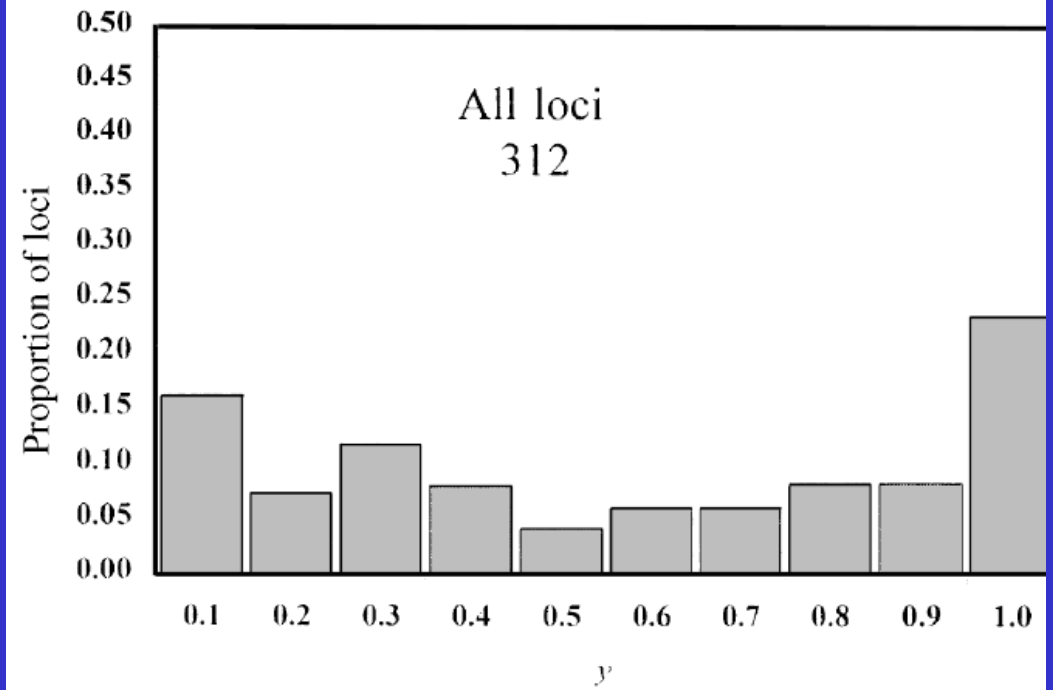
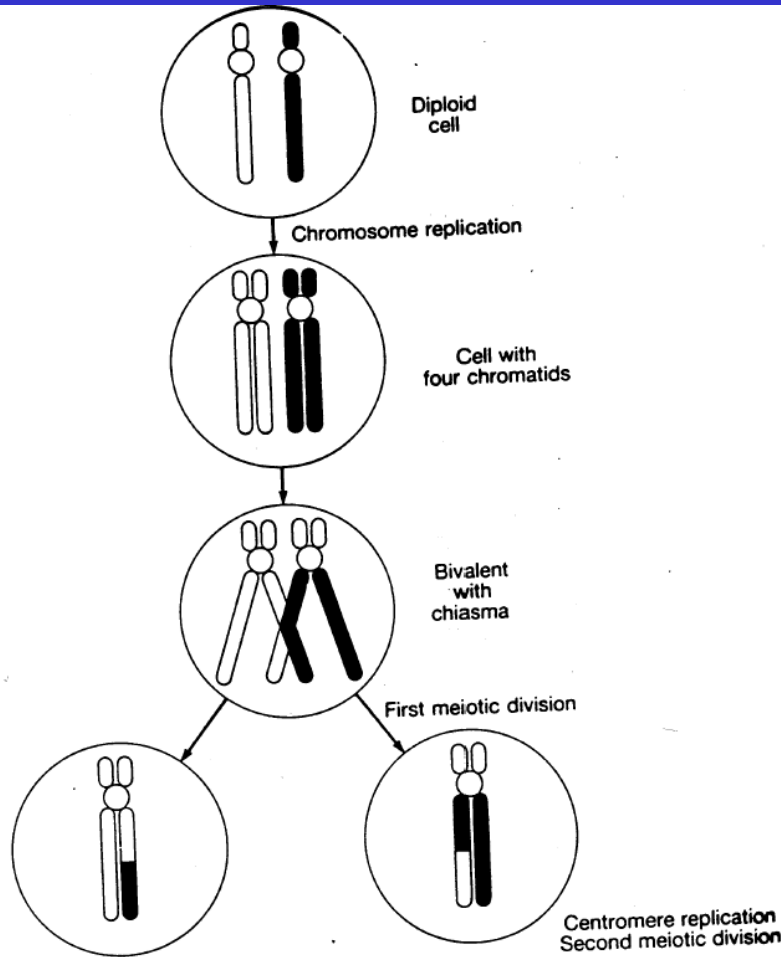
Fam	Parents		Progeny			het ^a	Θ
	Female	Male	*1111	*1112	*1122		
H06	*1111	*1122	14	266	9	0.920	0.242
H18	*1111	*1122	12	183	5	0.915	0.255
I15	*1111	*1122	1	92	3	0.958	0.126
I20	*1111	*1122	2	75	3	0.938	0.186
I24	*1111	*1122	0	72	1	0.986	0.042
K37	*1111	*1122	5	259	7	0.956	0.133
Total			34	947	28	0.939	0.184

^a Proportion of heterozygous (*1112) progeny.

18.4% tetrasomic inheritance
in males for this locus.

Gene-centromere mapping of 312 loci in pink salmon by half-tetrad analysis

K.R. Lindner, J.E. Seeb, C. Habicht, K.L. Knudsen, E. Kretschmer, D.J. Reedy, P. Spruell, and F.W. Allendorf



centromere → more distal

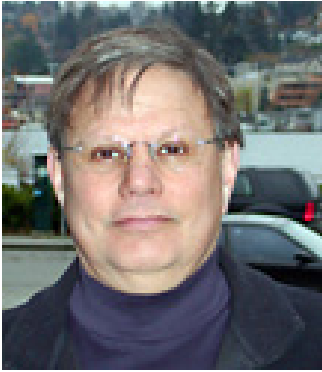
Transmission Genetics of Salmonid Fishes

Females

1. Greater recombination than males.
2. Little distal recombination with nearly complete crossover interference.
3. No homeologous recombination.

Males

1. Overall less recombination than females.
2. More distal recombination than females.
3. Homeologous recombination occurs within some populations (often of hybrid origin).



Eric Grau

Jim Seeb

Univ. Washington

Homeologue Hell

Conserved Region (97 bp)



```

ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATACGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATACGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATACGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATACGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACATGGAGGACAGCTACGGCCAGCAGTGGACATATGAGCGCAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .
ACCTGGAGGATAGCTATGGCCAGCAGTGGACTTATGAACAGAGAAAGATTGTGGAGTTCACCTGCCACACAGCATTCTTCGCCAG. . .

```

Sorted alignment of a ~140 bp contig in a chum salmon contains “polymorphisms” at 8 positions. Seven of these are differences between homeologues. True SNP is in blue.

Questions?

