hic sunt dracones

…. here be dragons!

Genetic and phenotypic architecture of complex traits

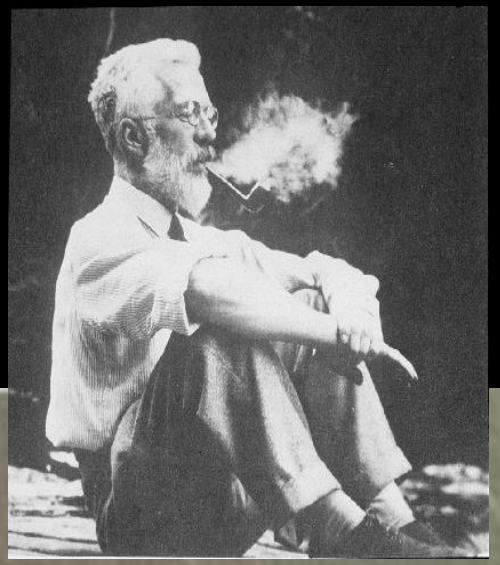
Number of genes
Dominance effects
Genetic (mutational) load

Expressivity, pleiotropy, plasticity
Interactions – gene/gene, gene/environment
Networks – regulatory and phenotypic

Epigenetic inheritance

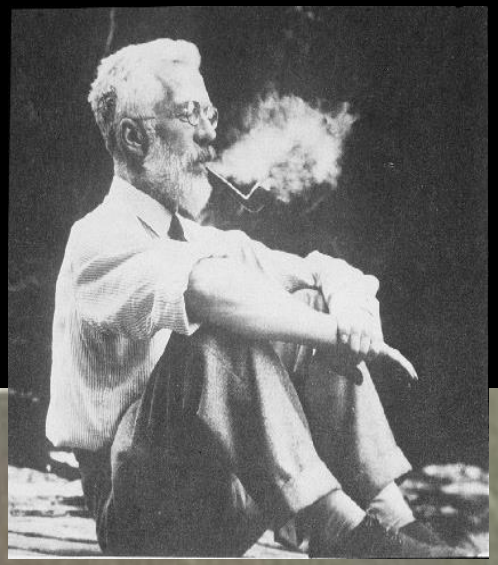
Complex traits:

……. *from Fisher et al*



$$P = G + E + GxG + GxE$$

0 0



epigenetics

and

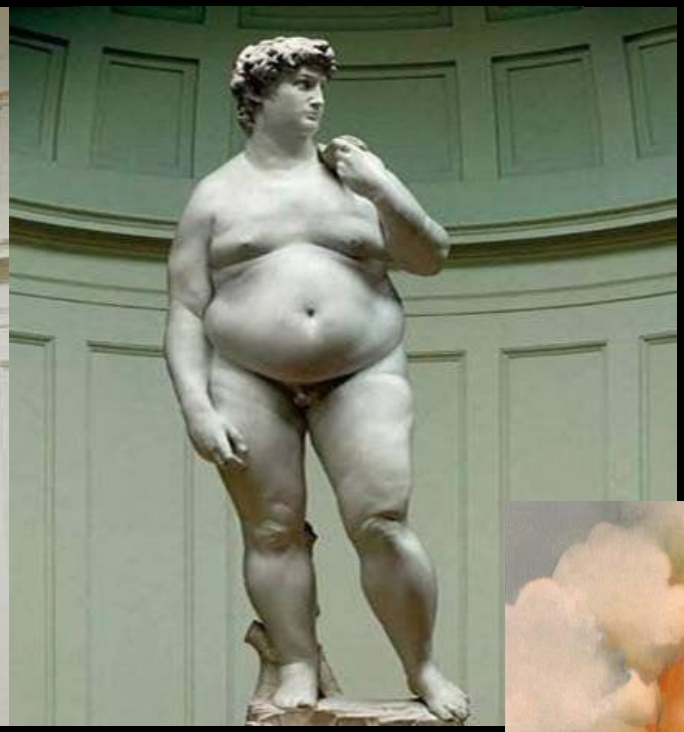
+

Three parts

Fractal genetics and gene discovery

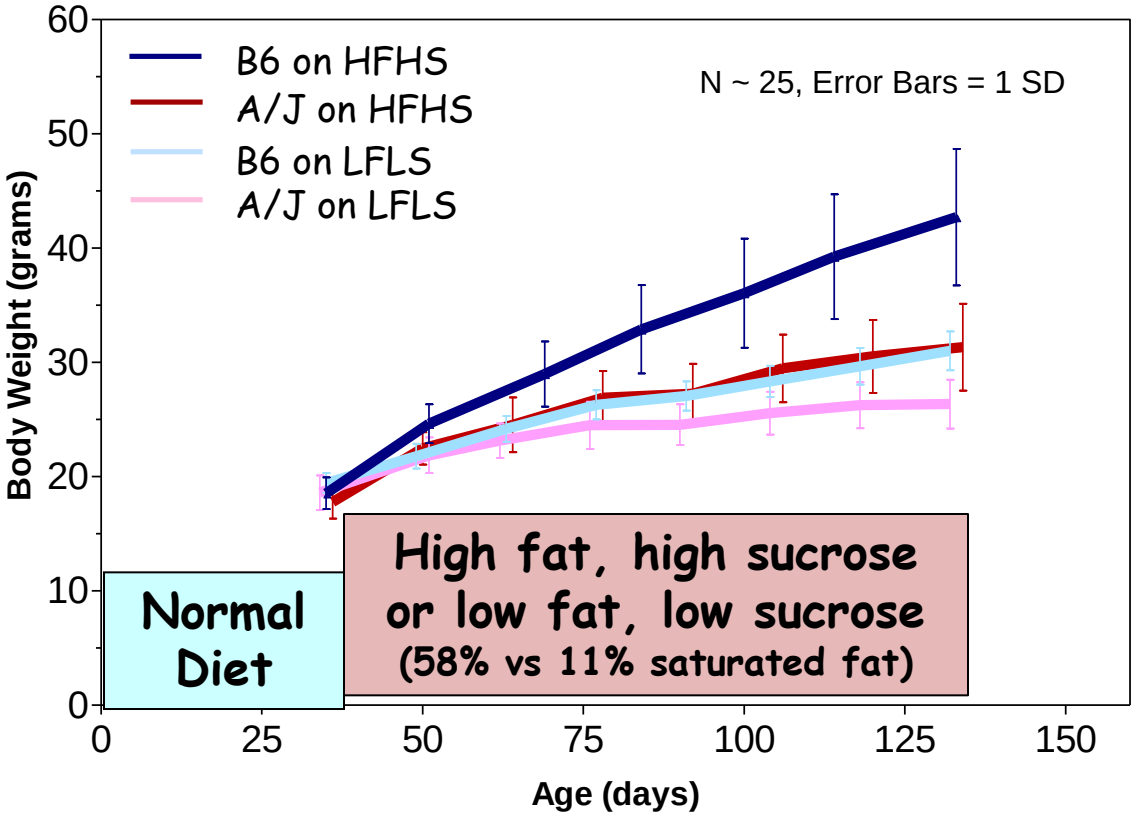
Epistasis and context-dependent effects

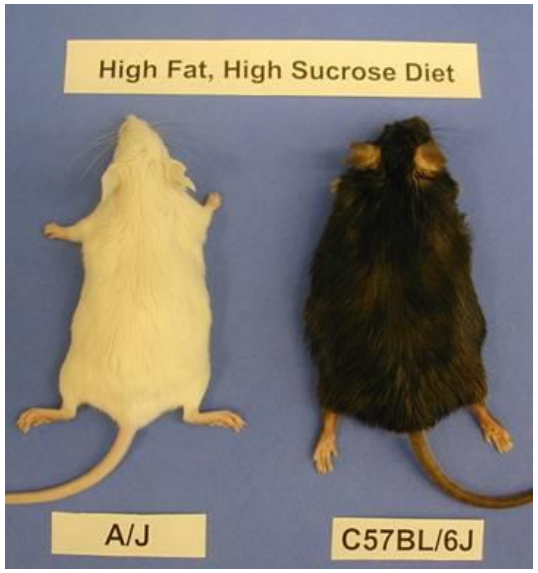
Epigenetic inheritance
transgenerational effects,
ancestral genetics,
and current disease risks

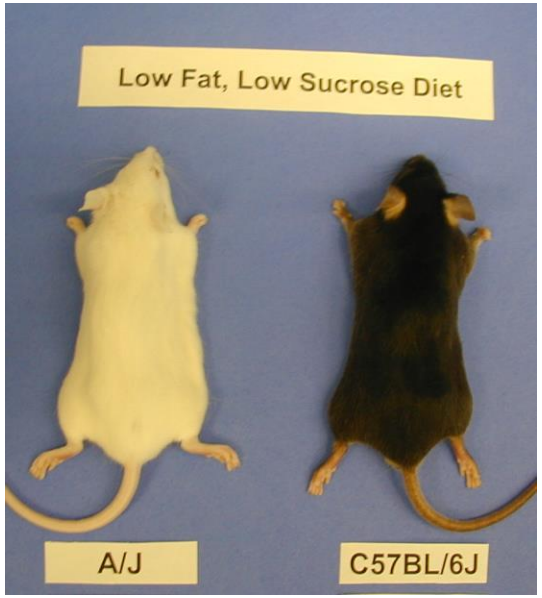


Diet-induced obesity:

Gene - diet interactions



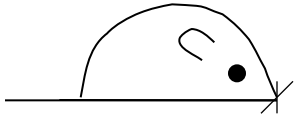




B6, obese only with a HFHS diet

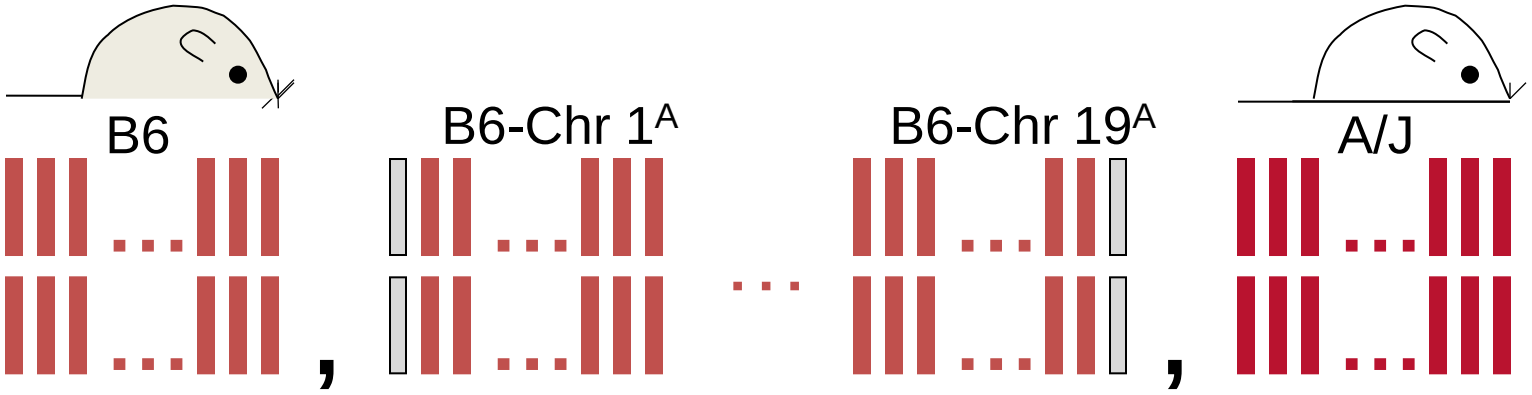
A/J, lean regardless of diet

B6 and A/J: Contrasting models of disease

On High Fat, High Sucrose Diet:	 B6	 A/J
Obesity	✓	X
Insulin resistance	✓	X
Hypertension	✓	X
Cardiovascular disease Risk	✓	X
Non-alcoholic steatohepatitis	✓	X
Hepatocellular carcinoma	✓	X
	Genetics of disease	**Genetics of health**

Nadeau and Topol, Nat. Genet. 2006; Shao et al. PNAS, 2008; Hill et al., Hum Mol Genet, 2009

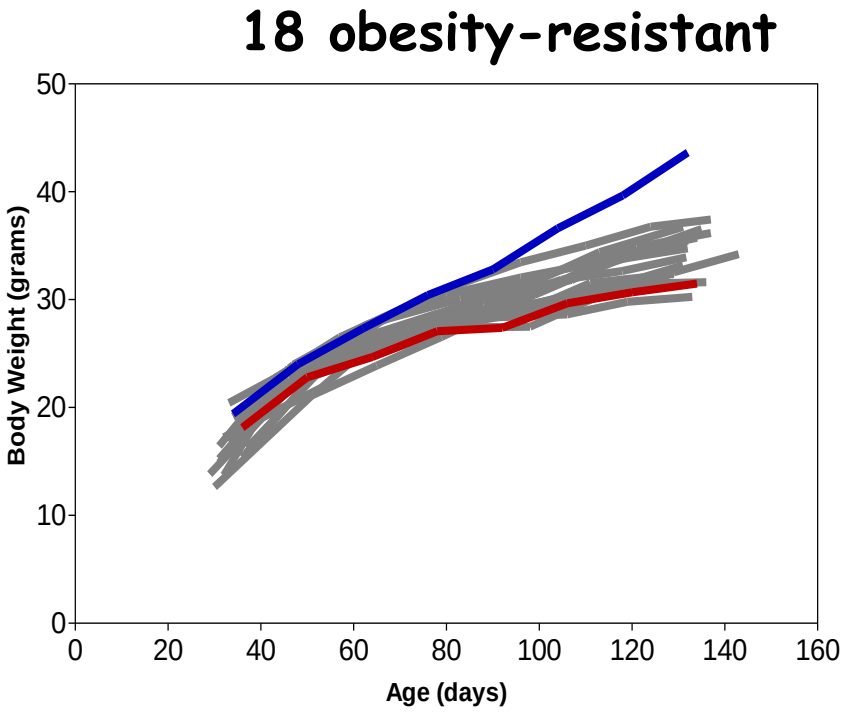
Chromosome Substitution Strains (CSSs): A genome survey of individual genotypes

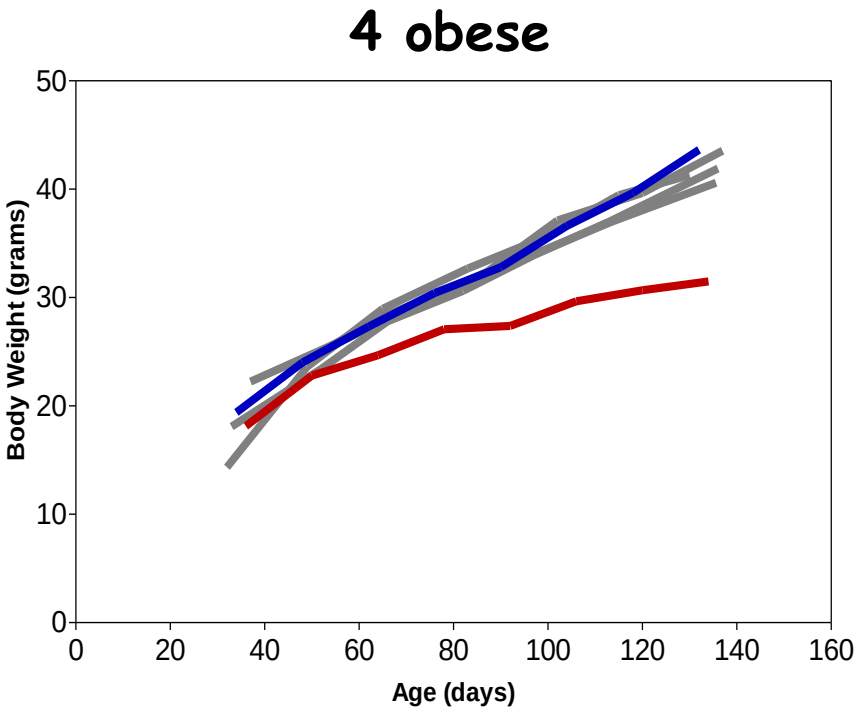


- CSSs partition the genome in a stable, defined and non-overlapping manner
- Genetic variation is controlled in a precise and reproducible manner

Singer and Hill *et al. Science* 2004, Shao et al. PNAS 2008

Many chromosomes confer resistance to diet-induced obesity



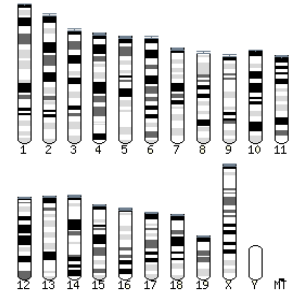


Summary: phenotypic variation in CSSs

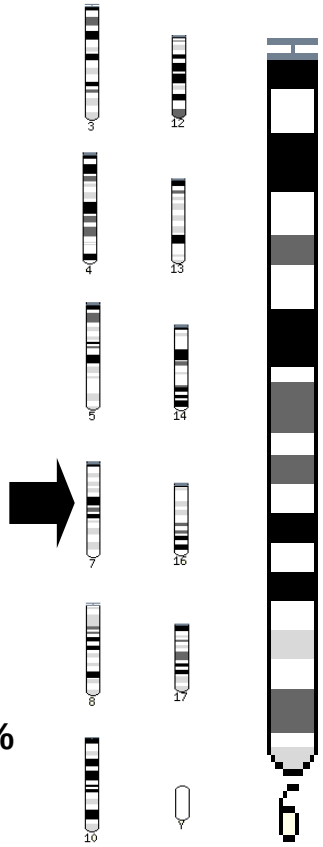
1. Many CSSs have QTLs
   - > 90 traits; > 700 QTLs
   - Average = 8 CSSs / trait
2. Unexpectedly large phenotypic effects
   - Average effect size in crosses: 6%
     (Flint et al., *Nat Rev Genet* 2005)
   - Average effect size in CSSs: 76%
3. Strong directional phenotypic shifts
   - 92% of QTLs shifted towards A/J

Shao et al. *PNAS* 2008, Spiezio et al. BMC Genetics 2012

Genetic and phenotypic complexity on a single chromosome



Genome – 100%
Effect size – 100%



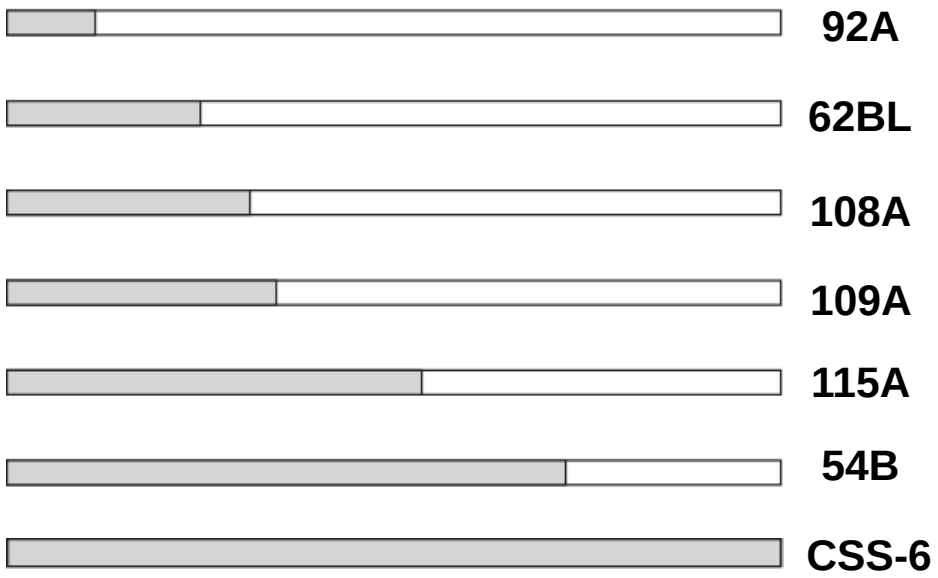
Genome ~ 5.0%
Average effect size = 53%
Chr. 6 effect size = 76%

Strain	Weight	BMI	Fat pads
B6	44.3	38.5	2.8
A/J	31.5***	31.3**	1.7*
A6	34.6***	33.4***	1.9*

*<0.05, **<0.01, ***<0.001

B6.A6 mice are obesity-resistant

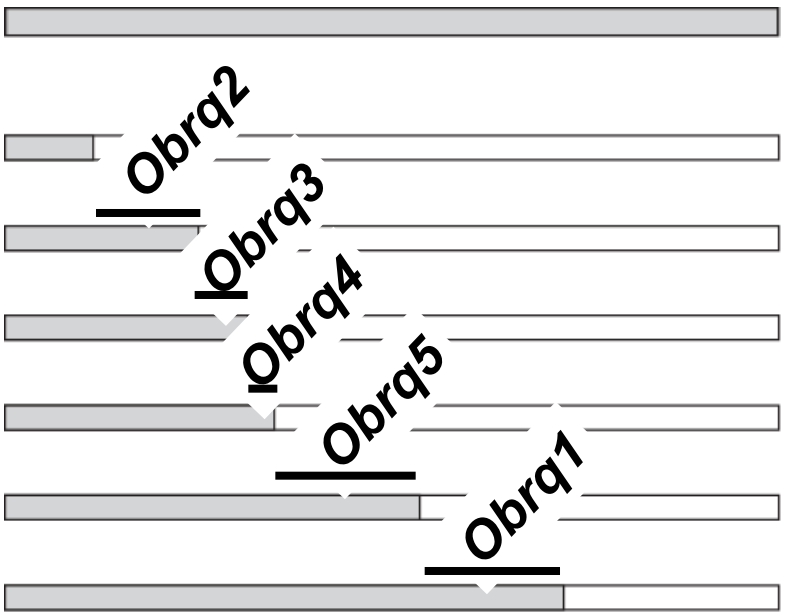
Chr 6 congenic strains



Shao et al. PNAS, 2008; Buchner et al. Physiol. Genomics 2008; Millward et al. Mammal. Genome2009

Phenotypes flip between alternative states

Chr. 6 congenics



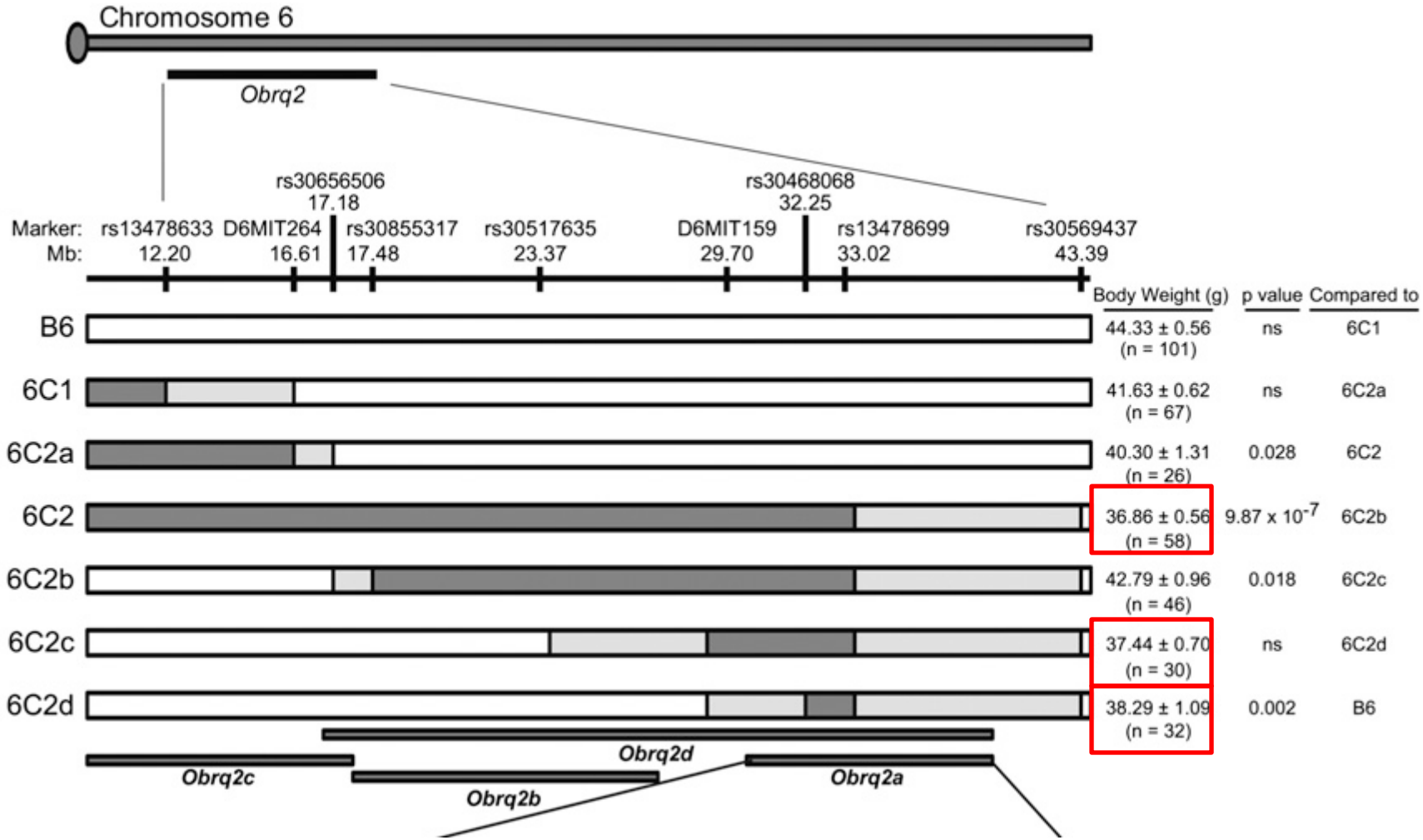
B6-derived sequence

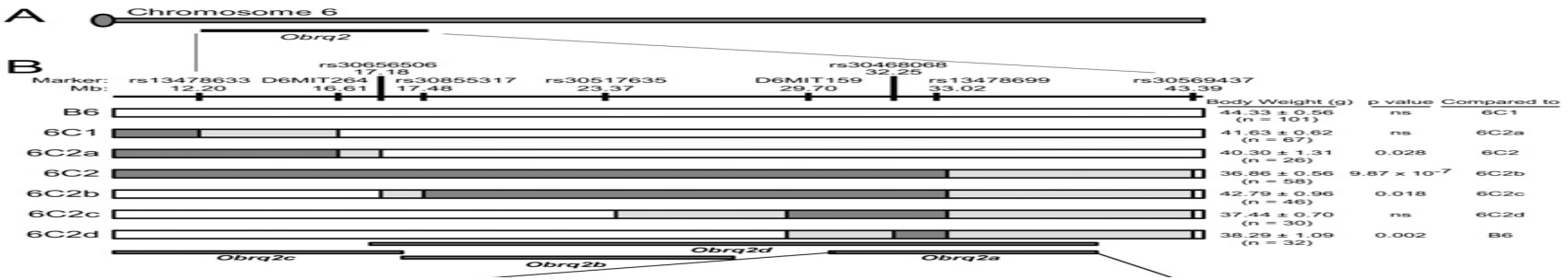
A/J-derived sequence

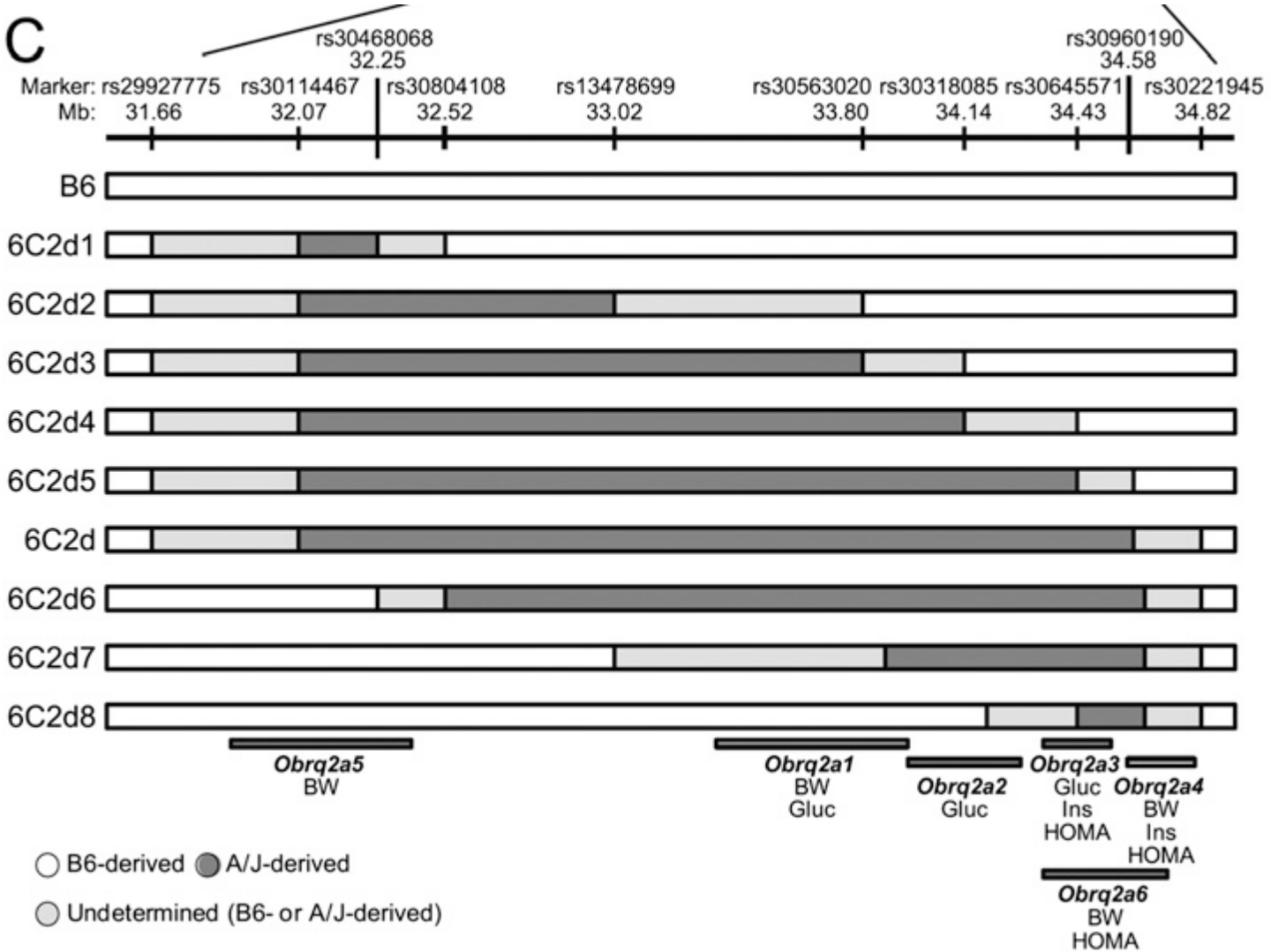
Strain	Final body weight (g)	Difference in body weight (g)	p value	Phenotype
CSS-6	37.7	-11.4	<0.001.	lean
92A	41.0	= B6	not sig.	obese
62BL	33.8	- 7.2	$< 10^{-5}$	lean
108A	42.6	+ 8.8	$< 10^{-8}$	obese
109A	38.4	- 4.2	$< 10^{-4}$	lean
115A	40.4	+ 2.0	< 0.03	obese
54B	38.2	- 2.2	< 0.03	lean

Many QTLs with large and contrasting effects

Also found for other traits and chromosomes


Chromosome 6
Obrq2
rs30656506
17.18
rs30468068
32.25
Marker: rs13478633 D6MIT264 rs30855317 rs30517635 D6MIT159 rs13478699 rs30569437
Mb: 12.20 16.61 17.48 23.37 29.70 33.02 43.39
Body Weight (g)
p value
Compared to
B6
44.33 ± 0.56 (n = 101)
ns
6C1
6C1
41.63 ± 0.62 (n = 67)
ns
6C2a
6C2a
40.30 ± 1.31 (n = 26)
0.028
6C2
6C2
36.86 ± 0.56 (n = 58)
9.87 x 10-7
6C2b
6C2b
42.79 ± 0.96 (n = 46)
0.018
6C2c
6C2c
37.44 ± 0.70 (n = 30)
ns
6C2d
6C2d
38.29 ± 1.09 (n = 32)
0.002
B6
Obrq2d
Obrq2c
Obrq2b
Obrq2a


A
Chromosome 6
Obrq2
B
Marker:
Mb:
rs13478633 12.20
D6MIT264 16.61
rs30656506 17-18
rs30855317 17.48
rs30517635 23.37
D6MIT159 29.70
rs30468068 32.25
rs13478699 33.02
rs30569437 43.39
Body Weight (g)
p value
Compared to
B6 44.33 ± 0.56 (n = 101) ns 6C1
6C1 41.63 ± 0.62 (n = 67) ns 6C2a
6C2a 40.30 ± 1.31 (n = 26) 0.028 6C2
6C2 36.86 ± 0.56 (n = 58) 9.87 x 10-7 6C2b
6C2b 42.79 ± 0.96 (n = 46) 0.018 6C2c
6C2c 37.44 ± 0.70 (n = 30) ns 6C2d
6C2d 38.29 ± 1.09 (n = 32) 0.002 B6
Obrq2c
Obrq2b
Obrq2d
Obrq2a


C
rs30468068 32.25
rs30960190 34.58
Marker: rs29927775 rs30114467 rs30804108 rs13478699 rs30563020 rs30318085 rs30645571 rs30221945
Mb: 31.66 32.07 32.52 33.02 33.80 34.14 34.43 34.82
B6
6C2d1
6C2d2
6C2d3
6C2d4
6C2d5
6C2d
6C2d6
6C2d7
6C2d8
Obrq2a5 BW
Obrq2a1 BW Gluc
Obrq2a2 Gluc
Obrq2a3 Gluc Ins HOMA
Obrq2a4 BW Ins HOMA
Obrq2a6 BW HOMA
B6-derived
A/J-derived
Undetermined (B6- or A/J-derived)

Fractal Genetics

Strain	QTL size (Mb)	# of genes	Effect size
A/J	2717	22,974	100%
CSSs	120	1,485	75%
Congenics	28	342	58%
Subcongenics	15	135	52%
Subsubcongenics	1	4	39%

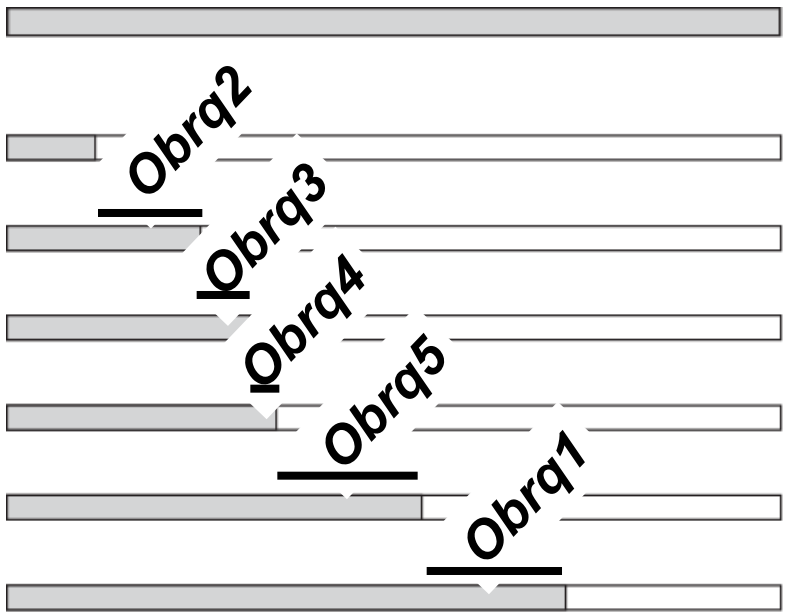
3,000-fold reduction in QTL size

5,000-fold reduction in gene content

2.5-fold reduction in effect size

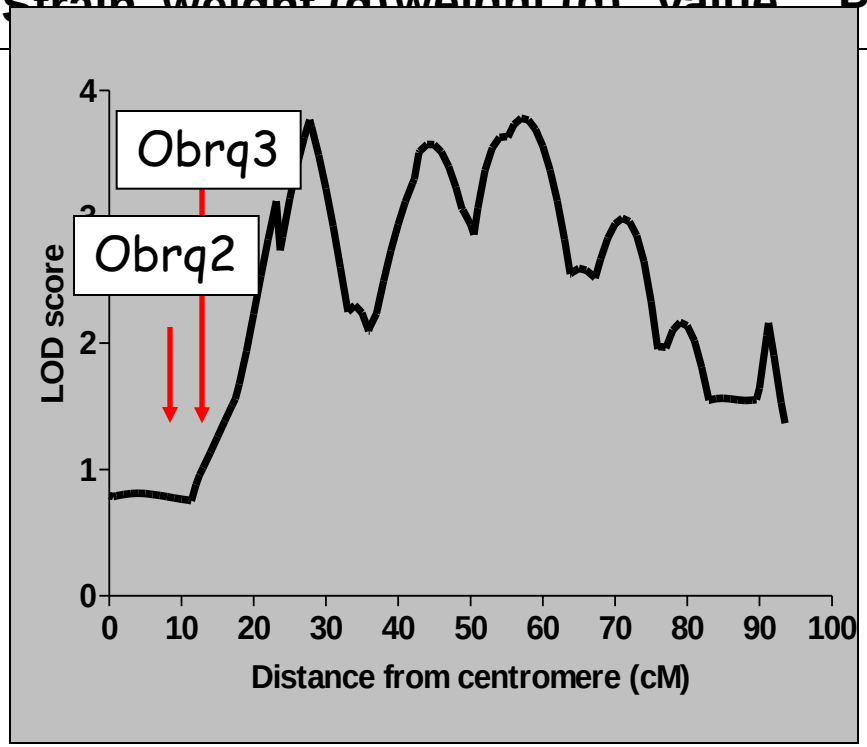
QTLs in congenic strains but not in crosses

Chr. 6 congenics



B6-derived sequence

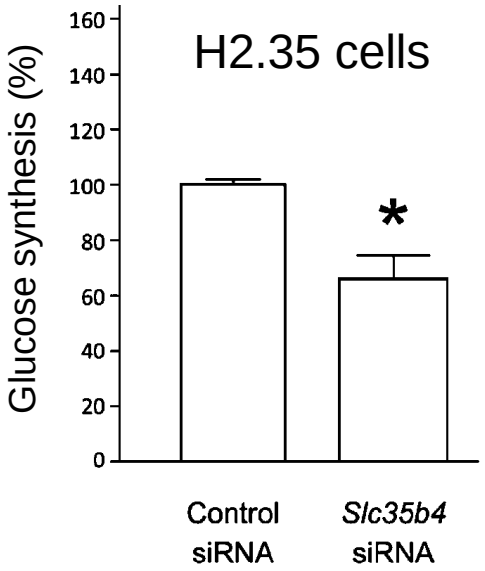
A/J-derived sequence



Strain	Final body weight (g)	Difference in body weight (g)	p value	Phenotype
				lean
				obese
				lean
				obese
				lean
				obese
				lean

Slc35b4 regulates body weight and glucose homeostasis

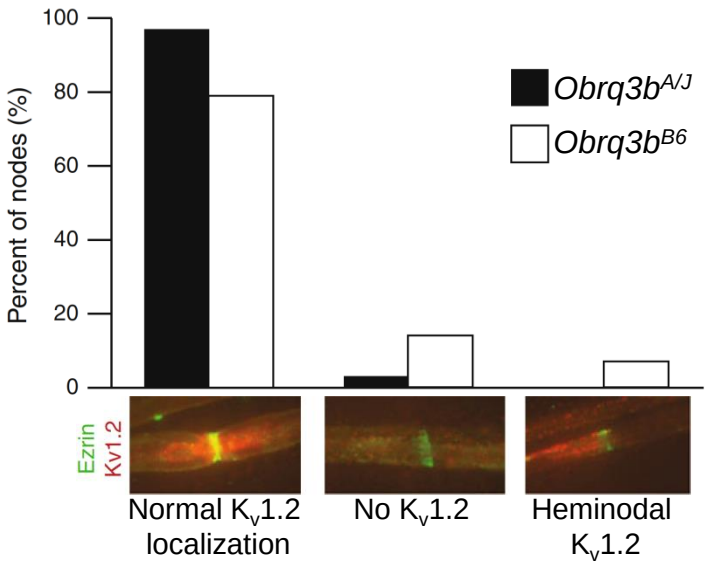
- *Obrq2a1*:
- 1 Mb interval on Chr. 6 (33-34 Mb)
- Phenotype:
  - Body weight differs on high-fat diet
    - 4 g, 9% of total body weight
  - Fasting glucose
  - Hepatic glucose production



- Genetics:
  - 3 genes located in QTL interval (*Exoc4*, *Lrguk*, *Slc35b4*)
  - No amino acid variants
  - Decreased hepatic *Slc35b4* expression associated with lower hepatic gluconeogenesis
    - Expression of all genes tested by qPCR in liver, pancreas, brain, WAT, muscle
  - *Slc35b4* knockdown in H2.35 decreases glucose synthesis *in vitro*

Juxtaparanodal proteins CNTNAP2 and TAG1

- *Obrq3b*:
- 3 Mb interval on Chr. 6
- Phenotype:
  - Body weight differs on high-fat diet
    - 5 g, 14% of total body weight
- Genetics:
  - 1 gene located in QTL interval (*Cntnap2*)
  - Missense mutation in evolutionarily conserved residue
    - H538Q
  - TAG1 and CNTNAP2 are both required for localization of K_v channels at juxtaparanodes
    - Impaired localization of juxtaparanodal K_v1.2 in *Obrq3b*^{B6}
    - Tag1 knockout mice were also found to be obesity-resistant



Three parts

Fractal genetics and gene discovery

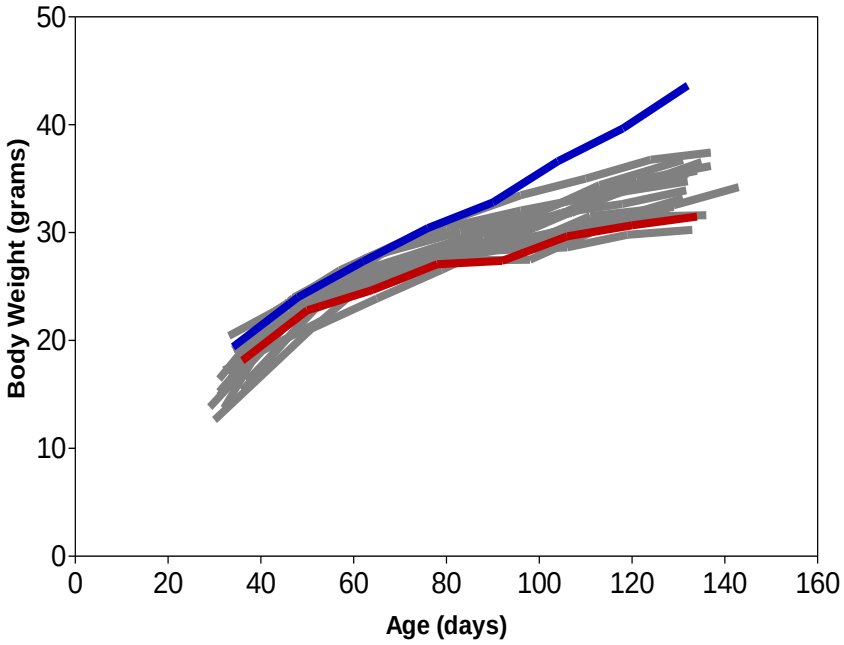
Epistasis and context-dependent effects

Usually tests for pairwise effects

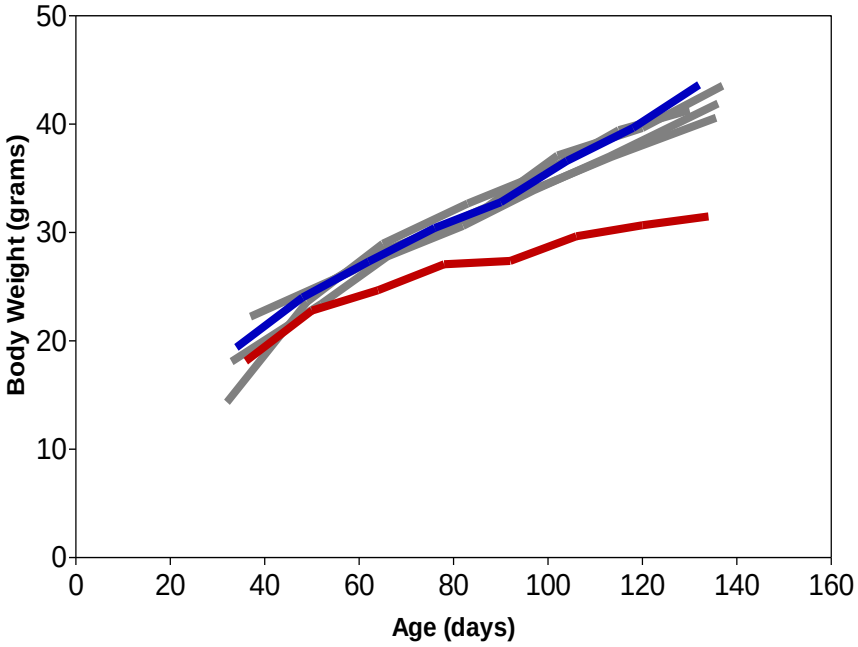
Transgenerational effects,
heritable epigenetic changes,
ancestral genetics,
and current disease risks

Too many CSSs have too large effects

18 obesity-resistant



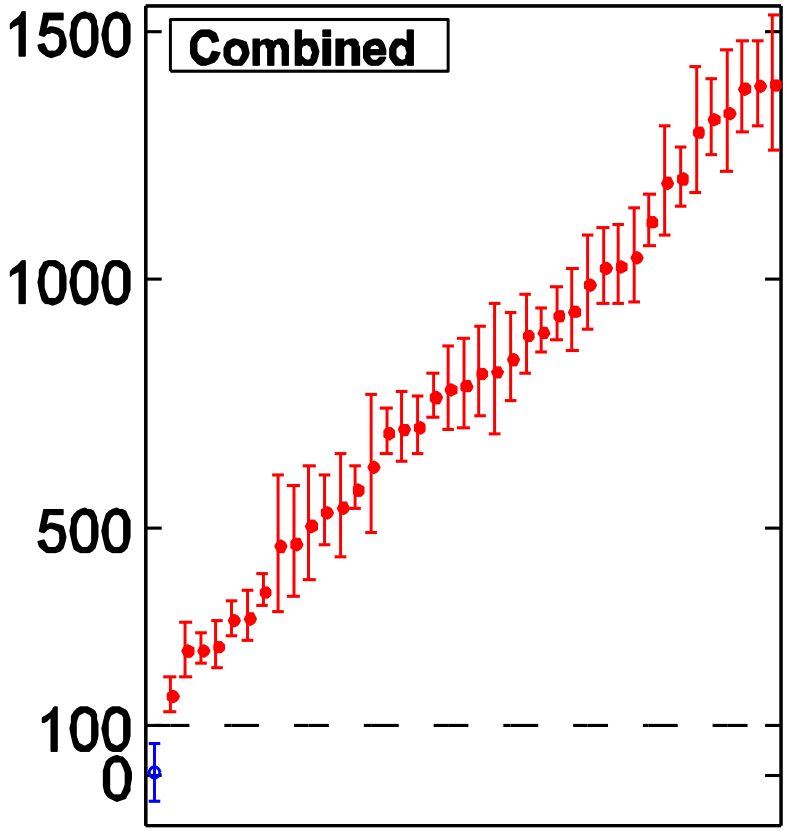
4 obese



Many CSSs are indistinguishable from A/J

Ave effect is 76% of the parental difference

Highly non-additive effects



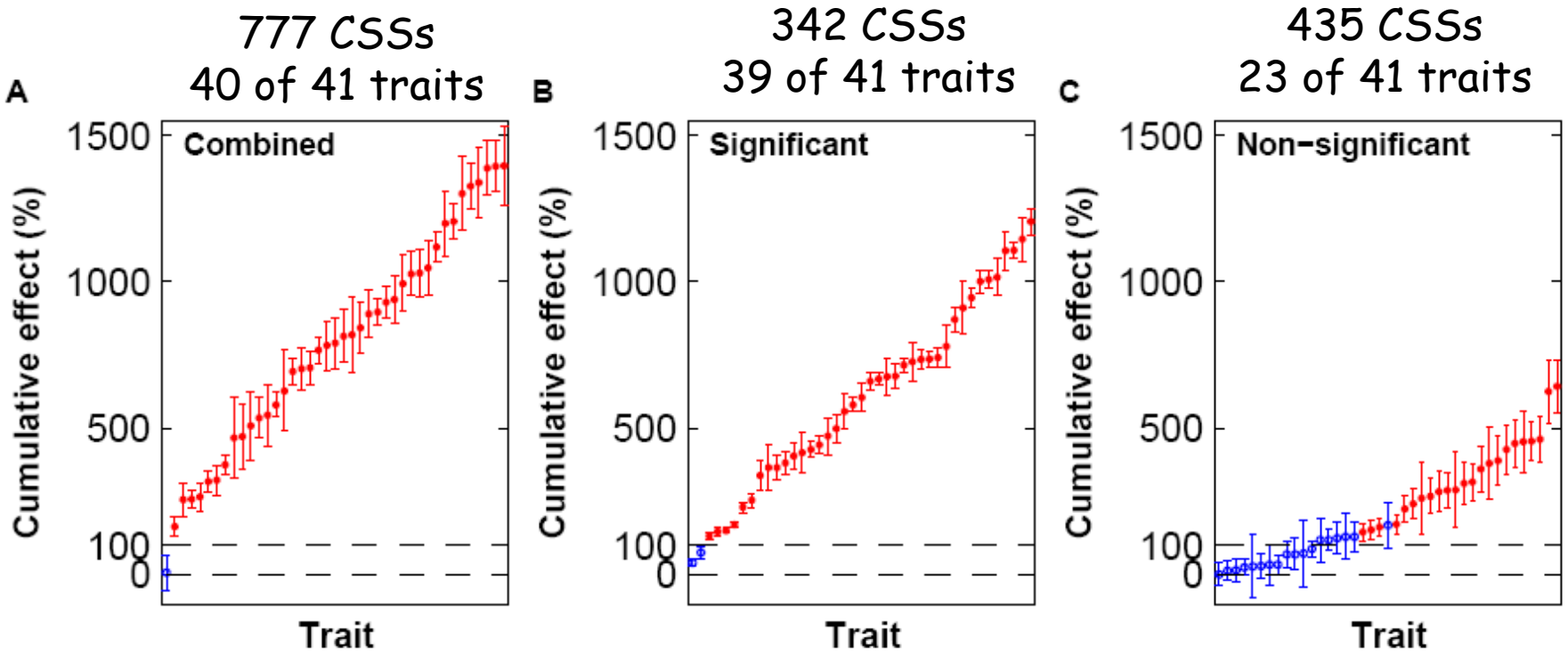
Sum of *signed* effects for *all* CSSs for each trait

If additive: sum ≤ 100%

If epistasis: sum > 100%

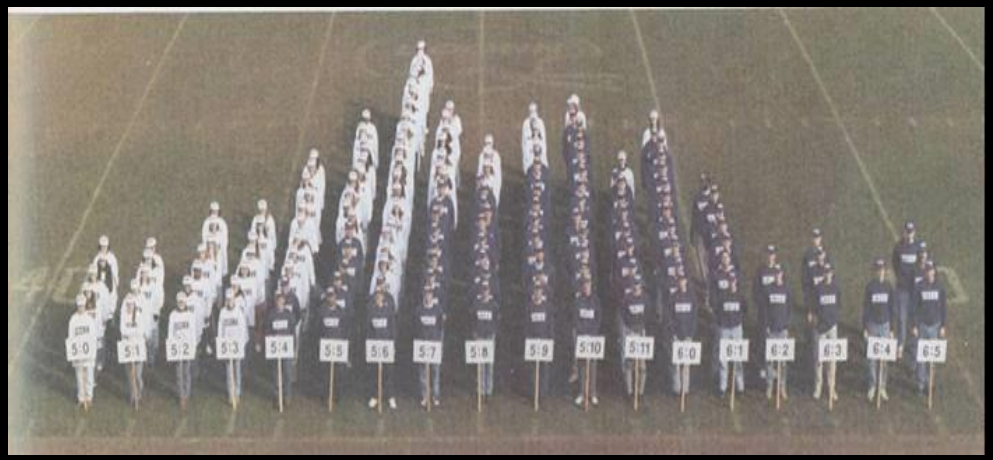
40 of 41 traits
Median cumulative effect: 803%
Range: 164% - 1,397%

Highly non-additive effects



9 CSSs affect cholesterol level on regular diet
Their average effect is 100% of the A/J – B6 difference
But A/J has all 9 genetic variants!

Reconciliation



Average effects

Individual effects

Model

Epistasis is pervasive

Organisms are non-random combinations of genetic variants that provide sufficient functions to survive and breed

Epistasis buffers physiological systems against environmental and genetic perturbations

Disease can result from dysfunctions in these networks of interacting genes

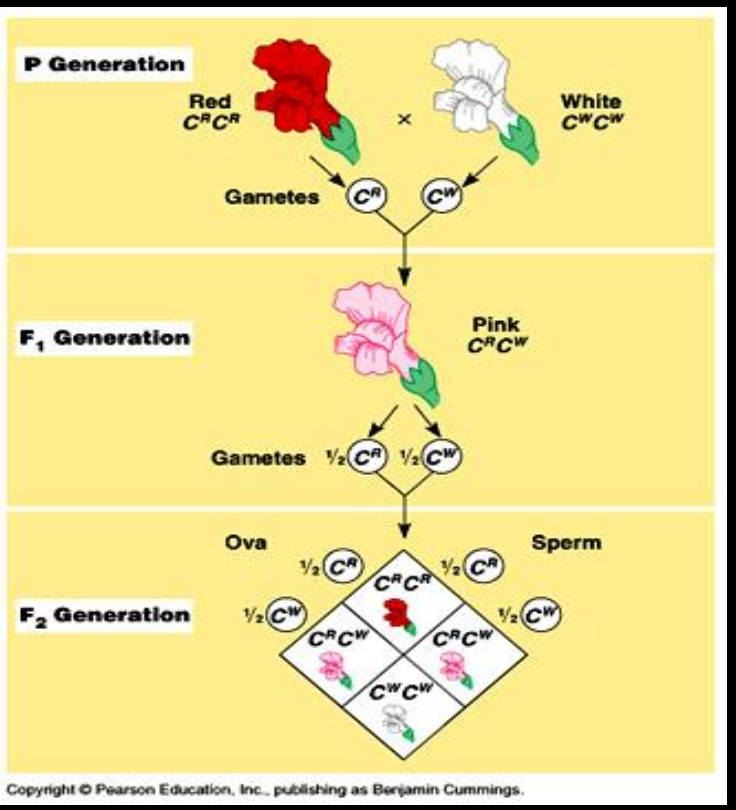
Three parts

Fractal genetics and gene discovery

Epistasis and context-dependent effects

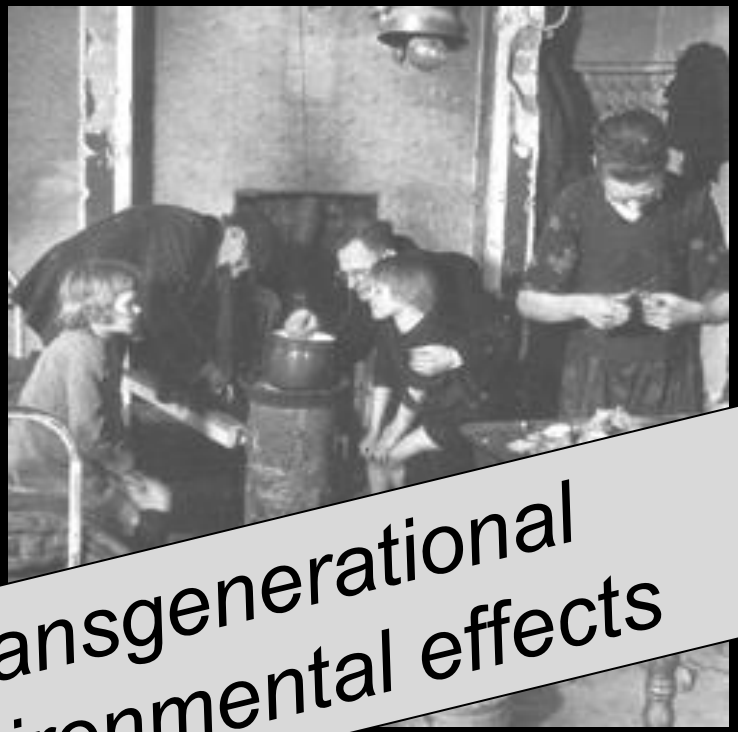
Transgenerational effects,
heritable epigenetic changes,
ancestral genetics,
and current disease risks

"Missing heritability"



Mendel's laws of inheritance

genotype – phenotype association
within individuals
is the foundation of most genetic studies



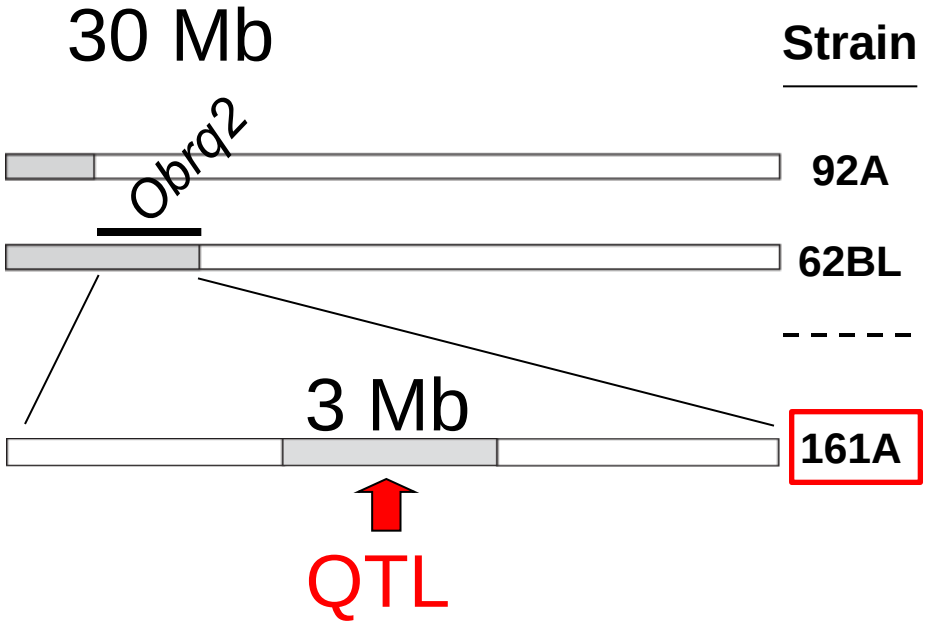
Transgenerational environmental effects

Transgenerational genetic effects

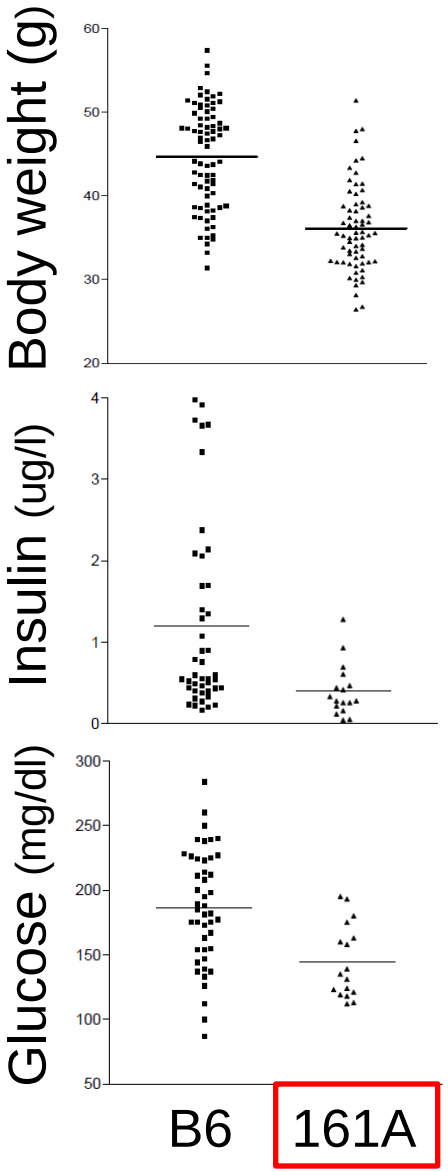
phenotypes and disease risk
result from genetic variants
in previous generations

Genetic origins, heritable and familial,
but genetic variants
are not in affected individuals

A QTL for transgenerational studies



8 of the 12 genes in the 161A interval maintain histone methylation in sperm



p < 0.0001

p < 0.001

p < 0.01

Parental effects on diet-induced obesity

(B6 x 161A)F1 x (B6 x 161A)F1

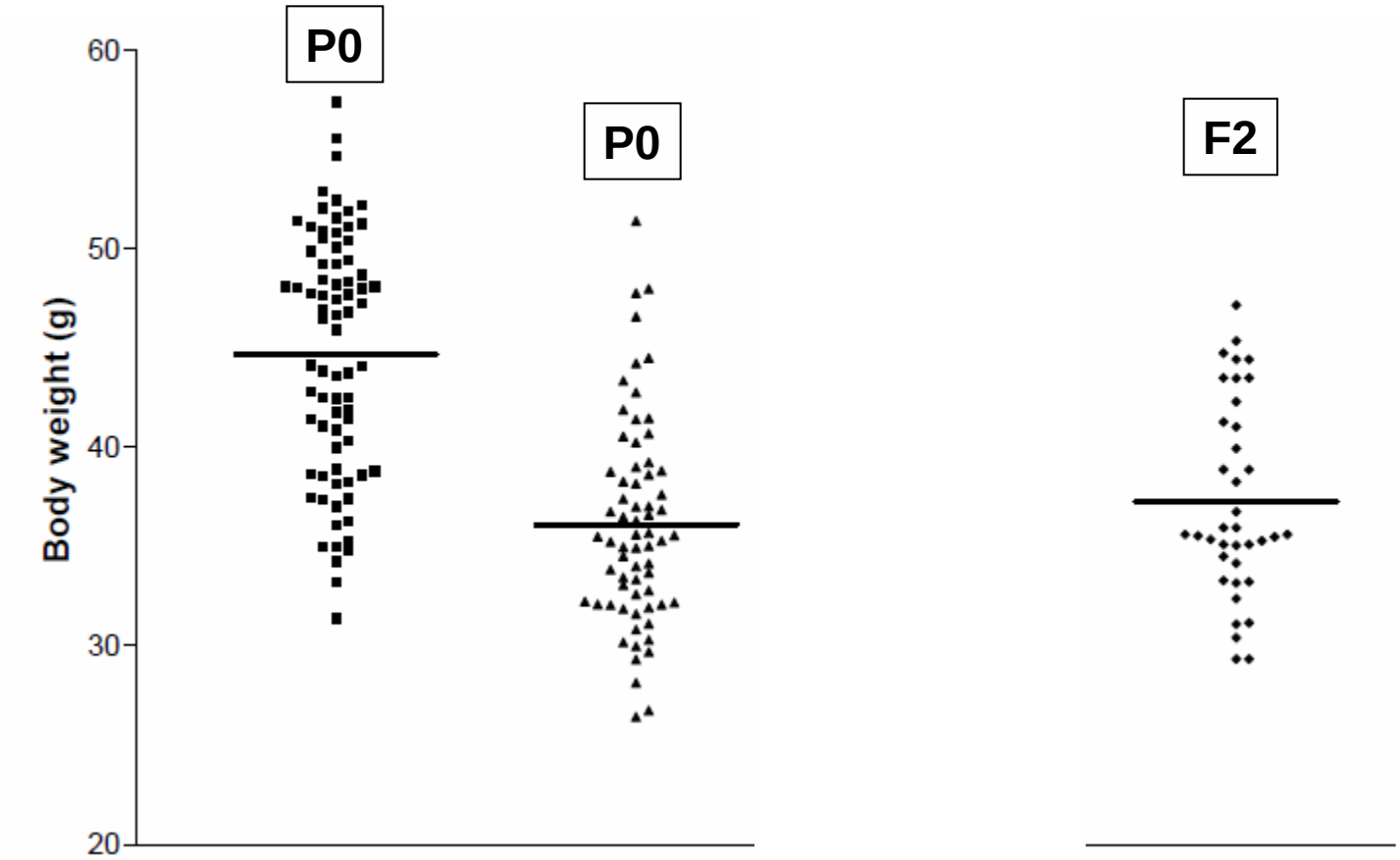
B6 ~~B6/161A~~ 161A

obese *lean*

if no transgenerational effects

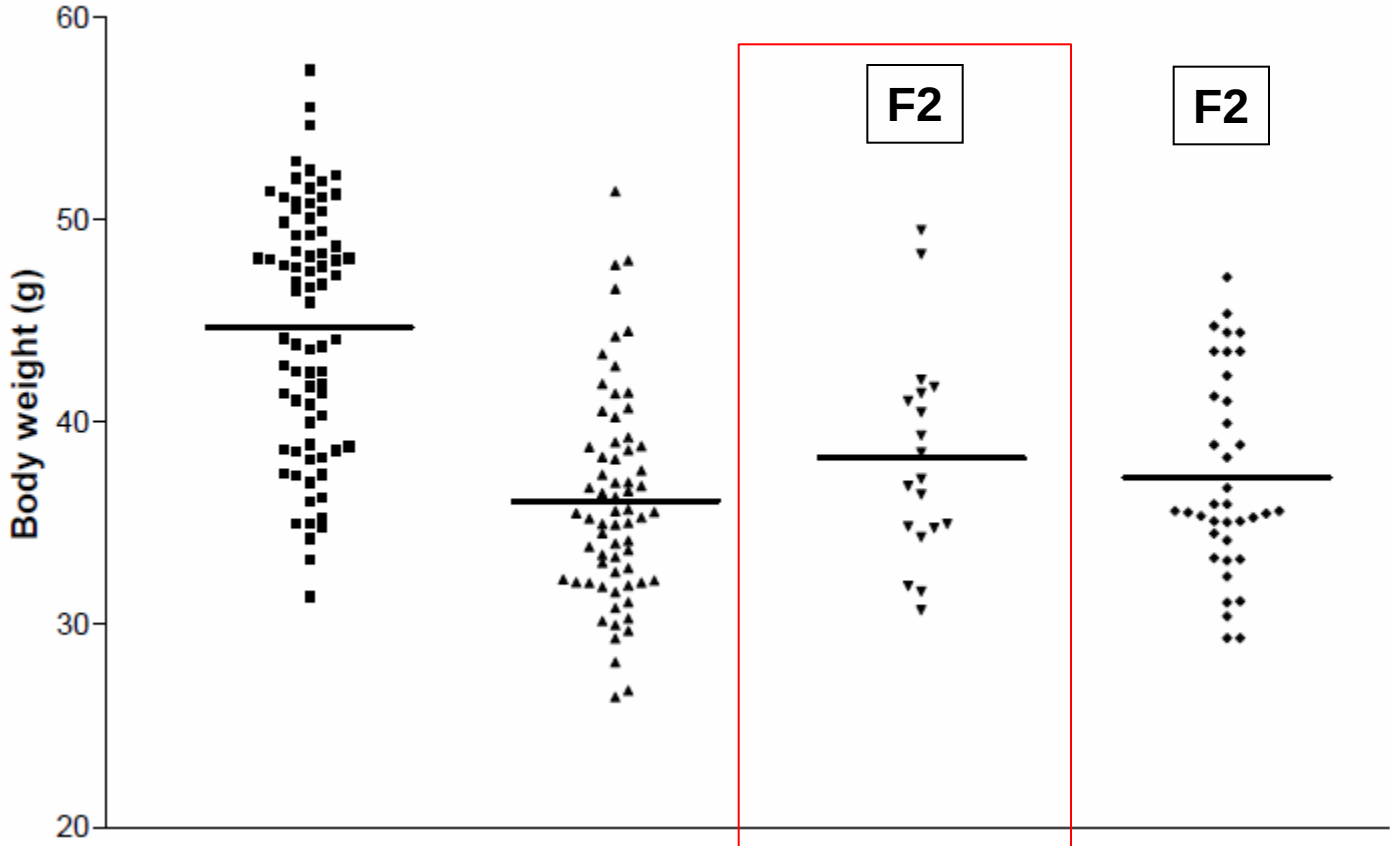
Breeders on standard diet
Test mice on high-fat diet

Transgenerational inheritance



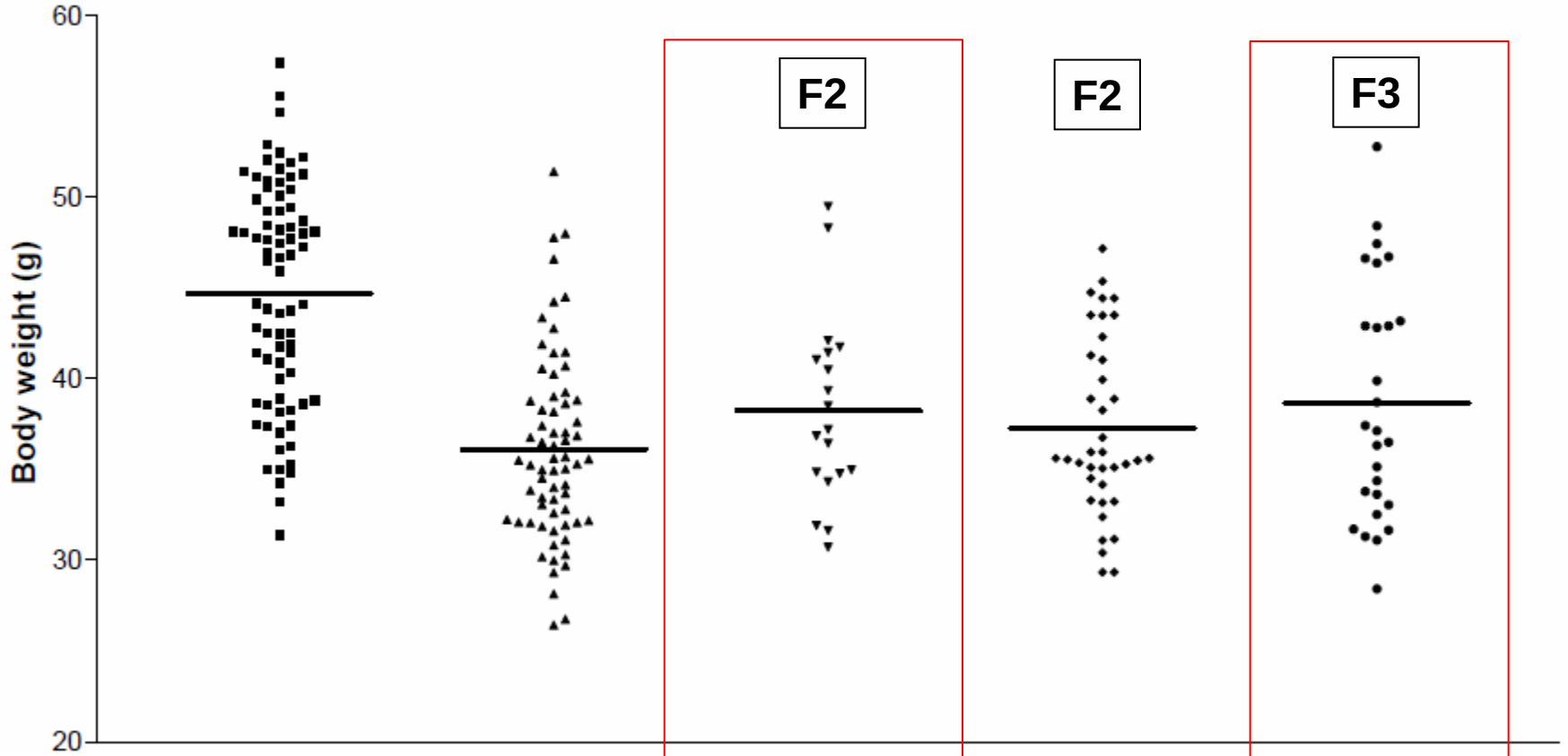
Genotype:	**B6**	**161A**	**161A**
Parents:	**B6**	**161A**	**(B6x161A)F1**
p value: (relative to B6)		**$< 10^{-8}$**	**< 0.0005**

Transgenerational inheritance to sons



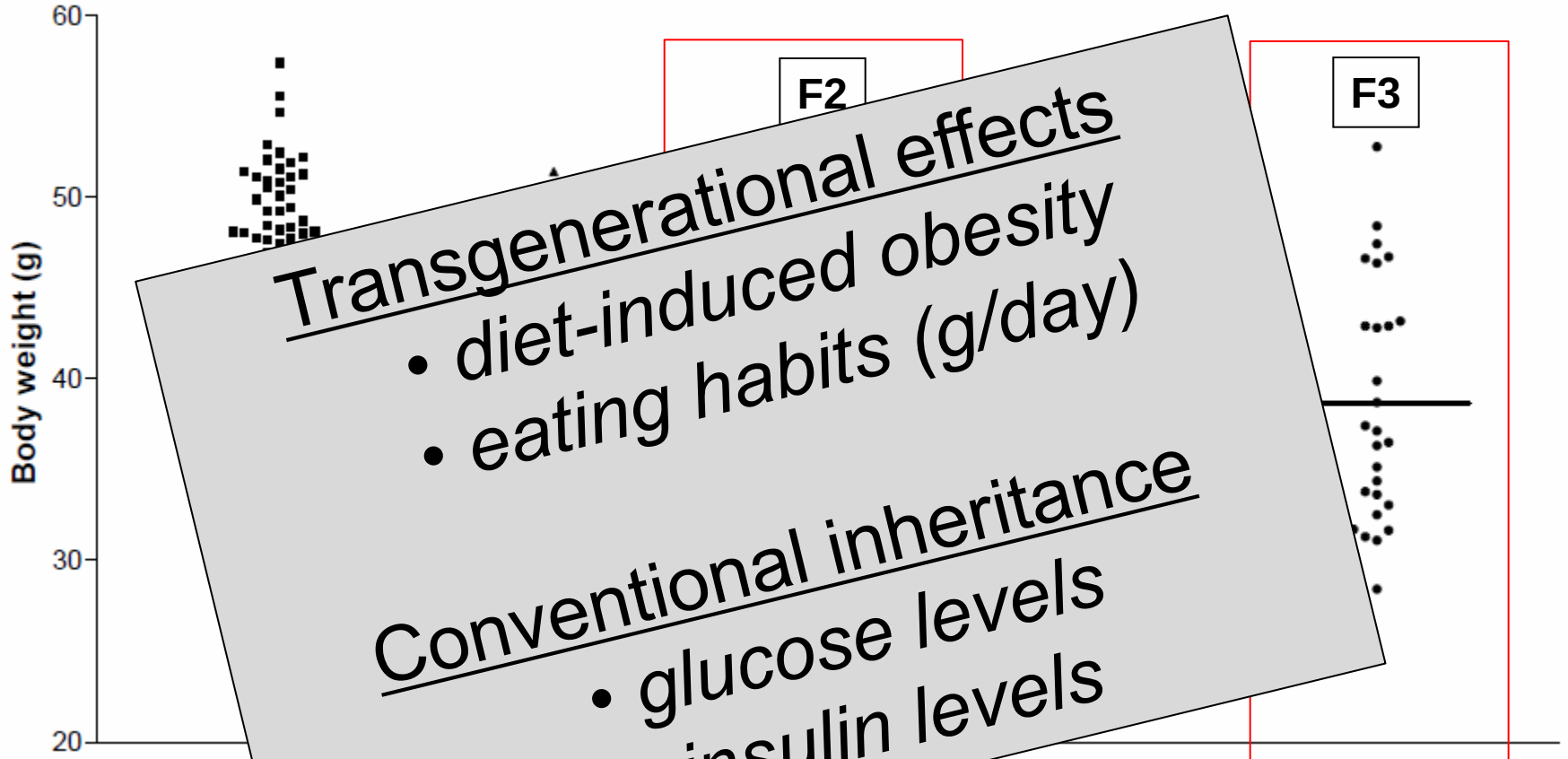
Genotype:	**B6**	**161A**	**“B6”**	**161A**
Parents:	**B6**	**161A**	**(B6x161A)F1**	**(B6x161A)F1**
p value: (relative to B6)		**$< 10^{-8}$**	**< 0.0005**	**< 0.0005**

Transgenerational inheritance to grandsons



Genotype:	**B6**	**161A**	**“B6”**	**161A**	**“B6”**
Parents:	**B6**	**161A**	**(B6x161A)F1**	**(B6x161A)F1**	**“B6”**
p value: (relative to B6)		**$< 10^{-8}$**	**< 0.0005**	**< 0.0005**	**< 0.0001**

Transgenerational inheritance to grandsons



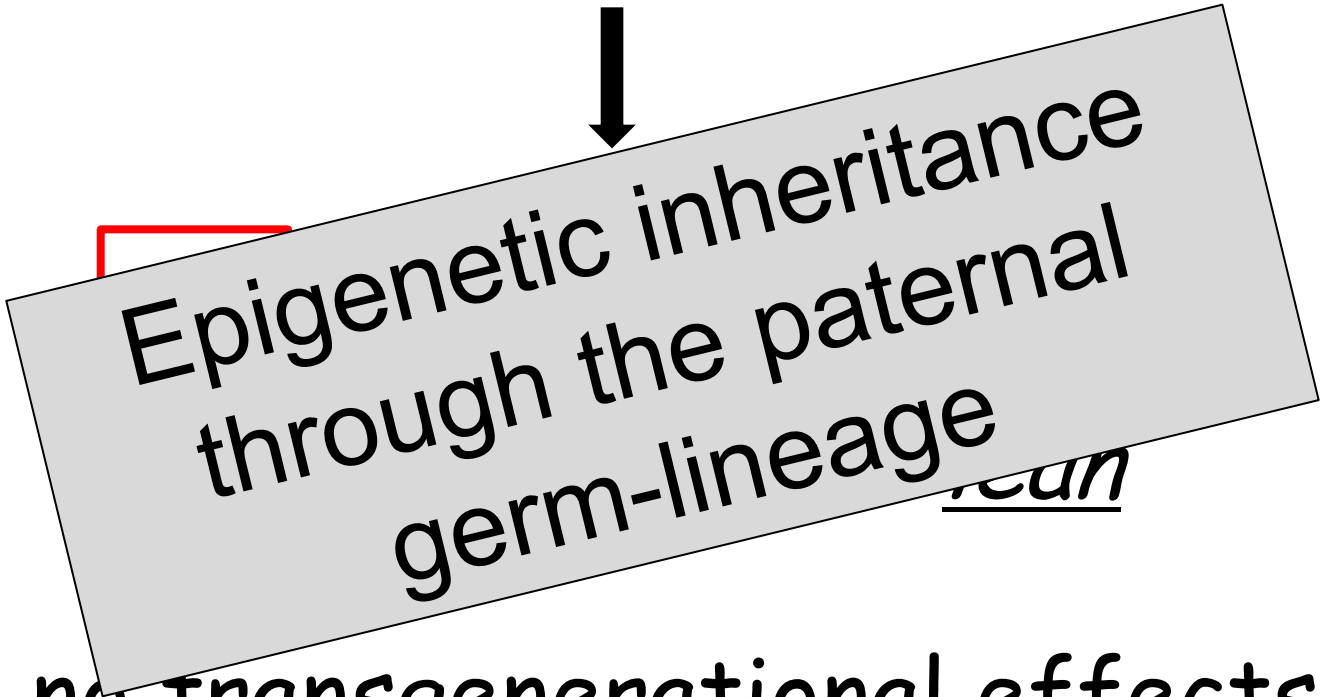
Transgenerational effects
- diet-induced obesity
- eating habits (g/day)

Conventional inheritance
- glucose levels
- insulin levels

Genotype:				161A	“B6”
Parents:	B	161A	(B6x161A)F1	(B6x161A)F1	“B6”
p value: (relative to B6)		$< 10^{-8}$	< 0.0005	< 0.0005	< 0.0001

Parental effects on diet-induced obesity

(B6 x 161A)F1 x (B6 x 161A)F1



if no transgenerational effects

Breeders on standard diet
Test mice on high-fat diet

Other examples of transgenerational genetic effects

Paternal Y chromosome effect on daughter phenotypes:

common and strong effects
paternal germ-lineage

Testicular cancer:

strong effects
enduring effects
maternal germ-lineage
reversed with paternal transmission

Persistence of memory

Environment Genetics

↘ ↙

Physiological stress

↓

Homeostatic and epigenetic response in soma

↓

Heritable epigenetic changes in germline

Genetic questions

1. The germline molecule that's not DNA

2. Mechanisms
   - *initiating epigenetic changes*
   - *changes in the germline*
   - *transducing changes in next generation*
   - *reversing epigenetic changes*

3. Embedding a lifetime of genetic and environmental exposures in epigenetic code

Computational questions

1. Rules for epigenetic inheritance
   interpreting, modeling, predicting

2. Testing for associations
   epigenetics and phenotypes
   epigenetics and genotypes

3. Distinguishing causes of variation
   genetics, environment, epigenetics

Three parts

Fractal genetics

Epistasis

Transgenerational genetic effects

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Jennifer Zechel
Steph Doerner
John Giesinger
Soha Yazbek
David Buchner
Ghunwa Nakouzi
Paola Raska
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Elaine Leung

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NCI and NIH Pioneer Award

Ευχαριστώ

Thank you