Why Mouse?

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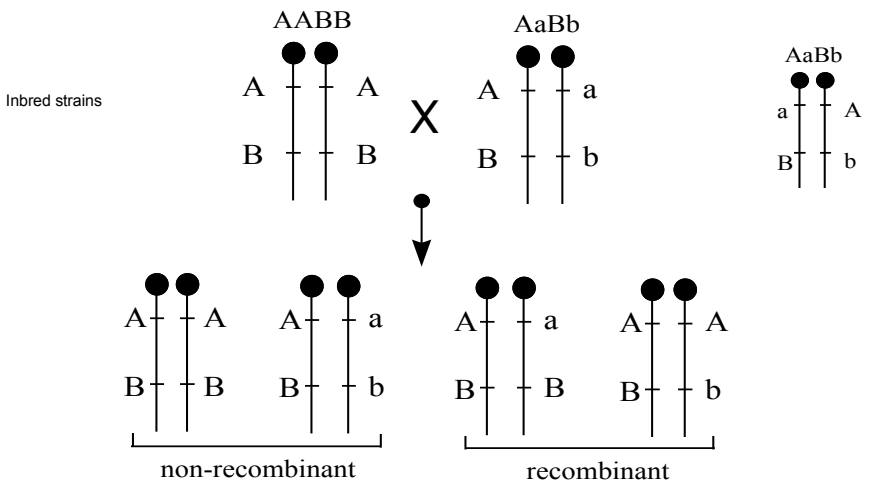
Comparative Genomics

1. Biology (mouse:man)
   - what is phenotype?
   - can mouse and human phenotypes be "the same"?
   - functions of a genome: development – metabolism – aging
   - evolution ("development" of species)

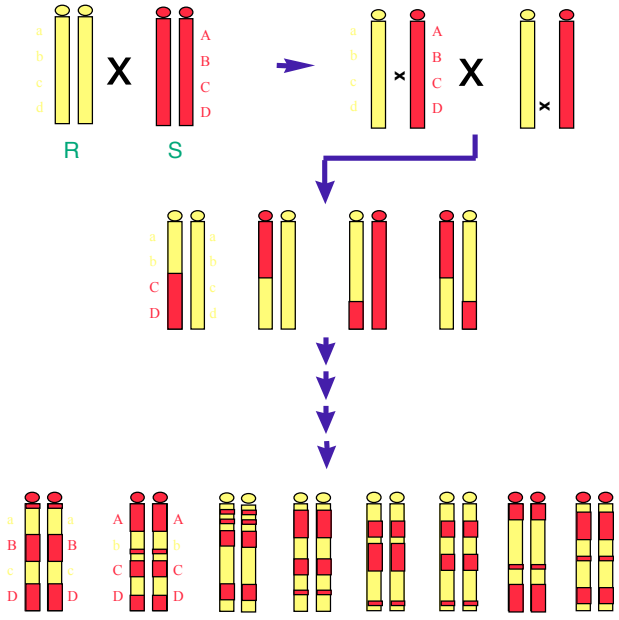
2. Learning from evolution
   - comparative sequence
   - comparative structure/ process

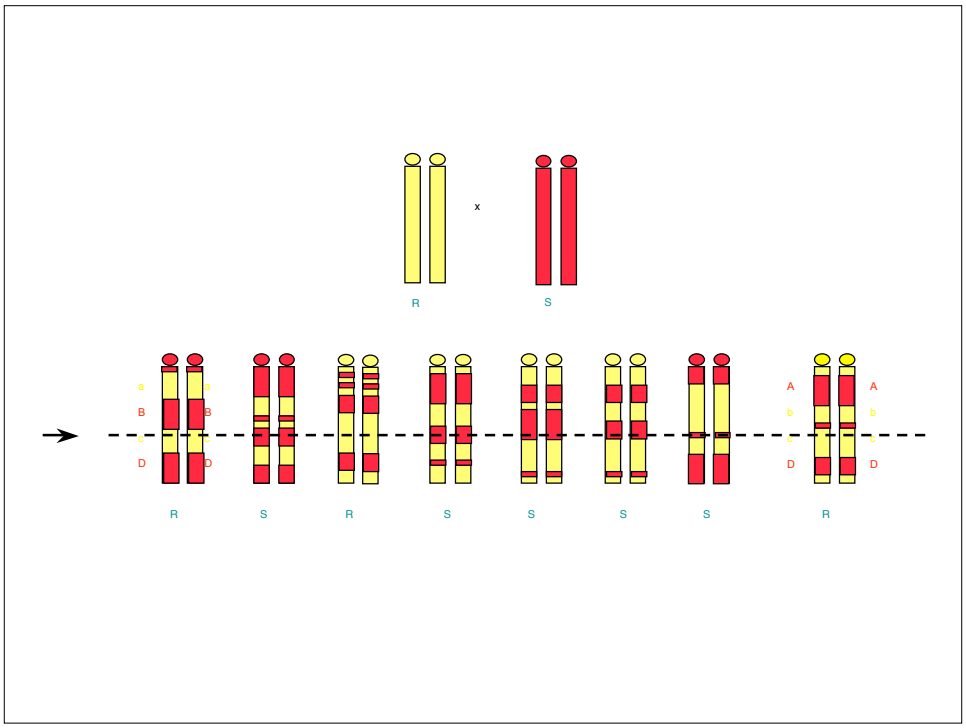
3. Why mouse?
   - genetics – ***unique genetic structures not found in nature***
   - ***ability to create new genotypes and phenotypes***
     - mutagenesis
     - transgenesis/ targeted gene modification/ chromosome engineering
   - real genetics - QTL/ modifiers

1. Special Genetic Structures in Mice



2. Recombinant inbred mouse strains – resource for genotype and phenotype.





Strain distribution patterns for Ctla6 and other loci on Chr 14 in the BXD RI strain set.

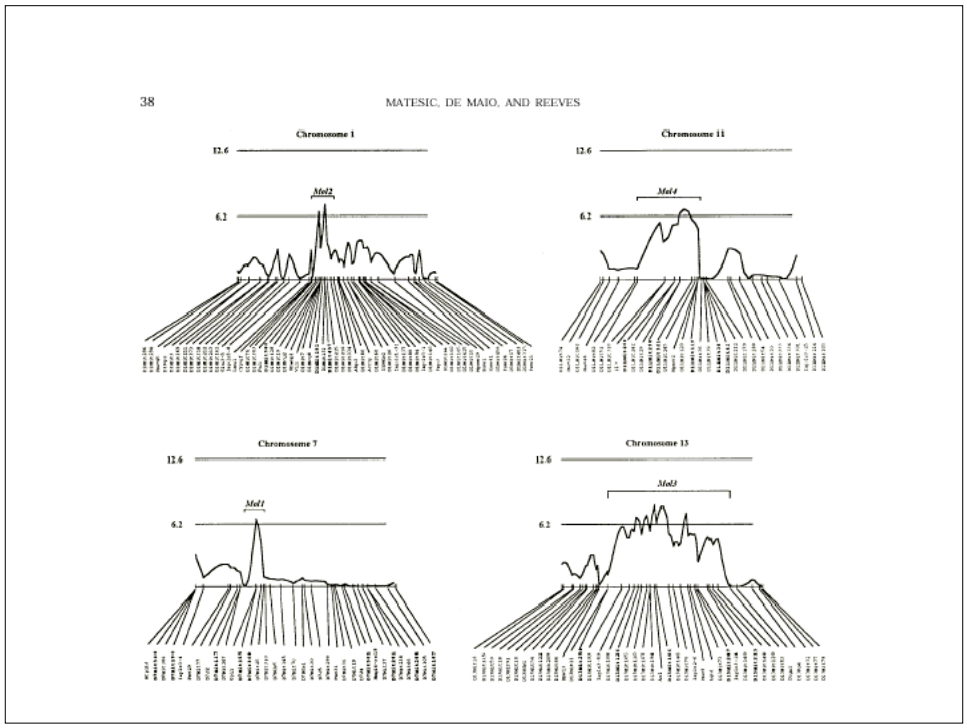
Locus	1	2	5	6	8	9	11	12	13	16	18	22
Np2	B	D	B	D	D	D	D	D	B	B	B	B
Tcra	B	D	B	D	D	D	D	D	B	B	B	B
Rib1	B	D	B	D	D	D	D	D	B	B	B	B
												x
Ctla6	B	D	B	D	D	D	D	D	B	B	B	D
								x		**x**	**x**	
Rb1	B	D	B	D	D	D	D	B	B	D	D	D
Es10	B	D	B	D	D	D	D	B	B	D	D	D

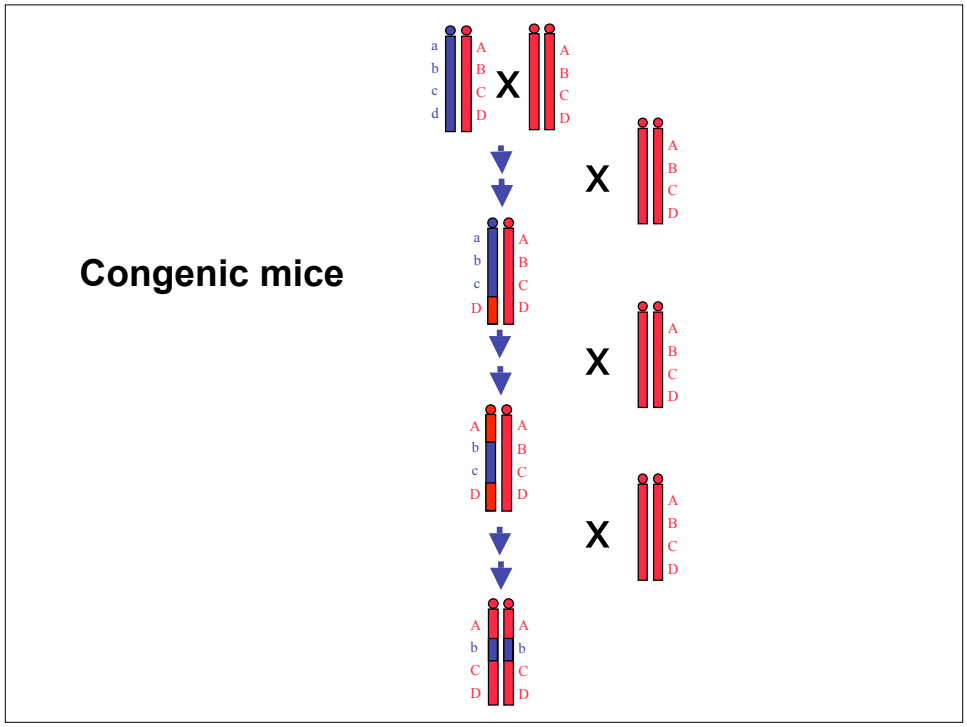
Benefits of RI strains:

- "Pre-genome scan"
- Reassay the "same" individual many times (find true mean and deviation for variable traits)
- Highly beneficial for quantitative traits
- Stock of genetic variation (recombinant congenic mice)

Limitations of RIs:

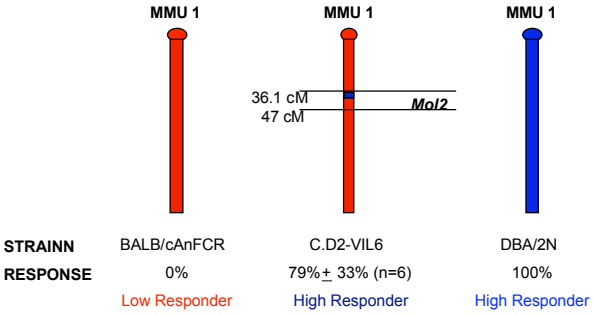
- Small strain sets have limited statistical power.
- Mapping is relatively low resolution on the first pass.


38
MATESIC, DE MAIO, AND REEVES
Chromosome 1
12.6
6.2
Mel2
Chromosome 11
12.6
6.2
Mel4
Chromosome 7
12.6
6.2
Mel1
Chromosome 13
12.6
6.2
Mel3


Congenic mice
a b c d
A B C D
X
A B C D
X
A B C D
a b c D
A B C D
X
A B C D
A b c D
A B C D
X
A B C D
A b C D
A b C D

Congenic mice can be used to confirm and refine localizations made with other approaches. Further, they demonstrate a contribution by an individual locus to a phenotype.

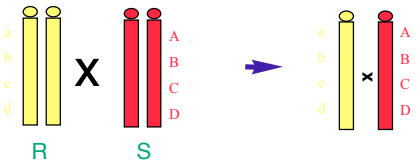
Independent confirmation of *Mol2* on Chr1

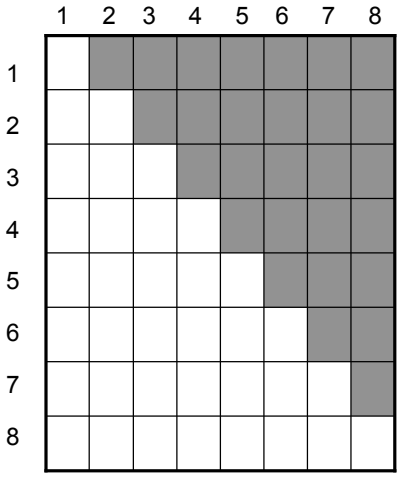


Matesic, L.E., A. De Maio, R.H. Reeves. 1999. Mapping LPS Response Loci in Mice Using Recombinant Inbred and Congenic Strains. Genomics 62:34-41.

Increased resolution from RI mapping

1. RIX - Threadgill

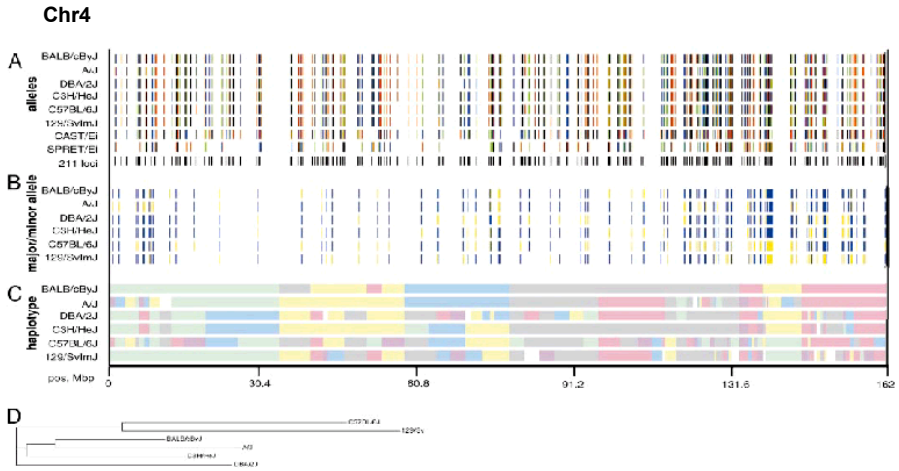




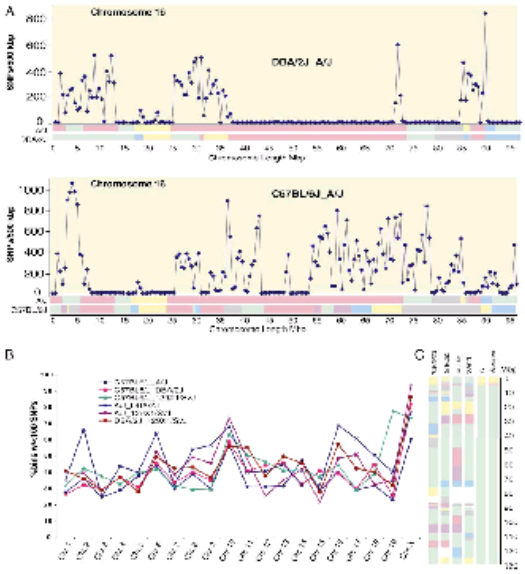
2. Complex Trait Consortium

- 8-way cross
- 2048 RI strains (256 on the shelf)
- www.complextrait.org/Workshop1.pdf

Haplotype structure in the world of inbred mice



Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/



Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/

Mapping with haplotypes: Tyr

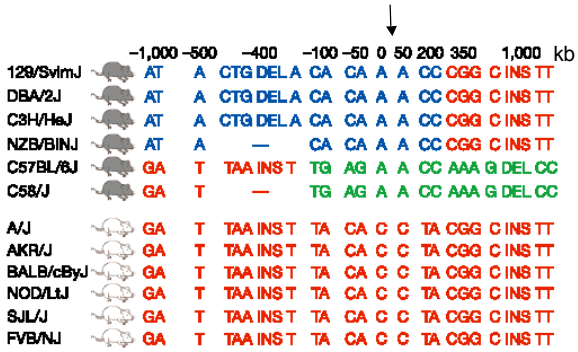


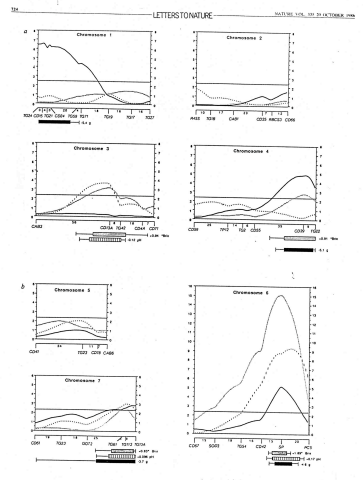
Figure 5 Association between a single haplotype and the albinism phenotype caused by a mutation at the tyrosinase locus[20]. Columns show SNPs discovered in ten 500-bp assays with positions (kb) relative to the centre of the genomic segment containing the gene (GenBank accession GI:12852585). The causal mutation (Cys103Ser) is located at +32.6 kb. The association between phenotype and ancestral haplotype for 12 strains would be sufficient to identify a haplotype background and 'critical region' of ~500 kb (including the assays from −100 kb before Tyr to 200 kb after Tyr) likely to contain the albinism mutation.

Wade et al., Nature 420:574-578 (2002)

Complex traits / polygenic traits / QTL/ genetic modifiers

Mapping traits determined by more than one gene

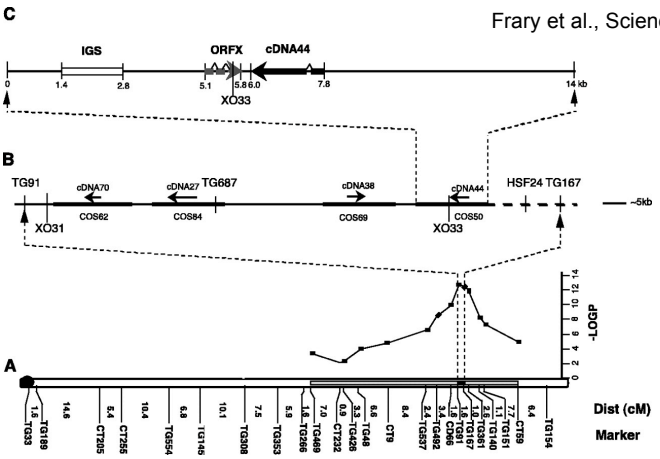
Many traits are influenced by more than one locus, a condition that can't be mapped using standard pedigree analysis. Experiments with maize and *Lycopersicon* demonstrate an approach to this problem that can be used to map such **quantitative trait loci**, or **QTLs**, using gene dense maps spanning the entire genomes of experimental organisms. This figure demonstrates linkage to three quantitative traits in tomato.



"PURE" positionally cloned QTL in *lypersicum*

Fig. 2. High-resolution mapping of the fw2.2 QTL. (A) The location of fw2.2 on tomato chromosome 2 in a cross between L. esculentum and a NIL containing a small introgression (gray area) from L. pennellii [from (8)]. (B) Contig of the fw2.2 candidate region, delimited by recombination events at XO31 and XO33 [from (8)]. Arrows represent the four original candidate cDNAs (70, 27, 38, and 44), and heavy horizontal bars are the four cosmids (cos62, 84, 69, and 50) isolated with these cDNAs as probes. The vertical lines are positions of restriction fragment length polymorphism or cleaved amplified polymorphism (CAPs) markers. (C) Sequence analysis of cos50, including the positions of cDNA44, ORFX, the A-T-rich repeat region, and the "rightmost" recombination event, XO33.

Frary et al., Science 289:85 (2000).



correspondence

From QTL to gene: the harvest begins

In the past decade, quantitative trait locus (QTL) mapping has identified hundreds of chromosomal regions containing genes affecting asthma, atherosclerosis, diabetes, hypertension, obesity and other complex phenotypes. The ultimate goal of QTL mapping is to identify the genes underlying these polygenic traits and to gain a better understanding of their physiology and biochemistry. But identifying the QTL genes has been slow and difficult. A commentary in *Nature Genetics* questioned the value of QTL mapping and proposed that mutagenesis strategies offer more promise for identifying the genes determining complex traits[1]. That pessimistic opinion was premature; we herein report that 29 QTL genes have been identified, almost half of them in 2001. We suggest that QTL mapping of complex traits is a promising technique and that the harvest of QTL genes is just beginning.

The 29 genes identified from mammalian QTL through the end of 2001 are from humans, mice, rats and pigs (Table 1; Fig. 1). We counted the genes identified for common complex diseases, whether the study in question used a quantitative measure (for example, bronchial hyperresponsiveness) or a qualitative evaluate (for example, clinically diagnosed asthma) to measure the trait. We counted any trait for which linkage was first found by QTL analysis or by human linkage studies showing several genes, even though the next stage of analysis involved reducing the trait to a mendelian pattern of inheritance by constructing a congenic strain (animal studies) or selecting only those families linked to a particular region (Alzheimer disease and breast cancer in human studies). These genes are related to many phenotypes, from cancer, asthma, obesity, diabetes and Crohn disease to hearing loss and meat quality. Some were identified because they were obvious candidates in a QTL region, whereas others required positional cloning (Table 1).

A total of only 8 genes were identified from 1991–1998, but 4 were identified in 1999, 7 in 2000 and 11 in 2001. This acceleration reflects the increasing availability of simple sequence length polymorphisms (SSLPs), which facilitated mapping; publication of the human and mouse genomic sequences, which eliminated the need to build contigs experimentally; and comparative genomics, which facilitated the identification of genes. The availability of microarray technology will probably accelerate QTL gene identification; already microarrays have been used to identify *CD36* and *C5* (*Hc*), genes underlying insulin resistance and asthma, respectively[2,3].

The analysis of QTL has also been made easier by recently developed statistical tools[4] for detecting gene–gene interactions. For example, gene association studies yielded conflicting results as to whether the vitamin D receptor affects bone density,

Table 1 • Genes identified from QTL studies

Polygenic trait	Year	Ref.	Gene	Species	pos	tg	ko	fu
Alzheimer disease	1991	9	*APP*	human				X
Alzheimer disease	1993	10	*APOE*	human				
Ovarian and breast cancer	1994	11	*BRCA1*	human	X			X
Breast cancer	1995	12	*BRCA2*	human	X			X
Insulin resistance	1995	13	*FABP2*	human				
HDL-cholesterol levels	1997	14	*LIPC*	human				
Intestinal cancer	1997	15	*Pla2g2a*	mouse	X	X		
Blood pressure	1998	16	*Atp1a1 / ATP1A1*	rat/human		X		X
Leptin levels	1999	17,18	*POMC*	human			X[a]	X
Asthma	1999	19	*Il4*	mouse	X	X		
Asthma	1999	19	*Il13*	mouse	X	X		
Insulin-mediated glucose uptake	1999	2	*Cd36*	rat		X		
Obesity	2000	20	*Ptpn1/PTPN1*	mouse/human			X[b]	X
Alzheimer disease	2000	21	*PSEN1*	human	X			
Diabetes	2000	22	*Il2*	mouse	X		X[b]	X
Gallstones	2000	23	*Abcc2*	mouse	X			X
Asthma	2000	3	*Hc*	mouse				
Muscle glycogen content	2000	24	*Prkag3*	pig	X		X[c]	X
Crohn disease	2001	25,26	*NOD2*	human	X		X[a]	X
Blood pressure	2001	27	*SCNN1A1*	human			X[a]	
Blood pressure	2001	28	*SCNN1G*	human			X[a]	
Blood pressure	2001	29	*Slc12a1*	rat				
Blood pressure	2001	30	*Cyp11b1*	rat				X
Bone density	2001	5	*COL1A*	human				
Left ventricular mass	2001	31	*Nppa*	rat			X[b]	X
Modifier of tubby hearing	2001	32	*Mtap1a*	mouse	X	X		X
Taste, saccharin response	2001	33	*Tas1r3*	mouse	X	X		X
Tumor susceptibility	2001	34	*Cdkn2a*	mouse	X		X[b]	X
Diabetes	2001	35	*B2m*	mouse		X	X	

pos, found by positional cloning; tg, transgenic insertion of normal gene changes phenotype to normal (for example, transgenic rescue); ko, knockout provides additional evidence (*human monogenic syndrome, [b]deletion of gene by homologous recombination produces a mouse with the phenotype typical of the disease, [c]knockout in yeast); fu, functional difference in candidate gene. *APP*, amyloid precursor protein; *APOE*, apolipoprotein E; *BRCA*, breast cancer gene; *FABP2*, fatty acid binding protein 2; *LIPC*, hepatic lipase; *ATP1A1*, α-Na,K-ATPase; *POMC*, pre-pro-opiomelanocortin; *Il*, interleukin; *Cd36*, fatty acid translocase; *PTPTB*, protein tyrosine phosphatase-1B; *PSEN*, presenilin 1; *Abcc2*, ATP-binding cassette, subfamily C2; *Hc*, hemolytic complement (*C5*); *Prkag3*, protein kinase, AMP-activated, γ3; *NOD2*, caspase recruitment domain-containing protein 15 (*CARD15*); *SCNN*, sodium channel, non-voltage gated; *Slc12a1*, Na,K,2Cl-cotransporter; *Cyp11b1*, 11β-hydroxylase; *COL1A*, collagen-1A; *Nppa*, natriuretic peptide precursor A; *Mtap1a*, microtubule-associated protein 1a; *Tas1r3*, taste receptor-3; *Cdkn2a*, cyclin-dependent kinase inhibitor 2a; *B2m*, β2-microglobulin.

Mapped QTLs

R Korstanje & B Paigen
Nature Genetics **31**, 235 - 236 (2002).

1. Genomic genetics- why is the genome complex?
How does it work (how do genes in many variant combinations in a population act together) to maintain homeostasis in the face of infinitely complex and dynamic challenges from the environment?
2. Plants have the lead. The tomato as a model for mammalian genetics.
Frary A, Nesbitt TC, Grandillo S, Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science. 2000. 289(5476):85-8.
3. Gene interactions

Genotype at *Hpi2*, Chromosome 5

Intercross analysis shows that Hpi1 is epistatic to Hpi2.

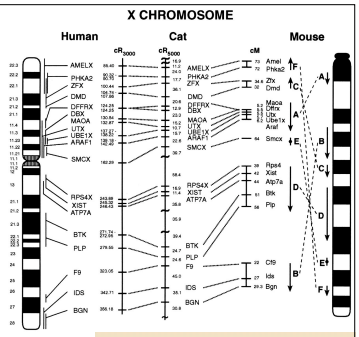
Genotype at *Hpi2*, Chromosome 5

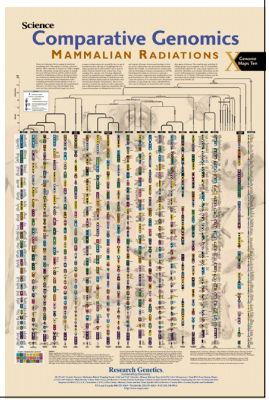
Genotype at *Hpi1*, Chromosome 13	A/A		A/B		B/B		Totals	
A/A	33.5 (9)	± 4.6	35.6 (12)	± 4.8	35.6 (8)	± 6.9	35.0	± 3.0
A/B	28.9 (11)	± 5.0	35.7 (40)	± 3.0	37.8 (11)	± 4.8	34.9	± 2.3
B/B[b]	42.5 (2)	± 4.1	44.7 (14)	± 5.3	69.9[c] (11)	± 5.5	54.8	± 4.3
Totals	32.0	± 3.2	37.6	± 2.3	49.0	± 4.3	39.5	± 1.9

[a] Avg. number of PMN per h.p.f. ± s.e. are given for (n) animals of each genotype class.[b] Mice with a B/B genotype at *Hpi1* showed significantly higher PMN infiltration values than other *Hpi1* genotypes (p=1.22 X 10-4, t-test assuming unequal variance)[c] Mice with a B/B genotype at both Hpi1 and Hpi2 showed significantly higher PMN infiltration than other genotype classes (p=7.83X10-5, t-test assuming unequal variance)
Matesic, LE, EL Niemitz, A De Maio, and RH Reeves. 2000. Quantitative trait loci modulate neutrophil infiltration in the liver during LPS-induced inflammation. FASEB Journal 14:2247-54.

Comparative Mapping

5. Comparative mapping is the prediction of gene location in one species based on the position of that gene in a different species. It derives from the observation that although chromosomes rearrange during evolution, they do not usually scramble. Thus, *orthologous segments* can be identified between species that demonstrate linkage of the same genes, often in the same order. The occurrence of a new gene in an orthologous segment implies its localization to the corresponding position in another species.



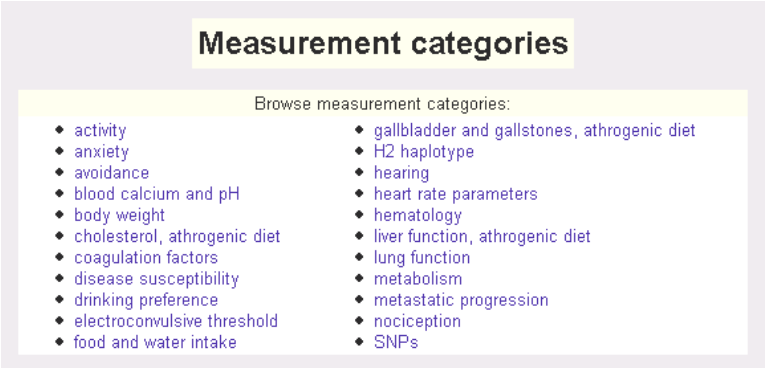


Genome maps 10. Comparative genomics. Mammalian radiations. Wall chart.

O'Brien SJ, Eisenberg JF, Miyamoto M, Hedges SB, Kumar S, Wilson DE, Menotti-Raymond M, Murphy WJ, Nash WG, Lyons LA, Menninger JC, Stanyon R, Wienberg J, Copeland NG, Jenkins NA, Gellin J, Yerle M, Andersson L, Womack J, Broad T, Postlethwait J, Serov O, Bailey E, James MR, Marshall Graves JA, et al.

Science. 1999 Oct 15;286(5439):463-78.

Mouse phenome project



www.jax.org/phenome

Can a mouse have a human disease?

- what is phenotype? can mouse and hum ?
- wh
dev g
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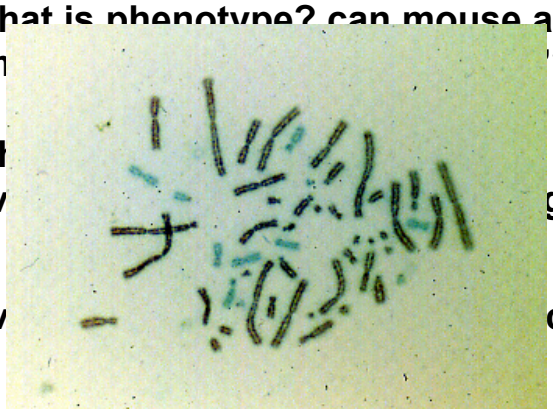


Transgenesis/ gene-targeting/ chromosome engineering

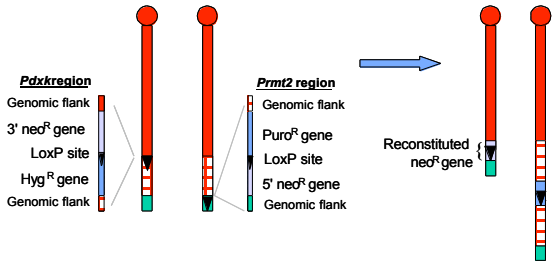
1. "Knock out" mice (null alleles)

2. "Knock-ins" (mutations, reporters), tissue-targeted and conditional mutations
   Shin MK, Levorse JM, Ingram RS, Tilghman SM. The temporal requirement for endothelin receptor-B signalling during neural crest development. Nature. 1999 Dec 2;402(6761):496-501.

3. Whole genome gene targeting
   Zheng B, Mills AA, Bradley A. A system for rapid generation of coat color-tagged knockouts and defined chromosomal rearrangements in mice. Nucleic Acids Res. 1999 27(11):2354-60.
   Zambrowicz BP, Friedrich GA, Buxton EC, Lilleberg SL, Person C, Sands AT. Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells. Nature. 1998 392(6676):608-11.

4. Chromosome engineering
   Ramirez-Solis R, Liu P, Bradley A. Chromosome engineering in mice. Nature. 1995 378(6558):720-4.

Fig. 8. Targeting lox sites asymmetrically by homologous recombination in ES cells provides target sites to create a reciprocal translocation by expressing Cre recombinase.



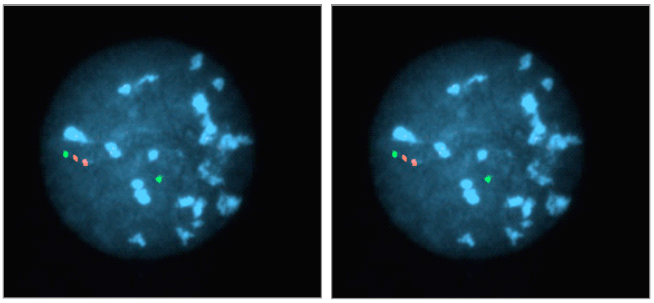


Fig. 11. Characterization of an ES line containing engineered MMU16s with a duplication or deletion of the "Down syndrome critical region. Left, metaphase FISH demonstrates the presence of a BAC proximal to the target region (green) on both copies of MMU16 but a signal from the dup/del segment (red) on only one copy of the chromosome. Right, interphase FISH with the same BAC probes shows the duplication (one green hybridization signal, two red signals) in a separate portion of the nucleus from the deleted chromosome (one green dot). These results confirmed marker selection, PCR and southern blot confirmation of the reciprocal asymmetric translocation.

Mapping Genome Function: Creating Phenotypes using Mutagenesis

Mutagenesis provides a means of generating new phenotypes in mouse.

1. Justice, M. J. 2000. Capitalizing on large-scale mouse mutagenesis screens. Nat Rev Genet 1: 109-15. Review.
2. MJ Justice in JJ Jackson and GM Abbott, Mouse Genetics and Transgenics: A Practical Approach, 2006 Oxford University Press, 299 pp.

1. Sources of mutations
spontaneous, frequency is 10-5/locus/generation; all types of mutations; radiation, frequency is dose dependent, primarily chromosomal rearrangement; chemical, ENU is highest giving point mutations at a frequency of 1/600 gametes per locus at som
2. Screens
specific locus test
MutaMouse/ Big Blue
SHIRPA
special targeted screens
dominant vs. recessive (1st vs. 3rd generation)
in combination with deletion (recessives in first generation)
3. targets/ mutation types
visible single gene dom. or recessive
allelic series
biochemical pathway
sensitization (Shedlovsky A, McDonald JD, Symula D, Dove WF. Mouse models of human phenylketonuria. Genetics. 1993 Aug;134(4):1205-10.)
4. mode of action, transfer ethyl group to a number of residues on all four nucleotides, including ethylation of phosphate groups that leads to mispairing; most frequent in mouse are AT -> TA (transversion) and AT -> GC (transition), but specific frequencies are locus specific ENU affects primarily spermatogonia (stem cells) – freq. in sperm and in females are low
5. Breeding schemes:
a. balancer;
b. recessive over deletion;
c. modifier (dominant mutation modifies another mutation)
d. sensitization (recessive mutations in genes that interact in a pathway/ allelic non-complementation)
6. Large centers, see Trans-NIH Mouse initiative http://www.nih.gov/science/models/mouse/index.html

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Mouse Genome Center, ENU Mutagenesis Programme, Harwell, http://www.mgu.har.mrc.ac.uk/mutabase/
German ENU Mutagenesis Center, http://www.gsf.de/isg/groups/enu-mouse.html

Enhancer, gene and promoter trapping

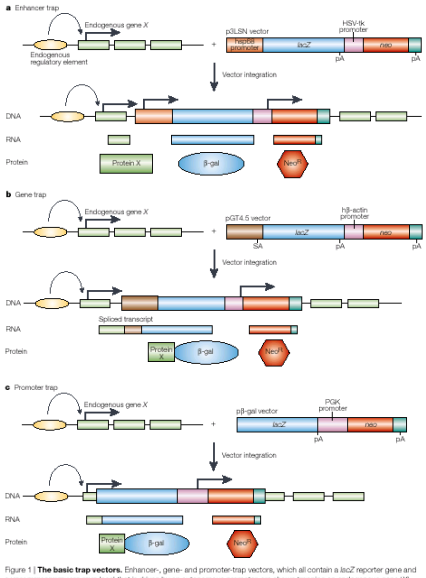
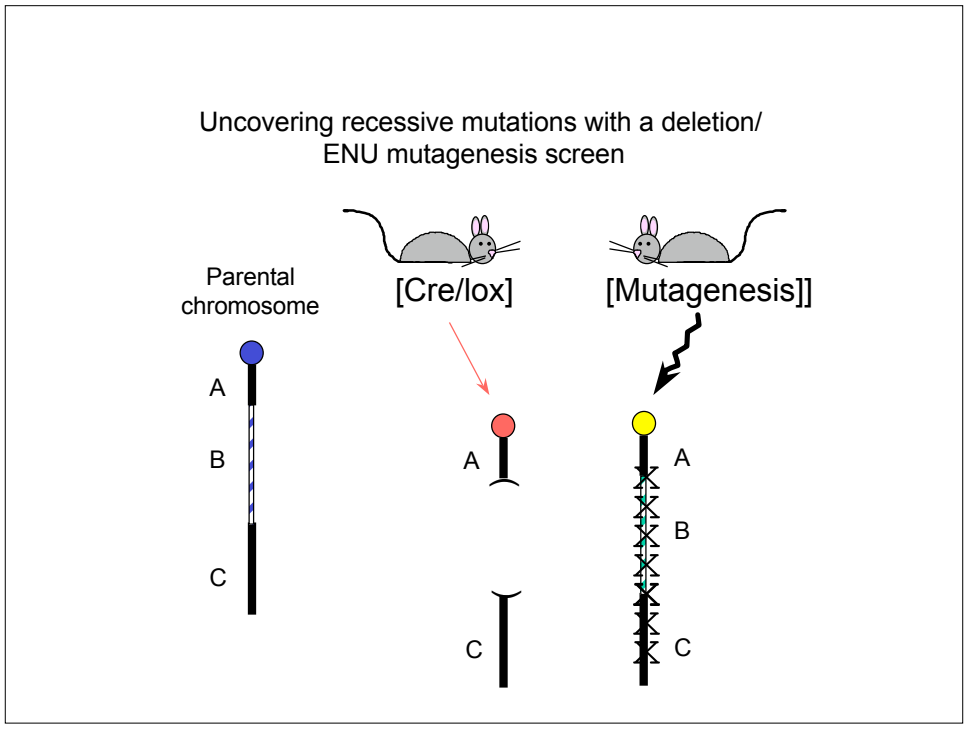
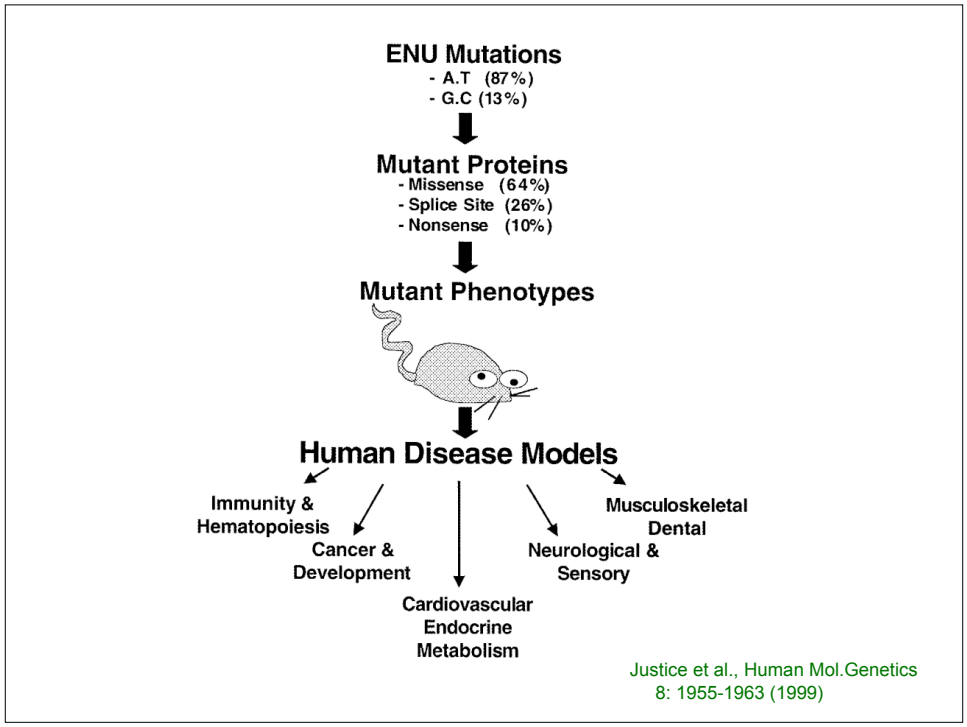
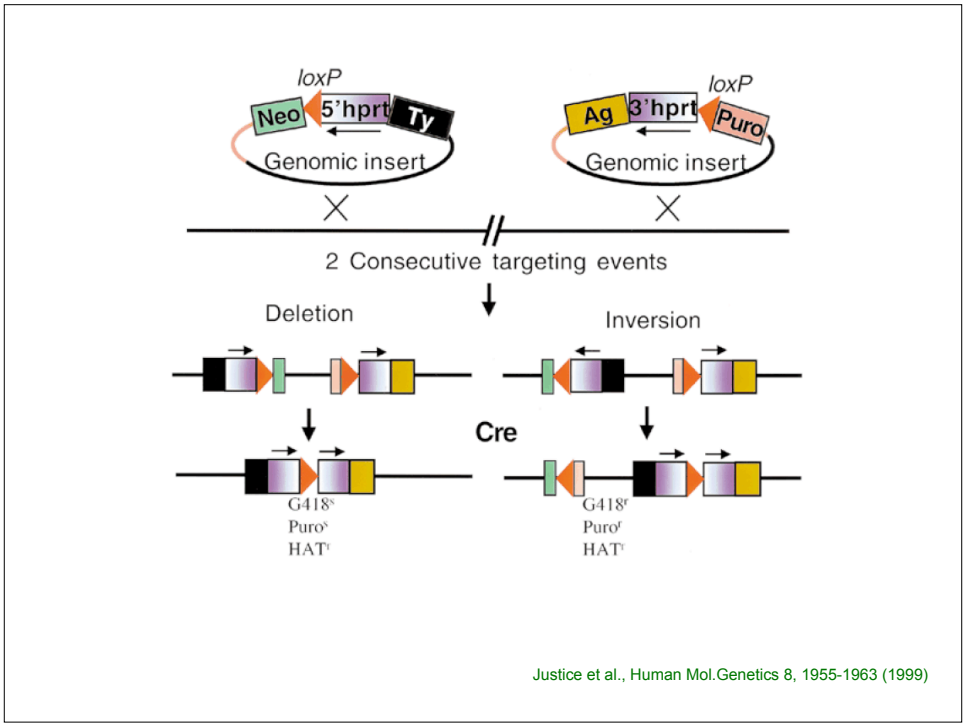


Figure 1 | **The basic trap vectors.** Enhancer-, gene- and promoter-trap vectors, which all contain a *lacZ* reporter gene and a NEOMYCIN RESISTANCE GENE (neo) that is driven by an autonomous promoter, are shown trapping an endogenous gene 'X'.

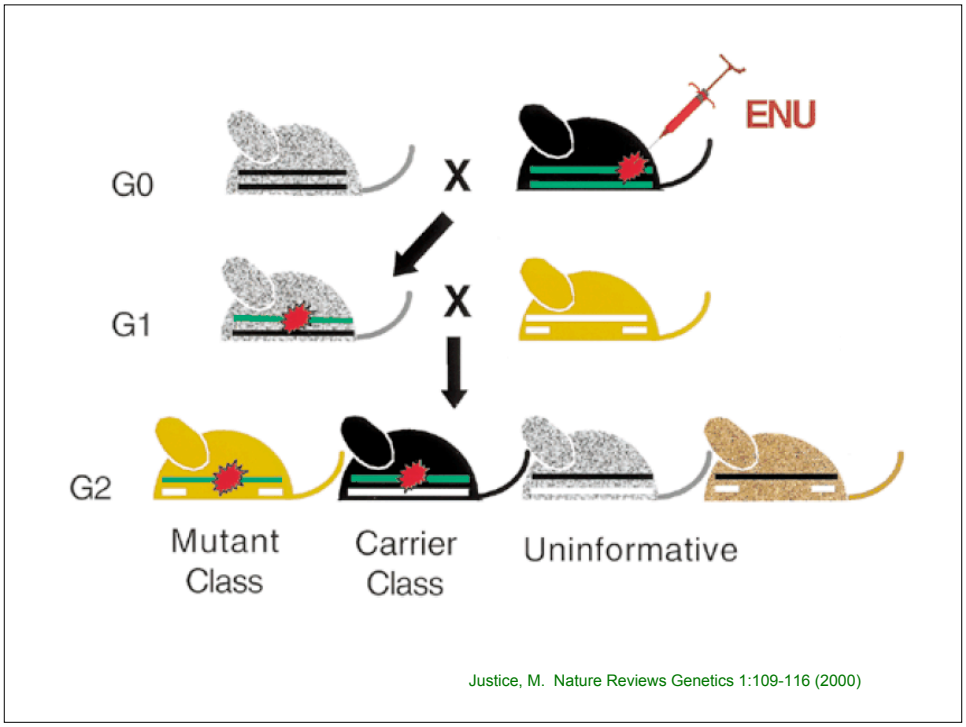
Stanford et al., Nature Reviews Genetics 2, 756-768 (2001)


Uncovering recessive mutations with a deletion/
ENU mutagenesis screen
Parental
chromosome
[Cre/lox]
[Mutagenesis]]
A
B
C
A
C
A
B
C

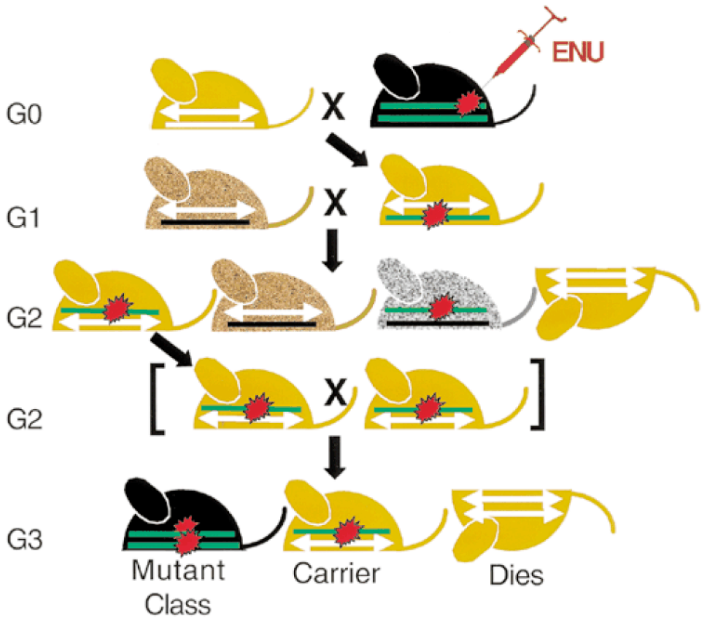

ENU Mutations
- A.T (87%)
- G.C (13%)
Mutant Proteins
- Missense (64%)
- Splice Site (26%)
- Nonsense (10%)
Mutant Phenotypes
Human Disease Models
Immunity &
Hematopoiesis
Cancer &
Development
Cardiovascular
Endocrine
Metabolism
Neurological &
Sensory
Musculoskeletal
Dental
Justice et al., Human Mol.Genetics
8: 1955-1963 (1999)



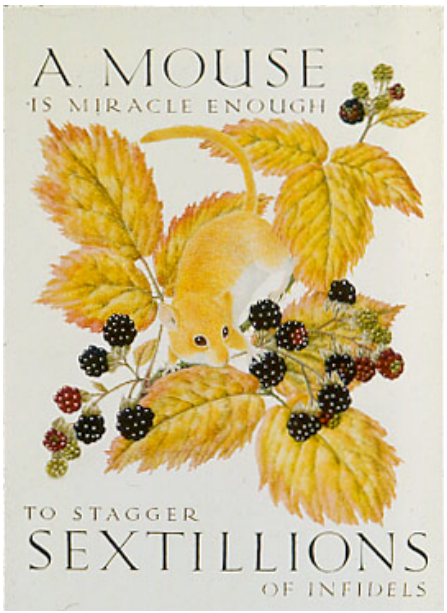
Justice et al., Human Mol.Genetics 8, 1955-1963 (1999)



Justice, M. Nature Reviews Genetics 1:109-116 (2000)



Justice, M. Nature Reviews Genetics 1:109-116 (2000)



Walt Whitman