MCB 140 – General Genetics

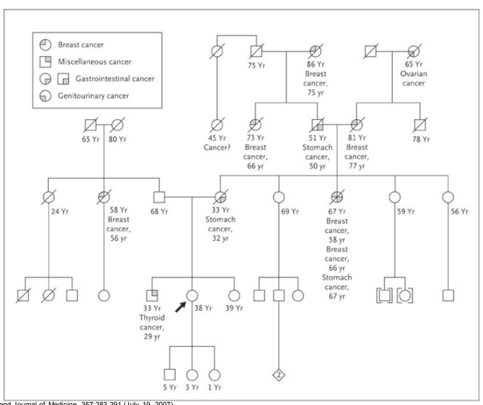
CASE RECORDS OF THE
MASSACHUSETTS GENERAL HOSPITAL

Case 22-2007 — A Woman with a Family History of Gastric and Breast Cancer

A 38-year-old woman was seen in the Gastrointestinal Cancer Genetics Clinic of this hospital because of a family history of breast and gastric cancer.

Approximately 15 months earlier, mild chronic gastrointestinal symptoms, including dyspepsia, heartburn, and midabdominal discomfort, increased in severity and began to occur daily. The symptoms did not resolve with antacid therapy. She had lost approximately 2.3 kg (5 lb) during this time, which she attributed to the stress of caring for her maternal aunt, **who was dying of gastric cancer**. Seven months before admission, an endoscopic examination of the upper gastrointestinal tract, performed at another hospital, was normal.

New England Journal of Medicine, 357:283-291 (July 19, 2007)



New England Journal of Medicine, 357:283-291 (July 19, 2007)

Gastric cancer

Gastric cancer is the second leading cause of cancer deaths worldwide. There are two major histologic subtypes of gastric cancer: intestinal and diffuse. The intestinal subtype is associated with environmental risk factors including *H. pylori* infection, smoking, and diets high in salted and cured foods … Only 1 to 3% of the cases are probably attributable to a high-penetrance genetic syndrome. Five entities confer a risk of gastric cancer (Table 1), all of which are inherited in an autosomal dominant manner.

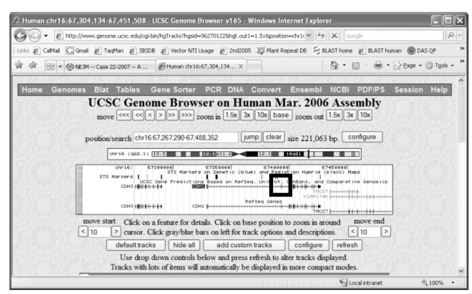
New England Journal of Medicine, 357:283-291 (July 19, 2007)

Table 1. Hereditary Cancer Syndromes That Include Breast Cancer, Gastric Cancer, or Both.

Syndrome	Gene	Cancers	Other Features
Hereditary breast and ovarian cancer	*BRCA1, BRCA2*	Breast, ovarian	Pancreatic cancer
Cowden's	*PTEN*	Breast, thyroid (nonmedullary), uterine	Mucocutaneous lesions (trichilemmomas, papillomatous papules, facial acral keratoses, mucosal lesions), macrocephaly, Lhermitte–Duclos disease, hamartomatous gastrointestinal polyps, mental retardation, fibrocystic disease of the breast, lipomas, fibromas, genitourinary tumors
Peutz–Jeghers	*STK11*	Esophagus, stomach, small intestine, colon, pancreas, lung, breast, endometrial, ovarian (sex cord), adenoma malignum of the cervix, testicular Sertoli-cell tumors	Hamartomatous gastrointestinal polyps, mucocutaneous pigmentation
Li–Fraumeni	*TP53*	Breast, sarcoma, brain, adrenocortical, leukemia	Childhood cancers
Familial adenomatous polyposis	*APC*	Colon, duodenal, and ampullary tumors	Adenomatous polyps of colon, fundic-gland gastric polyps, thyroid cancer, gastric cancer (rare)
Lynch (hereditary nonpolyposis colorectal cancer)	*MSH2, MLH1,* or *MSH6*	Colon, uterine, gastric	Ovarian cancer, renal pelvis and ureteral tumors, glioblastoma, sebaceous-skin tumors, small-bowel tumors, biliary-tract tumors
Hereditary diffuse gastric cancer	*CDH1*	Diffuse gastric, breast (typically lobular)	

New England Journal of Medicine, 357:283-291 (July 19, 2007)

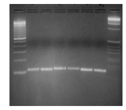
CDH1 – E-cadherin

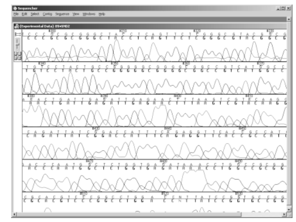


A particular exon of CDH1

>hg18_dna range=chr16:67401613-67401713
GCTGTGTCATCCAACGGGAATGCAGTTGAGGATCCAATGGAGATTTTGAT
CACGGTAACCGATCAGAATGACAACAAGCCCGAATTCACCCAGGAGGTCT

"Polymerase chain reaction" – PCR – "PCR primers" -- $16





A proper description of how this is done:
http://www.myriadtests.com/provider/doc/BRACAnalysis-Technical-Specifications.pdf

This woman's genotype for CDH1

When the patient's maternal aunt received a diagnosis of gastric cancer, she was offered tests to detect the CDH1 gene. These tests were performed … and showed an R732Q mutation resulting in a substitution of glutamine for arginine at amino acid 732. This information was known to the patient and to us at the time of her evaluation in our clinic. We offered this patient germ-line testing for the R732Q mutation that had previously been identified in the family; these tests showed the same mutation in our patient.

Mutations in E-cadherin, the protein encoded by the gene CDH1, result in a loss of normal adhesion and an increase in cellular migration and invasion.

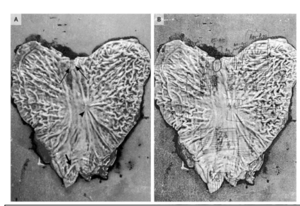
Management and solution

There are two major options for screening for gastric cancer in this patient: surveillance upper endoscopy with random biopsies and prophylactic gastrectomy.

In this 38-year-old patient with a *CDH1* mutation, we recommended prophylactic gastrectomy. If she declined, upper endoscopy every 6 months with random biopsies would have been recommended. She was initially hesitant to proceed with gastrectomy, so an upper endoscopy with methylene blue stain was performed. The examination was normal, and pathological examination of random biopsy specimens detected no cancer. After additional consultation with the surgeon, the patient elected to have a prophylactic gastrectomy.

The surgery

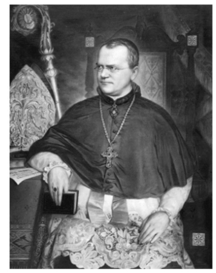
Dr. Sam Yoon: This patient was extremely well informed about the risks and benefits of prophylactic surgery through discussions with her physicians, nutritionist, and support groups. I performed a total gastrectomy and Roux-en-Y reconstruction consisting of a jejunal pouch and hand-sewn esophagojejunostomy. A study with diatrizoate meglumine and diatrizoate sodium on the fifth postoperative day showed no evidence of anastomotic leak, and she started a clear liquid diet. She was discharged on the eighth postoperative day, tolerating a soft solid diet. Five months after the operation, her weight had stabilized at 52 kg (115 lb) (decreased from 58 kg [128 lb]), and she was eating six to eight meals per day.



Words from the patient herself

"I always feared I would die young of stomach cancer, as my mother had, and the fear worsened after my three children were born. Learning that my aunt had the CDH1 mutation and helping care for her as she died, I became increasingly anxious. When I learned that I had the mutation, I was shocked to know that I was at great risk for the development of cancer, yet relieved I could do something about it — but it would be a radical choice. My husband researched the issue and helped us both realize that gastrectomy was the best option. It helped me tremendously to talk with others who had had this operation, and a support group for families with this diagnosis is available (http://health.groups.yahoo.com/group/HDGC/). I learned that recovery would be very difficult, but that I would be okay. My husband and I were honest with our children (1, 3, and 5 years of age), and reading a children's book with them helped the older ones understand.34 It was a very difficult recovery, but a year later, I feel almost normal, with even a 5-lb weight gain! When I consider that each of our children has a 50% chance of having this mutation, I know they at least have the same option I did, and I hope to show them what a livable solution it is."

Gregor Mendel (1822-1884)



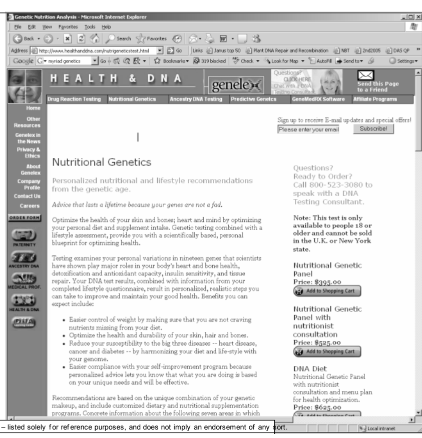
Mendel's garden in Brno

President Clinton Comes to Cal (Jan. 29, 2002)

"I was honored to be president at the time when the International Consortium of Scientists finished the sequencing of the human genome, something which has already yielded the two major variances that are high predictors of breast cancer, something that is leading us very close to unlocking the genetic strains that cause Parkinson's and Alzheimer's.

And quite soon, young women will come home from the hospital with their newborn babies in countries with good health systems with little gene cards that will say, 'Here are your child's strengths and weaknesses, and if you do the following ten things your baby has a life expectancy of 93 years.'

This is going to happen in the lifetimes, and in the childbearing lifetimes of those young people in this audience."



www.genelex.com – listed solely for reference purposes, and does not imply an endorsement of any sort.

Gene Name	Area of Activity
APOC3	Heart Health
CETP	Heart Health
LPL	Heart Health
eNOS	Heart Health
MTHFR	Heart Health; Vitamin B Use
MTR	Heart Health; Vitamin B Use
MS-MTRR	Heart Health; Vitamin B Use
CBS	Heart Health; Vitamin B Use
GSTM1	Detoxification; Antioxidant Activity
GSTT1	Detoxification; Antioxidant Activity
GSTP1	Detoxification; Antioxidant Activity
MnSOD	Heart Health; Antioxidant Activity
SOD3	Heart Health; Antioxidant Activity
VDR	Bone Health
COL1A1	Bone Health
IL-6	Heart Health; Inflammation; Bone Health
TNFa	Inflammation; Bone Health
ACE	Heart Health; Insulin Sensitivity
PPAR2	Insulin Sensitivity

"Apolipoprotein C-III gene (APOC3)

APOC3 plays an important role in lipid metabolism. It inhibits the break down of triacylglycerol, a lipid, by the enzyme lipoprotein lipase; leading to higher triglyceride levels (hypertriglyceridemia). The polymorphism 3175G is associated with a four-fold risk of hypertriglyceridemia and is linked to an increased risk of heart attack, atherosclerosis and cardiovascular disease." *(emphasis mine – fdu)*

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“Most ignorance is willful” (Bill Watterson)

The complexity of the truth
(stay tuned for Prof. Brem’s lecture)

1. SNP
2. Haplotype
3. Linkage disequlibrium
4. “Tags informative for multiple proxies”

→ the very significant scientific problem all of this – put together – creates for using linkage data as a tool for generating “nutrigenomics” guidelines based on a **particular individual’s** genotype at a **particular SNP**.

For now, read:
1. Naukkarinen et al, *Curr. Opin. Lipidol.* 17(3), p 285–290 (not required);
2. Haga and Willard *Nature Reviews Cancer* 206 – required

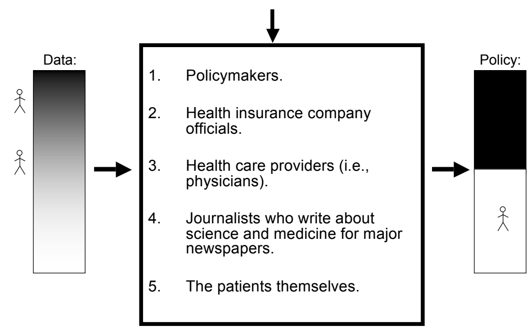
PubMed

A fact, and a problem

Fact: what we do is a function of what we know (and many other things, of course).

Problem: our knowledge comes in shades of gray, but actions tend to be black-and-white.

People with insufficient education in genetics AND statistics and not enough time to look at the primary data



Cancelled health insurance?

“Kevin McCormick called today. There’s another lawsuit from the Weller family. This time it’s the son of the deceased, Tom Weller. … Apparently, his health insurance got cancelled.”

“Because?”

“His father has the BNB71 gene for heart disease.”

“Gene Variant Is Linked to Common Type of Stroke” NYT 1/9/07

Japanese researchers have identified a gene variant that appears to predispose a person to strokes, but it seems more prevalent in Asians than in people of European or African descent.

In a paper to be published next month in the journal Nature Genetics, researchers write that the presence of the variant raised the risk of cerebral infarction, the most common type of stroke, by 40 percent.

Cerebral infarction occurs when blood supply to a part of the brain is obstructed, resulting in death or serious damage to brain cells. The obstruction can be caused by a blood clot, a buildup of fatty deposits in blood vessels or cancerous cells.

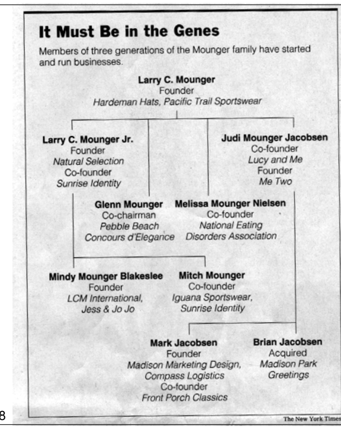
The researchers studied 1,112 Japanese and found that the variant of the gene PRKCH turned up more often in people who had had strokes. The variant also appeared to be linked to an enzyme, rendering it more active.

Gene for starting businesses

"If you belong to a certain extended family in Seattle, you're probably an entrepreneur. It seems to be about the only career many of the members ever considered. "It's in our blood" said Brian Jacobsen, president of Madison Park Greetings, a stationery and gifts company. Mr. Jacobsen's brother, mother, grandfather, two uncles, two cousins and an aunt all started and ran their own companies and say they cannot imagine any other livelihood.

Why are so many people in the same clan hooked? Some of them have a theory. They believe that somewhere in their chromosomes lurks an actual entrepreneurial gene -- that their bent for business really is in their blood."

New York Times, Nov. 20, 2003 – p. C8



New York Times,
Nov. 20, 2003 – p. C8

Gene for metaphors

"AG: *Many of your songs include clear, visual images. Do these images come from dreams?*

Suzanne Vega: My mind works in a metaphorical way. It's easier for me to say what I see than what I feel. The emotions are expressed in the images. I think it must be genetic, because my daughter, Ruby, thinks the same way. She'll see smoke coming out of the back end of a car and say, "The smoke is tap-dancing." And if you look at it, you can see what she means.

http://www.acousticguitar.com/issues/ag110/feature110.html

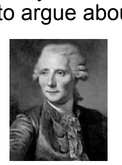
The God Gene

"Modern science is turning up a possible reason why the religious right is flourishing and secular liberals aren't: instinct. It turns out that our DNA may predispose humans towards religious faith. … Dean Hamer, a prominent American geneticist, even identifies a particular gene, VMAT2, that he says may be involved. People with one variant of this gene tend to be more spiritual, he found."

N. Kristof, New York Times, 2-12-05

The problem

"It is not necessary to understand things in order to argue about them"



Pierre de Beaumarchais:
The Barber of Seville (1775), *The Marriage of Figaro* (1784)

Ontology vs. epistemology

"The way things are vs. the way we go about understanding, how things are."

MCB 140 aims to educate MCB majors in not just key *facts* about the functioning of the genetic material in processes of heredity, ontogeny, and disease – but also in the power and the *limitations* of the methods that are used to obtain those facts.

What MCB140 is NOT

A “fun” time spent discussing “cool” stuff about, like, DNA and gene stuff. Dude. If you want that, go watch GATTACA.

Instead, it **is** a CHALLENGING, yet profoundly intellectually and (for some) emotionally gratifying experience of learning about the **methods** of the science of Genetics – methods that, by their elegance, sophistication, and, occasionally, simplicity, also offer the student a sense of intellectual gratification and excitement.

Important: any sort of gratification will only come from the application of considerable effort, and after the passage of time.

What to do so as to do well

1. Attend class.
   1. Note: reliance on the fact that many lectures are on the web, hence can be “crammed” at the last minute is a 100%-guaranteed recipe for failure.
   2. Further note: some of the exams will be open-book. This means that information is less important that understanding. Again, postponement of studying to the last minute is a recipe for failure. You have been warned.
2. Keep up with the reading.
3. Do all problem sets.
4. Attend discussion section.
5. Study hard and do well on all the quizzes.
6. Ask the GSIs questions.
7. E-mail the faculty: urnov ЭТ berkeley ДОТ edu

Part I – “classical genetics”

From a black box of “like begets like” to:

1. “Particles of inheritance” (genes) …
2. … that occur in pairs (alleles) …
3. … that lie on chromosomes …
4. … in a linear order …
5. … and control the development of traits.

Part II: methods in experimental genetics (Prof. Garriga)

- Gene interactions
- Mutations and mutagenesis
- “Genetic screen”:

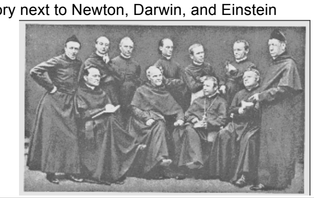
phenomenon → an understanding of mechanism

Section III: genomics and quantitative genetics (Prof. Brem)

1. We have sequenced the human genome, and many other genomes. Now what?
2. The genetics of “complex” traits.

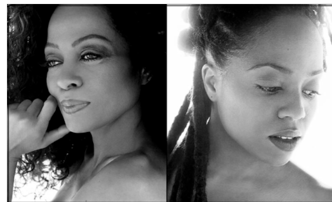
Gregor Johann Mendel

- Born to a peasant family in Brno (then Brunn) in Moravia
- Showed promise in school
- Studied at the University of Vienna, but could not get a degree, because of a psychiatric condition (exams made him nervous)
- Returned home, taught high school physics school
- Became an abbot at a monastery
- Bred peas for 8 years
- Presented the findings to his local “nature lovers” society
- Wrote to the leading authority of his time on plant hybridization, had his findings rejected as incorrect
- Died unknown, and remained so for 35 years
- Stands in history next to Newton, Darwin, and Einstein



Observable phenomena, explainable and not

1. Gravity – not understood at all.
2. The color of the sky – understood, but highly technical. $\sim\lambda^{-4}$ (elastic Rayleigh scattering)
3. Heredity – understood, and quite simple.




"It's All in the Genes"
New York Times, 5/2/04

Heredity: "blending inheritance"?

President W.J. Clinton

Senator H.R. Clinton

Their daughter, Chelsea

Phenomenon → explanation of mechanism

1. "Just so stories" (i.e., making up an explanation that "makes sense"). Encouraging (rare) example: Francis Crick's invention of tRNA. Discouraging (overhwelmingly so, in numbers) examples: theories of heredity before Mendel/C-T-dV.
2. Scientific method.

Just So Stories (R. Kipling)

- How the elephant got its trunk
- How the camel got its hump
- Etc.

R. Lewontin

"Accusers All; Going Negative: When It Works"
New York Times 8-22-04

"THIS was supposed to be the positive campaign. Late last fall, Democrats and Republicans alike predicted that a new campaign rule requiring candidates to appear in their own advertisements and take credit for them would discourage them from making negative ads. Yet it's not even Labor Day and President Bush has spent the majority of the more than $100 million he has spent on television advertisements attacking his Democratic opponent, Senator John Kerry. Mr. Kerry and the other Democratic primary contenders seemed to spend the fall and early winter in a contest to see who could jibe Mr. Bush the most."

“Accusers All; Going Negative: When It Works” New York Times 8-22-04 ctd

“Political consultants cite a strikingly consistent pattern when it comes to darker, more confrontational commercials. ''Focus groups will tell you they hate negative ads and love positive ads,'' said Steve McMahon, a Democratic strategist. ''But call them back four days later and the only thing they can remember are the negative ones.''

And studies have shown that not only are people more likely to remember attacks, it also takes fewer airings to remember them.

''There appears to be something hard-wired into humans that gives special attention to negative information,'' said Kathleen Hall Jamieson, director of the Annenberg Public Policy Center at the University of Pennsylvania. ''***I think it's evolutionary biology***. It was the wariness of our ancestors that made them more likely to see the predator and hence to prepare. The one who was cautious about strange new food probably didn't eat it, they sat back and watched other people die. There's a reason to be hesitant about that which is vaguely menacing.''

Emphasis mine – fdu.

Scientific method

1. Observe phenomenon.
2. Come up with an explanation for what accounts for it (=a hypothesis).

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3. Test the hypothesis by doing something (=perform an experiment).
4. Look at the data from the experiment.
5. Determine, whether the data are …
   a) … consistent with the hypothesis being true → 1
   b) … consistent with the hypothesis being wrong → 2
   c) … inconclusive → 3

Note: if you are unable to cross the red line, go give an interview to a newspaper. Journalists love conjecture. It sells more newspapers.

Problems 2.2 and 2.3 – **required** (write out the answer in essay form)

2.2 During the millennia in which selective breeding was practiced, why did breeders fail to uncover the principle that traits are governed by discrete units of inheritance (that is, by genes)? (required reading – Cobb, *Heredity Before Genetics: a History*).

2.3 Describe the characteristics of the garden pea that made it a good organism for Mendel’s analysis of the basic principles of inheritance. Evaluate, how easy or difficult it would be to make a similar study of inheritance in humans by considering the same attributes you described for the pea.

Before Mendel

5,000 B.C. - ~1650 A.D. – “just so stories”

1650 – 1760: flawed experiments

1760 – 1856: better experiments (Joseph Kölreuter, Carl Gärtner, but with flaws in experimental design, and deep flaws in interpretation); **heuristic** successes in breeding (Robert Blakewell).

1856-1866: Mendel’s experiments.

The significance of the “reverse cross”

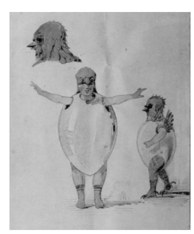
“Whatever the case, for the most recent part of humanity's history — that which has occurred since the rise of civilization — the involvement of both males and females in producing new life has been taken as a given. That did not mean, however, that the two sexes were considered to make complementary contributions, or that there was thought to be any consistent observable relation between parents and offspring. A classic assumption — which persists in much folklore today — turned the apparent prehistoric focus on women on its head, producing a male-centred view. Semen — the only immediately apparent product of copulation — was thought to be 'seed' ('semen' means seed in Greek); parents still talk to children about 'Daddy planting a seed in Mummy's tummy'.”

Cobb *NGR 7:* 953.

Surprisingly to the modern eye, no one in the seventeenth century argued that eggs and sperm represented complementary elements that made equivalent contributions to the offspring. Instead, the next 150 years were dominated by either 'ovist' or 'spermist' visions of what eventually became known as 'reproduction' (the term was coined only in 1745) (Ref. 7). Each view considered that only one of the two parental components provided the stuff of which new life was made, with the other component being either food (as the spermists saw the egg), or a force that merely 'awoke' the egg (as the ovists saw the spermatozoa).

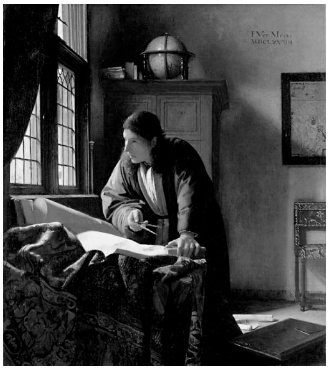
There were many reasons underlying this apparent scientific dead end. For example, in chickens, the two elements did not seem to be equivalent at all: there was a single enormous egg, which was apparently passive, whereas the 'spermatic animals' were microscopic, incredibly active, and present in mind-boggling numbers. Ultimately, however, the reason that late seventeenth-century thinkers did not realize what to us seems blindingly obvious — that both eggs and sperm make equal contributions to the future offspring — **was that there was no compelling evidence to make them appreciate this.**

The problem was not that thinkers did not look for similarities between the generations, but that they did, and were understandably confused by what they saw. Human families provided striking, highly contradictory and apparently inconsistent evidence — children sometimes looked like one parent, sometimes a mixture of the two, sometimes like neither and sometimes like their grandparents.

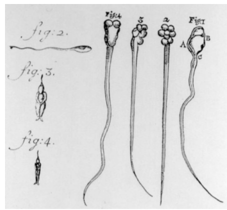


Victor Hartmann
-- the drawing that inspired Mussorgsky to write the “Ballet of the Unhatched Chicks” from *Pictures at an Exhibition*

Cobb *NRG 7:* 953.



1677



In a rare experimental study of resemblance, Leeuwenhoek provided yet another example of the way characters appeared in each generation, and added to the prevailing perplexity. Using what could have been a tractable model — rabbits — Leeuwenhoek was surprised to find that a grey male wild rabbit could give rise to only grey offspring. But Leeuwenhoek argued that spermatozoa were the sole source of the future animal, so his strange finding from rabbits became "...a proof enabling me to maintain that the foetus proceeds only from the male semen and that the female only serves to feed and develop it."9 In other words, there was no relation between both parents and the offspring, but simply between father and offspring, which was represented by the little animal in the male semen. The father was grey, so the offspring were inevitably grey, thought Leeuwenhoek.

It is tempting to imagine that if he had done the reciprocal cross, using a grey female wild rabbit, or if he had studied the grandchildren of his grey male, Leeuwenhoek might have paused for thought and the course of science might have been changed.

Cobb *NRG 7:* 953.

At the heart of agricultural practice is the assumption that, as Thomas Blundeville, an author with an interest in horse breeding, mathematics and navigation, put it in 1566: "...it is naturally geven to every beast for the moste parte to engender hys lyke."17 However, as Blundeville indicated, this was not always the case, and until the seventeenth-century studies on generation, it was not even clear that it applied to all organisms. More surprisingly, until the second half of the eighteenth century, there does not seem to have been any explicit attempt to exploit this phenomenon; selective breeding, in terms of a conscious decision to manipulate the stock of a domesticated organism, was not widespread, nor was it transformed into a theory. Breeders' 'knowledge' that like bred like was partial and entirely **heuristic**: they were concerned with what worked, not why18.

The difficulty with the breeders' basic assumption that like breeds like was that it was not always true. As Nicholas Russell has pointed out, when seventeenth-century English horse breeders tried to import animals from Arabia, the horses generally failed to flourish and rarely reproduced all the qualities that had made them attractive in the first place. As a result of many such experiences, "...most authors believed that the virtue of horses from exotic locations was only transmissible over generations while they remained in these places."18 Far from seeing the characters of their animals as having an innate, constitutional basis that could pass from one generation to another, breeders — like Aristotle and other thinkers — accepted that local conditions had a decisive role in shaping characters.

From the seventeenth-century, breeders tended to use the term 'blood' to describe the quality that apparently lay behind the characters of an animal. But, as with a royal 'bloodline', this was a vague, semi-mystical view of the power of an imprecise quality, rather than a recognition of the hereditary transmission of characters. This confusion was translated into practice: eighteenth-century racehorse breeders would not cross two successful racehorses, creating a 'thoroughbred' stock, but would instead cross racing stallions with local mares18.

Secretariat – to fans of horse racing, the analog of Ted Willams and Michael Jordan

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Word of the day: heuristic

"A method based on empirical information that has **no explicit rationalization**"

"A computational method that uses **trial and error methods** to approximate a solution for computationally difficult problems"

"Grrrrr"

Buffon was interested in the problem of hybrids, but chose to work with quadrupeds. It turned out to be difficult to do a controlled cross. For instance, during an attempt to mate a wolf with a dog, the female wolf ate the dog she was supposed to mate to, and then mauled the coachman.

Georges-Louis LECLERC, comte de BUFFON (1707-1788)
One of the great naturalists of all time

R. Olby *Origins of Mendelism*

Canis lupus

Joseph Kölreuter (1761)

Plant hybridization: 500 different hybridizations involving 138 species.

"The experimental study of genetics may be said to date from the work which Koelreuter described it."

Studied both F1 and F2 plants in crosses.

"When Kolreuter compared them, he found a striking contrast. F1 hybrids for any given cross were alike, and in most of their characters were intermediate between the two parental species. F2 and back-crossed hybrids were all different, and they tended to be less like their parental hybrids and more like one or other of the originating species."

R. Olby *Origins of Mendelism*

1761 - 1900

"The contrast between the two generations remained an enigma **until 1900** when Mendel's explanation was made generally known. Whereas Mendel explained the enigma on cytological and statistical grounds, Koelreuter explained it on bases which may be described as theological and alchemical. [He] looked upon the wonderful uniformity and exact intermediacy of F1 hybrids as evidences of Nature's perfection. The same cross repeated no matter how many times gave the same result. What caused the breakdown in the second generation? Surely, he reasoned, it must be man. Nature never intended that species should be crossed and to prevent it she had placed closely related forms far apart. Then came man mixing up nature's careful arrangement and cramming into the confines of his little garden species which formerly were separated by thousands of miles. … The strange motley of forms in the F2 generation was thus the direct result of tampering with nature."

R. Olby *Origins of Mendelism*

Mendel's most famous words

Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of the hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their numerical relations to each other.

(note: thank you, Christian Doppler)

Wer die Arbeiten auf diesem Gebiete überblickt, wird zu der Ueberzeugung gelangen, dass unter den zahlreichen Versuchen keiner in dem Umfange und in der Weise durchgeführt ist, dass es möglich wäre, die Anzahl der verschiedenen Formen zu bestimmen, unter welchen die Nachkommen der Hybriden auftreten, dass man diese Formen mit Sicherheit in den einzelnen Generationen ordnen und die gegenseitigen numerischen Verhältnisse feststellen könnte.

http://www.mendelweb.org/CollText/homepage.html

Newton, Darwin, **Mendel**, Einstein

(i) The simplicity, clarity, elegance, rigor, and power of Mendel's experimental approach to the problem of heredity.

(ii) The influence of his work on subsequent development of science.

What is Mendel proposing to do?

1. Let's generate hybrids, and after having done so, determine, how **many different types** of children (progeny) appear in the crosses.
2. Let us do this analysis generation-by-generation, in other words, analyze the parents, their children, and their grandchildren SEPARATELY.
3. Let us DETERMINE THE RATIOS: if, in a given generation, there is more than one type of child, let us ask, **what proportion of the whole** each type is.

Scientific reductionism

Put together – intelligently – an experimental setup that "isolates" a particular component of a phenomenon for study. One attempts to "reduce" a problem to its simplest possible form.

All previous hybridists – including such titans as Carl Linnaeus, the first *Homo sapiens*, and Charles Darwin himself! – looked at the transmission through generations of **all** the traits for a given species, or multiple traits at once.

Why?

It requires indeed some courage to undertake a labor of such far–reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the **importance of which cannot be overestimated in connection with the narrative of how living beings develop.**

Es gehört allerdings einiger Muth dazu, sich einer so weit reichenden Arbeit zu unterziehen; indessen scheint es der einzig, richtige Weg zu sein, auf dem endlich die Lösung einer Frage erreicht werden kann, **welche für die Entwicklungs-Geschichte der organischen Formen von nicht zu unterschätzender Bedeutung ist.**

http://www.mendelweb.org/CollText/homepage.html

Astonishing foresight

One might ask – why did Mendel spend 8 corageous, lonely years in backbreaking, painstaking work, planting peas, dissecting their flowers, crosspolinating them, tracking their progeny, counting seeds, replanting those, etc etc?

The answer, in part, seems to be: he was convinced that he was studying not an obscure phenomenon in an irrelevant setting (seed color in peas). He thought he would discover a key mechanism that operates in all living things!

Words to live by

"The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiment is conducted."

Der Werth und die Geltung eines jeden Experimentes wird durch die Tauglichkeit der dazu benützten Hilfsmittel, sowie durch die zweckmässige Anwendung derselben bedingt. Auch in dem vorliegenden Falle kann es nicht gleichgiltig sein, welche Pflanzenarten als Träger der Versuche gewählt und in welcher Weise diese durchgeführt wurden.

http://www.mendelweb.org/CollText/homepage.html

A universally applicable statement

Will your experiment generate data that will be of any use?

Well, a key determining factor in that is whether you chose the right material to do the experiment with.

Is the object of your study optimally suited to answer the question you are interested in?

What plant to pick

"The selection of the plant group which shall serve for experiments of this kind must be made with all possible care if it be desired to avoid from the outset every risk of questionable results.

The experimental plants must necessarily:

1. Possess constant differentiating characteristics.
2. The hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection."

http://www.mendelweb.org/CollText/homepage.html

Useful piece of experimental guidance for a geneticist

"Accidental impregnation by foreign pollen, if it occurred during the experiments and were not recognized, would lead to entirely erroneous conclusions."

Experimental genetics – from Mendel's days and to this day – heavily relies on crosses. It is critical, therefore, that the cross be a controlled one, i.e., that it occur between specific organisms as per the experimental plan.

The problem, of course, is most organisms on Earth mate naturally, and uncontrollably.

Nature, March 24, 2005: "Genome-wide non-mendelian inheritance of extra-genomic information in Arabidopsis" S. Lolle, R. Pruitt.

"*Arabidopsis* plants homozygous for recessive mutant alleles of the organ fusion gene HOTHEAD (HTH) can inherit allele-specific DNA sequence information that was not present in the chromosomal genome of their parents but was present in previous generations.

(in other words, hh plants, when crossed "to themselves," yield a surprisingly high frequency of Hh plants,)

"This previously undescribed process is shown to occur at all DNA sequence polymorphisms examined and therefore seems to be a general mechanism for extra-genomic inheritance of DNA sequence information. We postulate that these **genetic restoration events** are the result of a template-directed process that makes use of an ancestral RNA-sequence cache."

hh plant and its non-Mendelian offspring

"Startling Scientists, Plant Fixes Its Flawed Gene" – NYT 3/23/06

In a startling discovery, geneticists at Purdue University say they have found plants that possess a corrected version of a defective gene inherited from both their parents, as if some handy backup copy with the right version had been made in the grandparents' generation or earlier.

The finding implies that some organisms may contain a cryptic backup copy of their genome that bypasses the usual mechanisms of heredity. If confirmed, it would represent an unprecedented exception to the laws of inheritance discovered by Gregor Mendel in the 19th century. Equally surprising, the cryptic genome appears not to be made of DNA, the standard hereditary material.

Nature. 2006 Sep 28;443(7110):E8;

Plant genetics: increased outcrossing in hothead mutants.
Peng P, Chan SW, Shah GA, Jacobsen SE.

Lolle et al. report that loss-of-function alleles of the HOTHEAD (HTH) gene in Arabidopsis thaliana are genetically unstable, giving rise to wild-type revertants. On the basis of the reversion of many other genetic markers in hth plants, they suggested a model in which a cache of extragenomic information could cause genes to revert to the genotype of previous generations. In our attempts to reproduce this phenomenon, we discovered that hth mutants show a marked tendency to outcross (unlike wild-type A. thaliana, which is almost exclusively self-fertilizing). Moreover, when hth plants are grown in isolation, their genetic inheritance is completely stable. These results may provide an alternative explanation for the genome wide non-mendelian inheritance reported by Lolle et al.

reported by Lolle et al.
Initially, we constructed *hth-12 gl1-4* double-mutant plants in the Columbia ecotype, reasoning that *HTH* and *GL1* should revert independently because they are on different chromosomes. *hth-12* DNA carries a transfer-DNA (T-DNA) insertion (SALK_024611) and *gl1-4* is a guanine-to-adenine (G-to-A) transition mutation (like that shown previously to revert[1]) that changes the start codon of the trichome gene *GL1* (ref. 3) from ATG to ATA. Among 1,597 progeny of *hth-12 gl1-4* plants, 10 were phenotypically *GL1* (normal trichomes). Genotyping based on polymerase chain reaction showed that nine were heterozygous for *gl1-4*, and one was *GL1/GL1*. Surprisingly, the nine *GL1/gl1-4* plants were also heterozygous for *hth-12*, and the *GL1/GL1* homozygote was homozygous for *HTH*. These observations are most easily explained by pollen contamination (nine heterozygous plants) and seed contamination (one homozygous plant). We also found a single *hth-12* heterozygote that

The cross (a "self")_:
hh gg x hh gg

Find 10 plants that are phenotypically G (i.e., "reverted" to wild-type).

Genotype those.

Observe that they are Gg (one allele "reverted").

As a control, analyze the Hothead locus in those Gg plants.

Remarkably, find that ALL of them are also Hh.

Pull out Occam's razor.

I'm sorry, whose razor?

Occam's razor (also spelled Ockham's razor) is a principle attributed to the 14th-century English logician and Franciscan friar William of Ockham. (A heuristic maxim that advises economy, parsimony, or simplicity in scientific theories. Occam's razor states that the explanation of any phenomenon should make as few assumptions as possible, eliminating, or "shaving off", those that make no difference in the observable predictions of the explanatory hypothesis or theory. In short, when given two equally valid explanations for a phenomenon, one should embrace the less complicated formulation. The principle is often expressed in Latin as the lex parsimoniae (law of succinctness): "entia non sunt multiplicanda praeter necessitatem." (which translates to: entities should not be multiplied beyond necessity.)

This is often paraphrased as **"All things being equal, the simplest solution tends to be the best one."** In other words, when multiple competing theories are equal in other respects, the principle recommends selecting the theory that introduces the fewest assumptions and postulates the fewest hypothetical entities.

Table 1 | Outcrossing in *hth* mutants

	Number of phenotypically revertant plants	
Genotype	Mixed population	Isolated population
hth-12	19/245 (7.8%)*	0/295 (0%)
hth-12	18/415 (4.3%)†	0/637 (0%)
hth-8	156/994 (15.7%)‡	0/890 (0%)§
hth-5	22/1144 (1.9%)‖	0/913 (0%)§

Homozygous *hth* plants were grown in a room with plants of mixed genotype (mixed population) or in isolation (isolated population). Progeny from these two populations were scored for plants with the wild-type *HTH* phenotype. (Plants were cared for by Yu Li, Shawn Cokus, Lynn Jacobsen, Zhongliang Peng and Suwen Wang. *BIN2-1::GFP* seeds were provided by Jianming Li.)

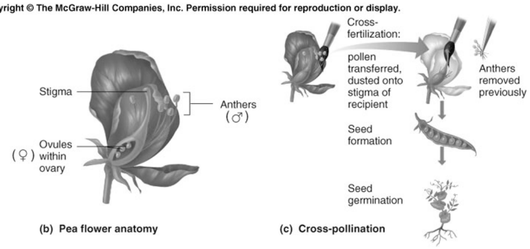
Why the pea?

"At the very outset special attention was devoted to the *Leguminosae* on account of their peculiar floral structure. Experiments which were made with several members of this family led to the result that the genus *Pisum* was found to possess the necessary qualifications.

Some thoroughly distinct forms of this genus possess characters which are constant, and easily and certainly recognizable, and when their hybrids are mutually crossed they yield perfectly fertile progeny.

Furthermore, **a disturbance through foreign pollen cannot easily occur**, since the fertilizing organs are closely packed inside the keel and the anthers burst within the bud, so that the stigma becomes covered with pollen even before the flower opens. This circumstance is especially important. As additional advantages worth mentioning, there may be cited the easy culture of these plants in the open ground and in pots, and also their relatively short period of growth. **Artificial fertilization is certainly a somewhat elaborate process, but nearly always succeeds.** For this purpose the bud is opened before it is perfectly developed, the keel is removed, and each stamen carefully extracted by means of forceps, after which the stigma can at once be dusted over with the foreign pollen."

http://www.mendelweb.org/CollText/homepage.html



(b) Pea flower anatomy (c) Cross-pollination

The garden pea (*Pisum sativum*) – a powerful "model system" for genetic experimentation

1. Can cross, in an entirely investigator-specified fashion, two organisms of defined phenotypes.
2. Can also cross an organism "to itself" ("a self-cross") – "selfing."
3. "Invert the direction of the cross" (take male gametes from a plant carrying trait A, and fertilize an ovum from a plant carrying trait A' – and then do the inverse, i.e., male A' crossed to female A).

Next time

What Mendel did.