

## Perspectives on Mouse Genetics

### MOUSE-2011 CONFEREES:

The Genetics Society of America sponsors this conference, as it has sponsored the journal GENETICS since 1916. One of the current features of this journal is the Perspectives column, now edited by Adam Wilkins. It occurs to me that, while traveling to the conference, attendees might be interested to browse certain of the Perspectives essays that address formative aspects of the field represented by MOUSE-2011. The attached PDF gives direct access to essays by Tibby Russell on the survival of JAX, Mary Lyon on the mouse map, Monica Justice on Oak Ridge and mutagenesis, Ken Paigen on the evolution of the field, and – especially for the Complex Trait Consortium – Jim Crow on Don Bailey and Recombinant-inbred strains.

If you browse over these Perspectives on the formation of our field, perhaps you will have a suggestion for future progress. As noted, Don Bailey's idea was simple but powerful. In the Chapman Lecture on Saturday, I shall share some specific suggestions from our lab in Wisconsin.

Looking forward! Bill Dove

p.s. when you are on the internet, you can scan the entire set of Perspectives essays from the 1987-2008 era, using the hot links on the pdf to benefit from the Open Source of GENETICS. Some of you may find material useful in teaching genetics. Feedback on how this series can be usefully indexed for the GENETICS website can be sent to [dove@oncology.wisc.edu](mailto:dove@oncology.wisc.edu) and will be greatly appreciated.

January 2009  
The University of Wisconsin  
Madison, Wisconsin

Geneticists,

We are pleased to be able to share with you an electronic compendium of the *Perspectives* series from 1987 to 2008. The attached PDF is composed of “hotlinks” to the GENETICS site. As you know, our professional journal has adopted a policy of open access, so that these links should function freely on your computer.

This compendium is a tribute to our field. Its stories, mini-reviews, and opinions have been offered by geneticists spanning the 20<sup>th</sup> century. The central core of the science of genetics is evident throughout, even as it has expanded over the past 30 years as a result of its marriage with genomics, enhancing the power to analyze the genotype. Genetics/genomics is now embarking on another union, as signaled by the 2008 Nobel Prize in chemistry: the enhancement of the power to phenotype by the use of reporter fusions to genes of interest. Finally, this compendium captures the early stirrings of population genetics and molecular evolution, bringing this amplified science of genetics into dialog with issues of our entire biosphere.

This year marks the end of an enjoyable venture for the two of us as editors of the *Perspectives* series in GENETICS. We are delighted that, under the leadership of the newly appointed Editor-in-Chief Mark Johnston, the *Perspectives* series will now be edited by Adam Wilkins from Cambridge UK. We have admired Adam’s work as Editor-in-Chief of BioEssays and his seamless and incisive writing style as represented by two books and a number of critical essays, some proposing original genetical hypotheses. On our part, we shall continue to observe and enjoy the radiation of the science of genetics over the coming years: JFC as a commentator on genetics in general and WFD in his streamlined research group completing the odyssey of genetically driven studies of controlled replication - from bacteriophage lambda, to the syncytial eukaryote *Physarum polycephalum*, and now to colon cancer in mice, rats, and humans. We expect that the time released from editing this series will enable us to respond to occasional requests for input from Editor Wilkins.

We two have steered this ship, enjoying the adventure into ocean that is genetics today. But the engine of the ship has been the individual geneticists who have taken time to share their stories, mini-reviews, and opinions. Take a look at any one of these essays, and you will see the fruit of many weeks (or even months or years!) of effort, often by an investigator who is also busy with primary research publications and ever-threatening grant proposals. The ship has also been kept “ship-shape” by the Editors-in-Chief of GENETICS with whom we have been privileged to work – Jan Drake, Beth Jones, and Mark Johnston. Each of these Editors has been effectively assisted: Pamela Drake, Leah Kaufman, and Tracey DePellegrin Connelly. Finally, our work at Wisconsin has been enhanced by the magnificent command over style of the late Dr. Ilse Riegel, by the effective copy editing of Ms. Kristen Adler, and by the skill of Ms. Linda Clipson with graphics.

We wish for each of you, for the *Perspectives* series, and for the field of genetics continuing success over the coming years.

Looking forward,  
JFCrow

WFDove

# Perspectives on Genetics

Anecdotal, Historical, and Critical Commentaries, 1987-2008

James F. Crow and William F. Dove, Editors

## Introduction

[Bird's Eye View: A Decade of Perspectives](#) by James F. Crow and William F. Dove

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- [Sewall Wright and Physiological Genetics](#) by James F. Crow
- [Paradox Found](#) by William F. Dove
- [Seventy Years Ago in GENETICS: H. S. Jennings and Inbreeding Theory](#) by James F. Crow
- [Twenty Years of Illegitimate Recombination](#) by Philip Anderson
- [Molecular Genetics of \*Mus musculus\*: Point Mutagenesis and Millimorgans](#) by William F. Dove
- [Twenty-Five Years Ago in GENETICS: Motoo Kimura and Molecular Evolution](#) by James F. Crow
- [The waxy Locus in Maize Twenty-Five Years Later](#) by Oliver E. Nelson
- [Doing Behavioral Genetics with Bacteria](#) by John S. Parkinson
- [Gene Recombination and Linked Segregations in \*Escherichia coli\*](#) by Joshua Lederberg
- [A Mouse Phoenix Rose From the Ashes](#) by Elizabeth S. Russell
- [“In the Air” – Theodosius Dobzhansky’s \*Genetics and the Origin of Species\*](#) by Jeffrey R. Powell
- [Quantitative Genetics in 1987](#) by B. S. Weir

## 1988

- [A Diamond Anniversary: The First Chromosome Map](#) by James F. Crow
- [The Year of the Fly](#) by William F. Dove
- [The Ultraselfish Gene](#) by James F. Crow
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- [Cytogenetics and Karl Sax](#) by Carl P. Swanson
- [H. J. Muller, Communism, and the Cold War](#) by Diane Paul
- [Eighty Years Ago: The Beginnings of Population Genetics](#) by James F. Crow
- [A Diamond in a Desert](#) by Herschel Roman
- [Unequal Crossing Over Then and Now](#) by Kenneth D. Tartof
- [The Genesis of Dysgenesis](#) by James F. Crow
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- [The Hawthorne Deletion Twenty-Five Years Later](#) by Ira Herskowitz

## 1989

- [Early Worms](#) by Jonathan Hodgkin
- [There’s a Whole Lot of Shaking Going On](#) by Barry Ganetzky
- [Replica Plating and Indirect Selection of Bacterial Mutants: Isolation of Preadaptive Mutants in Bacteria by Sib Selection](#) by Joshua Lederberg
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- [Evolving Theories of Enzyme Evolution](#) by Daniel L. Hartl
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- [M. R. Irwin and the Beginnings of Immunogenetics](#) by Ray Owen
- [The Linkage Map of Phage T4](#) by Frank Stahl

- [The Molecular Genetics of Differentiation](#) by Bert Ely and Lucy Shapiro
- [Intragenic Recombination in Drosophila: The rosy Locus](#) by Arthur Chovnick

## 1990

- [R. A. Fisher, A Centennial View](#) by James F. Crow
- [Regulating Tn10 and IS10 Transposition](#) by Nancy Kleckner
- [The Foundations of Genetic Fine Structure: A Retrospective From Memory](#) by M. M. Green
- [Studies of Yeast Cytochrome c: How and Why They Started and Why They Continued](#) by Fred Sherman
- [L. C. Dunn and Mouse Genetic Mapping](#) by Mary F. Lyon
- [The Role of Similarity and Difference in Fungal Mating](#) by Robert L. Metzenberg
- [Mapping Functions](#) by James F. Crow
- [Joy of the Worm](#) by H. Robert Horvitz and John E. Sulston
- [Genes and Development: Molecular and Logical Themes](#) by Sydney Brenner, William Dove, Ira Herskowitz and René Thomas
- [Sixty Years of Mystery](#) by Allan C. Spradling and Gary H. Karpen

## 1991

- [Our Diamond Birthday Anniversary](#) by James F. Crow
- [Haldane's Solution of the Luria-Delbrück Distribution](#) by Sahotra Sarkar
- [Fifty Years Ago: The Neurospora Revolution](#) by Norman H. Horowitz
- [Tomato Paste: A Concentrated Review of Genetic Highlights From the Beginnings to the Advent of Molecular Genetics](#) by Charles M. Rick
- [Impact of the Douglas-Hawthorne Model as a Paradigm for Elucidating Cellular Regulatory Mechanisms in Fungi](#) by Yasuji Oshima
- [The Regulation of Arginine Biosynthesis: Its Contribution to Understanding the Control of Gene Expression](#) by Werner K. Maas
- [Twenty-Five Years Ago in GENETICS: Electrophoresis in the Development of Evolutionary Genetics: Milestone or Millstone?](#) by R. C. Lewontin
- [Alfred Henry Sturtevant and Crosses Between \*Drosophila melanogaster\* and \*Drosophila simulans\*](#) by William B. Provine
- [The Gene \(H. J. Muller 1947\)](#) by Joshua Lederberg
- [Qualitative and Quantitative Genetic Studies of \*Arabidopsis thaliana\*](#) by Bruce Griffing and Randall L. Scholl
- [Twenty-Five Years of Cell Cycle Genetics](#) by Leland H. Hartwell

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- [Centennial: J. B. S. Haldane, 1892-1964](#) by James F. Crow
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- [Looking for the Homunculus in Drosophila](#) by Alan Garen
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- [On the Beginnings of Somatic Cell Hybridization: Boris Ephrussi and Chromosome Transplantation](#) by Doris T. Zallen and Richard M. Burian
- [Forty Years Ago: The Discovery of Bacterial Transduction](#) by Norton D. Zinder

- [Forty Years Ago in GENETICS: The Unorthodox Mating Behavior of Bacteria](#) by *L. Luca Cavalli-Sforza*
- [Unicorns Revisited](#) by *Franklin W. Stahl*

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- [N. I. Vavilov, Martyr to Genetic Truth](#) by *James F. Crow*
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- [The Gene, the Polygene, and the Genome](#) by *William F. Dove*
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- [A Notable Triumvirate of Maize Geneticists](#) by *Oliver E. Nelson*

### 1994

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- [The Transformation of Genetics by DNA: An Anniversary Celebration of Avery, MacLeod and McCarty \(1944\)](#) by *Joshua Lederberg*
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- [Terumi Mukai and the Riddle of Deleterious Mutation Rates](#) by *Peter D. Keightley and Adam Eyre-Walker*
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- [Development: Mendel's Legacy to Genetics](#) by *Iris Sandler*
- [Guido Pontecorvo \("Ponte"\), 1907-1999](#) by *Bernard L. Cohen*
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## 2001

- [The Other Fly Room: J. T. Patterson and Texas Genetics](#) by *Robert P. Wagner and James F. Crow*
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- [Discovery of the Transposable Element \*mariner\*](#) by *Daniel L. Hartl*
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- [Evolution by Jumps: Francis Galton and William Bateson and the Mechanism of Evolutionary Change](#) by *Nicholas W. Gillham*

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- [The Emergence of Hymenopteran Genetics](#) by *Robert E. Page, Jr., Jürgen Gadau, and Martin Beye*
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- [E. B. Lewis and the Bithorax Complex: Part II. From cis-trans Test to the Genetic Control of Development](#) by *Ian Duncan and Geoffrey Montgomery*
- [Richard Goldschmidt and the Crossing-Over Controversy](#) by *Marsha L. Richmond and Michael R. Dietrich*
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## 2003

- [One Hundred Years of Mouse Genetics: An Intellectual History. I. The Classical Period \(1902-1980\)](#) by *Kenneth Paigen*
- [The Centenary of the One-Gene One-Enzyme Hypothesis](#) by *Mark Hickman and John Cairns*
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- [RNA Processing: A Postdoc in a Great Laboratory](#) by *Sidney Altman*

## 2004

- [A Centennial: George W. Beadle, 1903–1989](#) by Norman H. Horowitz, Paul Berg, Maxine Singer, Joshua Lederberg, Millard Susman, John Doebley, and James F. Crow
- [Ira Herskowitz: 1946-2003](#) by David Botstein
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- [Did Demerec Discover Intragenic Recombination in 1928?](#) by Edward B. Lewis

## 2005

- [The Limits of Theoretical Population Genetics](#) by John Wakeley
- [Remembrance of Ching Chun Li, 1912–2003](#) by Eliot B. Spiess
- [The Favorable Features of Tryptophan Synthase for Proving Beadle and Tatum's One Gene-One Enzyme Hypothesis](#) by Charles Yanofsky
- [Reflections on a Path to Sexual Commitment](#) by Thomas W. Cline
- [Cornfests, Cornfabs and Cooperation: The Origins and Beginnings of the Maize Genetics Cooperation News Letter](#) by Lee B. Kass, Christophe Bonneuil, and Edward H. Coe, Jr.
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- [Theodosius Dobzhansky's Role in the Emergence and Institutionalization of Genetics in Mexico](#) by Ana Barahona and Francisco J. Ayala
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- [John R. S. Fincham \(1926-2005\): A Life in Microbial Genetics](#) by Alan Radford and R. H. Davis
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- [A Kingpin of Academic Inclusive Fitness: The History and Contributions of Bruce Grant](#) by Mohamed A. F. Noor and Norman A. Johnson
- [Norman Harold Horowitz, 1915-2005](#) by Robert L. Metzenberg

## 2006

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## 2007

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### A MOUSE PHOENIX ROSE FROM THE ASHES

**F**ORTY years ago, in October of 1947, the State of Maine experienced a short-term disaster which developed, at least for biomedical research and mammalian genetics, into a long-term benefit. To appreciate this situation, one must be aware of oddities of Maine weather, the status of biological research shortly after World War II, and the very special qualities of CLARENCE COOK LITTLE, mentioned in this column in January, 1987.

Down-easterners are fond of saying, "If you don't like Maine weather, wait a minute." Usually it does change frequently and usually there is plenty of rain and fog, but all of 1947 was dry, and in the fall the weather continued to be entirely too beautiful—nothing but glorious sunny days from late August until late October. Trees and bushes became dry tinder, and tiny brush smudges grew into raging forest fires. Most of the state was declared a disaster area, including Mt. Desert Island where both Acadia National Park and The Jackson Laboratory are located.

A brush fire started October 12 or 13 in a swamp about eight miles from the main Jackson Laboratory near Bar Harbor. I can remember seeing its small plume of smoke of October 14 while sitting in a staff meeting at nearby Hamilton Station, the newly acquired dog and rabbit branch of the Laboratory. We were told then that the fire was under control, but I also remember seeing this same fire, three days later, spreading to capture the crowns of successive pine trees, despite concerted fire-fighting efforts of local and imported fire engines. The tiny fire we had seen from the Hamilton Station continued to grow, fanned by strong winds, burning much of Acadia National Park and the middle of Mt. Desert Island. The fire extended to mountains east of the main laboratory, mercifully heading toward the sea.

On the afternoon of October 23, however, a sudden wind shift turned the flames directly back toward Bar Harbor and the Jackson Laboratory. The population of Bar Harbor was evacuated that evening in a car-convoy, driving 15 miles westward, often with burning trees on both sides of the road, to the only bridge

connecting to the mainland. Next day we learned that most of the Jackson Laboratory was gutted, with roof, inner partitions, floors, and mouse boxes completely gone.

Would there ever again be a Jackson Laboratory? C. C. LITTLE never doubted. He called us together in Ellsworth, on the mainland not far from the Island, assured mouse box-changers that they still had jobs, and assigned responsibilities to staff and research assistants. When he viewed the ashes around the wreck of the old Lab, he said, "Now we can see the sea." He saw to it that money appeared to continue building the new animal wing. Our needs were dramatic, and inspired both publicity and very welcome assistance.

In 1947, the young, small but growing Jackson Laboratory was emerging from serious financial struggles. It had been founded in 1929 by LITTLE with a group of seven researchers who had worked in the mouse genetics laboratory which he had maintained in Ann Arbor while President of the University of Michigan (1925–1929). The beginning of the great depression was not an ideal time to establish what the concerned group of Detroit industrialists had intended to be a research institute supported entirely by private funds. When anticipated support failed to materialize, LITTLE persuaded his coworkers to "live sparsely" while pushing forward with research on genetics and cancer.

Despite its precarious situation, the early Laboratory was a happy place. Prexy, as LITTLE was called, appreciated and supported everyone's research efforts. Every month, staff, box-changers and their families loved to go to the All-Lab Party. The Laboratory managed to survive, and in 1933 the entire staff jointly published an important scientific contribution ("The existence of non-chromosomal influences on the incidence of mammary tumors in mice," *Science* **78**: 465–466). LITTLE devoted great efforts to seeking money for the Laboratory and to persuading the federal government and the public that support of research was a national responsibility. Providing genetically controlled mice, gleaned from each research-

er's own colony to supply other institutions, became one of the Lab's means of support.

In 1947, the Jackson Laboratory was still a small place with 18 doctoral-level investigators plus a few research assistants and animal caretakers. College and pre-college students and visiting investigators came in the summer months. But the Laboratory showed potential for growth in the post-war expanding world of science. In the summer of 1947 foundations had been laid for a big new animal wing, with much of the construction supported by the new National Cancer Institute which LITTLE had helped found.

Living through the winter of 1947–1948 was quite an experience. Until the end of December we were all piled on top of one another in a hallway at the Hamilton Station. Most of the records, as well as the animals, were lost. I was very lucky because a fine assistant, KAY HAMILTON, had rescued an invaluable file of data on pigment granules. However, most of the others' research at that time involved waiting for cancers to appear. With treated and control mice destroyed, all experiments in progress had to be repeated from the very beginning. We also needed to build up animal resources to supply critical needs of researchers in other institutions. Where would the necessary mice come from?

Almost immediately after the fire, a very welcome pile of letters began to pour in. Investigators who had recently received pedigreed mice from the Jackson Laboratory, and geneticists who maintained inbred mouse colonies stemming from our stocks, wrote to offer "starts" of almost all the strains we had lost, plus some valuable new types. LITTLE assigned to me the exciting responsibility of accepting the most pertinent offers, and as quickly as possible we built up a common foundation colony from which both in-house individ-

ual research colonies and a separate animal resources colony were supplied on an equal basis. By spring of 1948, mice were moved into the new animal wing. Ground was also broken for a new research wing, with funding from the National Cancer Institute and the American Cancer Society. The Ladies Auxiliary of the Veterans of Foreign Wars provided us with a Summer Laboratory and living quarters for summer students. From that time on, the size and productivity of The Jackson Laboratory increased rapidly. During the next five years the research staff increased from 19 to 33, resulting in broader research programs and increased scientific publication. More and more mice were provided to outside investigators.

The 1947 fire came at a propitious time for the scientific community. Just as large numbers of researchers were coming to depend on animals from outside suppliers, disruption by the fire focused attention on the importance of selecting the right animals for a particular project. The Laboratory's losses in the fire, and rescue by gifts from other mouse geneticists, gave the staff a heightened sense of genetic responsibility. In addition to contributing through their own research, they now wanted to apply genetic know-how to guarantee ready availability and continuity of pertinent, genetically uniform, well-characterized mice for the growing biomedical research community. The Laboratory had added a new phase to its scientific mission.

Biomedical and genetic research are deeply indebted to the foresight of C. C. LITTLE in establishing inbred lines of mice. The Jackson Laboratory was both founded and rescued by his confidence, his personality, and his unfailing optimism.

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### L. C. DUNN AND MOUSE GENETIC MAPPING

THE year 1990 marks the 70th anniversary of the beginning of systematic mapping of the mouse genome. The first detection of linkage between two genes in the mouse, and also the first in any vertebrate, had occurred five years earlier, when HALDANE and his colleagues (1915) described the linkage of the coat color genes for *albinism* and *pink-eyed dilution*. However, it was not till 1920 that L. C. DUNN (1920a) published a paper on a systematic search for linkage among various coat color genes.

This was just one of many major contributions made by DUNN which laid foundations for the present highly detailed knowledge of the mouse genetic map. This map in turn makes the mouse now one of the key experimental organisms in genetics, particularly in relation to present efforts to elucidate the structure and function of the human genome. In the same year, DUNN (1920b) published what must have been the first paper on comparative mapping, in which he compared the linkage of *albinism* and *pink-eye* in mice and rats and investigated in detail factors, such as age and sex, which affect recombination between these genes in the mouse. Later, DUNN was involved in the development of inbred strains of mice, which are now very important in the mapping of DNA and protein variants. He was also one of the founders of the mouse Nomenclature Committee and of *Mouse News Letter*, which have provided the underlying framework for the databases which are now essential to successful mapping. In yet another contribution, DUNN was one of the first to use wild mice in genetic research (WAELSCH 1989). The wealth of genetic variation in wild mice, including species and subspecies closely related to laboratory mice, has led to the present most widely used mapping method, the interspecific back-cross (AVNER *et al.* 1988). DUNN's particular interest in wild mice was the insight they provided into understanding the *t*-complex on mouse chromosome 17 (WAELSCH 1989). The pioneering work of DUNN and his colleagues on this fascinating genetic variant has led to much recent molecular mapping, to the proximal half of chromosome 17 being now the most intensively mapped region of the mouse genome, and to

the cloning of a gene of major importance in vertebrate development (HERRMANN *et al.* 1990).

Progress in mouse genetic mapping has always been exponential (EICHER 1981), with the number of mapped genes doubling approximately every seven years. Thus, from our present vantage, the pace of advance in the early years appears slow. The first 50 years were spent in defining linkage groups. New genes were tested against all known genes or linkage groups, as in DUNN's (1920a) paper on independent genes. GRÜNEBERG's (1943, 1952) reference work of this time included tables showing which of these "tests for independent segregation" had already been completed. Also during this time, the statistical and theoretical backgrounds for the estimation of linkage or independence were much improved. In his 1920 papers DUNN used no statistics, but later FISHER (1946) published the maximum likelihood method for estimating recombination fractions, now the standard method. The detection of linkage between genes was put on a more rational basis by CARTER and FALCONER (1951), who put forward the concept of the "swept radius" or length of the genetic maps scanned in any given test for linkage, and thereby enabled the development of more rationally designed stocks for detecting linkage.

During the first 40 years or so, the genes mapped were mainly those producing some visible phenotypic effect, such as changes in coat color or texture, or in the skeleton or behavior. The work used "linkage testing stocks" of the type developed by CARTER and FALCONER. In the 1920s and 1930s much work went into the development of inbred strains (FESTING 1979; MORSE 1978, 1981). DUNN was the founder of the 129 strain, now widely used, but the great value of inbred strains in mapping studies was to emerge much later. The first biochemical genetic variant in inbred strains was found in 1941 (F. H. J. FIGGE and L. C. STRONG, quoted by MORSE 1981) and in the 1960s and 1970s emphasis shifted to the mapping of enzyme and other protein variants. It was then that the immense value of the inbred strains as a source of genetic variation became clear. Strains were typed for a wealth

of variants and RODERICK and his colleagues (RODERICK, STAATS and WOMACK 1981; RODERICK and GUIDI 1989) developed an extensive database of the alleles carried at particular loci in inbred strains. RODERICK and GUIDI (1989) provide a table of data on 338 loci in 246 strains, derived from a larger database at The Jackson Laboratory containing information on 426 loci in 569 strains. A breakthrough occurred when BAILEY (1971) developed the concept of recombinant inbred strains, subsequently developed further by TAYLOR (1978) for use in genetic mapping. In this method, two inbred strains with known genetic characteristics are crossed. The  $F_2$  offspring are then paired at random and their offspring are mated brother  $\times$  sister for 20 or more generations to form a new set of strains, the recombinant inbred or RI strains. In the formation of the new strains, genetically linked characters will tend to stay together and the number of cases of separation of two linked traits will depend on the recombination between them. Hence, if the set of recombinant inbred strains is typed, one can detect which traits are linked and the approximate recombination frequency between them. As a database of typed traits builds up, a given set of RI strains becomes steadily more powerful for linkage detection (TAYLOR 1989).

The study of biochemical variants led to increasing interest not only in inbred strains but also in wild mice. DUNN (DUNN and MORGAN 1952) had first used wild mice to search for new forms of the *t*-complex. He indeed found a range of different *t*-haplotypes and concluded that they are maintained as polymorphisms in the wild as a result of their abnormally high transmission from heterozygous males (DUNN and MORGAN 1952; DUNN 1957, 1964). Wild mice were then found to provide a rich source of biochemical genetic variation (CHAPMAN 1978) and numerous inbred strains were developed from various subspecies and species (BONHOMME and GUÉNET 1989). Another type of variation found among wild mice, and of key importance in genetic mapping, was karyotypic variation. For the first 50 years of mapping, mouse linkage groups could not be assigned to chromosomes. Many induced chromosome aberrations, mainly reciprocal translocations, had been studied (SEARLE 1989) and, following the pioneering work of SNELL (1946), had been shown to involve particular linkage groups. However, mouse chromosomes were not cytogenetically distinguishable until, in 1971, techniques for chromosome banding made possible the identification of individual chromosomes (MILLER and MILLER 1975). Knowledge of which linkage groups were associated with particular chromosome aberrations then enabled the assignment of linkage groups to chromosomes. The aberrations used included not only the induced reciprocal translocations, but also Robertsonian translocations found in wild mice, especially in

*Mus poschiavinus* in certain alpine valleys where some populations had up to nine pairs of Robertsonians (GROPP and WINKING 1981).

Thus, in 1972 the linkage map appeared for the first time with the linkage groups assigned to chromosomes (GREEN 1972). Mapping then entered a new phase involving the precise location of genes, both in terms of their recombination with other genes and in their physical location with respect to chromosome G-bands. A recent advance in methodology has again involved the use of wild mice. GUÉNET and his colleagues (AVNER *et al.* 1988) showed that, if subspecies or closely related species of mice are compared, restriction fragment length variants (RFLVs) can be found for nearly all probes with the use of only one or two restriction enzymes. Thus, if laboratory mice are crossed with a wild species, usually *Mus spretus*, and the  $F_1$  female is backcrossed to the laboratory strain (in a so-called interspecific backcross), all genes or other DNA markers can be mapped by their RFLVs. If DNA from individual backcross animals is stored, successive markers can be mapped, so that a panel of DNA from backcross animals becomes a resource which yields more detailed information as time progresses. DNA from animals with recombination within a certain interval can be used for further and finer mapping within that interval, thus enabling "homing in" on a region of interest as, for instance, in attempts to clone a gene. DUNN's interest in the *t*-complex has led to recent work which provides an interesting example of the use that can be made of a collection of rare recombinants in the region of interest. The *t*-complex is now known to involve a variant region of chromosome 17 (SILVER 1985; FRISCHAUF 1989; LYON 1990), which was first recognized by an interaction with the mutant gene for *brachyury*, *T*. Heterozygotes of *T* with wild type are short-tailed, whereas *T/t* heterozygotes are tailless. In mice heterozygous for a *t*-complex and a wild-type chromosome 17, recombination is suppressed over the region occupied by the *t*-complex as a result of four inversions carried in the *t*-complex (HAMMER, SCHIMENTI and SILVER 1989). However, rare recombinants are found. These recombinants have been kept and used to provide evidence for the genetic basis of the transmission ratio distortion and male sterility which are other features of the *t*-complex (LYON 1984, 1986). Further use of these rare recombinants has enabled the ordering on the chromosome of numerous DNA markers derived by microdissection of the proximal region of chromosome 17 (FOX *et al.* 1985; FRISCHAUF 1989). Together with cloned genes and DNA markers from other sources, there are now over 100 markers on chromosome 17, mainly in the proximal region (VINCEK *et al.* 1989). About 20 megabase pairs in three segments have been mapped by pulsed-field gel electrophoresis (BARLOW and LEHRACH 1989). In turn

this has facilitated the detection of candidate genes for the *distorter* and *responder* genes (RAPPOLD *et al.* 1987; SCHIMENTI *et al.* 1988) thought to be responsible for the transmission ratio distortion and male sterility, and also has led to the cloning of the *brachyury* gene (HERRMANN *et al.* 1990). The cloning of this gene is a major step forward because it plays a crucial role in the development of vertebrates. It is thought to be important in mesoderm formation and homozygotes fail to develop the notochord and the entire posterior part of the body.

With the detailed knowledge of comparative mapping now available, it should be possible to map the human homolog of *brachyury* very quickly. Although comparative mapping began at the same time as systematic mapping of the mouse itself with DUNN's paper on linkage in mice and rats, progress was relatively slow during the subsequent 50 years. Mapping was then largely restricted to markers with visible phenotypes and it was difficult to be sure of the homologies of particular syndromes. For instance, the mouse has many known genes for short tail or for polydactyly. Which of these might be the homolog of a gene with a similar effect in another species? With the use of protein variants, and even more when DNA markers became a standard tool for mapping, the determination of homologies could be made with much more confidence. Comparative mapping has since proceeded very rapidly, particularly in the comparison of mouse and human gene maps. The human homologies of nearly half the length of the mouse recombination map are now known (NADEAU 1989; SEARLE *et al.* 1989; LALLEY *et al.* 1989). Each mouse chromosome has homologies with from two to seven human chromosomes, and the known length of conserved segments ranges from <1 cM to >30 cM. Similarly, each human chromosome has homologies with up to six mouse chromosomes. So far, for instance, all known homologs of genes on human 17 are on mouse chromosome 11, but mouse 11 has homologies with five other human chromosomes (BUCHBERG *et al.* 1989). Knowledge of homologies is not only valuable in making chromosome assignments in other species, it is also important in identifying mouse homologs of human syndromes. Many such homologs of human syndromes are now known, particularly for those diseases in which the underlying protein or DNA defect is known. Some syndromes are clearly similar in man and mouse. Examples include the testicular feminization syndrome, due to a mutation in the androgen receptor (LYON, CATTANACH and CHARLTON 1981), and the hemoglobin mutants, in which comparable molecular changes produce comparable physiological effects (PETERS *et al.* 1985). In other cases the phenotypes of homologous mouse and human genetic defects may be rather different. The mouse mutant *mdx*, with a lesion in the dystrophin

gene *Dmd* (RYDER-COOK *et al.* 1988), has a much milder syndrome than is seen in Duchenne muscular dystrophy resulting from dystrophin defects in man. Similarly, the mouse gene *small-eye* (*Sey*) is apparently homologous with the aniridia defect of the Wilms-aniridia syndrome in man, but has a rather different effect (GLASER and HOUSMAN 1989; HOGAN *et al.* 1986). In these cases, knowledge that the mouse gene was appropriately located was important in finding the homology, and further homologous syndromes will no doubt be identified in this way in the future.

With the wealth of information now available on the mouse map, the importance of coordinated and widely available databases becomes clear. Here DUNN was once again a pioneer. Fifty years ago, in 1939, with GRÜNBERG and SNELL, DUNN founded the first Nomenclature Committee for the mouse, and produced the first gene list (DUNN, GRÜNEBERG and SNELL 1940). Ten years later, in 1949, DUNN, together with SALOME GLUECKSOHN-SCHOENHEIMER (now WAELSCH), edited the first edition of *Mouse News Letter* (now *Mouse Genome*) (SEARLE 1974). Both the Nomenclature Committee and *Mouse News Letter* have continued ever since. The Nomenclature Committee not only promulgates rules for genetic nomenclature but also promotes the dissemination of gene lists and maps with *Mouse News Letter* as its main organ of publication, and has sponsored the production of works of reference giving not only mapping data but also data on the necessary resources such as inbred strains, RI strains and wild strains (GREEN 1981; LYON and SEARLE 1989). The database underlying the mouse map was for many years maintained by M. C. GREEN (1966) and more recently by DAVISSON and RODERICK and their colleagues (DAVISSON, RODERICK and DOOLITTLE 1989). With the exponential growth of information, in the near future the maps are likely to become too detailed to be published regularly on paper, and there will be a move toward electronic publication. Already The Jackson Laboratory makes available on-line the set of databases known as Gbase, including a list of loci, mapping data, and lists of inbred strains and their variants (DAVISSON *et al.* 1989).

Indeed, in the future the previously exponential rate of increase in mapped genes may be surpassed. With the use of interspecific backcrosses, suitable RFLVs for mapping will be available for almost all genes. In addition, there will be anonymous DNA markers generated by microdissection, chromosome sorting and other means. However, in view of the wealth of RFLVs for known genes, anonymous DNAs will be relatively less important than in human genetic mapping. Other variants for mapping will be generated by induced mutagenesis. This may either use conventional agents such as chemicals or radiation, with mutants being collected from known loci or

regions (RINCHIK *et al.* 1986, 1990; RINCHIK, CARPENTER and SELBY 1990; RUSSELL, MONTGOMERY and RAYNER 1982; RUSSELL *et al.* 1979; HITOTSUMACHI, CARPENTER and RUSSELL 1985; SHEDLOVSKY *et al.* 1986; KING *et al.* 1989), or may involve insertional mutagenesis, with the advantage that the mutations will be tagged and thus cloning will be facilitated (GRIDLEY, SORIANO and JAENISCH 1987). Directed mutagenesis by homologous recombination will obviously be an important tool (CAPECCHI 1989). It is probable that the wheel will come full circle and that mutants with visible phenotypes will again become important, either as homologs of human syndromes or as genes with major roles in development.

For the first time, the true length in centimorgans of the mouse genome should become known. For this, convenient markers of centromeres and telomeres are needed and will surely become available. A problem is that this length may well be that, not of the laboratory mouse genome but of the  $F_1$  between *Mus spretus* and the laboratory mouse. Already, one small inversion has been found which differentiates these two genomes (HAMMER, SCHIMENTI and SILVER 1989) and there may well be others. Indeed, there may be differences among strains in small inversions or recombination hot spots, so that details of map lengths may vary from strain to strain. Insight will also be gained into chromosome structure and the genetic content of light and dark G-bands (BICKMORE and SUMNER 1989). At present there is strong variation in the density of markers on the map, from about 20 markers per 10 cM in some regions to fewer than one per 20 cM in others. Does this reflect real differences in the density of genes in different regions or variations in chiasma frequency, or is it merely an artifact of incomplete knowledge?

Thus, it is clear that the mouse has a key role in relation to the human genome mapping project. On the one hand it is by far the best mapped experimental vertebrate, and indeed ranks among the best mapped higher organisms. On the other hand there is detailed knowledge of the comparative mapping of the mouse and human genomes. The mouse is also an excellent organism, in both techniques and resources, for experimental manipulation and analysis of the genome. This means that the mouse will be of fundamental importance in elucidating the genetic basis of mammalian development and will be equally valuable in understanding the genetic basis of human disease.

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### **An Oak Ridge Legacy: The Specific Locus Test and Its Role in Mouse Mutagenesis**

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**W**ITHIN the last two decades, mouse genetics has undergone a revolution, an event initiated by breakthroughs in molecular biology and tissue culture techniques. Previous to this explosion, most scientists were content to puzzle over the thousand or so spontaneous mutants, deletion stocks, and specially designed strains of mice that currently existed. This analysis provided a wealth of insight into developmental biology, immunology, and mammalian genetics in general; nonetheless, the nature of the mutation and the gene that was affected often remained unknown. Today, gene targeting is in vogue, with investigators rushing to make “knock-outs” (disrupted alleles) of every cloned gene. This technique allows researchers to focus on specific genes of interest and to work backward to a phenotype, an approach opposite to studying spontaneous mutants. Gene targeting is indisputably a valuable tool for initiating a mutational analysis in the mouse (Capecchi 1989). The power of genetic functional analysis, however, lies in collecting an allelic series. A few rare examples do exist where gene targeting is used not to create a knock-out but rather a more subtle lesion, *e.g.*, Zeiher *et al.* 1995. For the most part, however, the field today does not often reflect that the early severe phenotype of a disrupted mutation can mask later functions and should only serve as a starting point—as opposed to an end-all—for gene analysis, especially when the allele results in embryonic lethality. Extrapolating from the rampant proliferation of gene targeting papers in the literature, one almost expects the 100,000 or so genes in the mouse to be mutated any day now. Ah, bliss: Every gene knocked-out and a phenotype ascribed to each mouse! In increasing numbers, however, neo-classical geneticists are stepping forward to ask everyone to rethink the analysis.

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**Backstory:** Last November represented the 50<sup>th</sup> anniversary of the arrival of mouse geneticist William (Bill) Lawson Russell to Oak Ridge, Tennessee. The move was coincident with the great fire in Bar Harbor, Maine, that destroyed most of The Jackson Laboratory, Russell’s previous employer (E. S. Russell 1987). At the urging of Alexander Hollaender, Bill—and later his wife Liane (Lee) Brauch Russell, who was finishing her doctorate at the University of Chicago—joined the Biology Division at Oak Ridge National Laboratory to add to Hollaender’s vision of determining the effect of radiation on genetic systems (von Borstel and Steinberg 1995). The research legacy of the Russells is vast but centers on the creation of a specially designed mouse strain called the *T* (test) stock that was used as a genetic screen for the mutagenic testing of radiation and chemicals.

The *T*-stock mouse is a unique genetic tool packed with seven recessive, viable mutations affecting easily recognizable traits. Six influence coat color: *a* (nonagouti, chromosome 2), *b* (brown, chromosome 4), *c<sup>ch</sup>* (chinchilla at albino, chromosome 7), *d* (dilute, chromosome 9), *p* (pink-eyed dilution, chromosome 7), and *s* (piebald-spotting, chromosome 14); one controls ear morphology: *se* (short-ear, chromosome 9). Russell created the strain from a stock of NB mice that already harbored six of the recessive alleles. This strain, however, was so highly inbred that its fecundity and viability were dropping fast, and the line risked becoming extinct. To save the stock, Russell had to outcross NB to another mouse. With the recent burning of The Jackson Laboratory, however, mice were next to impossible to locate (E. S. Russell 1987). Any spare animals that existed were being shipped to Bar Harbor to reestablish the mouse colony. Persistent, Russell located a professional photographer in Florida who also dabbled as a “garage” mouse geneticist and breeder. He supplied Russell with animals that carried three of the six recessive alleles and also provided the *s* mutation.

Invigorated by this outcrossing, the seven-locus *T*stock was created in April 1948 (Russell 1989). By packing all of these mutations into a single mouse, Russell built a valuable tool for simultaneously following the genetics of seven traits.

Russell's initial goal at Oak Ridge was not to determine whether radiation caused hereditary changes in mice because George Snell and others had shown a decade earlier that chromosomal changes induced by X rays had phenotypic consequences (reviewed in Russell 1954). Rather, he wanted to calculate a rate for heritable gene mutations induced by radiation in germ cells. By using the *T*stock, he proposed to study a defined set of loci to see how often they mutated. His approach was simple: Wild-type males were divided into two groups, one set irradiated with various doses of X rays and the second set used as controls. All of these males were crossed to his *T*stock females. Because of the recessive nature of the mutations, the progeny would appear wild type ( $a/+$ ;  $b/+$ ;  $pc^{ch}/++$ ;  $dse/++$ ;  $s/+$ ). However, a mutation at any one of the specific loci would be immediately recognized in the progeny. This approach, called the specific locus test (SLT), allowed Russell to score the number of specific mutations and to calculate a radiation-induced mutation rate in mammals.

It is important to stress the significance of the *T* stock mouse. The experiment could have taken several different approaches that did not employ a specially designed tester mouse. In fact, alternative ideas were suggested by H. J. Muller and Russell's thesis advisor Sewall Wright during a closed-door meeting in Hollaender's office (Russell 1989). Wright thought it would be more appropriate to measure the vital statistics, *e.g.*, weight, longevity, and fertility, of the offspring of irradiated males. Muller feared an SLT in mice would be too difficult to conduct and suggested examining recessive lethals over a larger segment of the genome. Russell argued that was disadvantageous because it would require three generations of breeding. The SLT with the *T*stock mouse, Russell boasted, would allow an individual to rapidly score in the first generation 2000 loci per hour by focusing on the coat color and ear shape. More importantly, however, the SLT would provide better data on gene mutation rate for comparison with *Drosophila*. This was critical to him. The human risk-estimates of radiation at that time were almost exclusively based on fly studies, and Russell wanted to make as precise a correlation between *Drosophila* and mouse as possible. He reasoned that it would be too difficult to compare mutation rates for all dominant visibles in a fly with those in a mouse owing to the "the virtual impossibility of equating morphological and physiological levels of detectability in the two species" (Russell 1951). Instead, the SLT in mice would provide a specific mutation rate for defined loci, a feature especially important because no one had any idea how comparable the two genomes of a mouse and

fly would be. Finally, the SLT allowed the capture and propagation of all of these new mutations in one generation, even those that were lethal when homozygous, allowing many different alleles to be acquired for each locus. By examining each allele individually and in comparison with others, Russell hoped to gain information on the nature of the mutations, initiating a detailed functional genetic analysis in mice.

Russell examined over 85,875 offspring (data rarely matched by today's mouse geneticists) for his first paper on the subject (Russell 1951). In the experiment, he collected 53 new alleles for the seven loci and two spontaneous mutations in the control group, allowing him to calculate a radiation-induced mutation rate per locus for mammals that was 10 times higher than that for *Drosophila*. In his results, however, Russell discovered a wide range in mutation yield among the seven loci and realized that the data could not be quantitatively extrapolated to the entire genome: Some genes just appeared to be more mutable than others. Because most of the seven loci in Russell's SLT were originally discovered by mouse fanciers and pet owners, they might have an intrinsically higher mutation rate, which would explain why they were easily isolated by amateur breeders (like the photographer in Florida). To test this, a team in Harwell, England, constructed a new *T*stock, using different loci: *bp* (brachypodism), *fz* (fuzzy), *ln* (leaden), *pa* (pallid), and *pe* (pearl). None of these are known to have been originally collected by mouse fanciers, even though some of them produce coat colors similar to those in Russell's loci. This Harwell-test stock was subjected to the same X-ray treatments that Russell employed. While examining a smaller data set (about 26,000 offspring), the researchers calculated an averaged radiation-induced mutation rate that was four to five times lower than Russell's but still much higher than that of *Drosophila* (Lyon and Morris 1966). The value of the SLT approach, however, was not that it could extrapolate to whole-genome mutation rates but that it provided a rapid and defined assay to address the parameters affecting mutagenesis.

The Russells continued to use the *T*stock in numerous applications to estimate the genetic hazards of radiation to humans. A seminal paper demonstrated that radiation-induced mutations were dependent on the dose rate, a result in stark contrast to *Drosophila* studies. In a Herculean task of raising over half a million mice in an SLT, the Russells found that animals exposed to a chronic dose of radiation produced markedly lower numbers of mutations than mice given the same radiation as an acute dose (Russell *et al.* 1958). To reconcile this effect with *Drosophila*, they proposed that in mammals some mutations were "reparable" by an as yet unknown mechanism. Having earlier shown that mouse genes were over 10 times more mutable than those of *Drosophila*, the Russells had now demonstrated that "the genetic hazards at least under some

radiation conditions may not be as great as those estimated from the mutation rates obtained with acute radiation" (Russell *et al.* 1958).

Several hundred specific locus mutations were scored and collected in a few decades of radiation mutagenesis. Thanks to the foresight of the Russells, many of these were propagated and maintained for analysis. With dozens of independently induced alleles at each locus, Lee Russell conducted complementation tests that identified sets of overlapping, nested deletions (Russell 1971). This organized the alleles into complementation groups and localized functional units, pioneering a new mapping strategy in the mouse (reviewed in Rinchik and Russell 1990).

The SLT had other valuable spin-offs. First, radiation-induced translocations between autosomes and the X-chromosome were made visible when coat color markers showed variegated patterning owing to the influence of X-inactivation. Such mutants helped propel the single-active X-chromosome hypothesis (Lyon 1961; Russell 1961). Second, specific locus markers allowed Lee to develop the spot test, an assay that quickly scores somatic genetic events such as point mutations, deletions, recombination, and chromosome loss. In the spot test, embryos heterozygous for four of the coat color loci from the SLT were mutagenized and upon birth screened for somatic mutations at these loci by looking for spots of colored fur patches in the pups (Russell and Major 1957). Today, the spot test is still the only general primary *in vivo* screen for mitotic recombination in the mouse and is likely to be revived for today's interest in DNA repair and recombination. Finally, mosaics recovered from the SLT are providing surprising insights into the timing of spontaneous mutations that arise in the germline (Russell and Russell 1996). The analysis of such mice will be invaluable in understanding the basis of human mosaic disease syndromes (reviewed in Kent-First 1997).

**Chemical mutagenesis:** In addition to study of radiation, the SLT could also be used to assay for harmful effects caused by chemicals. It was already known that certain compounds injected into mice had genetic consequences, *e.g.*, Falconer *et al.* (1952), and concerns for human safety were raised by J. B. S. Haldane's "plea" to examine "the mutagenic effect of substances which are frequently added to human food as preservatives" (Haldane 1956). However, most of the early experiments testing a variety of compounds (from caffeine to diethyl sulfate) provided ineffective at inducing mutations in spermatogonia (reviewed in Ehling 1978). Procarbazine, a drug used in the treatment of Hodgkin's disease, was the most effective chemical found to cause any type of significant spermatogonial mutagenesis in an SLT, yet even this rate was still only one third of the maximum Russell attained with X rays (Ehling and Neuhauser 1979). It was almost beginning to appear as if mouse sper-

matogonia were strongly protected against chemical insults or were highly efficient at repairing such lesions. Even diethylnitrosamine (DEN), a compound known to be strongly mutagenic in *Drosophila*, was completely ineffective in mice (Russell and Kelly 1979). To be mutagenic, however, DEN is enzymatically converted into an alkylating agent, and it was possible either that this activation process was not occurring in mammals or, if it was, that the short-lived metabolite was not capable of reaching the testis in time to be effective. To circumvent this complication, Ekkehart Vogel suggested that Russell try the experiment again using ethylnitrosourea (ENU), a chemical that forms the same alkylating species as DEN but does not require metabolism. Russell, still disappointed by the DEN results, thought ENU was going to be another long shot, but he set up a small pilot experiment.

A single dose of ENU was injected into a group of male mice, which then underwent temporary sterility owing to massive killing of spermatogonia. Upon recovery about 10 wk later, however, 90 males were crossed to *T*-stock females and sired 7584 pups. Among this small set of offspring, 35 were mutant for one of the seven loci, yielding an induced mutation rate five times higher than the maximal rate obtained with X rays (Russell *et al.* 1979). Encouraged by these findings, Russell's group showed that if, instead of one large dose, the ENU was fractionated and injected on a weekly schedule to permit a higher total dose to be tolerated, then the mutation frequency jumped to 12 times that of X rays, 36 times higher than procarbazine, and over 200 times the spontaneous rate. When averaged across all seven loci, ENU was now inducing mutations at a frequency of one per locus in every 700 gametes (Russell *et al.* 1982a, 1982b; Hitotsumachi *et al.* 1985). Because the spermatogonial stem cells were being affected, the genetic lesions were not restricted to transient stages but could be recovered indefinitely (at least as long as the mutagenized male survived). Additionally, the ENU mutants were slightly different from the ones induced by X rays. First, the phenotypes sometimes appeared intermediate between the wild-type and *T*-stock alleles. Second, there were never any mutations simultaneously affecting the two closely linked *d* and *se* loci. Third, the number of mutations for the *T*-stock loci that were lethal when homozygous was very low (Russell 1982). ENU apparently caused subtle intragenic lesions (instead of the X-ray-generated deletions) and was heralded a "supermutagen," deigned the "mutagen of choice for the production of any kind of desired new gene mutations in the mouse" (Russell *et al.* 1979).

**ENU as a genetic tool:** The earliest application of ENU to create new mouse mutations was in detecting electrophoretic mobility variants of blood proteins, an efficient screen that could easily assay 21 different loci from a single preparation (Johnson and Lewis 1981).

Because the primary structure of many of these proteins had already been biochemically determined, it was of interest to characterize the structure of the ENU-induced variants to identify the molecular basis of mutagenesis. In the first analysis of a hemoglobin variant, a single amino acid substitution was discovered, and it was proposed that ENU had induced an A to T transversion in a histidine codon (Popp *et al.* 1983), supporting the idea that ENU acts as a point mutagen in mice.

Vernon Bode at Kansas State University and William Dove and Alexandra Shedlovsky at the University of Wisconsin used ENU to dissect the properties of the mouse *t-region*, a bizarre genetic locus with many distinctive traits including interaction with *T* (Brachyury) to produce tailless mice, transmission ratio distortion, and male sterility in compound heterozygotes. The analysis of *t* was complicated by the fact that recombination at *t-region* was strongly suppressed, disallowing the locus to be genetically dissected by crossovers. Thus, to study individual functional units, ENU mutagenesis was used to saturate the area and make discrete intragenic lesions (Bode 1984; Justice and Bode 1986, 1988; Shedlovsky *et al.* 1986, 1988).

Realizing the value of the mouse as a model for human diseases, it was now feasible to mutagenize an animal with ENU and screen for phenotypes resembling clinical disorders. Phenylketonuria, one of the first in-born errors of metabolism characterized in humans, was chosen by Bode as a disease to reproduce in the mouse with chemical mutagenesis (McDonald *et al.* 1990). Besides creating new mouse models, one of the interesting results from this study was the dramatic frequency at which mutations in the phenylketonuria pathway were collected: an astounding one mutant for every 175 gametes examined (Shedlovsky *et al.* 1993). This value, close to 10 times better than the frequency of other loci, may mean that different genes could have very different induced mutation rates, as Russell's group had noticed in the SLT (Hitotsumachi *et al.* 1985).

The value of ENU alleles and the different types of screens used to capture them are diverse:

1. In the positional cloning of complex genetic lesions, ENU-induced mutations can confirm the functional identity of candidate genes, as was done for the *kreisler*, *quaking*, *eed*, and *Clock* loci (Cordes and Barsh 1994; Ebersole *et al.* 1996; Schumacher *et al.* 1996; King *et al.* 1997b).
2. The easiest screen is a hunt for dominants. These will inherently fall out of any ENU experiment and can yield diverse phenotypes from circling behavior to neoplasia disposition (Moser *et al.* 1990). *Clock*, probably the most famous example, is a dominant, antimorphic, ENU-induced allele captured by carefully assaying mice for abnormal well-running activity, resulting in the first cloned mouse mutation to

disrupt circadian rhythm (Vitaterna *et al.* 1994; Antoch *et al.* 1997; King *et al.* 1997a, 1997b).

3. Alleles of an already known mutation can be recovered by conducting an SLT similar to Russell's, where a mutagenized male is crossed to a female homozygous for the test locus (*m/m*). ENU mutations specific to the locus (\*) will be uncovered and recognized in the F<sub>1</sub> generation (*\*/m*). To fully characterize any one mouse gene, this technique should be applied to any disrupted allele made by gene targeting because a functional analysis can be appreciated only by examining an allelic series. While null mutations are necessary, subsequent alleles generated by point mutations including hypermorphs, hypomorphs, antimorphs, and neomorphs can yield vastly different phenotypes. For example, *eed* is a mouse mutation that causes early embryonic lethality. A hypomorphic allele of *eed* induced by ENU, however, allows the mouse to survive embryogenesis. The hypomorph shows skeletal transformations along the vertebral column and provides insight into *eed* as a regulator of homeotic genes (Schumacher *et al.* 1996). Thus, a knock-out database for the mouse genome should be considered only as a starting point; additional alleles are mandatory to complete the functional analysis.
4. Besides structural mutations, ENU will also induce lesions in regulatory elements, a feature not considered in most gene targeting studies.
5. By exploiting nonallelic noncomplementation, it may be possible to conduct sensitized screens in mice. An induced mutation at another locus that happens to interact with the specific locus of interest might fail to complement (*\*+; +/m*) yet still yield a phenotype reminiscent of the original homozygous mutant (*m/m*). This approach, reiterated with each new mutation captured, might generate an extensive functional map of genetic interactions.
6. Another application of ENU is in saturation mutagenesis at defined deletions, yielding discrete functional units at any chromosomal site. Eugene Rinchik designed elegant screens exploiting coat color genetics to provide a fine-structure functional analysis for the *c* and *p* loci deletions originally produced in Russell's X-ray treatments in the SLT (Rinchik *et al.* 1990, 1995). Because deletions in the mouse can now be quickly generated in any part of the genome (Ramirez-Solis *et al.* 1995; You *et al.* 1997), the merging of this technology with chemical mutagenesis will undoubtedly be one of the most productive phases of functional genomics starting off the next millennium.

We now stand at an exciting crossroad in mouse genetics. The field has exploded with an infusion of molecular biologists applying their "tricks-of-the-trade" to manipulate the genome. For a while, chemical mu-

tagenesis fell out of favor. It seemed as if knowing the nucleotide lesions in a mutation would be necessary to produce any value to understanding the biology of genetics. Though ENU mutagenesis may produce interesting variants, the nature of its own power, that being a point mutagen, frightened many people who were obsessed by the fear of never being able to clone the affected gene. Instead, pushes were made to sequence genomes, and the concern over function and phenotype would come later. Well, folks, it's later. The tremendous sequencing projects are starting to pay off by now, providing molecular landmarks throughout the mouse genome that can serve as launching points to sequence new mutations. The pendulum has started to swing back to ENU mutagenesis to generate and collect those interesting mice in phenotype-driven screens that will allow an in-depth study of any gene, chromosomal region, or biological system, thanks to the legacy of the Russells on their 50th Anniversary in Oak Ridge.

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### **One Hundred Years of Mouse Genetics: An Intellectual History. I. The Classical Period (1902–1980)**

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**T**HE year that just ended marked the 100th birthday of mouse genetics. In light of the explosion of interest in recent years in using genetics to understand mammalian physiology and development, especially human disease processes, it is worth recounting the evolution of this field over its first century and the contributions it has made.

If not for Bishop Anton Ernst Schaffgotsch of Austria, we could soon be celebrating the 150th, rather than the 100th, anniversary of mouse genetics (HENIG 2000). The Augustinian monastery in Brünn (Brno) that Mendel entered as a monk was a politically liberal center of scientific thought and investigation, and its abbot was in conflict with his conservative, anti-intellectual bishop. Although the bishop was unsuccessful in his effort to close the monastery, he did succeed in forbidding Mendel from continuing his nascent efforts to study inheritance using the coat color traits of mice. Mendel, who had begun his research breeding mice in cages he kept in his two-room quarters, had to turn to his garden when his bishop insisted that it was not appropriate for a monk to share his living quarters with creatures that had sex and copulated! Fortunately for Mendel, his bishop was rather ignorant of botany, and fortunately for his brother monks, the transition must have had a rather favorable impact on the aroma of the monastery.

Although it is not likely that the history of our science would have followed a different course if Mendel had derived his laws by studying albino *vs.* pigmented, rather than smooth *vs.* wrinkled, there is a subjective warmth in knowing that the Father of Genetics could easily have been the Father of Mouse Genetics as well.

When Correns, Devries, and Tschermak independently reported their rediscoveries of Mendel's laws in 1900, each had worked with higher plants as their experimental material. The question whether Mendelism applied to animals as well as to plants immediately arose, and the answer was not long in coming. By 1902, Lucien Cuénot in France, in the first of a series of articles, demonstrated Mendelian ratios for the inheritance of

coat color characters in mice (CUÉNOT 1902); Gregory Bateson and E. R. Saunders published their work showing that the Mendelian laws applied to the inheritance of comb characteristics in chickens; and Archibald Garrod, with Bateson's advice (BEARN 1994), was able to suggest that alcaptonuria in humans represents a rare homozygous recessive condition. Cuénot's experiments with mice went further. In 1905, he described what proved to be the first lethal mutation discovered, the *A<sup>y</sup>* allele of the agouti locus, although it must be said that an understanding of Cuénot's unusual segregation ratios was not placed on solid experimental grounds until 1910, when W. E. Castle and C. C. Little (CASTLE and LITTLE 1910) confirmed that crosses involving a lethal gene gave predictably aberrant Mendelian ratios in which the missing mice were replaced by dead embryos. Additionally, in his original report on *A<sup>y</sup>* in 1904, Cuénot described the first case of multiple alleles at a locus, although, again, the significance of this was not appreciated until nearly a decade later when, in 1913, Alfred Sturtevant clearly stated the significance of his own finding of multiple alleles at the *white* locus in *Drosophila*. It was, of course, the existence of multiple alleles at a single locus that eventually served to disprove Bateson's early theory that dominant and recessive alleles represented the presence or absence of a gene. (A more in-depth description of Cuénot's efforts will appear in a forthcoming *Perspectives* by M. Hickman and J. Cairns.) Once it was understood that more than two alleles could exist at a single locus, this convenient explanation was no longer tenable, although it took another 50 years before geneticists generally came to accept the view that recessiveness and dominance are not genetic phenomena *per se*, but rather the physiological outcome of the action of gene products.

Despite these beginnings by Cuénot, mouse genetics did not start on the course it was to follow for the next half century until 1909, when two important events occurred. E. E. Tyzzer published a crucial paper (TYZZER 1909) on the inheritance in mice of resistance to

the growth of transplanted tumors, and Little made the first matings to construct an inbred mouse strain. Indeed, Little, recognizing the importance of being able to make a reproducible genetic cross, began his matings in an effort to provide the experimental material he saw as essential to extending Tyzzer's observations. It was in these efforts that the application of mouse genetics to the analysis of mammalian physiology, biochemistry, and pathology began in the sense that we now understand it. Cuénot was a very talented scientist, and his work was quite important in the emerging science of genetics, but it was in the work of Tyzzer and Little that mouse genetics made the first of its contributions to our understanding of mammalian biology.

#### THE CANCER PROBLEM AND THE FIRST 50 YEARS

Tyzzer's and Little's efforts originated from earlier work by J. Loeb showing that tumors arising in a particular strain of Japanese waltzing mice could be successfully transplanted to all animals of that strain, but that "common" mice were completely resistant to tumor transplantation. Tyzzer attempted to study the genetic basis for this difference by crossing the Japanese and common mice and found that, whereas all of the first generation  $F_1$  hybrid mice were susceptible to tumor growth, none (0/54) of the second generation  $F_2$  animals were susceptible. Reasonably enough, he concluded that tumor susceptibility was not inherited as a Mendelian trait. It was the desire to continue these experiments that led Little, who was then a graduate student, to begin the crosses leading to the construction of the first inbred strain of mice, the animals we now call DBA. He was impelled to this by the fact that, although the Japanese waltzing mice were relatively uniform genetically, being the product of many generations of limited inbreeding by mouse fanciers and hobbyists, the other stock used in the cross was rather heterogeneous. Little's goal was to create a genetically uniform stock that could be used as the second partner in making a reproducible cross with Japanese waltzing mice.

Privately, Little was driven by the belief that Tyzzer's results could be explained by Mendelian mechanisms. In 1914, the year Little received his doctorate, he published a theoretical article (LITTLE 1914) offering his alternative explanation. In brief, Little suggested that a large number of genes were involved in determining whether a mouse would accept or reject a transplanted tumor and that for each of these genes there were two alleles, one dominant and one recessive. For a mouse to accept a tumor transplant, it had to carry at least one copy of the dominant allele at every one of the loci involved. If the mouse were homozygous for the recessive allele at even one of these loci, it would reject the tumor transplant. All of the  $F_1$  animals were susceptible because they all received a dominant allele at every

locus from the Japanese parent that provided the tumor. However, because of random assortment of the large number of genes involved, only a very rare  $F_2$  animal would receive a dominant allele at every single one of these loci and would become susceptible to transplantation. In 1916, using the same tumor, LITTLE and TYZZER (1916) reported the results of a much larger experimental series in which 3 of the 183  $F_2$  animals were susceptible. They forwarded this as a demonstration that the ability to accept a tumor transplant was indeed a Mendelian trait and estimated that 14–15 separate genes were involved. Genetic chutzpah indeed!

The boldness of Little's efforts hardly stopped with these experiments. He went on to become a university president (twice), founded and directed The Jackson Laboratory, which over the years has served as home base for mouse geneticists, and managed in the course of all this to discover the maternal inheritance of mammary tumor susceptibility. His very colorful life has been well profiled by Jim Crow in another of these *Perspectives* (CROW 2002).

Over the next 10 years, work by Little, Leonell Strong, and John Bittner tested various tumor and strain combinations, seeking further support for the Mendelian interpretation of tumor transplantation (LITTLE 1941). By the end of the 1920s, Strong had reported a case in which only a single gene was involved and clear Mendelian ratios were obtained and another case in which one of the genes involved showed sex linkage, and Bittner was able to explain in Mendelian terms the inheritance pattern of ability to accept transplantation of a tumor that arose in an  $F_1$  animal. By the early 1930s, the Mendelian interpretation was generally accepted, Little's early faith in a genetic basis for tumor transplantation was vindicated, and the challenge now lay in explaining the mechanism involved.

The groundwork for that explanation came in 1936, when Peter Gorer established the immunological basis of tumor resistance, which had been postulated by J. B. S. HALDANE (1933) 3 years previously. Despite earlier failures by others, Gorer succeeded in demonstrating the existence of red cell antigens in mice similar to those already known in humans. Obtaining antisera against two immunologically distinct red cell antigens, he found these present in some mouse strains and absent from others (GORER 1937a,b). Gorer went on to show that a single gene determined the presence of one of these antigens (erythrocyte antigen 2) and that this gene cosegregated with one of the genes determining resistance to tumor transplantation. Moreover, animals rejecting tumors developed antibodies recognizing the same red cell antigen. Gorer had succeeded in demonstrating two crucial points: that a gene determining resistance to tumor transplantation acts by determining the presence of a cellular antigen and that tumor rejection is associated with formation of antibodies against that

antigen. The major histocompatibility complex, *H2*, had been discovered.

It was now possible to explain the early experiments on tumor transplantation. If a tumor carrying a particular antigen is transplanted into a mouse lacking that antigen, the recipient will mount an immunological reaction against the antigen and reject the tumor. If the recipient carries the antigen, it will be tolerant to that antigen and unable to reject the tumor. In the original experiments of Tyzzer and Little, many such antigens and their genes were involved, and an  $F_2$  animal had to receive an allele for the presence of every one of these antigens to accept a tumor transplant. If even a single one of the antigens was missing in an  $F_2$  animal, the animal would be capable of mounting an immune response against that antigen and thus become capable of rejecting the transplant.

One additional step had to come before it was possible to unravel the intricacies of *H2*; this was George Snell's introduction in 1948 (SNELL 1948) of co-isogenic or, as they came to be later called, congenic strains as a means of eliminating the complexities introduced by the presence of so many other histocompatibility loci. In essence, Snell's idea was to make an  $F_1$  hybrid between two strains, backcross these hybrids to one of the parents (the recipient), and choose progeny retaining the *H2* type coming from the other parent (the donor). By repeating this process for many generations, the eventual result would be a mouse whose genome came almost entirely from the recipient strain except for a small segment of chromosome containing the *H2* locus derived from the donor strain. In practice it was not so simple, because Snell had to alternate each backcross generation with an intercross generation to produce mice that were homozygous for the donor *H2* allele to select a mouse that would transmit the allele in the next backcross. Nevertheless, using a common recipient strain and various donors, he was able to construct a series of strains carrying different alleles of *H2* and other histocompatibility loci on a common genetic background. The construction and study of these strains proved crucial in the analysis of *H2*, and that, in turn, led to the finding of the human major histocompatibility locus, *HLA*. For his pioneering work in this endeavor, Snell later received a Nobel Prize in 1980. J. KLEIN (2001) has provided an essay in this series on Snell's early work.

From 1916 onward, while studies of the genetic basis of tumor transplantation were proceeding apace, many of the same group of geneticists were concerned with the other side of the problem, the genetic factors underlying spontaneous neoplasia. A number of the inbred mouse strains in common use today were developed during that period, either as strains exhibiting a very high incidence of spontaneous neoplasia or as strains that provided necessary low-incidence controls. The A strain with a high incidence of lung adenomas and the C3H strain with a high mammary tumor incidence were

bred by Strong; the high leukemia strains AKR and C58 were bred by Jacob Furth and Carleton MacDowell, respectively.

A key finding in the genetic analysis of spontaneous tumor incidence came in 1933 when, under C. C. Little's leadership, the entire staff of the nascent Roscoe B. Jackson Memorial Laboratory (as The Jackson Laboratory was then called) published a note in *Science* (ROSCOE B. JACKSON MEMORIAL LABORATORY STAFF 1933) reporting that the propensity to form mammary tumors in mice is maternally inherited; the genetic origin of the fathers was irrelevant. These facts were independently established by Korteweg in the Netherlands (KORTEWEG 1936), and the explanation for maternal inheritance came 3 years later when Bittner foster-nursed newborn mice on susceptible and resistant mothers and discovered that the factor being transmitted was in the mother's milk (BITTNER 1936), not in the genome. By 1942 the milk factor was recognized as a virus (BRYAN *et al.* 1942; VISSCHER *et al.* 1942); by 1968 the mouse mammary tumor virus (MMTV) was an established entity, and the concept of germ-line transmission of provirus was understood (MÜHLBOCK and BENTVELZEN 1968; VARMUS *et al.* 1972). Conceptually similar experiments led to an understanding of the viral etiology of murine leukemia, except that here the virus was transmitted by experimental inoculation of newborn mice, rather than spontaneously through the milk. From those experiments came our understanding of mammalian retroviruses and an understanding of their ability to induce tumors by attaching an active viral promoter of gene transcription to an adjacent chromosomal proto-oncogene.

Two themes, then, dominated the first 50 years of mouse genetics. One was the study of genetic factors determining susceptibility to transplanted tumors, which eventually led to the discovery and analysis of the major histocompatibility complex. The other was the effort to analyze the genetic basis for differences in the incidence of spontaneous neoplasms, which eventually led to the discovery of retroviruses and their role in neoplastic transformation. Related by the cancer problem, these two lines of research provided the original motivation for establishing inbred mouse strains and later stimulated several of the other technical developments of the mouse as a genetic system. The conceptual goal, one that intensely motivated many of the early workers on a personal level, was an understanding of cancer, and, as always, methodology was developed in response to experimental needs. Cancer was the driving force that carried mouse genetics through its first 5 decades and greatly influenced the development of the mouse as a genetic system. The pressure to solve an important medical problem resulted in the creation of a new experimental system that was to have far wider application in the years to come. For the cancer problem itself, the eventual outcome proved to be one of those

recurrent ironies of scientific history. While the study of spontaneous neoplasms led to the discovery of retroviruses and oncogenes and has brought us to the brink of a deep understanding of the biological basis of cancer at a molecular level, the studies of tumor transplantation, which started it all, had no significant impact on our understanding of cancer. Rather, in leading to the discovery of the major histocompatibility complex, these studies inadvertently initiated the description of a molecular complex central to the operation of cellular immunity.

#### THE EXTENSION OF MOUSE GENETICS TO MAMMALIAN BIOLOGY AT LARGE

Beginning about 1960, a series of quite different subjects began to appear in the mouse genetics literature with increasing frequency. The genetic systems that had been developed for the analysis of the cancer problem were proving powerful enough to be turned to new uses, and these soon moved to the forefront.

**Sex determination and dosage compensation:** One of these uses was in providing an explanation of how mammals cope with having two X chromosomes in females and only one in males. In 1961, Mary Lyon proposed the now widely accepted inactive X mechanism to resolve the X chromosome dosage dilemma (LYON 1961). Her hypothesis derived from observation of the expression of X chromosome mutations with visible phenotypes and suggested that the problem of having two copies of the X chromosome in females and only one copy in males is solved in mammals by having one of the two female X chromosomes randomly inactivated for the life of the organism in each cell of early embryos. This is in contrast to flies, in which both X chromosomes are active in all cells, but at a reduced level compared with the single X present in males.

Almost simultaneously, studies of human and mouse chromosome abnormalities made it clear that the Y chromosome determines sex. It rapidly became apparent that in mammals sex determination and dosage compensation of the X chromosome occur by mechanisms fundamentally different from the classic explanations originally derived from *Drosophila* studies. Mammalian sex is determined by the presence or absence of a Y chromosome and not by the relative numbers of X chromosomes and autosomes, as occurs in flies. The crucial observation was that among mammals, XO individuals are females, whereas among flies they are males (albeit sterile). We now understand that the driving factor on the Y chromosome is the *Sry* gene, coding for a DNA-binding protein.

**Biochemical genetics:** The contemporary study of biochemical genetics in mice developed out of work in K. Paigen's laboratory on the  $\beta$ -glucuronidase gene (PAIGEN 1961a,b; SWANK *et al.* 1973) establishing that there are genetic determinants closely linked to the

structural gene for this enzyme deciding its tissue-specific pattern of expression, intracellular location, and responsiveness to hormonal regulation, thereby demonstrating for the first time the existence of mammalian genetic regulatory systems at the molecular level. During the 1960s and '70s a number of laboratories analyzed increasing numbers of genetic variants with altered enzyme activity, until a fairly comprehensive picture of the kinds of genetic variation likely to lead to changes in enzyme activity emerged. Two salient features were noted. One was the independent (so-called codominant) expression of the two alleles of a gene, a finding that was important in clarifying the physiological basis of recessive *vs.* dominant inheritance. The other was that, almost without exception, regulatory differences were *cis*-acting, mapping to the structural genes themselves. No regulatory systems akin to those discovered in *E. coli* were found. It will be interesting to see how our rapidly evolving abilities to study mammalian regulation at the molecular level modify these insights.

**Mammalian physiology:** The last 25 years have also seen the steady accumulation of physiologically and biochemically interesting mutants of the mouse, mutants that allowed mammalian geneticists to enter entirely new areas of research. Many came from The Jackson Laboratory, where mouse handlers in the production department were trained to recognize and save any mouse showing exceptional appearance or behavior; from the MRC Radiobiology Unit (now the Molecular Genetics Unit) at Harwell; and from the observations of numerous investigators elsewhere. There ensued a steady flow of useful new mutants with immune deficiencies, endocrinological defects, blocks in specific differentiation pathways such as hematopoiesis, and neurological and behavioral abnormalities of all kinds. All of this was in addition to a variety of mutants whose physiological bases at that time could only be guessed (GREEN 1981). The early analysis of mutants provided important genetic as well as physiological insights. One with far-reaching implications was the demonstration by Douglas Coleman and Katrina Hummel of the importance of the genetic background and the modifier genes it may contain on the expression of a single major locus determining a pathological trait (HUMMEL *et al.* 1972). They found that the *obese* and *diabetic* mutations, which produced quite different phenotypes in the two inbred strains in which they arose, actually produced the same phenotype if they were present in the same genetic background. This observation, the importance of strain background and modifier genes, has been observed repeatedly and, indeed, has become a major theme in contemporary experimentation. COLEMAN (1978) went on to show by parabiosis experiments that, although the two mutations gave the same phenotype, they were physiologically quite distinct. *Obese* determines a circulating satiety factor (later identified as leptin) and *dia-*

*betic* determines its site of action (later identified as the leptin receptor).

#### THE MOUSE AS A GENETIC SYSTEM

The development of the mouse as a genetic system for the analysis of mammalian biology was driven by research requirements.

**Inbred strains:** The first major development and the one that determined the course of mouse genetic research more than any other was, of course, the development of inbred strains of mice. Beginning with Little's first crosses in 1909, workers had been continuously developing new inbred strains, until by 1980 over 300 such strains existed (STAATS 1980). Many of the most commonly used strains originated in the 20 years following Little's initial breeding efforts as a response to the need for genetically uniform stocks in the study of the cancer problem. In no other eukaryote have such a variety of genetically uniform stocks been available as the starting point for genetic work. The genotypic and phenotypic diversity across these strains is quite remarkable, often exceeding that of the entire human population and serving as a starting point for the identification of the underlying genetic elements. The sources of this variation are likely twofold. The common laboratory mouse is not, strictly speaking, a species in the Linnaean sense, but rather a mixture of genomes from at least four species and subspecies, if not more. It is likely that, in the course of laboratory-directed brother-sister matings, sets of once compatible alleles at multiple genes were disrupted and arbitrary new sets were created. And all of the buffering effects of heterozygosity were removed when strains were made completely homozygous. Although this variation was not created intentionally, it is now of great utility, and a phenome project has developed to characterize and record this diversity for its scientific utility (<http://www.jax.org/phenome>).

**Genetic maps:** Every genetic system ultimately rests on the availability of a useful set of "markers," genes or DNA sequences whose alleles can be conveniently typed in crosses to track the inheritance of chromosomal regions. The map, describing the linear arrangement of these sites along with estimates of the distances between them, is an essential genetic tool. Together, the markers and map allow us to locate new genes and manipulate them in experimentally useful ways. To the outsider, the geneticist's obsession with markers and maps may appear amusing, even pedantic, but the insider knows that we live by our markers and maps. The more complete and detailed these are, the more precise and elegant our efforts.

The first genetic markers of the mouse go back to antiquity. The term for a spotted mouse appears in the earliest Chinese lexicon dating back to 1100 BC, and waltzing mice have been known since 80 BC. For 1300 years, beginning in the fourth century AD, the Chinese

government kept records of the finding of wild albino mice. In Japan, the mouse was admired as the symbol and messenger of the God of Wealth, Daikoku, and old Japanese woodcuts clearly show such familiar mouse mutations as albino, non-agouti, dominant and recessive spotting, and pink-eyed dilution. These ancient mutations, preserved by mouse fanciers, provided the earliest markers of mouse genetics.

In 1915, Haldane, Sprunt, and Haldane described the first genetic linkage in the mouse, between albino and pink-eyed dilution (HALDANE *et al.* 1915), to create linkage group I, which in later years was located to mouse chromosome 7. Initially, progress was slow; it was 12 years to the next linkage, and by 1935 only 11 markers had been collected into five linkage groups. But mapping efforts continued to accelerate, and for a long time the map grew exponentially with a doubling time of about 8 years. Mary LYON (1990) has provided an illuminating description of these efforts. Initially, the maps were assembled by Margaret Dickie, but for many years the cartographer of this effort was Margaret C. Green, who patiently collated the accumulating data and annually revised the map.

The genetic markers came in three waves. Initially, there were morphological mutants whose changes in coat color or skeletal characteristics were obvious to the naked eye. Then came the biochemical variants, primarily alternate electrophoretic forms of enzymes that could easily be stained in gels. Finally, we saw the introduction of DNA sequence polymorphisms that can be detected with molecular technologies, which in the post-1980 period took us to a doubling time of 2–3 years. The first molecular markers were restriction fragment length polymorphisms (RFLPs), then simple sequence length polymorphisms (SSLPs), and finally single nucleotide polymorphisms (SNPs). Several million SNPs are present in the mouse (and human) genomes, providing an inexhaustible supply of densely spaced markers. Now, with the mouse genome sequence virtually complete, we can know their physical as well as genetic location.

Assigning genetic linkage groups to physical chromosomes occurred very rapidly once the requisite technical advances had occurred. In essence, making these assignments involved three experimental steps. First, cytogenetic techniques, especially quinacrine staining, were developed, enabling each chromosome to be recognized by its unique banding pattern. Then a series of chromosome rearrangements were obtained. These were either translocations that produced new physical connections between parts of chromosomes or chromosome fusions that attached two previously separate chromosomes. Finally, genetic crosses were carried out to determine which linkage groups were affected in each case. Outstanding among the laboratories involved in the tedious work of finding and characterizing rearrangements were the groups of T. C. Carter, Mary Lyon, and A. G. (Tony) Searle at Harwell, England. Much of

the work of correlating linkage groups with physical chromosomes (FRANCKE and NESBITT 1971; NESBITT and FRANCKE 1971; MILLER and MILLER 1972) was done in the laboratories of D. A. and O. J. Miller. Eva Eicher, who provided the first such attachment, has provided an intriguing history of the multiple efforts (EICHER 1981). The process of assigning all 20 linkage groups to chromosomes was completed by 1980.

**Congenic strains:** As already mentioned, Snell, in 1948, had introduced the concept of congenic strains into his studies of histocompatibility genes as a way of examining one gene at a time. Nowadays, so-called "speed congenics" can be constructed in about four generations by using genome-wide marker scans to take advantage of chance variation among backcross offspring to minimize the transmission of unwanted donor strain genes.

**Recombinant-inbred lines:** Constraints of generation time and population size originally limited genetic mapping efforts in the mouse. Later, the development of recombinant-inbred (RI) lines drastically increased these capabilities. The first use of such lines was reported by Bailey in 1971 (BAILEY 1971), following up on a theoretical suggestion published 40 years earlier by J. B. S. Haldane and C. H. Waddington. The basic principle is straightforward. Two mouse strains are crossed, and from the  $F_2$  generation, mating pairs are used to establish a new set of inbred lines by repeated generations of brother-sister matings. Within a line, each pair of chromosomes is homozygous for a mosaic of alternating DNA stretches derived from the two parents, and each of the new lines has a randomly different mosaic arrangement. In any set, the number of recombinant-inbred lines that are concordant for the segregation of two genes will be greatest when the two genes are very close to each other, and the degree of discordance is a measure of the distance between them. On average, closely linked markers show a fourfold increase in the apparent genetic distance between them because of the multiple opportunities for recombination over the course of inbreeding.

The elegance of the RI strain approach to mapping becomes apparent when we realize that each of these new inbred lines can be maintained indefinitely as the equivalent of an "immortal" segregant in a cross; a new gene tested for its segregation pattern now can be compared for genetic linkage with every gene that was ever scored by any laboratory in the same set of strains, and it can eventually be compared for linkage with any gene that is tested in the future. Initially, this approach was carried forward extensively by Ben TAYLOR (1978) and since then has been widely adopted. Over time, approximately 20 sets of recombinant-inbred lines arising from various pairs of progenitor strains were constructed and mapped for hundreds of markers (<http://www.informatics.jax.org>), making it quite probable that, when a new

gene is tested for its segregation pattern, a genetic linkage and map position will be forthcoming.

Today, although any DNA sequence can be mapped instantly by reference to the mouse genomic sequence, RI resources still have a powerful utility in mapping the genes underlying phenotypes whose molecular basis is still unknown and are especially useful for complex phenotypes, such as developmental processes, regulatory phenomena, or disease progression, that cannot be determined on a single mouse. Such phenotypes can be determined across multiple animals within each member of a set of RI lines, searching for concordant distribution with previously typed molecular markers. For a single-gene effect, typing 14 or more lines is enough to provide a defined map location with a resolution of a few centimorgans.

**Variations on the recombinant-inbred lines:** Peter Demant, in an effort to reduce genetic complexity when multiple genes underlie a phenotypic difference, in his case tumor susceptibility, extended the basic concept of RI lines by constructing RI lines he called recombinant congenics, in which one parent contributes 1/8 and the other 7/8 of the composite genome (DEMAN and HART 1986). In yet another variation, Ariel Darvasi has suggested construction of "advanced intercross lines," in which multiple generations of random mating precede interbreeding as a means of increasing recombination and hence the resolving power of genetic crosses (DARVASI and SOLLER 1995).

**Chromosomally engineered lines:** Several additional approaches have been developed to provide a more orderly, less random means of assigning genetic determinants to phenotypes. Although developed post-1980, it is appropriate to mention them here. One is chromosome substitution lines (called consomics), in which one chromosome at a time in a recipient strain is replaced by its homolog from a donor strain, a strategy first carried out by Jean-Louis Guénet, who introduced chromosomes from *Mus spretus* into a *M. musculus* laboratory mouse strain. Another, which reduces the size of introgressed DNA even further, is the construction of a set of congenic strains carrying pieces that systematically cover the entire genome, each about one-fourth of a chromosome in size.

#### THE WINTER OF 1980–1981

Numerous historical accounts describe various aspects of this classical period of mouse genetics to which the interested reader is directed for further insight (LITTLE 1941; HESTON 1949; STAATS 1966; POTTER and LIEBERMAN 1967; KEELER 1973; KLEIN 1975; MORSE 1978). Then, at the end of 1980, in a period of a few months, an entirely new era in mouse genetics began, with the creation of the first transgenic mice, initiated by the abrupt and then continuing entry of molecular biological techniques into what had, until then, been a

classical genetic system. What ensued was an explosion of knowledge when a myriad of new biological and molecular insights appeared over the following years. Although certainly built on the past, the new science quickly developed a life of its own and deserves its own chapter.

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### **One Hundred Years of Mouse Genetics: An Intellectual History. II. The Molecular Revolution (1981–2002)**

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To a Well-Connected Mouse

*(Upon reading of the genetic closeness of mice and men.)*

Wee, sleekit, cow'rin, tim'rous beastie,  
Braw science says that at the leaſtie  
We share full ninety-nine per cent  
O'genes, where'er the odd ane went.

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A previous *Perspectives* (PAIGEN 2003) described the development of mouse genetics from its beginnings in 1902 until the beginning of the molecular revolution. Here, we pick up the story and follow it through to the end of its first century.

Understanding human biology in both its normal and pathological aspects requires experimental material. Despite their best efforts, clinical scientists face some intrinsic difficulties. Deliberately inducing pathology or toxicity is unacceptable; patients are understandably reluctant to provide serial tissue samples; a critical portion of human life, the embryonic/fetal period, is not very accessible; genetically defined lines cannot be created; the generation time is too long for extended genetic studies; and individuals exhibit a stubborn tendency to choose their own mating partners, frustrating geneticists.

In searching for an experimental surrogate, we are fortunate that the basic features of mammalian biology have changed little over the 75+ million years since the major orders of mammals diverged. This is true not only of gross anatomy and the major physiological systems, but at the molecular level as well. The recent genomic sequencing efforts suggest that we share 99% of our genes, and hence our molecular building blocks, with another mammal, the mouse.

*In vitro* and cell culture systems are very powerful, but they do not allow us to study physiological systems in their entirety, and certainly not their interactions, which are many. For these we need the whole animal.

In choosing a mammalian model, we want a creature that breeds rapidly, can be inbred (not all mammals

can), whose husbandry is facile, and that is small and hence cheap to maintain. This last characteristic is of no small importance. Enter the mouse. Sixty years of classical genetics (PAIGEN 2003) resulted in the development of a very sophisticated genetic system with hundreds of inbred (RI) strains, even more mutants, a dense genetic map, sophisticated mapping resources such as recombinant inbred lines and their derivatives, and a well-established system of husbandry.

The dramatic transformation of mouse genetics into a truly powerful system for understanding mammalian biology at the molecular level and its relevance to the human state came when the newly emerging techniques of molecular biology were added to the well-established genetic systems of mice. We can date the birth of that union to a few months centered around New Year's Day of 1981, when the first transgenic mice were created. Since then, two complementary lines of experimentation have dominated, developing either our ability to begin with a known DNA sequence and answer questions about its functions in the organism or our ability to begin from a particular phenotype and discover the genes required for its normal or abnormal expression. Enhancing both genotype- and phenotype-driven approaches has been the ability to do this iteratively, first discovering genes underlying phenotypes and then using the powers of molecular gene manipulation to study their function and search for additional related genes or, conversely, beginning from the DNA sequences themselves and deducing from directed mutations the range of phenotypes controlled by each gene.

## FROM GENOTYPE TO PHENOTYPE

**Transgenic mice:** Five laboratory groups more or less simultaneously showed that viral or mammalian DNA injected into mouse oocytes could be incorporated into the genome (GORDON *et al.* 1980; BRINSTER *et al.* 1981; COSTANTINI and LACY 1981; HARBERS *et al.* 1981; WAGNER *et al.* 1981). Chronologically, Jon Gordon and his colleagues (GORDON *et al.* 1980) were the first, but the experiment that forced a dramatic change in our thinking was that of T. E. Wagner and associates (WAGNER *et al.* 1981). The latter group showed that an intact rabbit  $\beta$ -globin gene introduced into the mouse genome was expressed in a correct tissue-specific manner, resulting in the presence of rabbit protein in mouse erythrocytes. The implications of this finding were stunning. Not only did it make clear that correct expression of a gene is not limited to the environment of a unique chromosomal location, but for the first time we saw that developmental, tissue-specific regulatory systems are conserved and can function across orders of mammals, implying that the function and regulation of human as well as rabbit genes could be studied in an experimental species as tractable as the mouse. This was soon followed by a dramatic demonstration that captured the imagination of the lay public as well as scientists: Richard Palmiter, Ralph Brinster, and colleagues fused the rat growth hormone gene to a mouse metallothionein promoter and inserted this as a transgene, obtaining very high levels of gene expression and giant mice that made the cover of *Nature* (PALMITER *et al.* 1982). A flood of experimentation followed from many laboratories, and Palmiter has provided a detailed review of those early years (see PALMITER and BRINSTER 1986).

**Homologous recombination and knockouts:** The next dramatic advance, which opened the field to much broader applications, came a few years later when gene replacement became possible in mice. Initially, this led to the creation of absolute knockouts and later to tissue-specific and conditional knockouts. Two lines of experimentation had to merge for this to become possible. One was the development of cultured pluripotent embryonic stem cells (ES cells), which could be reimplanted into mouse blastocysts, giving rise to chimeric mice whose adult tissues, including their germ line, were derived from the inserted ES cells and thus were capable of transmitting ES cell genetic material to their offspring. The other line of experimentation was the achievement of homologous recombination in ES cells in a manner that left them pluripotent and germ-line competent. Together, these made gene-specific, genetic engineering possible. This molecular revolution took nearly a decade, and it is a tribute to the perseverance of all involved (as a reading of the articles published during this period makes clear) that the ultimate goal was apparent from the outset.

ES cell cultures were first obtained in 1981 by Martin

Evans and Matt Kaufman (EVANS and KAUFMAN 1981) and by Gail Martin (MARTIN 1981), following on the intellectual and experimental foundations laid by Roy Stevens and Barry Pierce. When these cells were introduced into blastocysts, they gave rise to a variety of adult tissues, including the germ line (BRADLEY *et al.* 1984). In 1986, two groups showed that genetically altered ES cells, either neomycin-resistant mutants (GOSSLER *et al.* 1986) or cells carrying retroviral insertions (ROBERTSON *et al.* 1986), could transmit these traits to offspring. In the following year, HPRT-deficient mice were created from ES cells selected for this mutation (HOOPER *et al.* 1987; KUEHN *et al.* 1987).

The challenge then was to achieve targeted homologous recombination in ES cells. In 1985, Smithies and his group (SMITHIES *et al.* 1985) successfully targeted a normally resident gene, the human  $\beta$ -globin locus, in other cultured cells, and two years later the Smithies (DOETSCHMAN *et al.* 1987) and Capecchi (THOMAS and CAPECCHI 1987) groups both achieved gene targeting in ES cells. Two technical advances, positive/negative selection (MANSOUR *et al.* 1988) and the development of an appropriate enhancer/promoter system (THOMAS and CAPECCHI 1987), brought the efficiency of targeting up and enhanced selection in ES cells, and in 1989–1990 three groups reported the first gene-targeted mice (KOLLER *et al.* 1989; THOMPSON *et al.* 1989; THOMAS and CAPECCHI 1990).

A practical system had come into existence, and a virtual land rush began to knock out genes of biological interest. Many of these early experiments are well described in the addresses given by Evans, Smithies, and Capecchi when they jointly received the 2001 Lasker Award (EVANS *et al.* 2001).

The early knockout results were sobering; the phenotypes of homozygous null mutations were often quite unexpected. When *MyoD*, a gene thought to be essential for muscle differentiation (WEINTRAUB *et al.* 1989) was knocked out, there was little if any reduction in muscle mass (RUDNICKI *et al.* 1992). *MyoD* turned out to be redundant to *Myf-5*, and both must be inactivated to affect muscle differentiation (RUDNICKI *et al.* 1993). Similarly, when *p53*, the gene most frequently mutated in human tumors and presumed to be essential for orderly cell division, was knocked out, it was found not to be a developmental lethal (DONEHOWER *et al.* 1992; JACKS *et al.* 1994). The resulting homozygotes were born alive and well. The importance of *p53* was vindicated when the mice came down with multiple tumors months later. Despite these frequent lessons in humility to the research community, knockouts have provided an extraordinarily powerful research tool, making possible the analysis of many physiological processes in ways that were previously unimaginable.

That power soon stimulated refinements to overcome the developmental lethality many knockouts exhibited. The Cre/loxP recombinase system was stolen from bac-

terioophage P1 and introduced into mammalian systems to allow tissue-specific gene inactivation. When mice received a target gene flanked by loxP sites from one parent and Cre recombinase under the control of a tissue-specific promoter from the other, the target gene is deleted from chromosomes only in tissues where the recombinase is expressed (LAKSO *et al.* 1992; ORBAN *et al.* 1992). The microbial FLP system has been similarly employed (WIGLEY *et al.* 1994).

Later, to satisfy the desire to modulate gene function in a reversible manner, target genes were put under the control of the *tet* repressor, again a regulatory system borrowed from microorganisms, allowing them to be reversibly turned on and off by the presence or absence of tetracycline analogs in drinking water (FURTH *et al.* 1994). An early demonstration of just how powerful tissue-specific reversible control could be appeared in the experiments of MAYFORD *et al.* (1996), who examined the role of CaMKII in the molecular basis of learning and memory by reversibly controlling the presence of this protein in specific neural tissues.

The recent discovery of RNA interference (dubbed RNAi), in which small, double-stranded RNA sequences attenuate the expression of genes containing the same sequence, holds the promise of advancing matters further by offering a much simpler technology for eliminating the functions of particular genes. Obtaining homozygotes of conventional knockouts requires isolating homologous recombinants in ES cells, injecting these into blastocysts that are then implanted in pseudo-pregnant females, obtaining chimeric offspring, and then breeding these for at least two generations to obtain homozygotes. If transgenes expressing the requisite RNA sequences as palindromes can be used as dominant negative regulators of gene function through RNA interference, this would provide a technically far easier means of generating knockouts. Combining this with tissue-specific and/or regulatable promoters would provide a very facile experimental system. Already, success has been achieved in cell culture systems (ELBASHIR *et al.* 2001; BRUMMELKAMP *et al.* 2002; PADDISON *et al.* 2002; SUI *et al.* 2002), and it seems likely that we shall soon see success in mice.

**Lessons:** Transgenic and knockout technologies have been critical in answering many questions about the role of specific genes in the biology of the organism. Beyond these individual answers obtained over the past 20 years, there have been some general lessons. One was, of course, the recurring difficulty of predicting phenotype. Another was just how pleiotropic mutations are, emphasizing what some classical geneticists had been saying for years: we do not have genes “for” a particular phenotype, but rather a particular phenotypic change is likely to be only one among the many consequences of the mutation in question, a point well understood by human geneticists and reflected in the fact that so many human gene defects are called “syndromes.”

## FROM PHENOTYPE TO GENOTYPE

**Spontaneous and induced mutations:** The obvious requirement of gene to phenotype studies is knowing which gene to begin with. And so there was a major simultaneous push to go in the other direction, starting from mice with altered or different phenotypes and then identifying the genes responsible.

This began with efforts to clone already existing mouse mutations by using a “positional” cloning strategy. The logic was straightforward. Carry out genetic crosses on a scale sufficient to refine the location of a mutation down to molecular dimensions, something less than a megabase, and then explore the genes within that region for sequence and/or expression changes in the mutant. The fact that these mutations arose on inbred strain backgrounds meant that the only sequence or expression differences present should be related to the original mutation, and having multiple alleles at the mutant locus was a definite assist.

An early and important success came with the cloning of the *W* and *Steel* mutations, showing that *W* encodes the c-kit tyrosine kinase receptor (CHABOT *et al.* 1988; GEISLER *et al.* 1988) and *Steel* encodes its ligand (BRANNAN *et al.* 1991; FLANAGAN *et al.* 1991). This was a fine confirmation of earlier transplantation studies between these two mutants and with wild-type animals showing that *Steel* and *W* determine, respectively, the ability to send and receive signals essential for hematopoietic stem cell differentiation. Elizabeth RUSSELL (1979) has provided a review of these initial efforts.

Other early successes encouraged others by showing that gene cloning can help explicate biological phenomena. Two examples were the cloning of the *agouti* (*A*) gene in Rick Woychik’s laboratory (BULTMAN *et al.* 1992) and the *obese* (*ob*) gene in Jeff Friedman’s laboratory (ZHANG *et al.* 1994). The latter identified leptin, a new peptide hormone acting as a satiety factor. Shortly thereafter, the *db* gene was also cloned, proving to be the leptin receptor. Together, these confirmed Doug Coleman’s original hypothesis regarding these mutations (PAIGEN 2003).

Many additional mutations were soon cloned; many were classical mutations, and others arose as accidental consequences of transgene insertions. Indeed, a small industry quickly emerged, providing multiple new insights into old biological problems. (For a review of these many efforts, see MEISLER 1992; BEDELL *et al.* 1997; and WOYCHIK and ALAGRAMAM 1998.) So successful were these efforts that researchers soon sought a broader supply of mutants to challenge their efforts, especially mutants altered in specific phenotypes. They turned to ethylnitrosourea (ENU), a powerful mutagen in male mice whose very high mutation rates make it feasible to seek changes in specific phenotypes. The discovery that ENU is an exceptionally powerful mutagen in mice had come some years earlier in the work

of the Russells at Oak Ridge, using the “seven locus” test for chemical mutagenesis (RUSSELL *et al.* 1979; DAVIS and JUSTICE 1998). Early success showing that it was now feasible to screen for and then clone mutations with specific phenotypes came from Vernon Bode’s laboratory (BODE 1984; McDONALD *et al.* 1988). Soon the Dove laboratory created and cloned a mutation causing colon cancer (MOSER *et al.* 1990, 1993), which proved to be the ortholog of the human *Apc* gene with the same phenotype, and Joe Takahashi’s laboratory, working with Dove, created and cloned the *Clock* mutation affecting circadian locomotor activity (VITATERNA *et al.* 1994; KING *et al.* 1997).

These efforts had a considerable impact on thinking within the research community. In 1998 the National Institutes of Health (NIH) convened the first of several meetings of the mouse genetics community to recommend important new initiatives that NIH might undertake to further the use of mice in biological research. Stimulated by the early successes with ENU, one of the major recommendations was the undertaking of large-scale mutagenesis programs. (For a full report of that and later meetings go to <http://www.nih.gov/science/models/mouse/>.) Similar mutagenesis initiatives were launched elsewhere; more than a dozen such efforts are under way in Germany, the United Kingdom, Australia, Japan, Canada, and the United States, providing hundreds of new mutants per year. Many of these mutations affect phenotypes that were not possible to search for previously, such as seizure susceptibility, hypertension, fear-potentiated startle response, immune responses, and blood chemistry, phenotypes that would be too difficult or too expensive to test for without the very high mutant frequency provided by ENU.

**Natural polymorphisms:** The other phenotype-driven strategy for finding the genes and their products underlying complex traits depends on exploiting the remarkable phenotypic diversity found among inbred mouse strains, a diversity that often exceeds that of the entire human population. Indications that such diversity might exist came in earlier, smaller strain surveys and has become abundantly apparent with the onset of a systematic effort, the phenome project, to characterize a broad array of phenotypes across a standard set of inbred strains and then provide these data in the form of a searchable database (<http://www.jax.org/phenome>). The data on blood cholesterol levels serve to illustrate this diversity: among the tested set of inbred strains, total cholesterol levels vary fivefold, HDL-cholesterol levels tenfold, and HDL cholesterol as a percentage of total cholesterol ranges from 5 to 75%.

It is interesting to speculate on the sources of this exceptional diversity. Two obvious possibilities suggest themselves. First, inbred strains of laboratory mice represent a mosaic of genetic material from at least three wild species or subspecies, *Mus musculus musculus*, *M. m. domesticus*, and *M. m. castaneus*, and perhaps others

(BECK *et al.* 2000). They represent a geographic range from the Atlantic shores of Western Europe to Japan. It may well be that what were once physiologically compatible sets of alleles at multiple loci in the original wild populations have been scrambled into less compatible, new arrangements in the course of the forced inbreeding used to create our present-day inbred strains. Second, our inbred mice are exactly that, homozygous at virtually every gene, having lost all the physiological buffering effects of heterozygosity that are obtained in natural populations and strain crosses.

This diversity among inbred strains can be exploited to find the genes determining physiologically important phenotypes, especially those relevant to human health. The conceptual basis for doing so stems from the landmark paper by Eric Lander and David Botstein (LANDER and BOTSTEIN 1989) proposing a methodology for using a dense array of genetic markers to localize the genetic determinants for a polymorphic quantitative trait to particular chromosomal regions. The paper from John Todd’s group (TODD *et al.* 1991) examining diabetes susceptibility not only initiated efforts to identify such quantitative trait loci (QTL) in mice, but also introduced two major advances in the approach. One was technical, for the first time employing as genetic markers in mice the polymorphic microsatellite markers (simple sequence repeats) detected the year before in human populations (LITT and LUTY 1989; TAUTZ 1989; WEBER and MAY 1989). The other advance was conceptual, breaking a complex phenotype into subphenotypes that are likely to have a simpler genetic causality. In this case, the subphenotype was insulinitis, which turned out to be determined by one, but not the other, of the two new diabetes loci they had discovered.

Since these initial efforts, a wide variety of phenotypes representing multiple physiological systems have been mapped in mice (PAIGEN 2002). Initially, molecular identification of the underlying genes was difficult, but that is changing rapidly with the availability of the mouse genomic DNA sequence and the use of large-scale expression assays relying on arrays, chips, and especially real-time PCR. The year 2001 saw almost as many naturally polymorphic genes successfully cloned as in all previous years combined (KORSTANJE and PAIGEN 2002).

**Human-mouse concordance:** For years it has been an open question whether the genetic polymorphisms governing phenotypic diversity in the mouse and human populations would be the same. One can make theoretical arguments for either answer. The recently emerging experimental data, which have enormous implications for understanding our own species, indicate that a rather limited set of genes is responsible for most of the natural polymorphisms underlying many complex pathologies and that the salient loci are highly concordant in their chromosome locations between mouse and humans.

In the mouse, 27 chromosome regions affecting plasma HDL-cholesterol levels have been mapped, many recurring in crosses between different mouse strains. From the known map relationships between the human and mouse genomes, it is apparent that 18 of the 22 human QTL so far identified occur at corresponding chromosome locations (WANG and PAIGEN 2002). Recognizing that human variation largely occurs among the same set of genes requires that most of the important genes have already been identified in both species; *i.e.*, not more than a few dozen genes account for most of the genetic variation in both the mouse and the human.

Concordance is not limited to HDL levels, but is also observed for hypertension (SUGIYAMA *et al.* 2001) as well as asthma, inflammatory bowel disease, non-insulin-dependent (Type II) diabetes, and osteoporosis (B. PAIGEN, personal communication). The significance of this generality is great. If the same limited set of genes is responsible for most population variation in the human and the mouse, this suggests that these genes represent a special subset of all genes that could potentially influence a phenotype and that this subset codes for proteins that play critical regulatory roles. Because we already know that variation in these proteins does affect phenotype, these naturally polymorphic proteins present particularly inviting targets for drug intervention.

Finding and eventually cloning these genes in humans are now both difficult and expensive, either requiring extensive family studies, which are inefficient when a gene is only one among the several that contribute to the phenotype, or requiring linkage studies on populations, looking for statistical association between phenotype measures and genetic markers. Since haplotype blocks in non-African populations are on the order of 20 kb in size, a genome-wide scan for one individual could easily cost upward of \$50,000 and for a statistically useful population, many millions. Finding these natural polymorphisms in mice and then using that information to guide human studies presents a low-cost, rapid shortcut.

#### WHAT HAVE WE LEARNED?

The union of molecular biology, genomics, and mouse genetics has led to major advances in our understanding of mammalian biology. A few of the many notable examples include insights into the molecular circuitry underlying embryonic and fetal development, the existence of multiple stem cell types in the adult organism, the genetic changes in tumorigenesis, and the emergence of molecular pathology as a full-fledged discipline exploring a wide variety of human ills. Detailed discussions of these are far beyond the scope of this *Perspectives*, but several points deserve comment.

**Evolutionary conservation:** The power of model systems for understanding human biology obviously depends on their degree of similarity to the human state.

We have long known that all mammals share their basic anatomy and physiology. Now, as our knowledge of genomics has advanced over these last 20 years, we have learned just how conservative evolution has been at the molecular level as well. It has been startling to realize that not only are metabolic pathways conserved over evolutionary time, but genetic regulatory systems, the intracellular signaling pathways, and the molecules that carry out these functions are conserved as well. We are less surprised, then, when a comparison of the genomic sequences of mice and humans indicates that we share 99% of our genes and confirms earlier genetic studies showing that the arrangement of genes along chromosomes is largely preserved among mammals. Additionally, the discovery of strong sequence conservation in some noncoding regions is proving a useful means of identifying sequences whose evolution has been constrained by the need to preserve important regulatory and chromosomal structural elements encoded in DNA.

**Imprinting:** The first indication that the maternal and paternal genomes contributed to the zygote do not function equivalently came from nuclear transplantation experiments (MCGRATH and SOLTER 1984). Diploid zygotes created by combining a male and a female pronucleus gave rise to viable embryos, but combining two male or two female pronuclei did not. The nature of the abnormalities depended on the parental source of the pronuclei, making clear that there is a reciprocal, essential difference between the two. This was soon confirmed in genetic crosses that used translocations to produce animals in which both copies of a chromosome segment came from the same parent (CATTANACH and KIRK 1985) and was put on a molecular basis when the *Tgf2r* (BARLOW *et al.* 1991) and *H19* (BARTOLOMEI *et al.* 1991) loci were shown to be expressed from only one parental allele. We now understand that the physical basis for imprinting is differential DNA methylation, which is replicated during mitosis and reset in the germ line. Almost simultaneously with these studies in mice, parallel investigations showed imprinting to be a significant phenomenon in human genetics (HALL 1997).

**Informatics:** The average quantity of data generated per scientist per year has been increasing exponentially for decades. This is true in many areas of science and is apparent whether we look at the numbers of new organic molecules synthesized, genes mapped, protein X-ray crystal structures solved, or megabases of new DNA sequence deposited in the GenBank. One suspects that this is true because a substantial fraction of our total research budgets are spent on the research and development of the research process itself, developing new technologies as well as increasing the efficiency of older ones. DNA sequencing is the example that immediately comes to mind. In not much more than a decade we have gone from laboriously sequencing a few hundred base pairs at a time by hand to implementing automated machines that deliver tens of kilobases of sequences in

a day. Storing the new volumes of information, making them accessible in a useful manner to researchers, and mining them to gain insight into biological processes require computers, very large, sophisticated databases, and a new science of computational biology. Providing some sense of scale, BAXEVANIS (2002) has provided summaries of 335 databases relevant to molecular genetics available at <http://nar.oupjournals.org>. More specific access to mouse-related information is available at <http://www.informatics.jax.org>.

**Phenotyping:** At the present time, the major limiting factor in our ability to apply the powers of mammalian genetics toward understanding mammalian biology is our capacity to measure phenotype. True, new experimental resources and improvements in genetic technologies that will further improve our discovery tools are on the way, but what limits their application is the ability to measure appropriate phenotypes. Developing means for measuring atherosclerotic plaques in mice opened a new field of research, as did learning to measure intraocular pressure, hyperactive airways, and fear-potentiated startle responses. The steady miniaturization of physiological measuring techniques and the introduction of imaging technologies (MRI, CT, PET, DEXA, and the like) are making it increasingly feasible to use mice and their genetic systems for the exploration of phenotype, and we can expect this to continue.

**Pharmaceuticals:** All of the drugs now in common use are targeted at fewer than 400 proteins, and these are largely surface receptors. In this context, consider that the number of mammalian genes is currently estimated at 30,000, that splice variants raise the potential number of polypeptides to something approaching 100,000, and that our knowledge of molecular pathology, while growing, is still rudimentary. All this suggests that genetic approaches directed at furthering our understanding of the molecular basis of disease are likely to have a profound impact on the development of new and more effective classes of drugs. Genetics will also help to resolve the problem that what is ostensibly one disease to the pathologist can arise from a variety of molecular causes, that each of these may require its own therapy, and that we also vary in our responses to pharmaceuticals.

#### THE PRESENT STATE OF AFFAIRS

We largely understand the basic mechanisms of genetics, how traits are passed from one generation to the next, as well as the fundamental mechanisms of gene action. The major challenges we now face are in deciphering the regulatory circuits that control gene expression; understanding the relationship between gene function and the higher order, three-dimensional structure of chromosomes; and continuing to improve our technical capabilities in multiple ways.

Importantly, mouse molecular genetics has moved

beyond being a subject of research to becoming a research tool in its own right. Our existing capabilities make it possible to manipulate the genome and analyze its contributions in determining phenotype, both normal and abnormal. The great challenge is in applying these capabilities to improving the human condition. It also seems reasonable to expect that, as our knowledge of the molecular regulatory systems underlying development increases, so will our ability to alter developmental processes, providing additional and potentially powerful means of perturbing biological systems for discovery purposes.

Our present circumstances are virtually unique. Traditionally, advances in scientific knowledge do not come at a steady pace. Long periods are spent consolidating sporadic major advances, exploiting the new possibilities until the next breakthrough arrives. As time passes, advance becomes increasingly constrained until either a new technology emerges, opening up entirely new realms of investigation (think of the first light and electron microscopes), or a conceptual advance provides a new way of thinking about old problems (think of Mendelian genetics or the DNA double helix). But mouse genetics now finds itself in the unprecedented circumstance of being neither technically nor conceptually limited. We face highly productive years, even using only what we already understand conceptually and what we already know how to do technically. Further, the absence of either limitation is generating a positive feedback loop, stimulating both new conceptual frameworks and new technologies. It is a circumstance virtually unprecedented in the history of the biological sciences.

In the past few years, we have been introduced to multipotential adult stem cells, insulator sequences, and haplotype analysis, and on the technical side we have seen the introduction of gene expression profiling, SNPs and RNAi, and now have nearly the entire mouse genomic DNA sequence available. There is no expectation of a placid future, and if anything, the rate of change is accelerating. One can appreciate this sea change via a simple thought experiment. Place yourself mentally in the year 1988 and ask how far back in time it was possible to go and still be able to reasonably predict the 1988 state of affairs. Then do the same in 1995, and then now. This expansion of possibilities goes beyond our intellectual desire to understand biological systems; it goes to the heart of the reason society supports our efforts: understanding the nature of our physical being toward the goal of improving the quality and duration of human life.

#### WHERE IS ALL THIS TAKING US?

Certainly, we are moving toward a far more profound understanding of molecular pathology: how disease comes about at the cellular and molecular level, what the critical molecular circuits and events are, and how

we differ from each other in our genetic bases. It is also driving us to move beyond the very reductionist approach of one gene, one protein, one phenotype that has been so powerful the last half-century and increasingly to frame our thinking around entire physiological systems considered at the molecular level.

As an outcome, we can expect dramatic changes in our strategies for human intervention, both therapeutic and preventive. Some of these will be idiosyncratic to the particular ill they address, but others will be generic. These latter are likely to include drugs directed at the control of gene expression, manipulation of the immune system, and the use of stem cell derivatives in replacement therapy. At the least, we can expect conventional drugs that are directed at much more rationally chosen protein targets. Much of this effort will be directed, in one form or other, at modulating the regulatory systems controlling physiological systems.

We are also coming to an increased understanding of our genetic/environmental interactions. Genetics makes sense only in an environmental context, and while we cannot readily alter the genomes our parents gave us, we can alter our lifestyle, diet, and exposure to toxins.

It is difficult to predict in which physiological systems our knowledge, and hence our ability to intervene in terms of therapy and prevention, will advance most rapidly. But considered across the broad spectrum of human disease, we can confidently expect remarkable advances in our fundamental understanding.

Enormous challenges lie in translating this understanding into societally acceptable, practical advances and, even more, in accommodating the altered perceptions that will arise in our views of normality and pathology, for both physiology and behavior. In this translation of basic knowledge into practice, it is not clear that our present pharmaceutical/biotech, for-profit structure will entirely suffice. It may well be that for some classes of problems the nonprofit sector will need to assume additional roles. And as to our cultural perceptions, consider only how we will address the issue of personal responsibility for antisocial, aggressive behavior if, as seems likely, that turns out to contain a significant genetic component. Conflicts between new, scientifically based insights and historic cultural norms can be painful to resolve: witness over a century of tension between evolutionary concepts and religious beliefs and our present societal conflicts over reproductive rights and stem cell research.

#### CODA

It would be hard to devise a more emblematic close to the first century of mouse genetics than the December 5, 2002, issue of *Nature*, containing as it does a detailed annotation of the public draft sequence covering some 96% of the mouse genome (WATERSTON *et al.* 2002),

a very broad and deep survey of mouse cDNA clones extracted from a wide variety of embryonic and adult tissue samples (OKAZAKI *et al.* 2002), and a provocative sampling of the haplotype structure of inbred mouse strains (WADE *et al.* 2002). These descriptions of the mouse genome are followed by two prophetic surveys of gene expression for nearly all the mouse orthologs of the genes on human chromosome 21, where trisomy leads to Down syndrome (GITTON *et al.* 2002; REYMOND *et al.* 2002).

The details of the sequence annotation are fascinating, and several highlights are exceptional (WATERSTON *et al.* 2002):

Some 99.5% of mouse genes have a clear human counterpart, and nearly all fall within the predicted syntenic interval.

In support of previous evidence, the two genomes are indeed organized in a highly parallel fashion. Something over 90% of the respective genomes are contained in 216 conserved syntenic blocks whose median size is 23 MB; within each block there are occasional rearrangements giving rise to a total of 326 segments, each with entirely conserved gene order. Evolutionary conservation goes further. Some 5% of the genome shows significant sequence conservation, suggesting common functions in the mouse and human; yet, at most, only 2.5% is accounted for by coding and associated sequences. What is the other conserved 2.5%?

The two genomes have not been evolving at equivalent rates. As judged from divergence among members of lineage-specific repeat families, since the mammalian radiation the average rate of base substitution has been twice as high in the rodent lineage, and this ratio may be as high as 5:1 in the recent past. However, after correcting for generation times the picture reverses, and the rate per generation in primates is probably an order of magnitude higher than that in rodents. Since nearly all human point mutations arise in males (CROW 2000), probably because of the larger number of cell divisions in the male germ line, it is possible that the difference between mice and humans simply reflects our relative size and time to sexual maturity.

The mammalian genome is hardly uniform in its properties. Beyond variation in base composition, as WATERSTON *et al.* (2002) note: "It is clear that the mammalian genome is evolving under the influence of non-uniform local forces." There is substantial variation across the genome in neutral base substitution rates, SNP density (in humans), insertion and deletion rates, and the local frequency of meiotic recombination. Remarkably, these rates are correlated (but not with base composition), reflecting as yet unappreciated features of chromosome organization.

The FANTOM consortium (OKAZAKI *et al.* 2002) re-

ported their analysis of 61K full-length cDNA sequences clustered into 33K “transcriptional units” providing alternate splice forms. Derived from over 200 embryonic and adult tissue samples, many normalized, this is by far the most extensive sampling of the mouse transcriptome so far. Among its highlights is the suggestion, supported by the genomic sequencing data, that a significant class of genes whose ultimate products are not proteins, but rather RNAs with novel functions, may exist.

The provocative suggestion in the report of WADE *et al.* (2002) on the haplotype structure of inbred mice is the possibility that our inbred mouse strains may each be mosaics of a very limited number of ancestral haplotypes, perhaps only two, representing *M. m. musculus* and *M. m. domesticus*. To the extent that this is true (and additional data are urgently required), it means that once they are haplotyped, the existing inbred mouse strains can be used as if they were a very large set of recombinant inbred strains with at least an order of magnitude increase in genetic resolution over existing RI lines. However, to the extent that this is true, it also requires that the progenitor populations of *M. m. musculus* and *M. m. domesticus* are virtually monomorphic, which would be quite remarkable.

The past 100 years have taken us from the first demonstration of Mendelian inheritance in mice to a nearly complete catalog of all mouse genes (many of whose functions are known), the mouse genomic sequence, and an extraordinarily powerful experimental system for understanding mammalian (*i.e.*, human) biology and pathology. And the pace of advance has steadily accelerated. It is impossible to predict what discoveries will come in the next 25, much less the next 100 years; the prospect is awesome.

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# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

### **Haldane, Bailey, Taylor and Recombinant-Inbred Lines**

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**T**HAT mouse Mecca, the Jackson Laboratory, has repeatedly pioneered in bringing mouse genetics to its present state. There was George Snell's Nobel-Prize-winning work on histocompatibility, Roy Stevens' work on embryonal carcinoma, Tibby Russell's on hematopoiesis, and many others (reviewed by PAIGEN 2003a,b). It has also been the site of several important methodological innovations. First and most important, C. C. Little had the foresight to establish inbred lines (CROW 2002). His first line was started in 1909; by 1980, there were >300 (PAIGEN 2003a). Another innovation was the development of congenic strains—inbred lines with a small foreign chromosomal region introgressed by repeated backcrossing into the line (SNELL 1948). A third was chromosome substitution (consomic) strains. These have a single chromosome introgressed into an inbred line (SINGER *et al.* 2004). The fourth innovation, in many ways the cleverest, was recombinant inbred (RI) lines. These innovations each required many years of advance work before they could be utilized effectively. Such projects certainly would not fare well as grant applications today. Only in an organization with a long-time commitment, such as the Jackson Laboratory, could such projects be carried through.

The idea of RI lines arose sometime in the 1950s or 1960s in the fertile mind of Donald Bailey. Don is a quiet, low-key scientist who has not made a big splash in the genetic world at large. But within the Jackson Laboratory and with others who know his work, he has long been revered. He is knowledgeable and creative—the person to go to for help with a technical problem or to search for a new idea.

The principle of RI lines is simple (BAILEY 1971). In retrospect it has a “why didn't I think of it” quality: Two inbred lines are crossed and the hybrids are intercrossed to produce F<sub>2</sub> progeny. Pairs of the F<sub>2</sub> mice are then

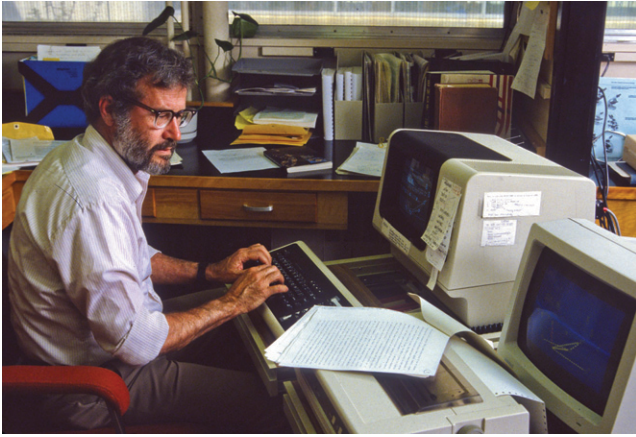
mated to establish inbred lines through repeated sib-mating. The genomes of each of these lines are a homozygous mosaic of chromosomal regions from the two founding inbreds. These RI lines are then typed for the genotypes and phenotypes that differed between the two founders.

Sets of RI lines have a number of advantages. Since each RI line is nearly homozygous, its genotype is reproducible and individual genetic variation is minimized. Replications average out the effects of environmental influences and measurement errors. Furthermore, once a line has been genotyped, this information can be used over and over. Unlinked loci largely randomize during the process, even though inbred lines can show “linkage disequilibrium” for loci on different chromosomes (GRABER *et al.* 2006), but linked genes retain some of the linkage disequilibrium that characterized the two founding inbred strains. Furthermore, there are several meioses in the F<sub>2</sub> and during the inbreeding stage, with the result that the amount of recombination is increased fourfold; this is now called map expansion and is very advantageous for mapping closely linked loci. For the history of linkage studies in the mouse, see LYON (1990).

Bailey started with 12 RI lines from a cross of BALB/cBy and C57BL/6By (designated C × B6). Of these, 7 survived for 30 generations of sib-mating. Bailey identified 11 loci and classified them as to the strain of origin. Three were coat-color genes and 8 were histocompatibility factors. The power of the method was shown by the immediate discovery that some phenotypically similar histocompatibility factors mapped to different locations. Despite the small number of RI lines, Bailey and his associates were able to discover some 20 linkages in the next 5 years (TAYLOR 1978).

The next person to enter the RI story was Ben Taylor. Ben joined the Jackson Laboratory in 1969 and immediately started generating RI lines and developing the theory. He, like Don, is soft spoken and reticent, with a manner that belies his sharp mind.

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Donald W. Bailey (courtesy of the Jackson Laboratory).

Some of the background mathematics had been done by HALDANE and WADDINGTON (1931), who worked out the detailed consequences of repeated brother-sister mating. This involved the kind of extensive algebraic manipulations that most people hate, but which Haldane loved. When I read the article I was overwhelmed. For two linked autosomal loci, this involved no less than 22 linear recurrence equations, which Haldane and Waddington were able to solve for the equilibrium values. Remember that this work, published in 1931, involved hand calculations. This was long before the development of high-speed computers.

HALDANE and WADDINGTON (1931) includes only four references, all from 1921 and earlier, and the summary reads: "Formulae are given for the amount of crossing over which is found in the final population when organisms heterozygous for linked genes are inbred according to various systems" (p. 374). Readers who were seduced by the innocent-sounding title, "Inbreeding and Linkage," and the three-line summary did not realize what an algebraic morass they were getting into. It is interesting and perhaps significant that Waddington later left transmission genetics and studied development. He frequently expressed the opinion that mathematical population genetics was a fruitless endeavor. I wonder if experience with the exhausting and tedious algebra in this article sensitized him forever against any further work in this field.

Ben Taylor was familiar with the Haldane and Waddington article, having studied it as a graduate student at the University of Wisconsin in connection with a research project on radiation effects in rats. Following Haldane and Waddington, he pointed out that the map expansion was a factor of 4 for sib-mating, 3 for an X-linked locus, and 2 for selfing. He also found a remarkable result. Despite very strong positive interference in meiosis, the number of exchanges in RI lines agreed closely with the Poisson distribution; in other words, there was no measurable interference. This can be understood by noting that in RI lines there are several

meioses in succession. Following an exchange, if a different exchange occurs in the same chromosome in a later meiosis, in effect this is a double crossover. But the two exchanges are largely independent of each other and produce an interferenceless double exchange. This means that the simple Haldane mapping function is appropriate for RI lines. Later we shall see that the situation is more complicated.

Taylor made use of another Haldane idea. HALDANE (1956) undertook to develop a method for measuring the recessive lethal mutation rate in mice following irradiation. The idea was to discover a lethal linked to a known recessive marker, detected by the absence of the marker phenotype when the mating system rendered the linked lethal homozygous. As the probability of remaining linked to the marker decays exponentially with distance, Haldane asked for an equivalent region with the probability of detecting the lethal and called this the "swept distance." He hoped that this would be roughly comparable to the powerful *Drosophila* methods, such as Muller's CIB. (See the APPENDIX for an account of a curious Haldane mistake.) Taylor calculated the swept distance on either side of a marker in RI crosses. He found that with seven RI strains the swept distance within which no exchanges occur is 9.3 cM compared to 23.9 cM in a corresponding backcross. Actually Haldane's idea of a swept distance has found only limited usage for mutation studies in the mouse (CARTER and FALCONER 1951). But similar schemes for finding recessive lethals in a specified chromosomal region, such as using appropriately spaced markers and taking advantage of the near-complete interference for short distances, have been fruitfully applied (*e.g.*, SHEDLOVSKY *et al.* 1988).

Taylor developed a number of RI sets, which were used for a variety of molecular traits. One of the earliest uses was identification of genes affecting the group-specific antigen of the murine leukemia virus (TAYLOR *et al.* 1971).

In the ensuing years, 20 or more sets of RI lines were developed in the mouse and hundreds of markers were mapped. Of course, any known DNA sequence can now be mapped by reference to the mouse genome sequence. But this technique is not applicable to many phenotypes. RI lines are particularly useful for genes causing phenotypes whose molecular basis is not known, including components of quantitative traits. Some sets of RI lines have been cryopreserved.

The techniques have spread to other species. RI lines have been useful for studying insecticide resistance (COCHRANE *et al.* 1998). Of course *Drosophila melanogaster* is not to be left out. RI lines have been especially helpful in identifying quantitative trait loci (GIBSON and MACKAY 2002). As the fields of genetics and genomics have moved from gene identification to gene expression, so have applications of RI lines. For example, they have been used to study gene expression in brain tissue

in mice, hematopoietic tissues also in mice, and in fat and kidney tissues in the rat (summarized by BROMAN 2005b).

Plants have a number of advantages for RI analysis. Two of the most important are the ready availability of large numbers and the possibility of self-fertilization, which greatly shortens the necessary time of inbreeding. For example, to lower heterozygosity to 0.016 of its original amount requires only 6 generations of selfing but 20 generations of sib-mating. RI lines have been developed for rice, sunflowers, soybeans, tomatoes, wheat, maize, Brassica, and undoubtedly others. Not surprisingly, RI lines have been extensively used in *Arabidopsis*. By 1993 a set of 100 RI lines involving 64 RFLP probes at  $\sim 20$ -cM intervals were being used to map 500 loci (LISTER and DEAN 1993). Finally, one maize group has created a set of 5000 RI lines by crossing 25 diverse maize inbreds to a single common inbred and deriving 200 RI lines from each cross. The set is being genotyped at 1500 marker loci and the 26 parents are all being sequenced ([http://www.panzea.org/info/RIL\\_phenotyping\\_press\\_rel.html](http://www.panzea.org/info/RIL_phenotyping_press_rel.html)).

Early in the game, two modifications of RI lines were suggested. One was to increase the number of foundation inbred lines from two to four or eight; for the mating diagram, see TEUSCHER and BROMAN (2007). A drawback of having only two lines is that the analysis is restricted to genes in the two parent inbreds; to some extent this defect is repaired by having a number of sets of RI lines. This does not compare alleles in different sets, but by expanding the RI set to more lines this difficulty is partially overcome. This also increases greatly the opportunity for study of epistatic interactions. A complication is that epistasis in RI lines may differ from that in crosses of the parental strains. The second suggestion is, in the interest of further map expansion, to interpose one or more generations of random mating before inbreeding starts. Yet the effect is slight. With eight foundation lines and sib-mating, the map expansion increases only from 7.0- to 7.5-fold (TEUSCHER and BROMAN 2007).

The COMPLEX TRAIT CONSORTIUM (2004) recommended developing a set of eight-way RI lines for mice and this has been started. It would require 23 generations of sib-mating to achieve a 99% reduction of heterozygosity. This is a heroic undertaking and would involve some 1000 strains, each needing to be typed. If accomplished, this could provide a valuable tool for mouse research, especially for difficult phenotypes or quantitative traits. It would also permit study of many two- and three-way interactions. A limitation of all RI lines is that they do not give any information on heterozygotes without additional crosses. But intercrosses between RI lines are also proposed by the Consortium. Whether this program will be accomplished and, if so, whether it will live up to its great expectations will be decided in the future.

Meanwhile, the theoretical work has gone on apace. BROMAN (2005a) extended the Haldane and Waddington method to four and eight lines, for both sib-mating and selfing, giving expressions for map expansion, interference, and clustering of breakpoints. With a three-point cross of tightly linked loci, there is actually negative interference. (This was actually foreshadowed by Haldane and Waddington.) The coincidence for tightly linked loci in two-way RI lines, where the effect is most pronounced, is  $\sim 1.75$ .

The negative interference, at first glance, is surprising. Yet it has a ready explanation. It is comparable to the negative interference found in bacteria and phage crosses (ROTHFELS 1952; VISCONTI and DELBRÜCK 1953). The number of single and double crossovers occurs randomly and, if divided by the number of individuals in which these occur, would show a coincidence of one. But there are a number of individuals in the RI line that have become homozygous for this region and exchanges are irrelevant. When these are included in the denominator, there is a seemingly high coincidence.

More recently, TEUSCHER and BROMAN (2007) have discovered a remarkable simplification, a real *tour de force*. They were able to reduce Haldane and Waddington's 22 equations to a much smaller set that is readily solved. They also solved three-point haplotype probabilities for four- and eightfold RI lines, which previously had been done numerically by large computer runs. The theory is in good shape; the task ahead—to put the theory to good use—is harder.

For several years Don Bailey has led a quiet life in retirement. More recently, Ben Taylor has also retired. I hope and trust that both of them are finding satisfaction in the recent progress and great popularity of the methods that they pioneered a quarter century ago.

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#### APPENDIX: HALDANE'S ERROR

For many years, William Russell, at the Oak Ridge National Laboratory, studied radiation-induced mutations occurring at specific mouse loci. This mega-mouse

project involved enormous numbers and great expense, and it yielded few mutations, although it eventually led to important findings, both quantitative and qualitative. In the mid-1950s, Haldane wrote an article for the *Bulletin of the Atomic Scientists* stating that this approach was all wrong; what Russell should be doing, Haldane wrote, is measuring *all* the mutations in a chromosome or chromosome segment, as was done with *Drosophila*. Haldane, following his standard practice of insulting people, went on to write that undoubtedly geneticists and statisticians at Oak Ridge were kept in separate cages or they would have followed the *Drosophila* pattern. He suggested a mating scheme by which recessive lethals linked to a marker gene could be identified. He wrote that, from this mating system, he could define a chromosomal region around the marker that was “swept” in that all the lethals in that region could be identified. Haldane also wrote that space did not permit his deriving the method, but “Sewall Wright could do it in 20 minutes.” The article made the rounds at a meeting of the Biological Effects of Atomic Radiations (BEAR) committee (CROW 1995). Needless to say, Bill Russell was incensed (RUSSELL 1989). The article was not published.

Wright eagerly accepted the challenge. He and I attended the BEAR meeting and occupied adjacent berths on a sleeper train back to Wisconsin from the New York meeting. We both worked on the problem. Wright solved it first, but it took much longer than 20 min. I got an answer later by a different method. Wright and I agreed, but differed from Haldane. It turned out that Haldane had had a rare mental lapse. He had made a rather elementary conceptual error (CROW 1989).

Wright wrote Haldane, giving the correct solution. Haldane sent back a casual acknowledgment; it was clear that he had not read Wright's letter. The reason was obvious. As several Wisconsin secretaries can attest, Wright's handwriting was atrocious. Aware of this, he sent a second letter, this time typewritten, whereupon Haldane answered appreciatively and gave the correct formula in his published paper (HALDANE 1956). This is the article that Ben Taylor studied to determine swept distance for markers in RI lines.