Mapping the genes underlying phenotypic changes of interest

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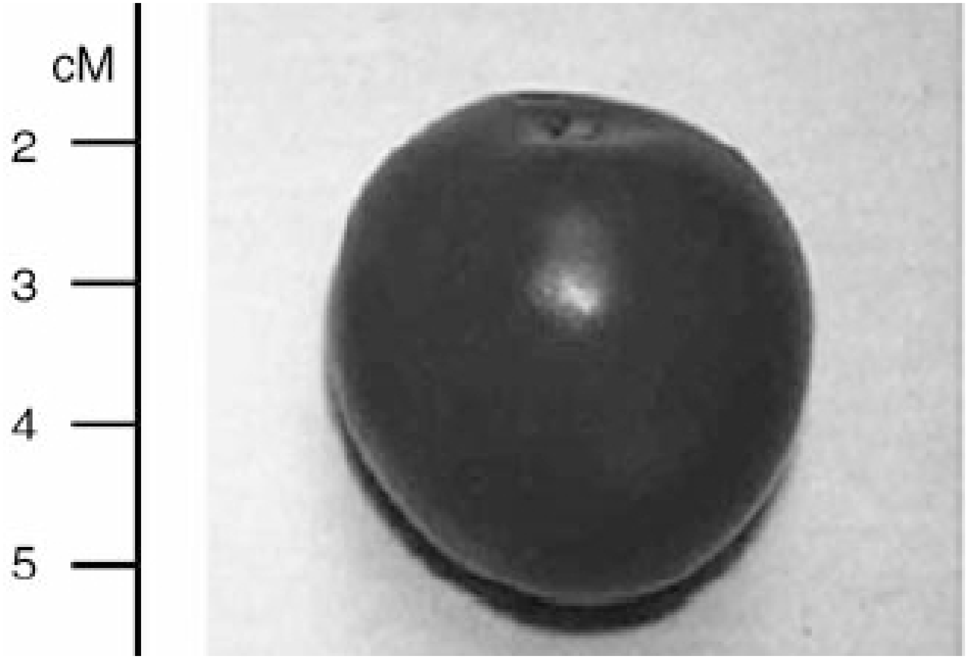
Tomato shape

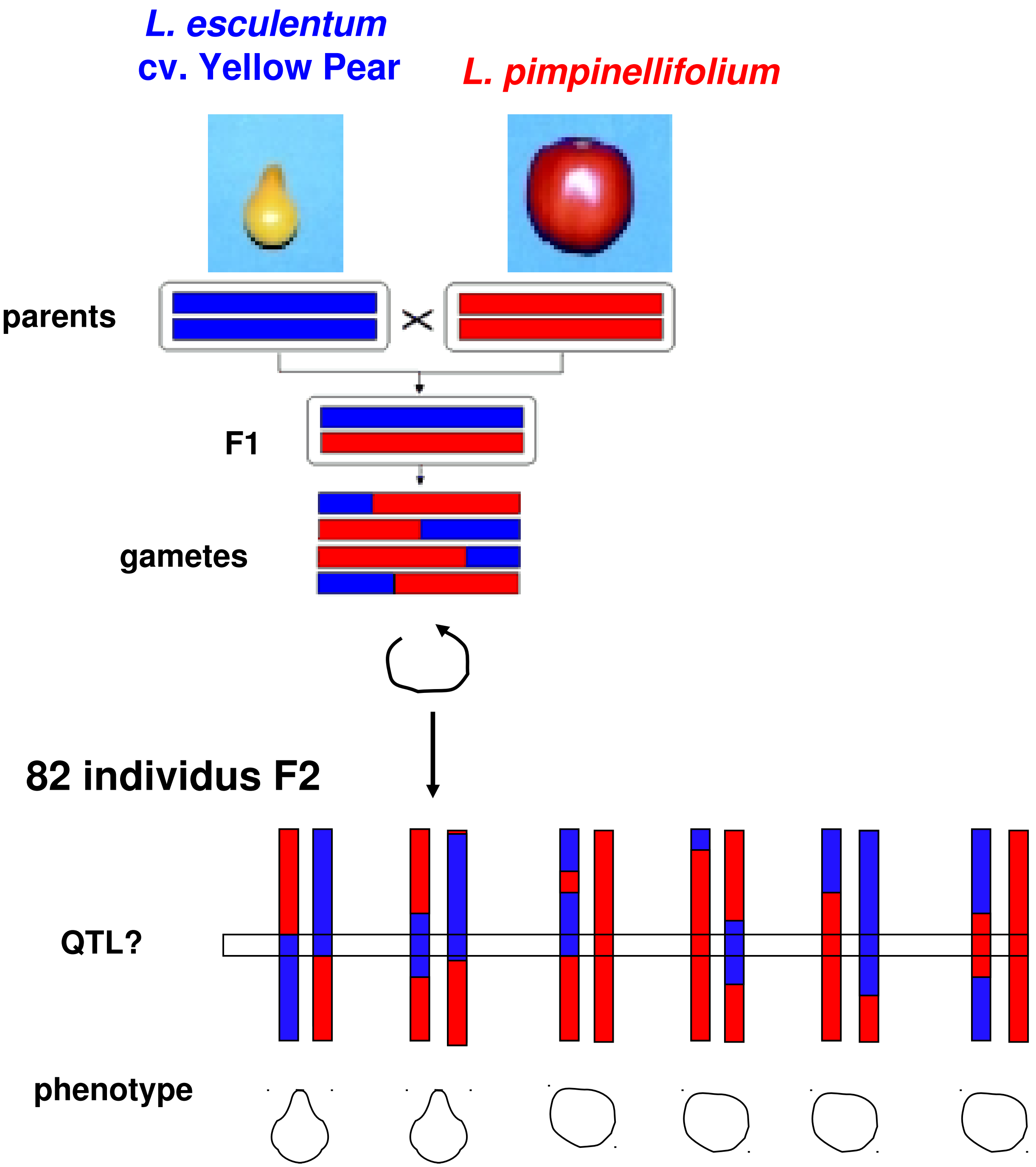


Lycopericon esculentum

***Lycopersicon esculentum* cv. Yellow Pear**

(Ku et al., 1999; Liu et al., 2002)

QTL mapping



L. esculentum
cv. Yellow Pear

L. pimpinellifolium

parents

×

F1

gametes

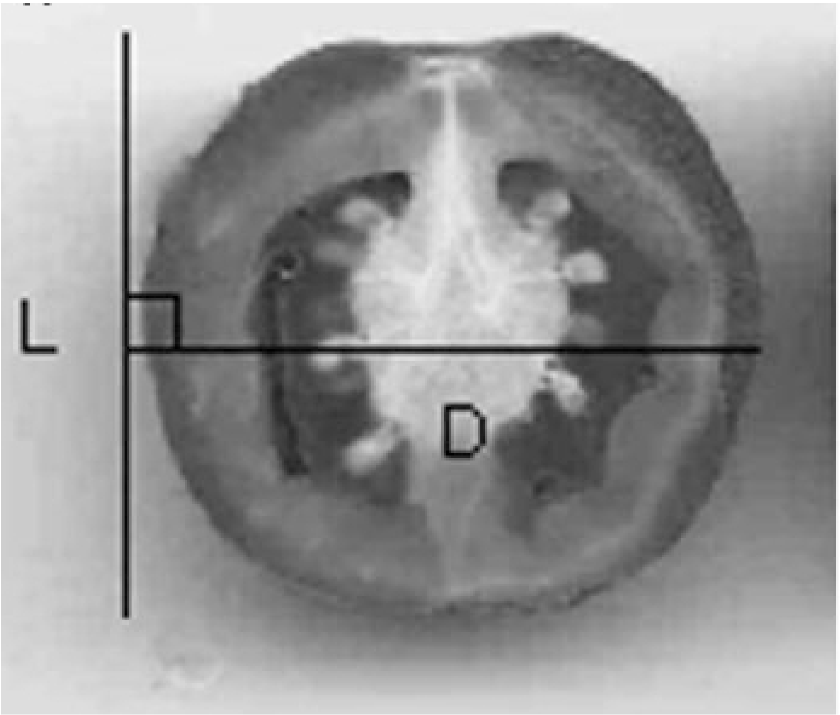
82 individus F2

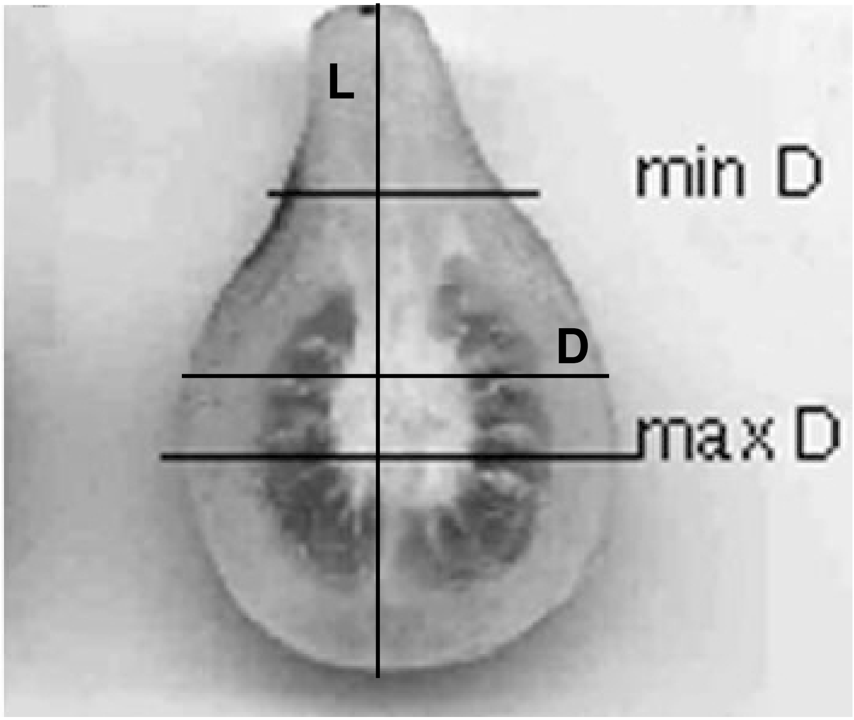
QTL?

phenotype

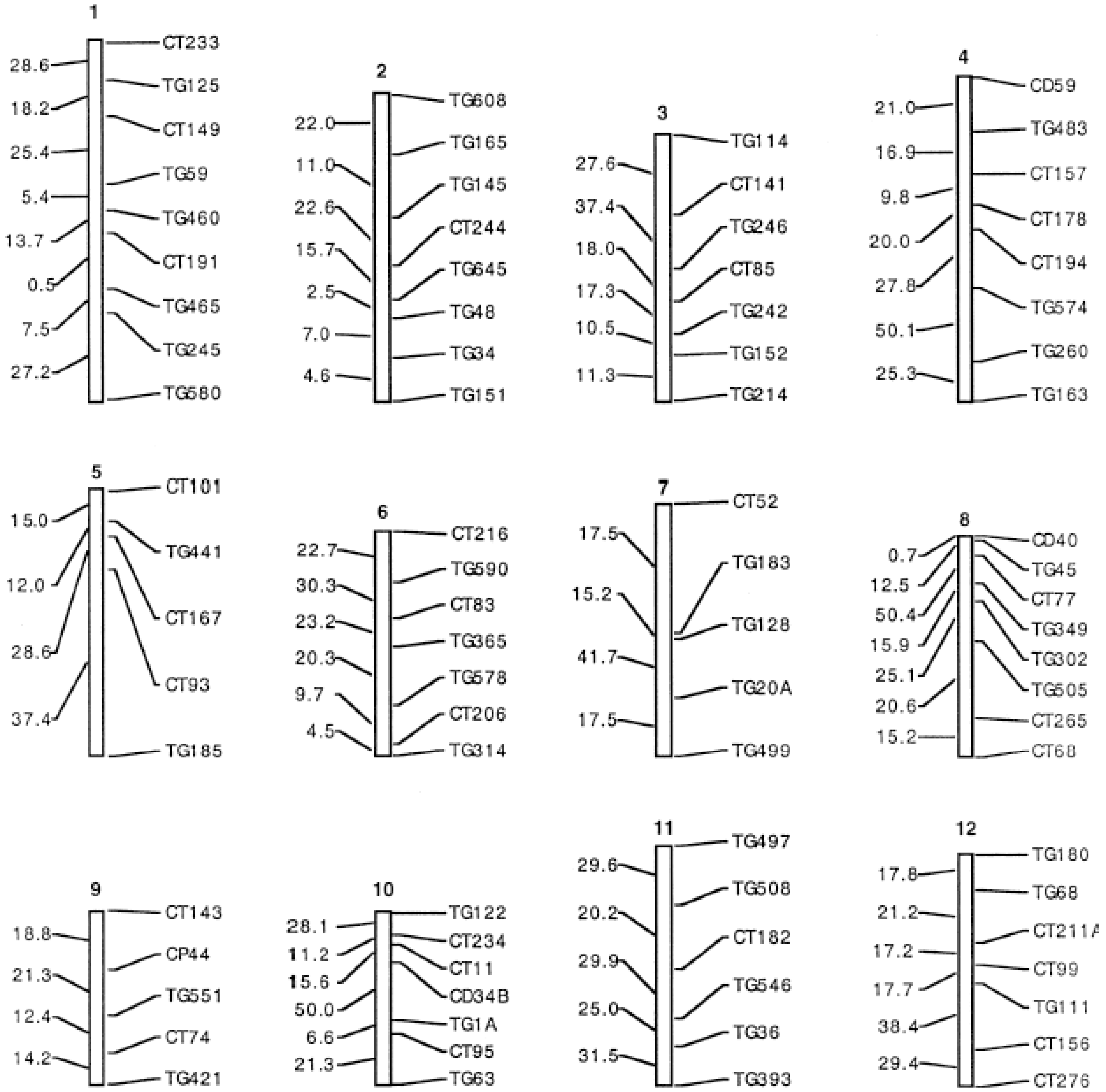
Quantitative measure of the phenotype

Measure of 2 indexes L/D and Dmin/Dmax for 10 fruits per plant
L/D : L= length, D = diameter at equator
Dmin/Dmax



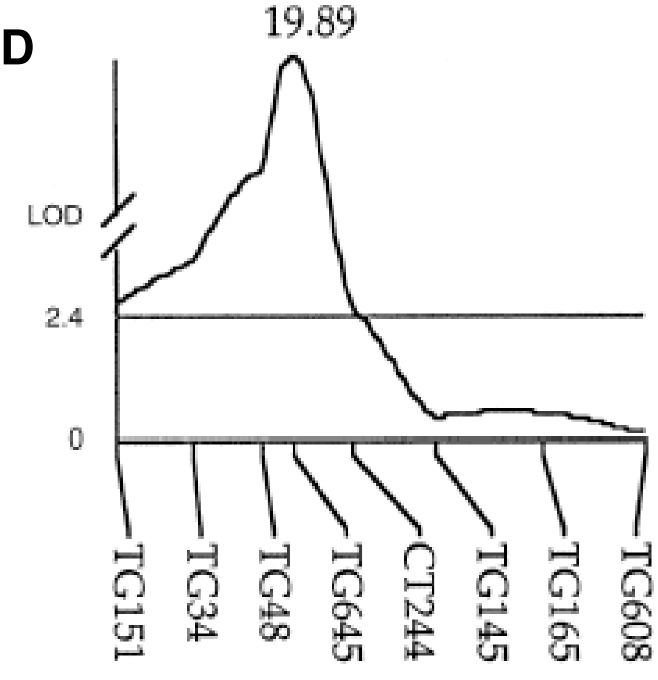


82 molecular markers on the 12 tomato chromosomes

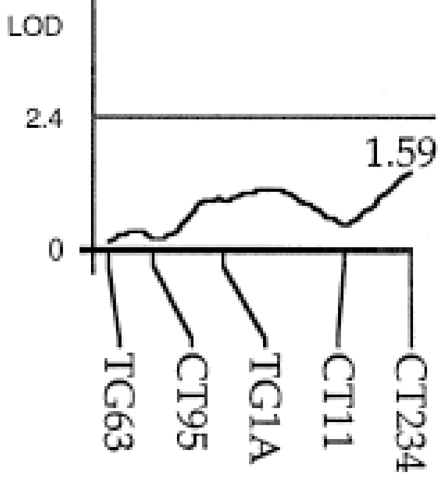


One major locus near marker TG645

L/D

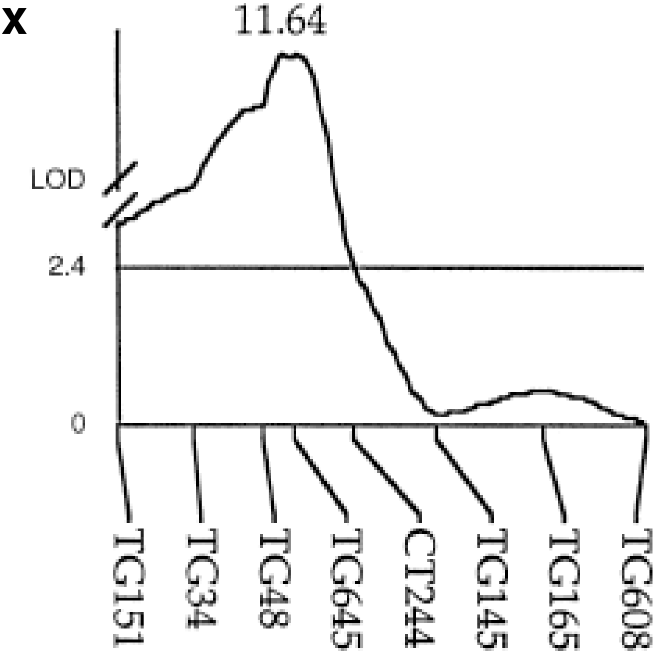


Chromosome 2



Chromosome 10

Dmin/Dmax



Chromosome 2

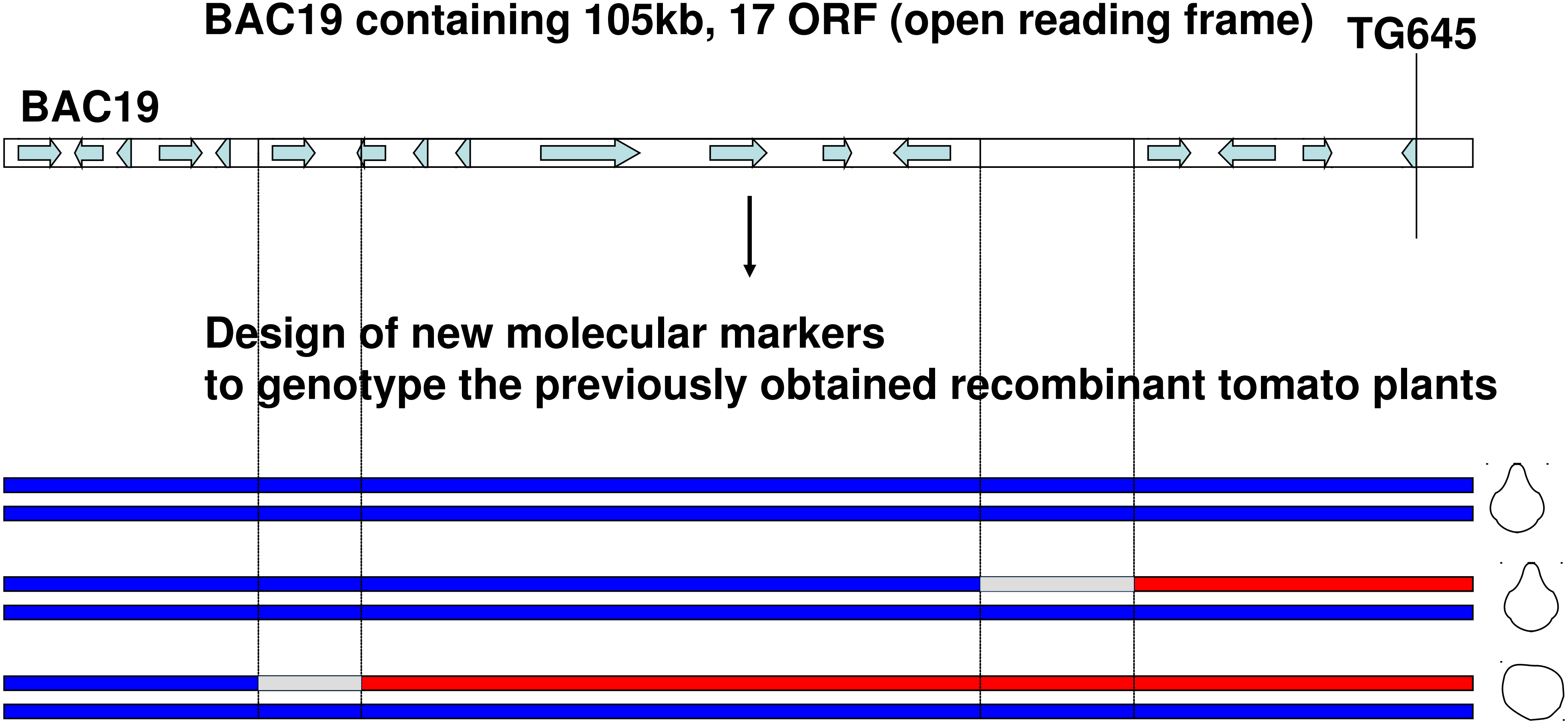
responsible for 67% of L/D variance

allele YP = recessive

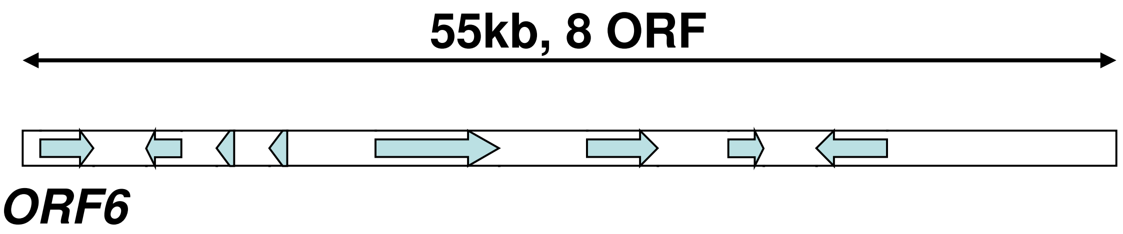
BAC library (Bacterial Artificial Chromosomes)
contains genomic DNA fragments of 100-350kb

Screen of the library with marker TG645

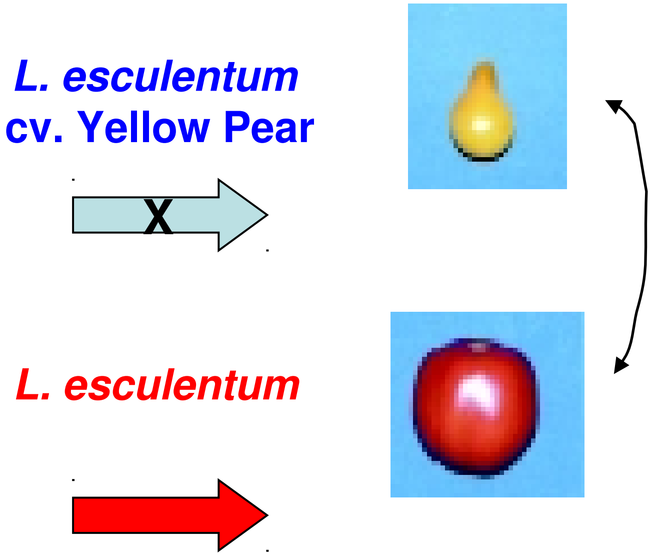
BAC19 containing 105kb, 17 ORF (open reading frame)



Design of new molecular markers
to genotype the previously obtained recombinant tomato plants



Sequencing of the region in the 2 tomato varieties



L. esculentum
cv. Yellow Pear

1 SNP (single nucleotide polymorphism) et 1 indel (insertion-deletion) of 2bp in non-coding regions

L. esculentum

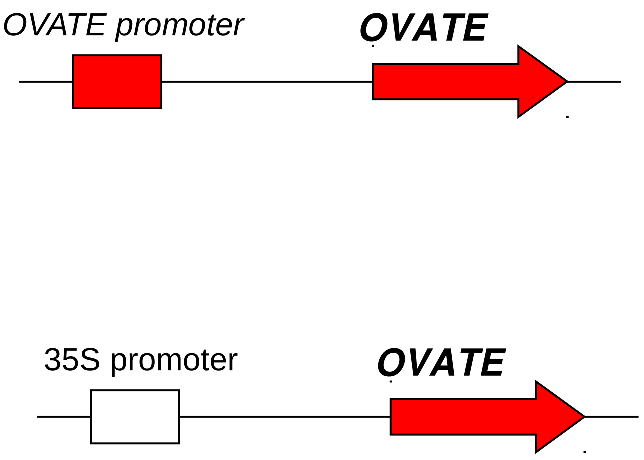
1 SNP in *ORF6* : G496T, stop codon stop, truncated protein with last 75 amino acids missing

Hypothesis: the causing gene is *ORF6* = *OVATE*

The causing gene is *OVATE*/*ORF6*

Same mutation in 3 other pear tomato varieties

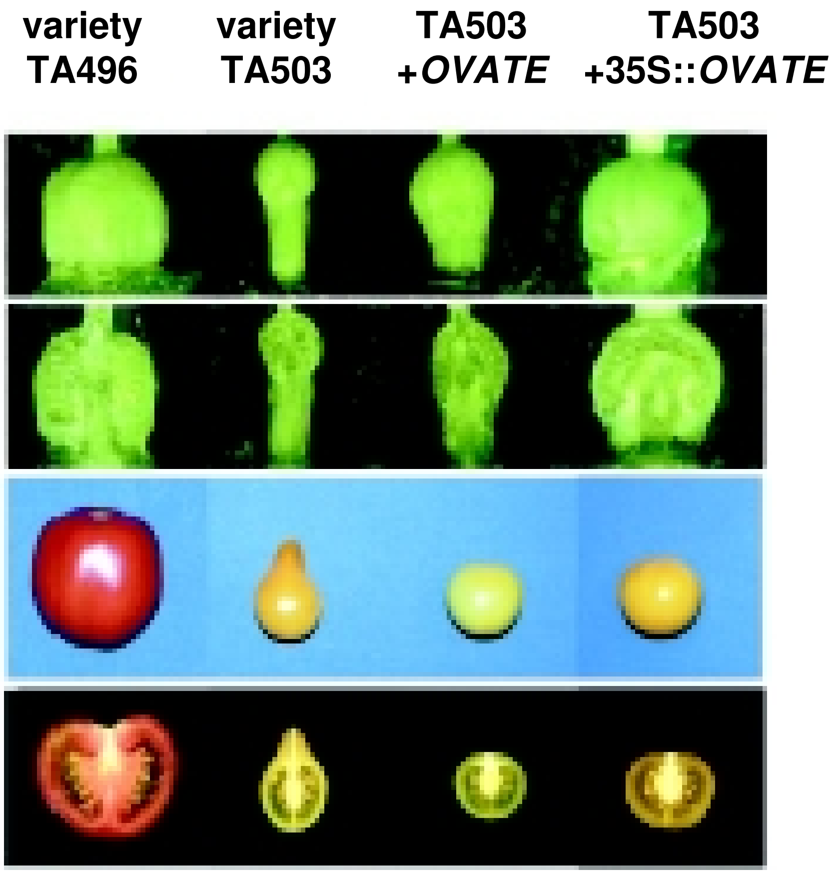
Complementation of the mutation by transgenesis


OVATE promoter

OVATE

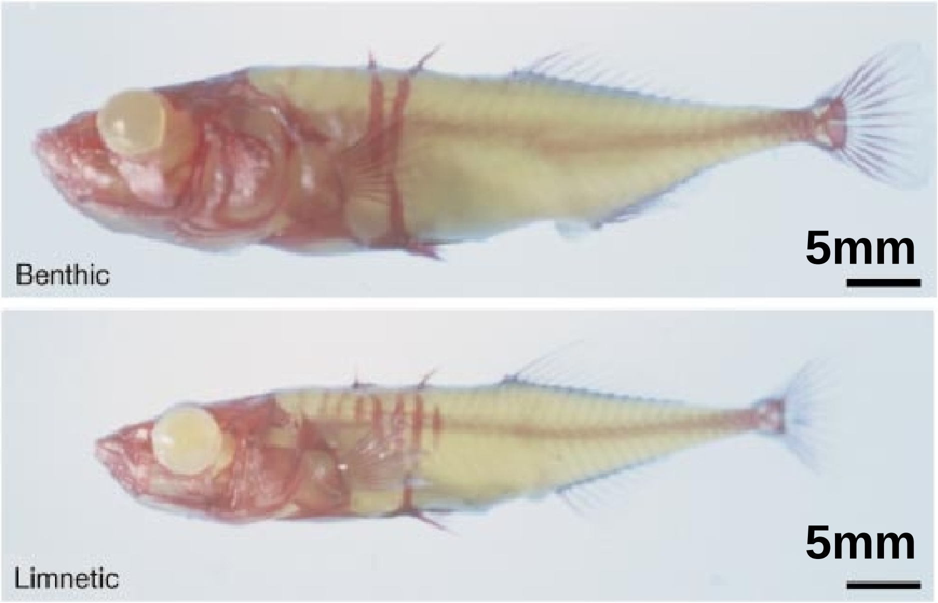
35S promoter

OVATE



OVATE = protéine à domaine NLS (nuclear localisation signal), fonction inconnue, exprimée dans les fruits en développement mais pas dans l’appareil végétatif

Evolution of morphology in threespine sticklebacks



marine

Paxton Lake, Canada

Gasterosteus aculeatus

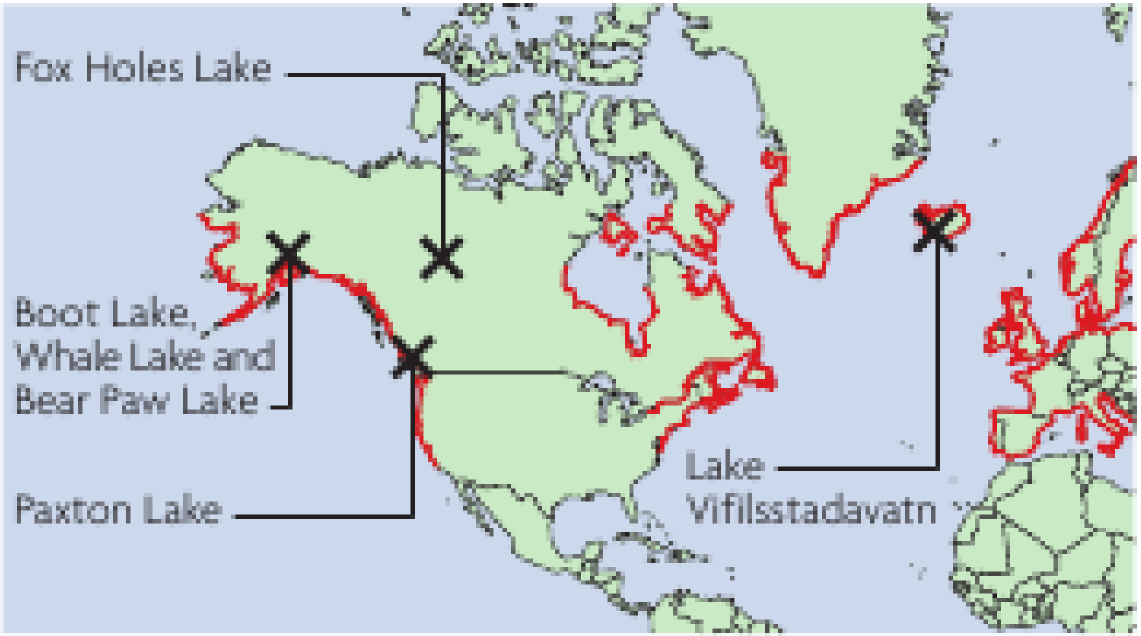
(Peichel et al., 2001 ; Shapiro et al, 2004 ; Chan et al. 2010)

Marine fishes with robust pelvis = ancestral

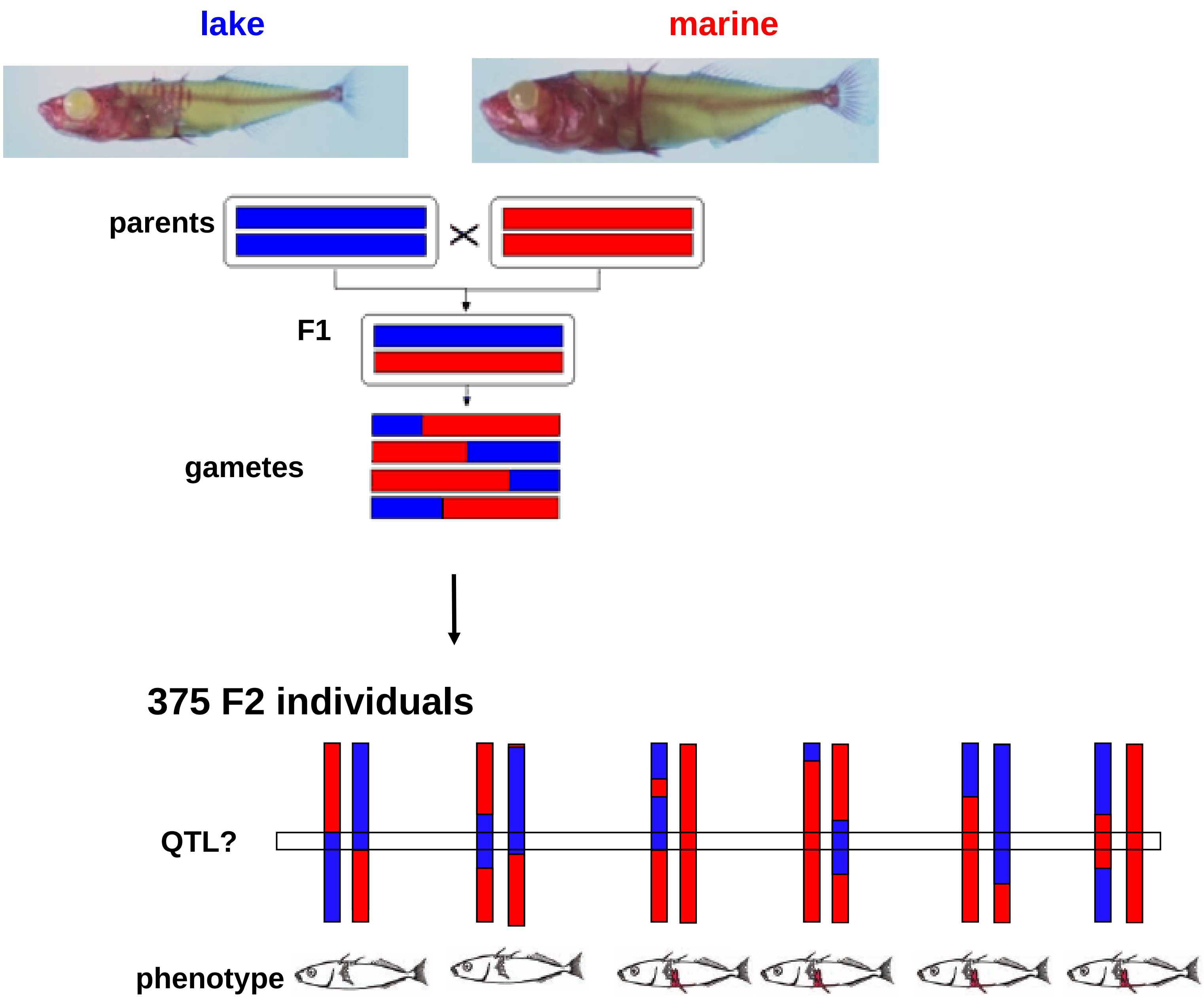
Freshwater fishes with reduced pelvic structures = derived, independently at least 20 times

- **limited calcium availability**
- **absence of gape-limited predatory fishes**
- **predation by grasping insects**

Last glacier retreat = 10 000 – 20 000 years ago



QTL mapping



lake

marine

parents

×

F1

gametes

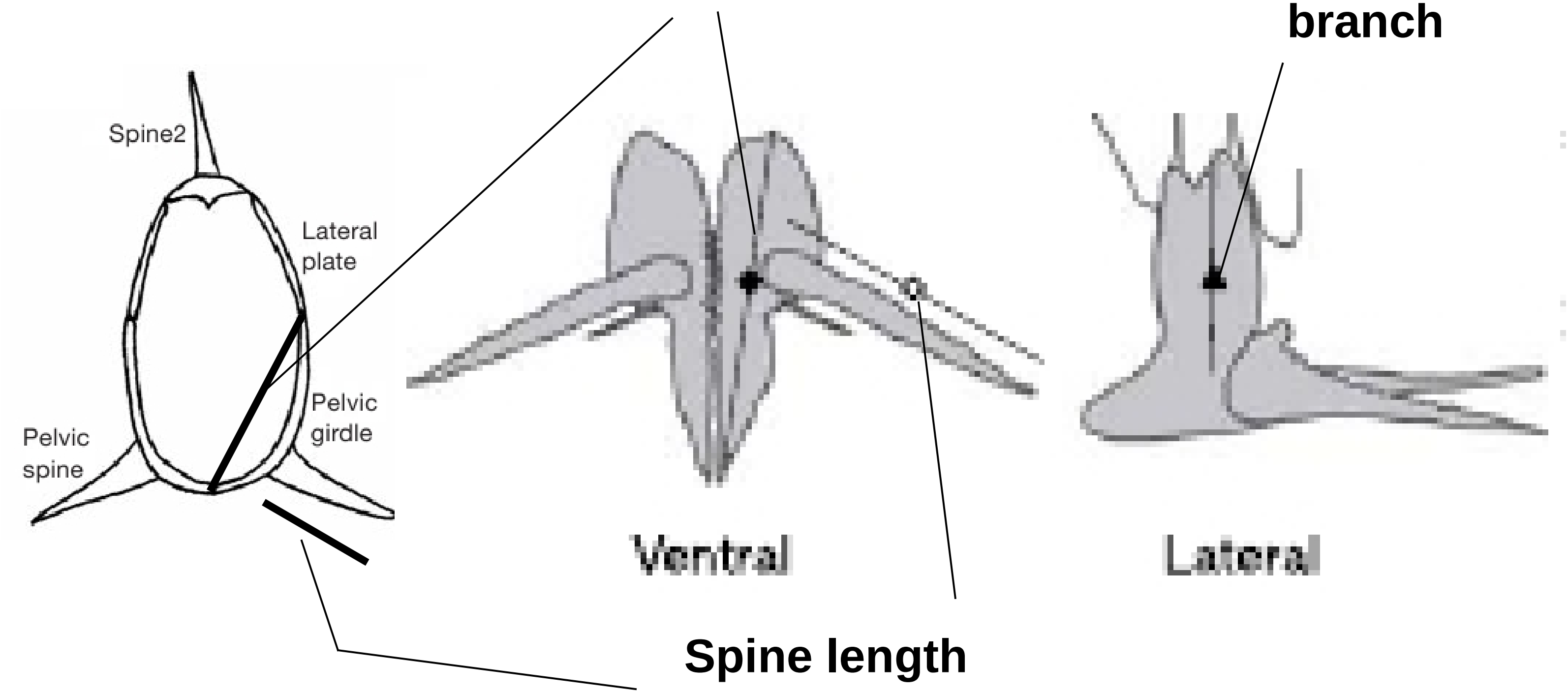
375 F2 individuals

QTL?

phenotype

(Shapiro et al., 2004)

Quantitative measurement of the phenotype



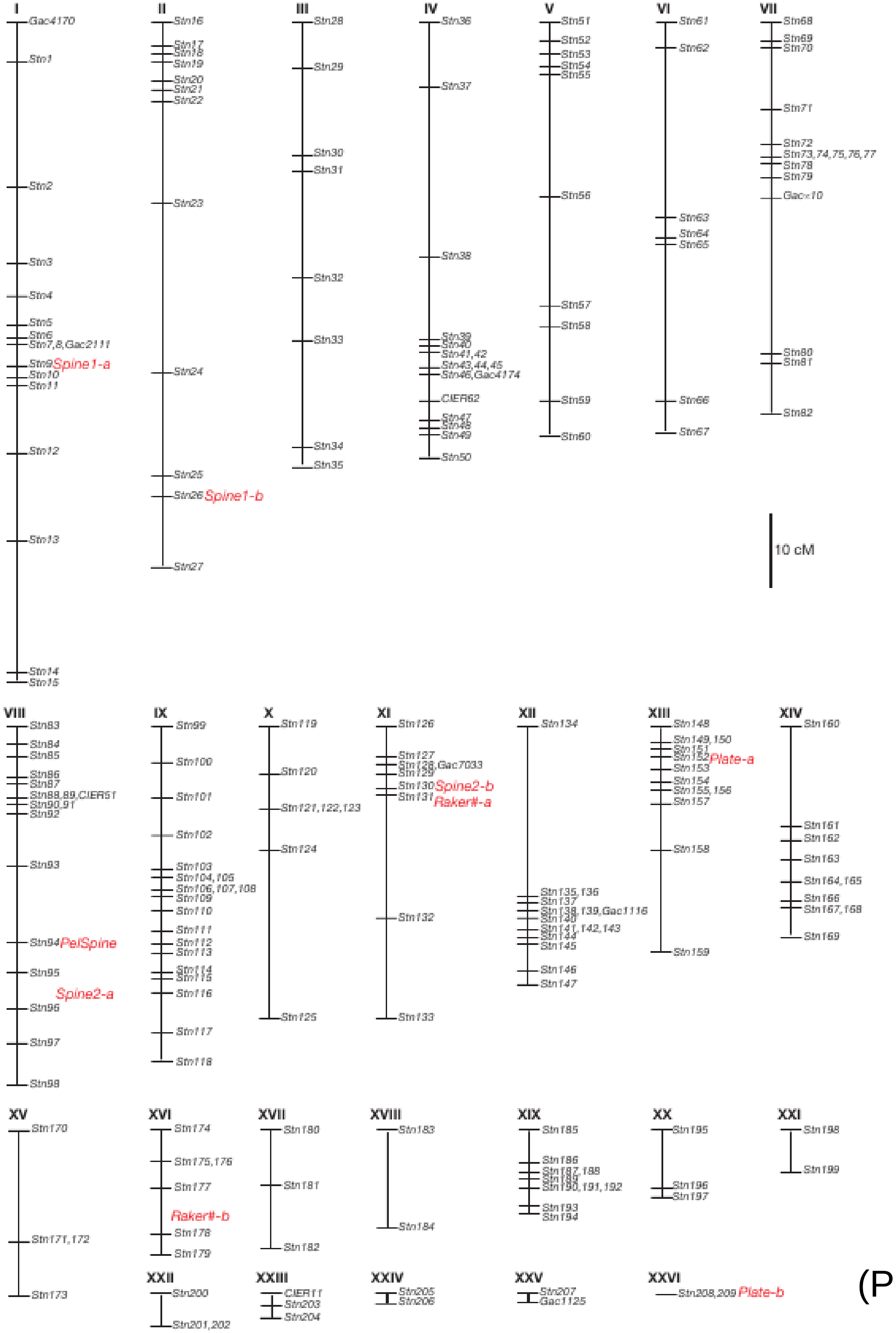
Length of pelvic girdle

Height of ascending branch

Spine length

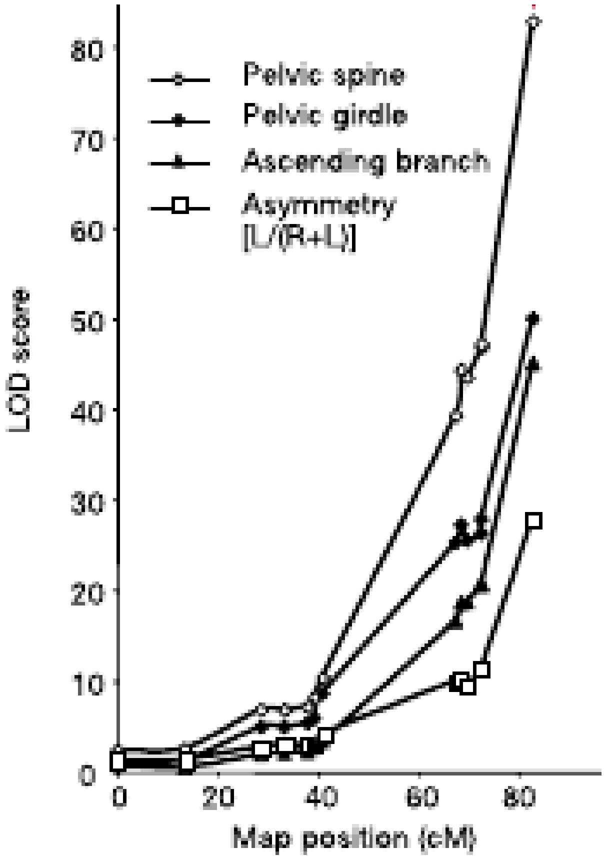
1000 microsatellite markers

26 linkage groups



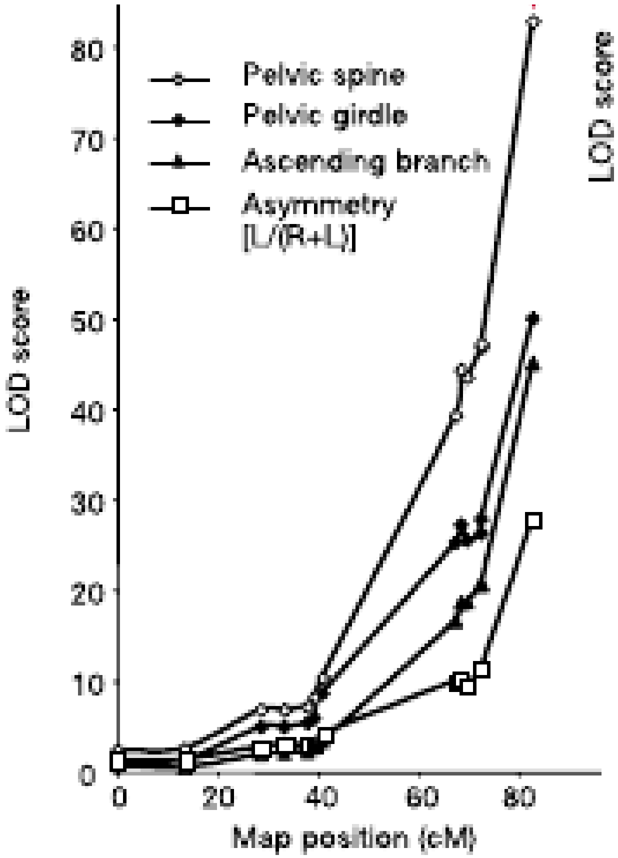
(Peichel et al., 2001)

One major locus at the end of linkage group 7

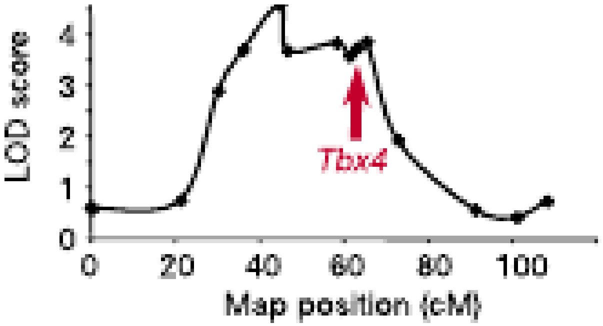


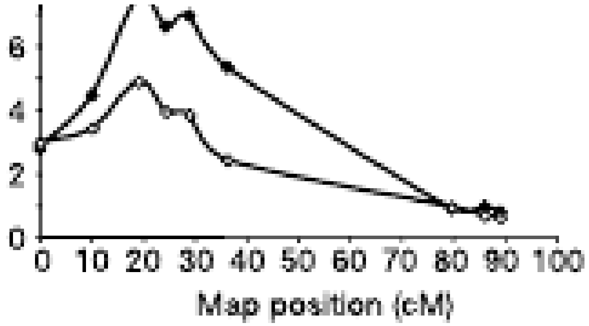
Major locus responsible for 65% of the variance

One major locus at the end of linkage group 7



A few minor loci





Major locus responsible for 65% of the variance

Three candidate genes: *Pitx1, Pitx2, Tbx4*

↓

Screen of a BAC library (Bacterial Artificial Chromosomes)
containing 100-350-kb fragments of stickleback genomic DNA

↓

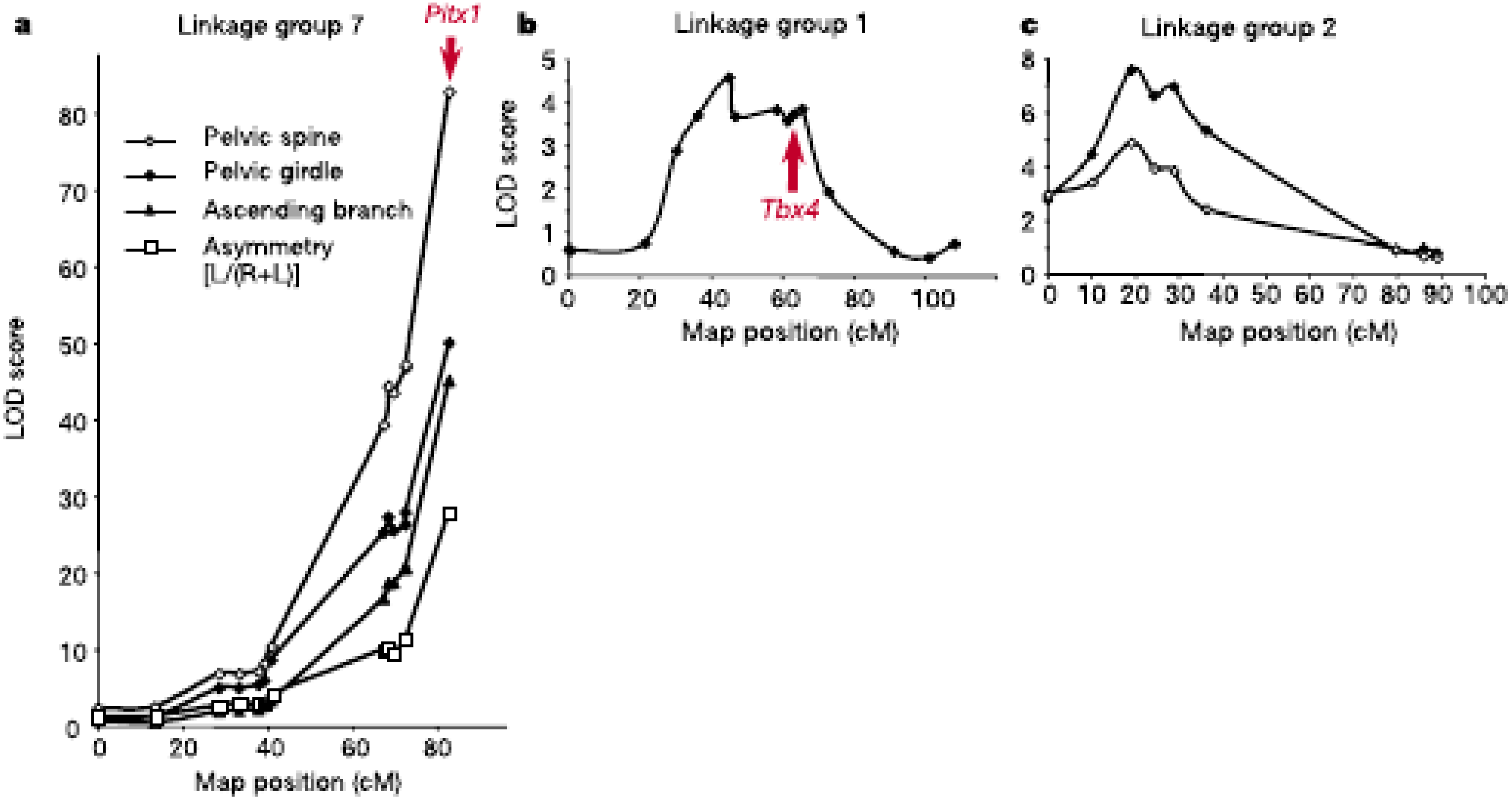
Partial sequencing and generation of new markers

↓

Genotyping of 375 F2 individuals

One major locus at the end of linkage group 7

A few minor loci



***Pitx1*, responsible for the phenotypic change?**

Pitx1 null mutations in mice
(pelvis reduction, stronger on right side)

QTL mapping

Same coding sequence in lake and marine forms

Pitx1 expressed at stage 29 in marine individuals but not
in marine individuals

Pitx1, responsible for the phenotypic change?

Pitx1 null mutations in mice
(pelvis reduction, stronger on right side)

QTL mapping

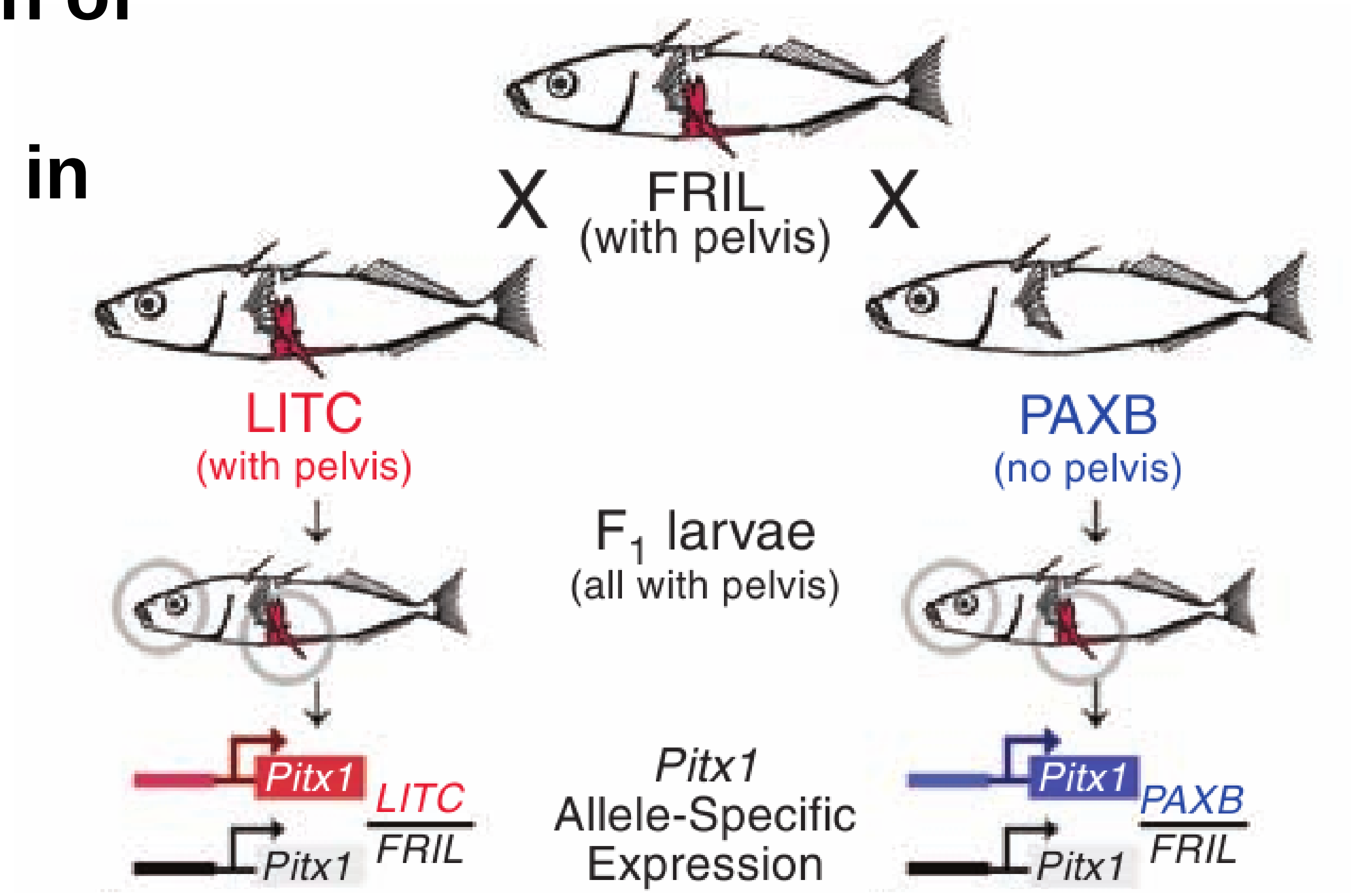
Same coding sequence in lake and marine forms

Pitx1 expressed at stage 29 in marine individuals but not
in marine individuals

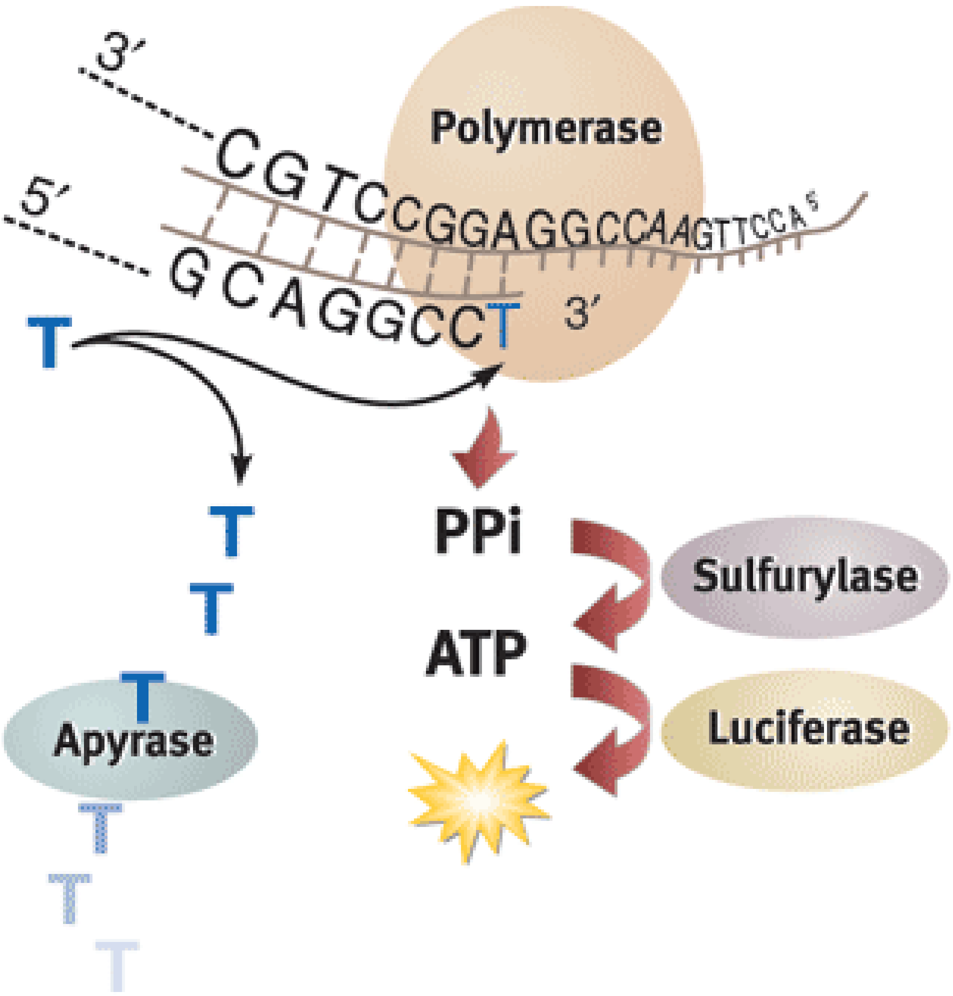
BUT

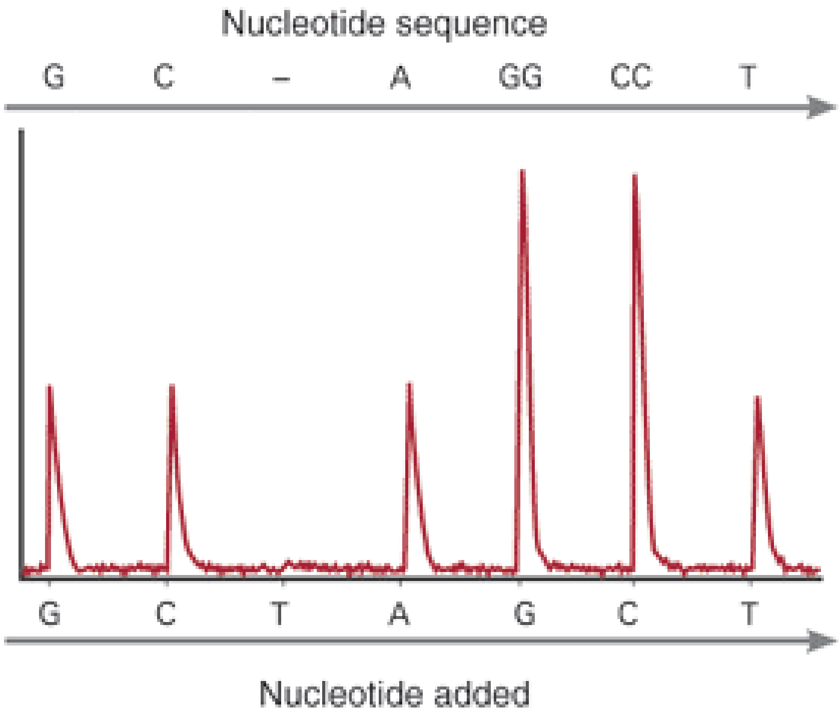
The decrease in *Pitx1* expression levels might
have evolved due to mutations in an upstream
regulatory gene

Comparison of allele expression in hybrids

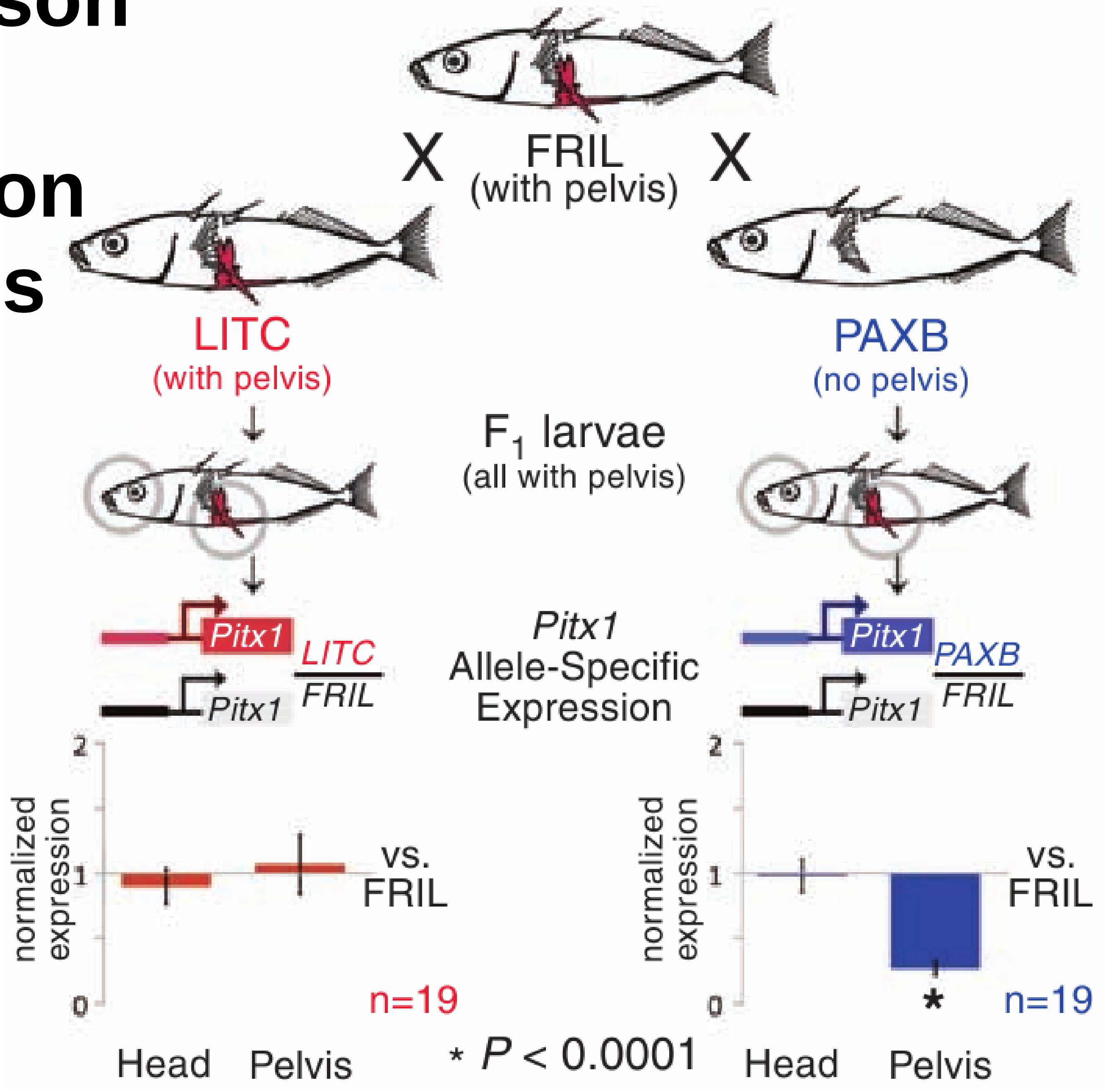


Pyrosequencing

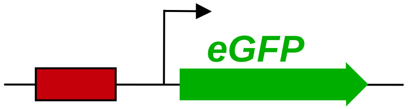


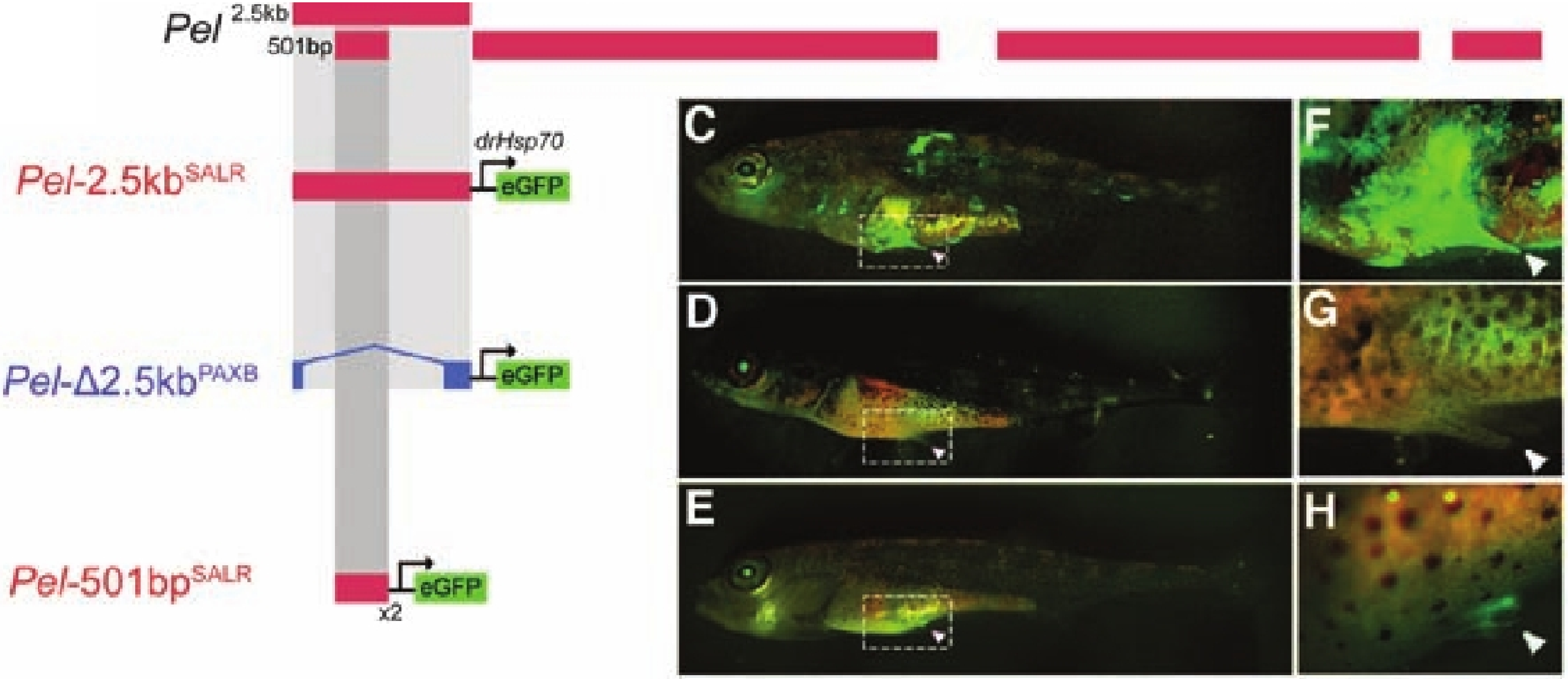


Comparison of allele expression in hybrids

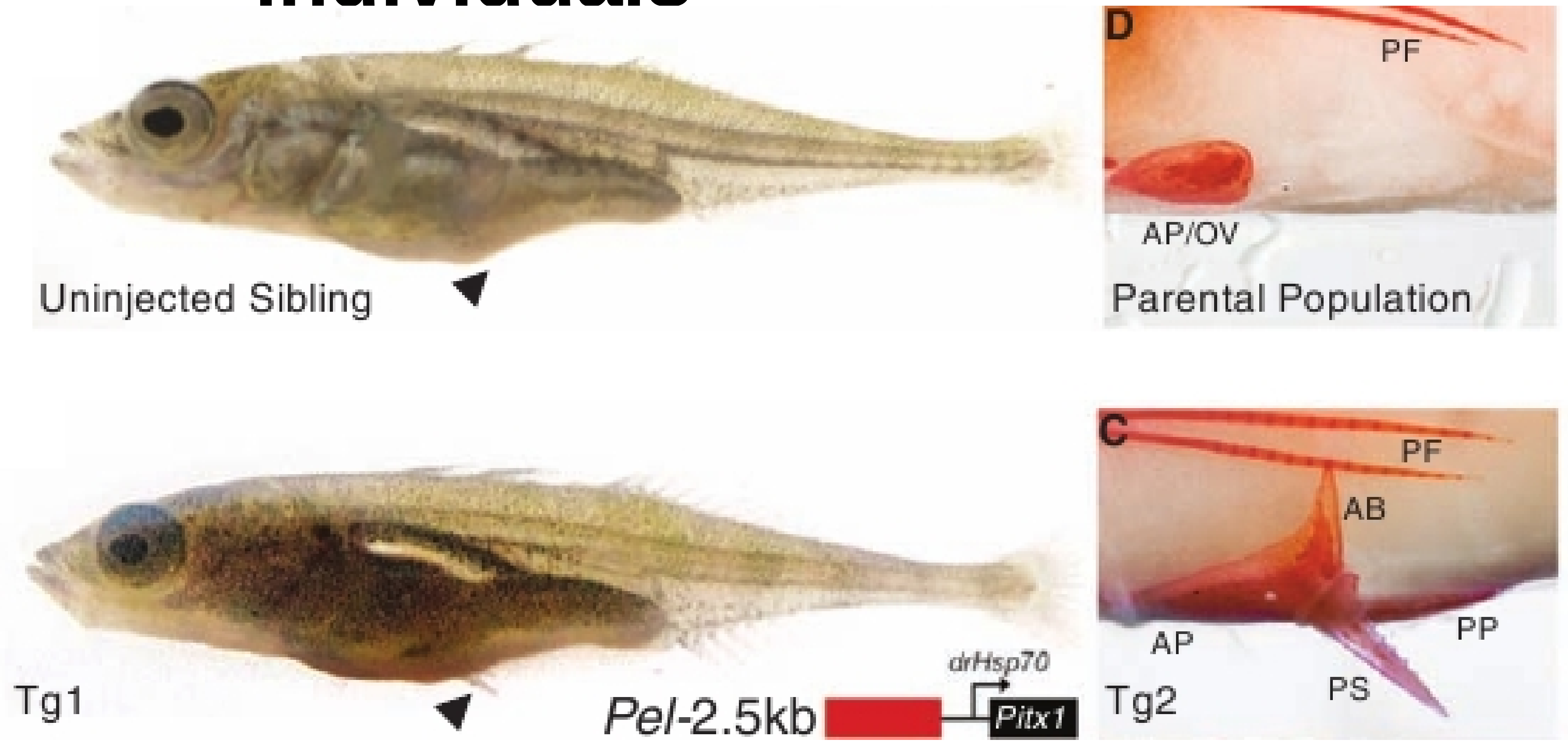


Test of *Pitx1* cis-regulatory regions





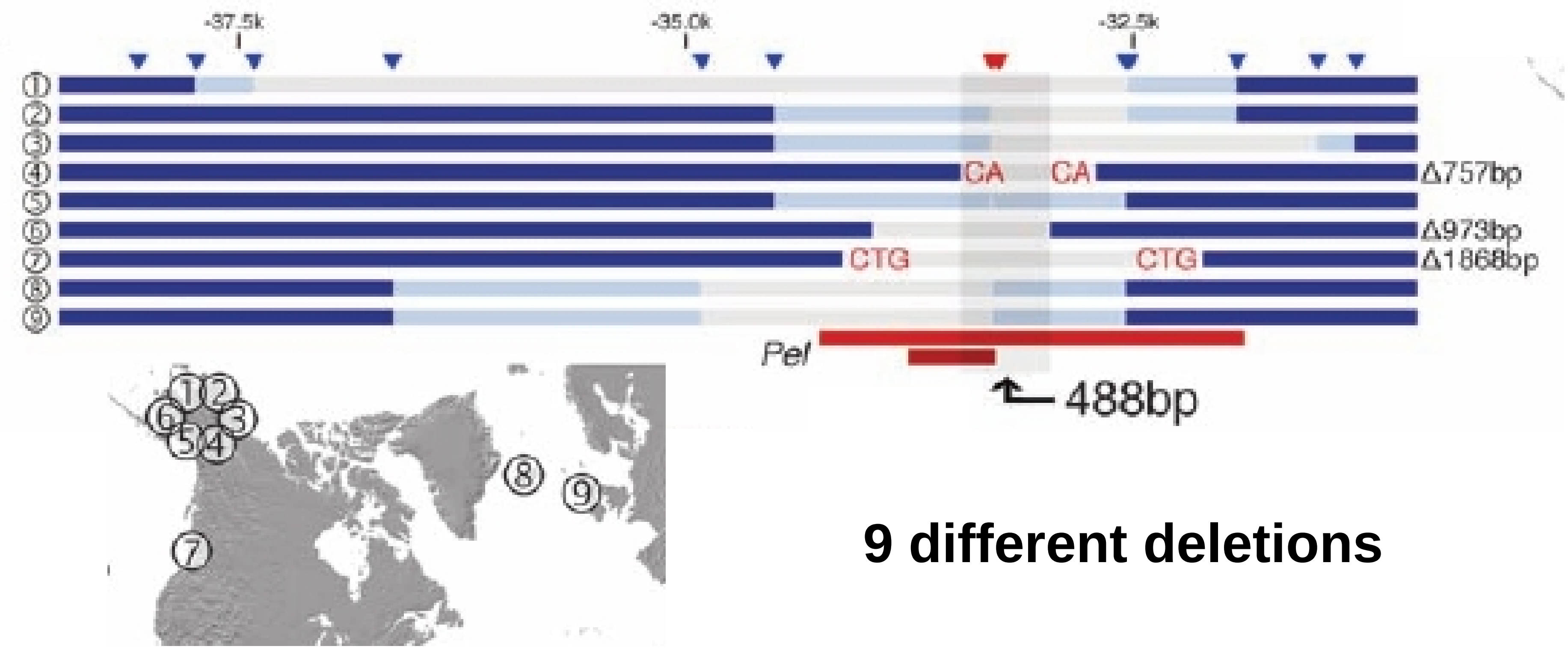
Rescue of a pelvis in freshwater individuals



Several independent deletions in the cis-regulatory region of *Pitx1*

Region sequenced in two lake pop[ulations: a 2-kb deletion in one
and a 757-bp deletion in the other one

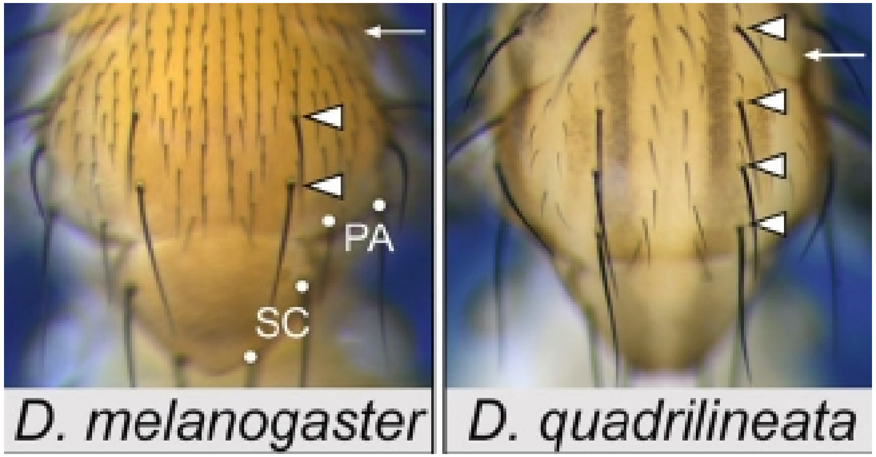
SNP genotyping in 13 populations with reduced pelvis
and in 21 populations with complete pelvis



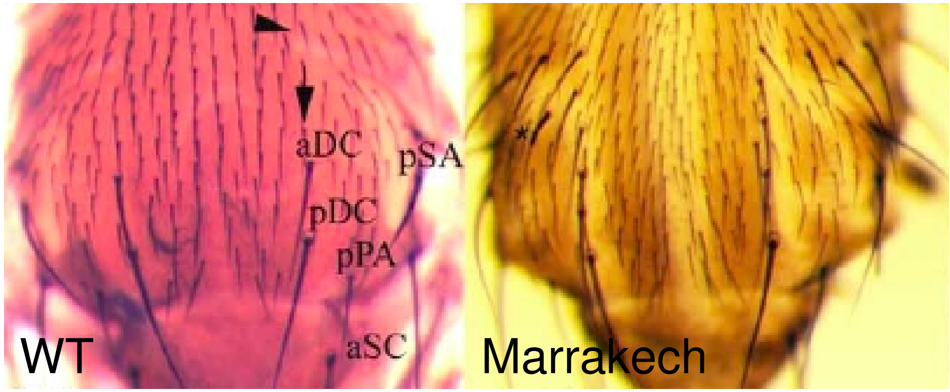
9 different deletions

Evolution of extra bristles

Interspecific change
in *D. quadrilineata*

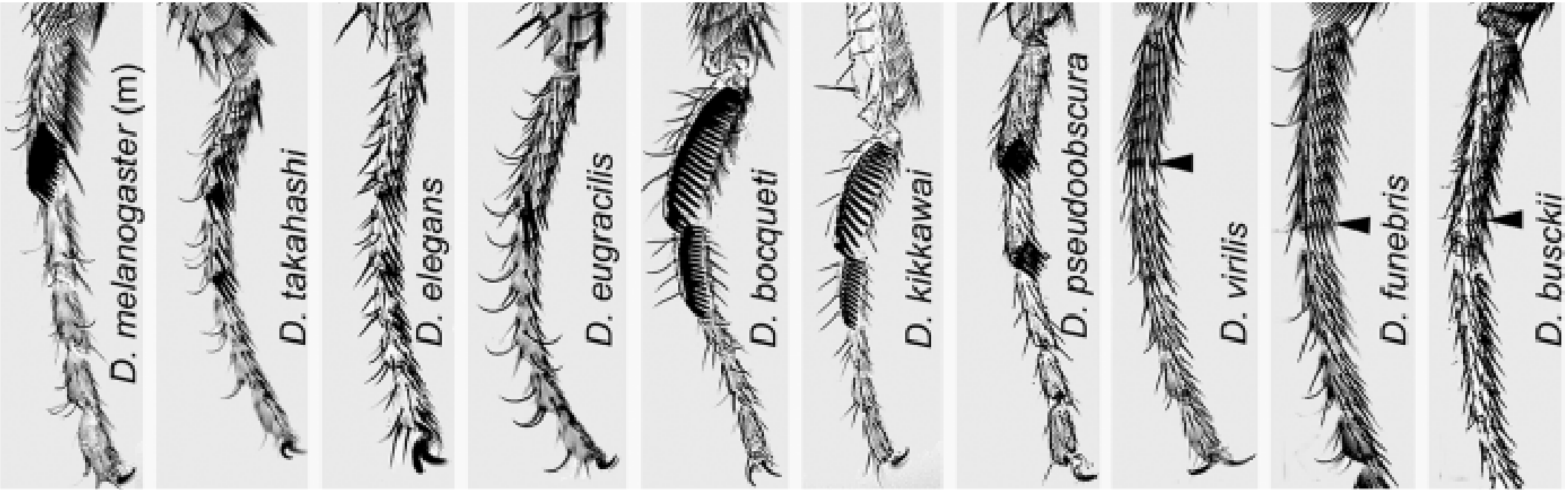


Intraspecific change
in *D. melanogaster*

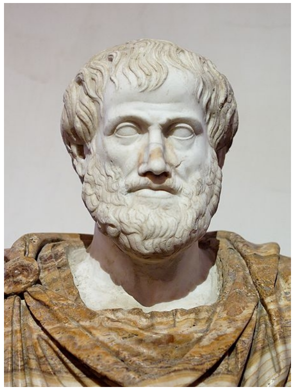


Marcellini and Simpson 2005, Gilbert et al. 2005

Finding genetic rules on bristle evolution



Randsholt and Santamaria 2008



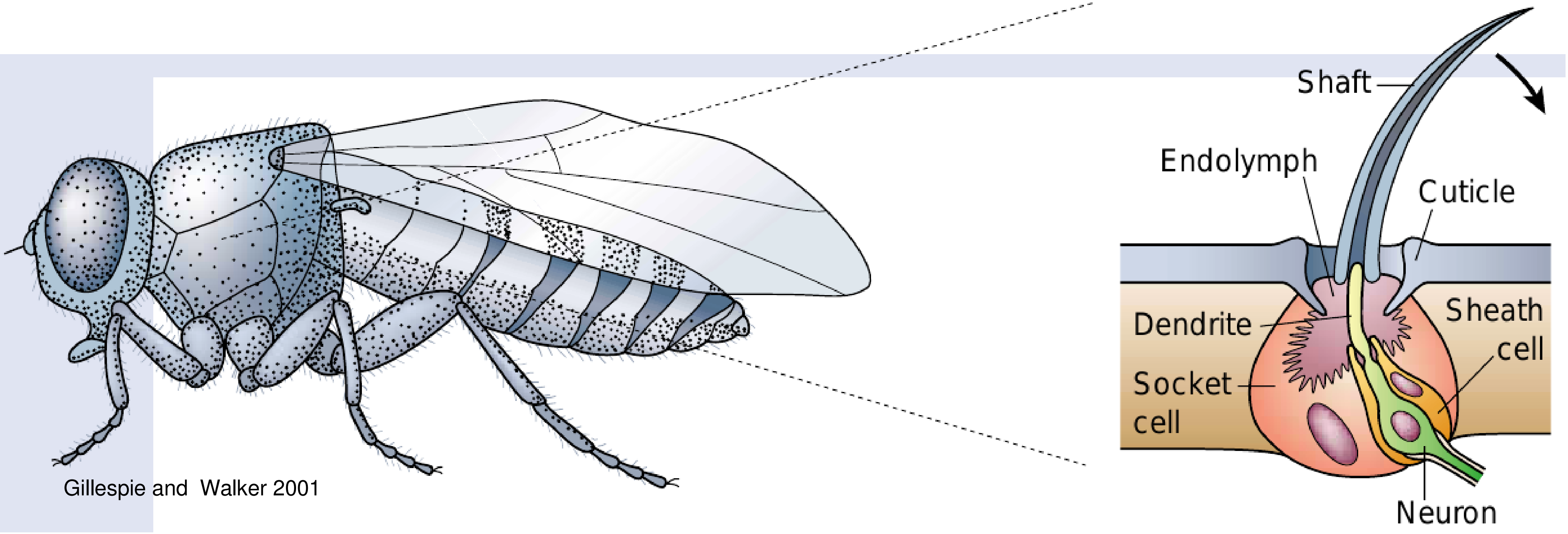
Aristotle, Historia animalium, book I, 2, 300BC

color, type, orientation
shape and size
presence/absence
position

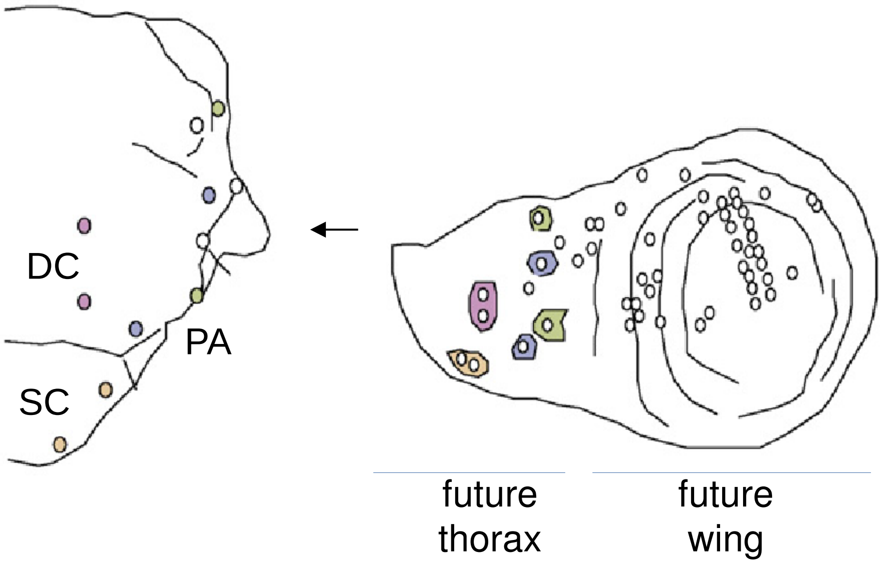
CRE mutations in *achaete-scute*

Stern and Orgogozo 2009

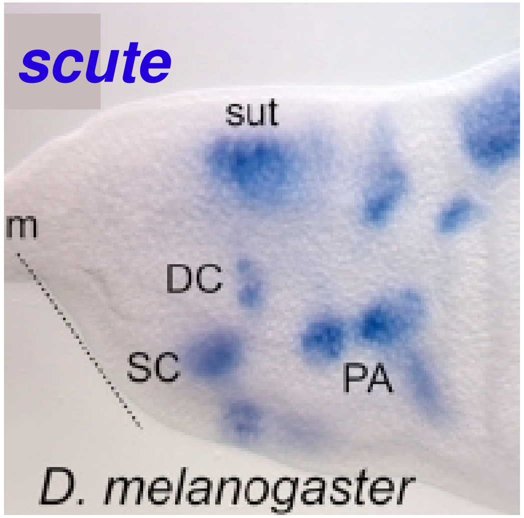
Bristle development



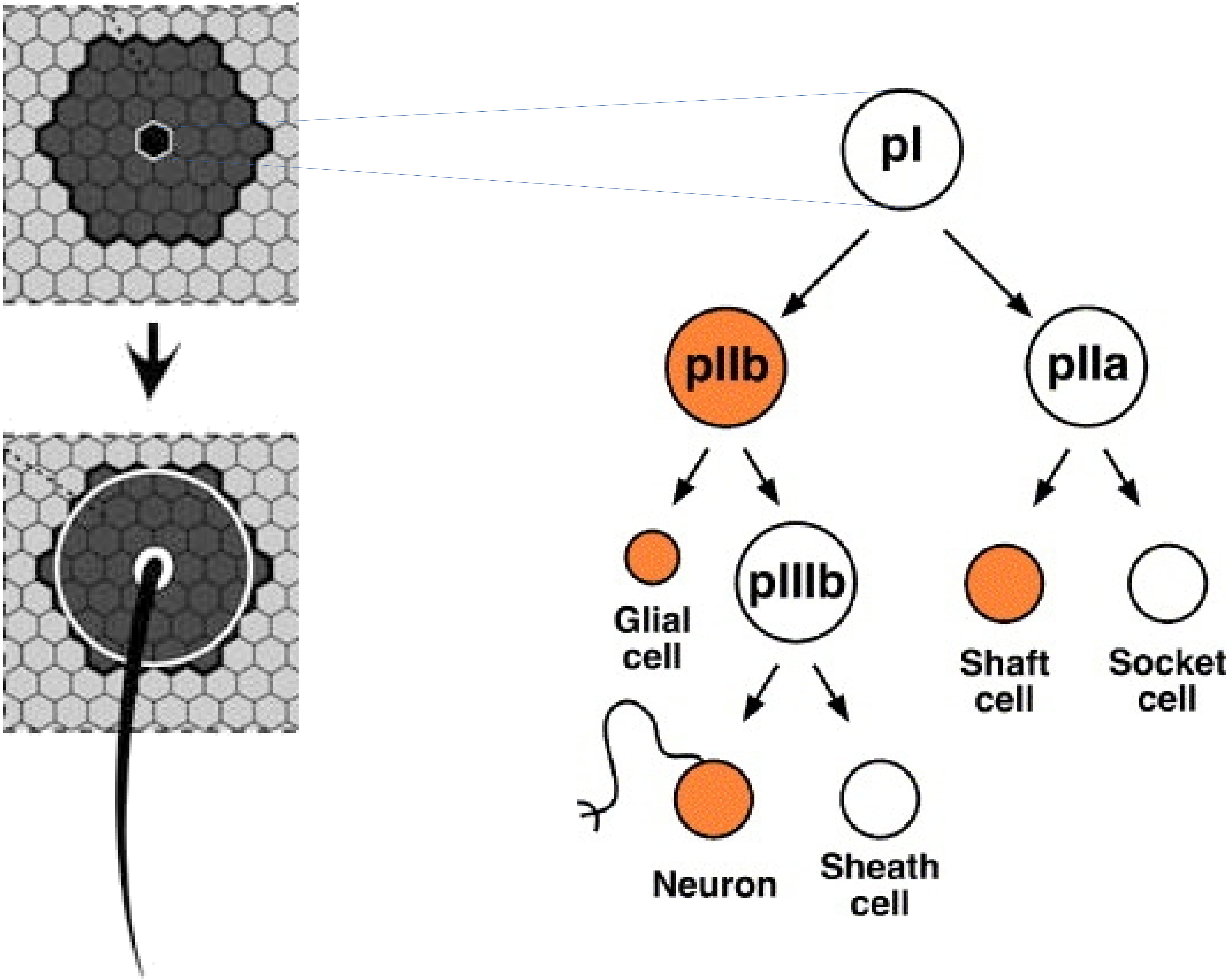
Gillespie and Walker 2001



Simpson 2007

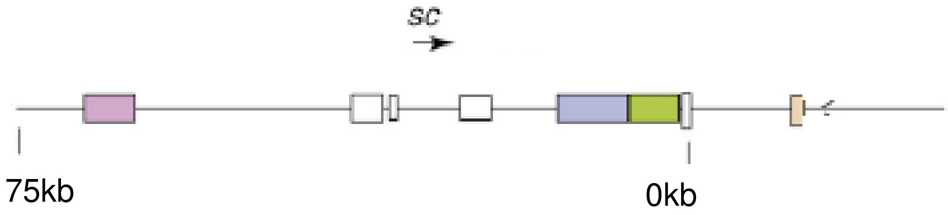


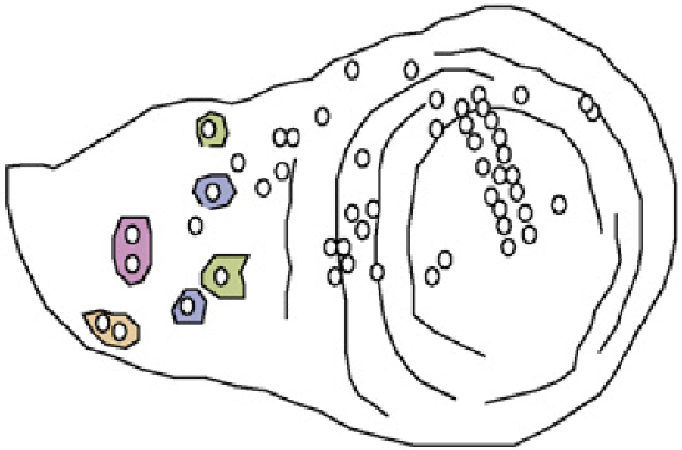
Marcellini 2006 - PloS Biology



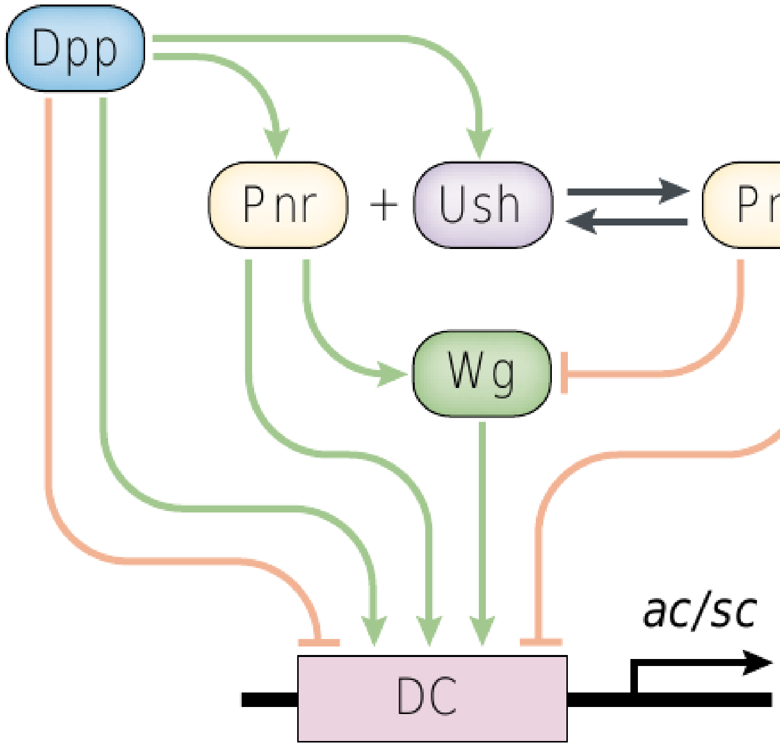
Lewis - Imaginal discs

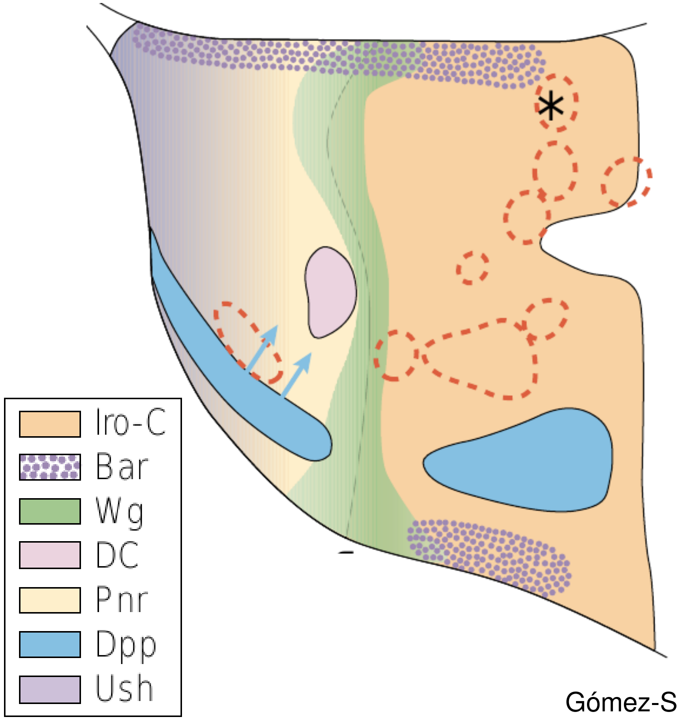
scute cis-regulatory elements are “master switches”





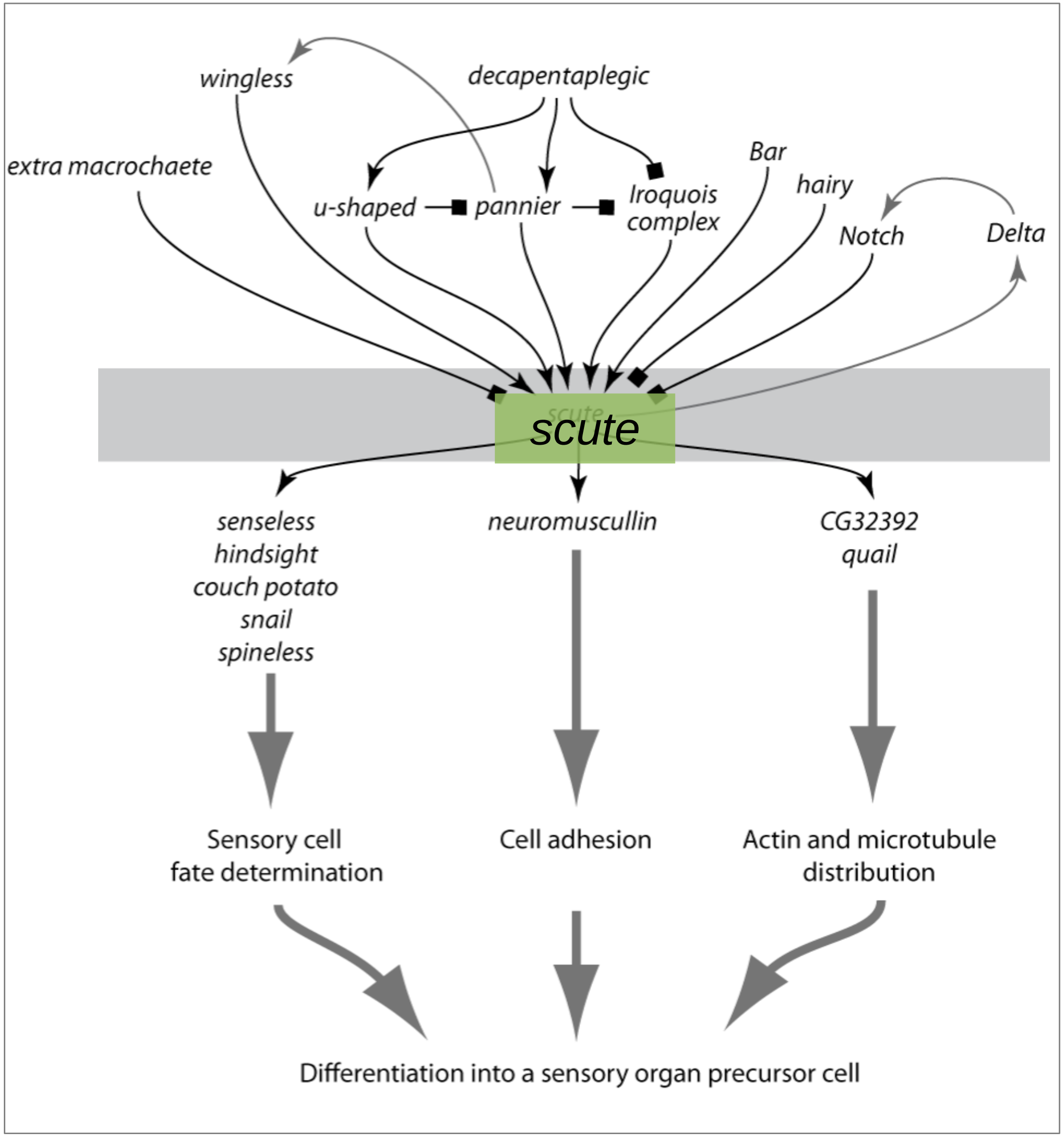
Simpson 2007

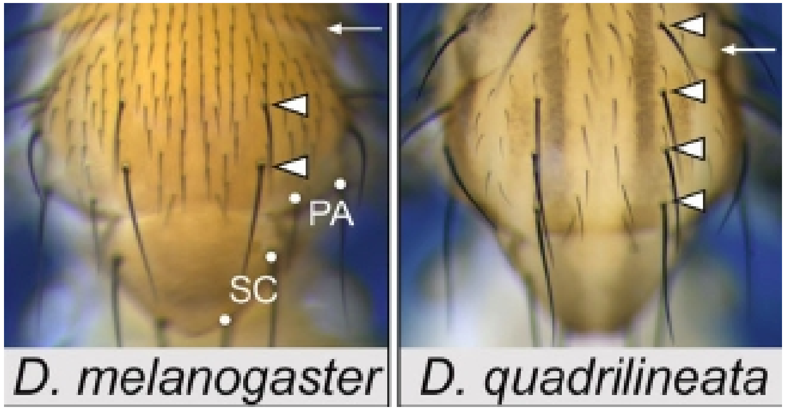


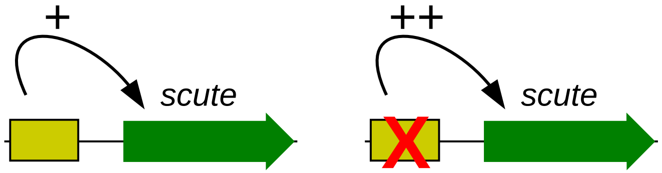


Gómez-Skarmeta 2003

Genetic evolution is partly predictable

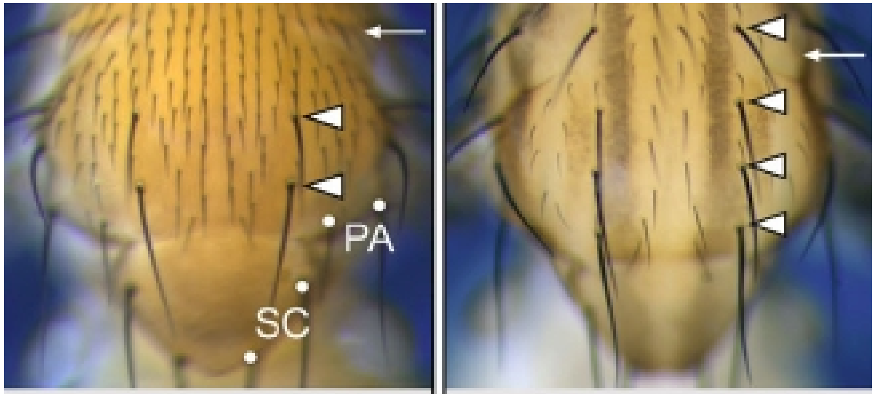




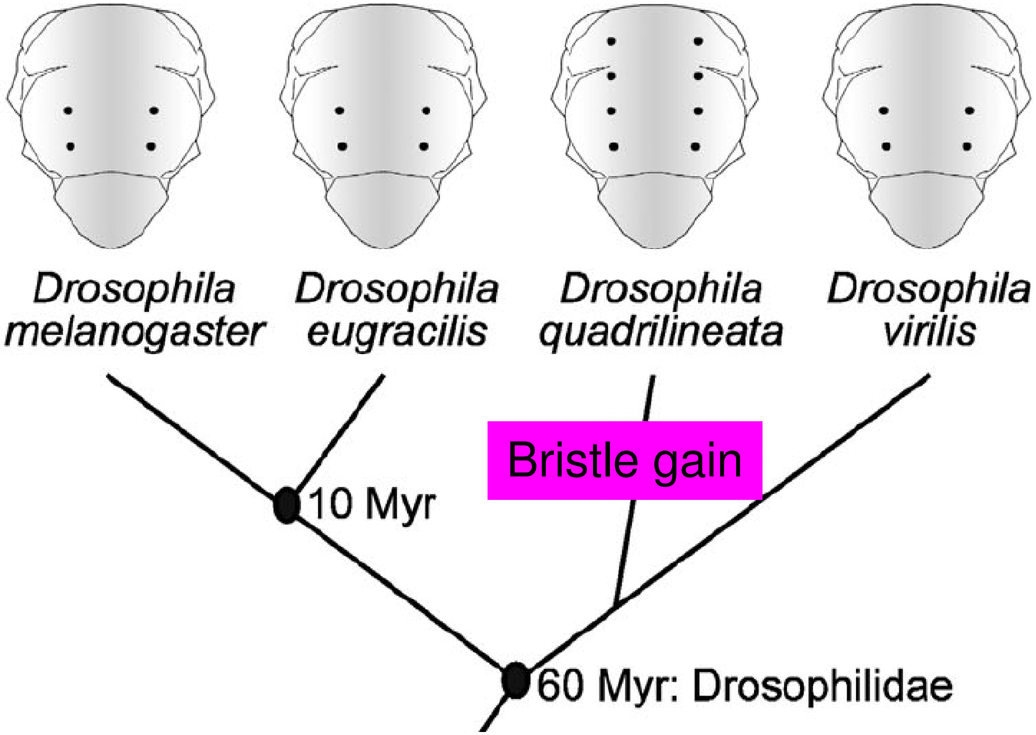


Stern and Orgogozo 2009 Science

Extra bristles in *D. quadrilineata*



D. melanogaster *D. quadrilineata*



Drosophila melanogaster

Drosophila eugracilis

Drosophila quadrilineata

Drosophila virilis

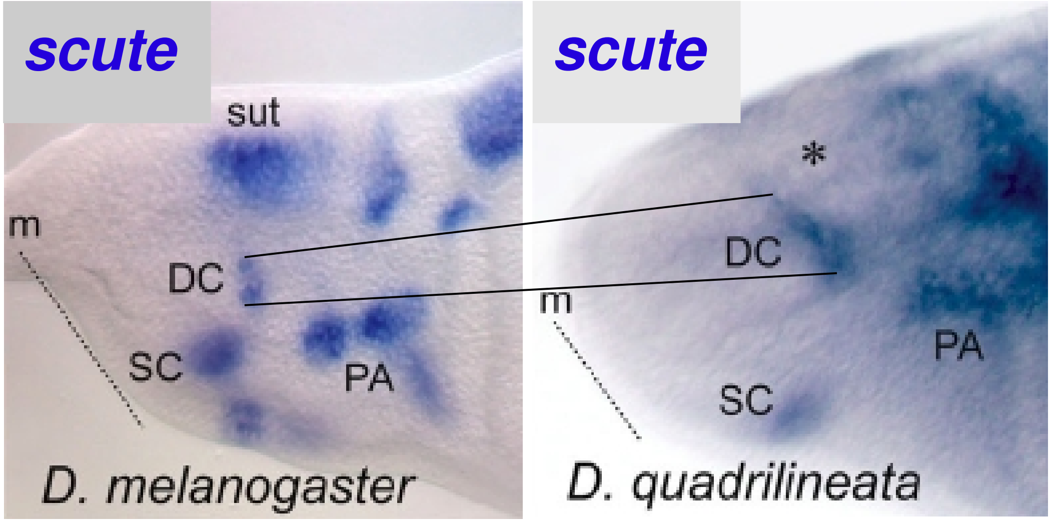
Bristle gain

10 Myr

60 Myr: Drosophilidae

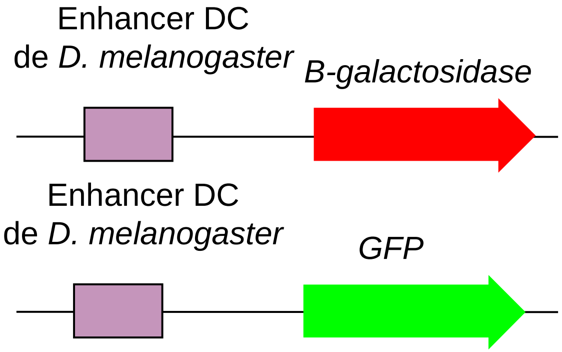
Extra bristles in *D. quadrilineata* correlate with larger *scute* expression domain

In situ hybridization



Test for a cis-regulatory change (1)

D.melanogaster
transgenics

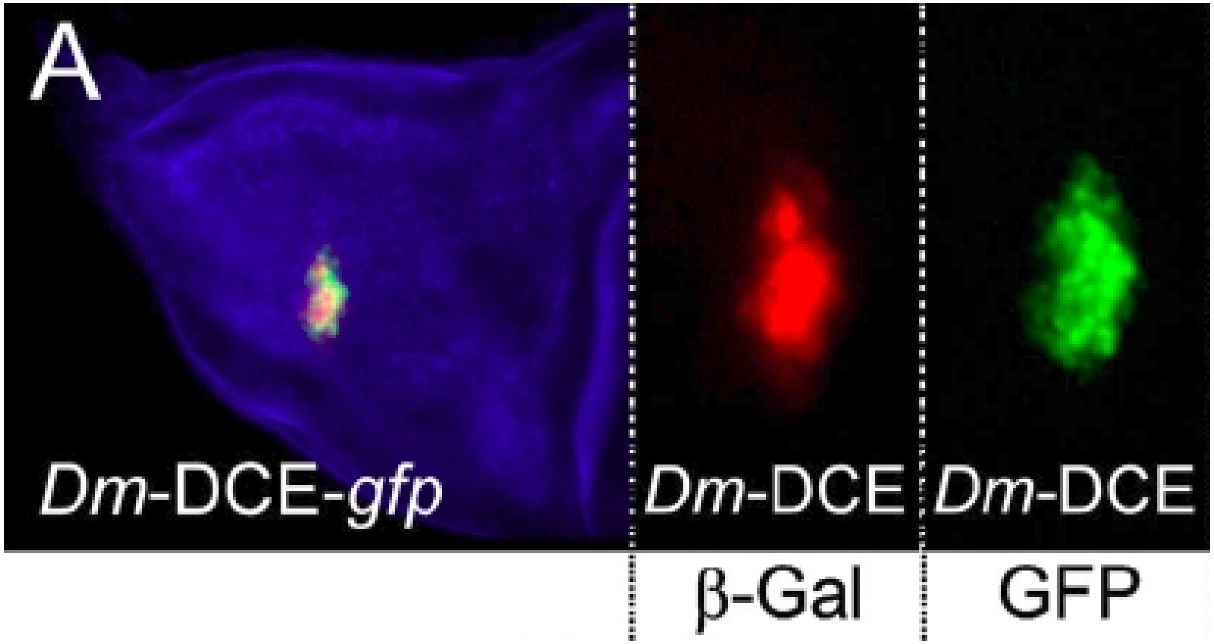


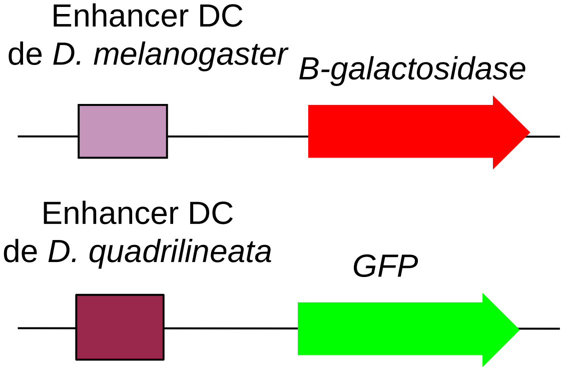
Enhancer DC
de *D. melanogaster*

B-galactosidase

Enhancer DC
de *D. melanogaster*

GFP



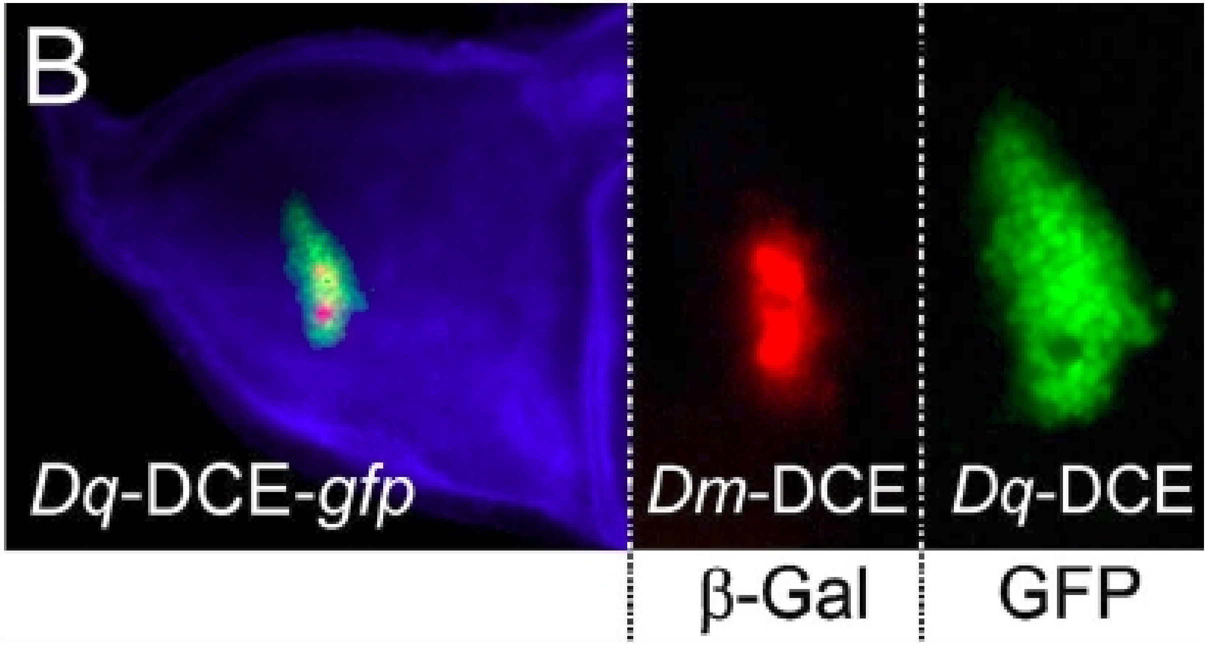


Enhancer DC
de *D. melanogaster*

B-galactosidase

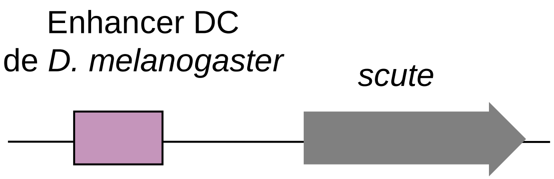
Enhancer DC
de *D. quadrilineata*

GFP



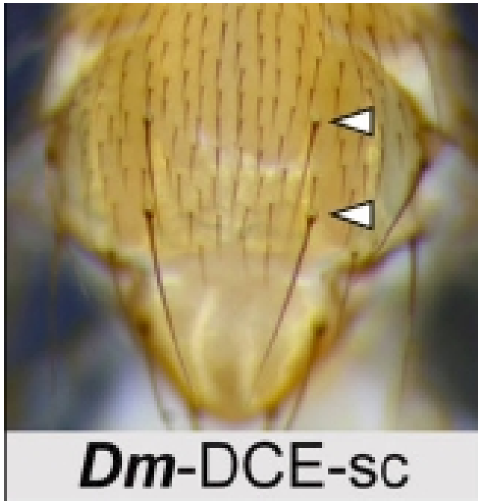
Test for a cis-regulatory change (2)

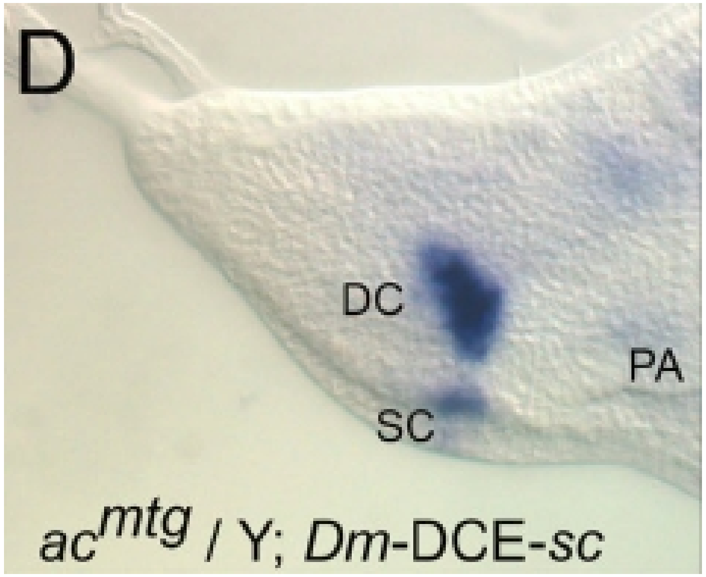
D.melanogaster
transgenics

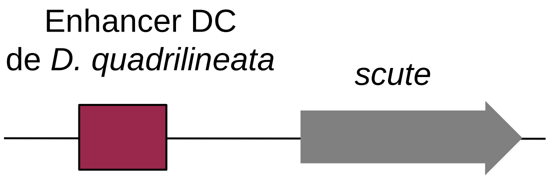


Enhancer DC
de *D. melanogaster*

scute

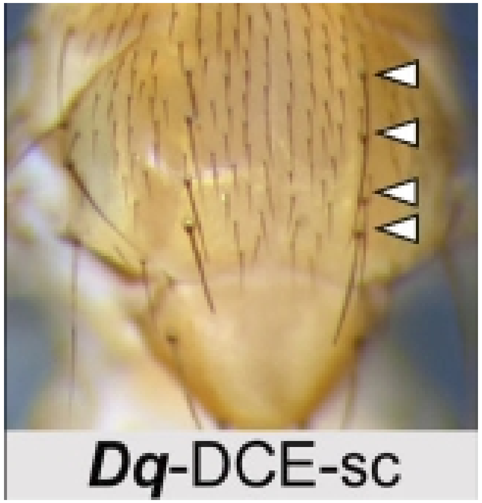


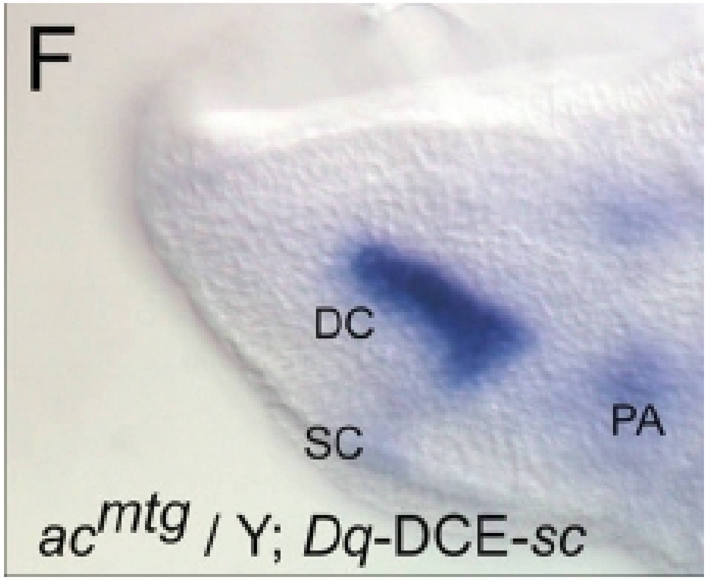




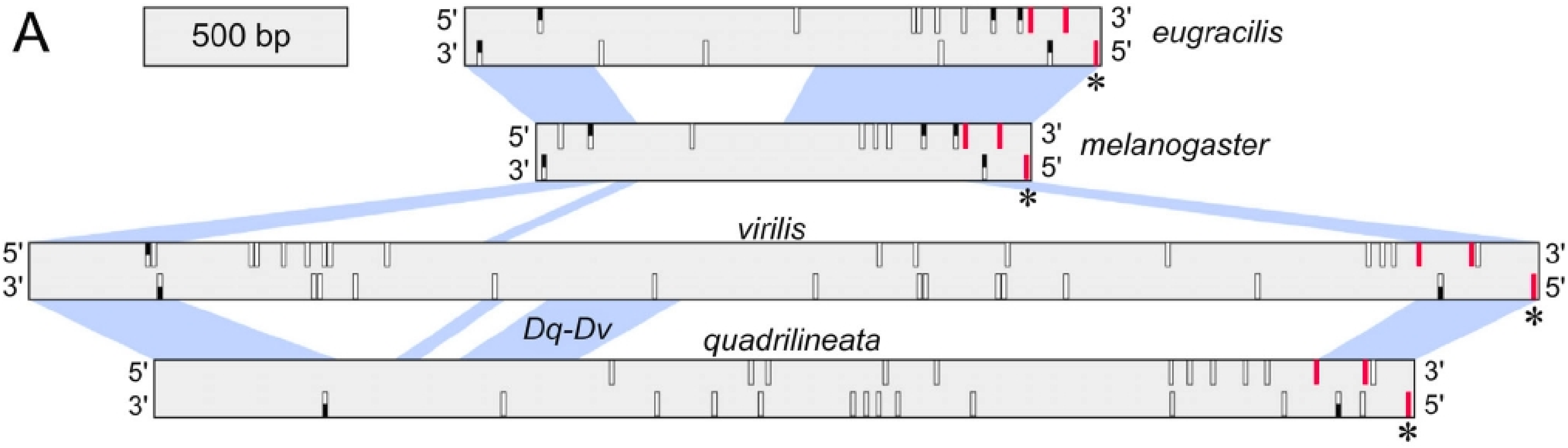
Enhancer DC
de *D. quadrilineata*

scute

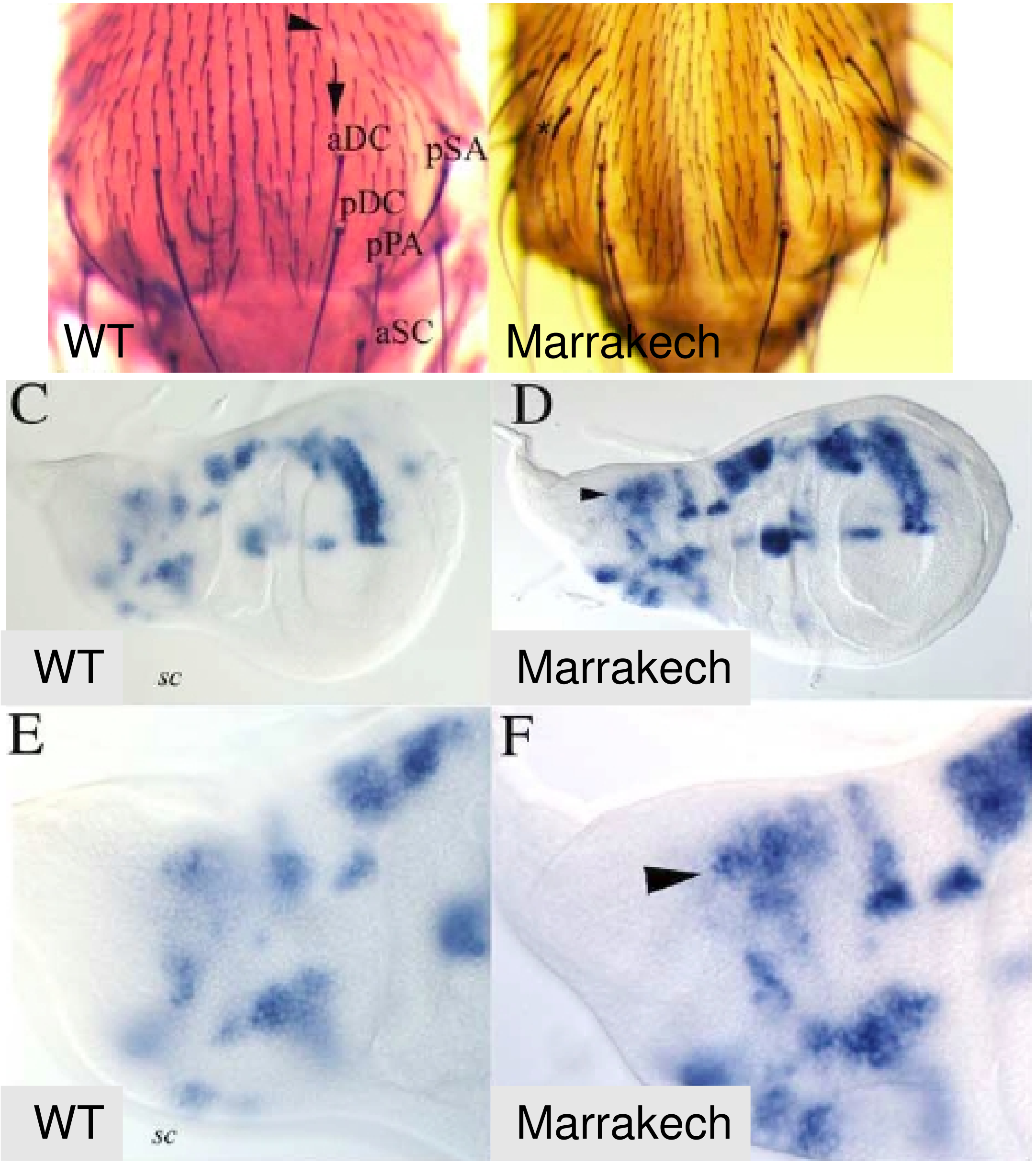




Alignment of the DC region



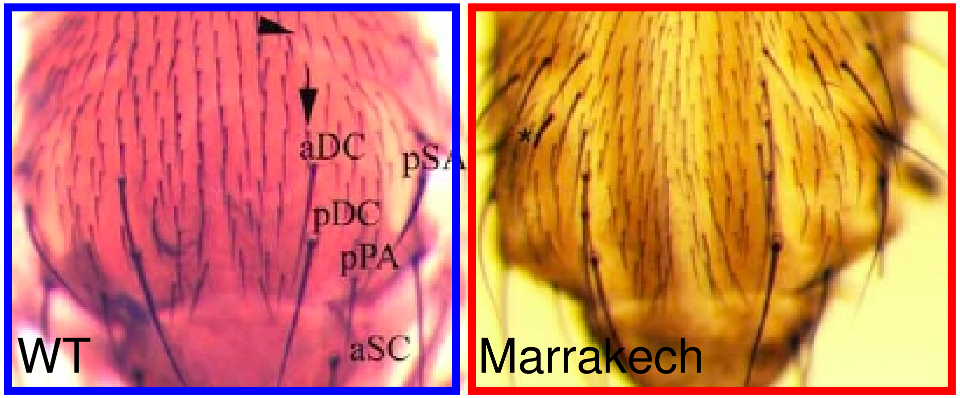
Extra bristles in *D. melanogaster*-Marrakech correlate with larger *scute* expression domain

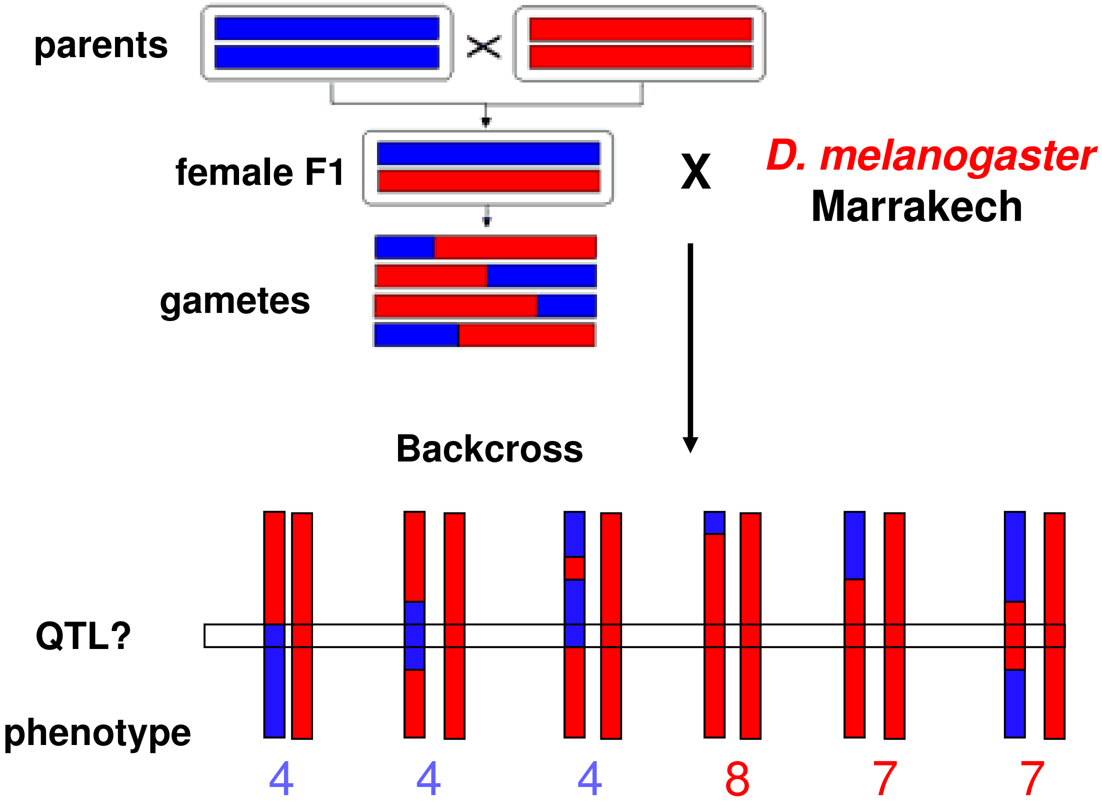


scute

Gibert et al 2005

Extra bristles in *D. melanogaster*-Marrakech due to mutation(s) in *poils-au-dos*




parents

×

female F1

X

D. melanogaster
Marrakech

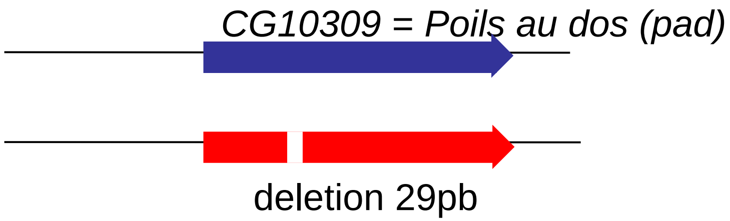
gametes

Backcross

QTL?

phenotype

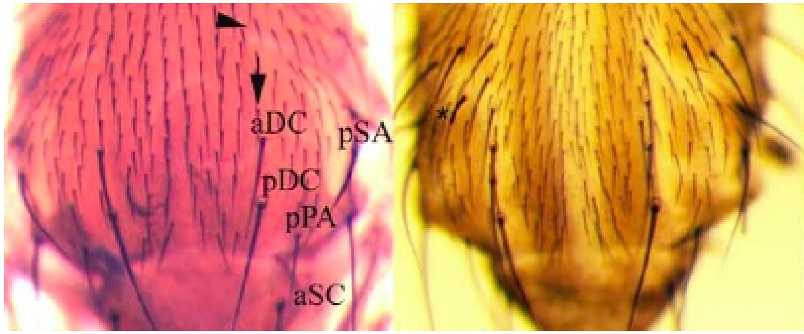
4 4 4 8 7 7


CG10309 = Poils au dos (pad)

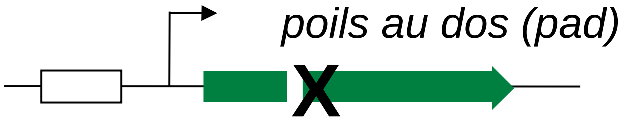
deletion 29pb

Gibert et al 2005

Short-term evolution…



D. melanogaster ***D. melanogaster* variant**



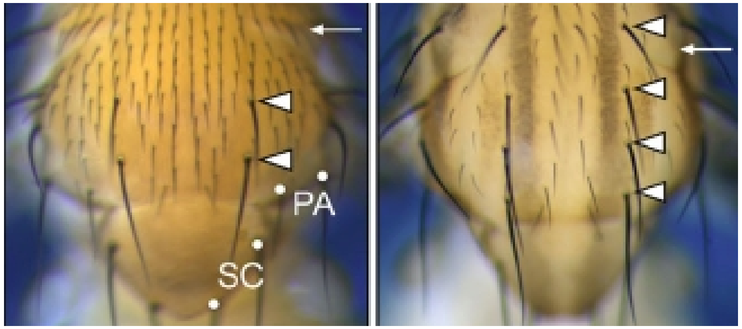
poils au dos (pad)

29bp deletion

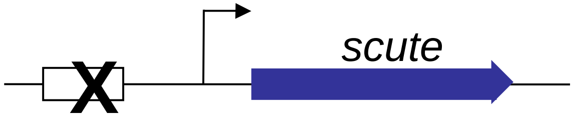
null mutation in coding region
change in thorax and wing

(Gibert et al., 2005)

…versus long-term evolution



D. melanogaster ***D. quadrilineata***



scute

cis-regulatory mutation
change in the thorax only

(Marcellini et al. 2006)

Methods to identify the genes and the mutations responsible for phenotypic evolution

Two types of appraoches

Genetic mapping

Candidate gene

no a priori, fewer biais
long and tedious
rarely ends with identification of the gene

only with strains/species which produce fertile hybrids

Based on an a priori idea
can be fast and efficient

will only find known genes

In both cases, genes with small effect are more difficult to identify

Various methods

Genetic

which chromosome (ex: autosomal versus sex)
QTL mapping
Genetic association studies
Complementation tests

General biology

General knowledge of the genes involved in the phenotype
Similarity with a known phenotype
Correlation with a change in gene expression level/pattern

Final test of protein activity

in vitro in *E. coli*, by transgenesis in the studied species or the closest model organism (ex: *beta-defensin* of dogs tested in mouse)

Final test of cis-regulatory regions

- with reporter constructs, transgenesis, comparison of both regions
- comparison of allele expression levels in hybrids (pyrosequencing)

QTL Mapping

4 steps: crosses, genotyping, phenotyping, statistical analysis

(Association study: no crosses, sample of a natural population)

Crosses for QTL mapping

Backcross with one line
Backcross in both directions
F2
Crosses for several generations
Introgression lines
Recombinant Inbred Lines
…

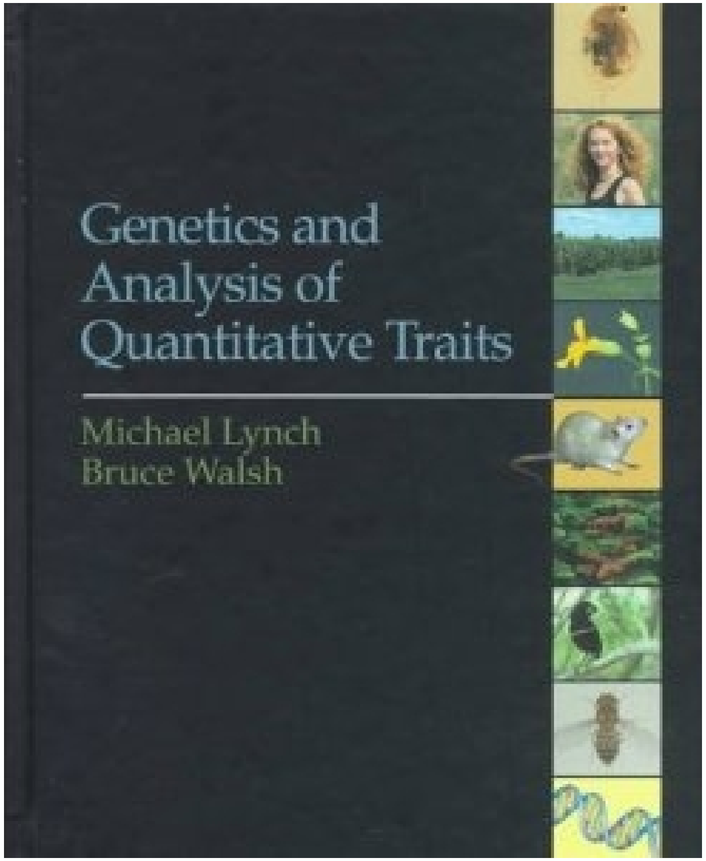
Always try to maximize the number of recombination events

Markers for QTL mapping

yes-no PCR
<u>PCR length polymorphism</u>
<u>Sequencing</u>
Pyrosequencing
Probe hybridization
Microarray
RAD…

How many markers?

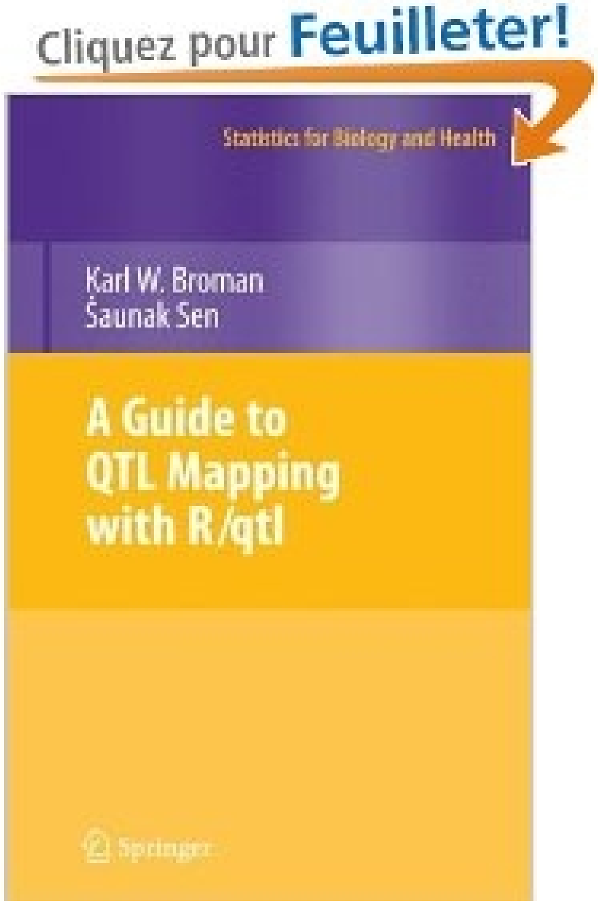
theory



Genetics and Analysis of Quantitative Traits

Michael Lynch
Bruce Walsh

practice



Cliquez pour Feuilleter!

Statistics for Biology and Health

Karl W. Broman
Śaunak Sen

A Guide to QTL Mapping with R/qtl

Springer