Good science in maize breeding and genetics: Is serendipity involved?

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Scientists should ask important questions and then follow the leads that might come from serendipity. Examples of serendipity in my research include: 1) An initial goal of developing maize strains with various knob combinations led to the identification of an NOR duplication, providing the material for an unanticipated objective to locate the rRNA genes of maize, and to estimate the number of copies at 8000. 2) A goal of understanding the genetics of a rough-textured kernel that was shown to be due to a brittle-mutable allele controlled by the Spm/En system; interestingly the material was then used to clone the Brittle-1 gene. 3) The technology for the regeneration of whole corn plants from tissue culture involved the use of the proper tissue, culture medium, and genotype. Unexpected was the use of this technology for the genetic engineering of maize. 4) Crosses of maize and oats were made to produce haploid oat plants. About one-third of the embryo-rescued progeny had the haploid number of oat chromosomes and, unexpectedly, one or more maize chromosomes. This led to a high-throughput method of mapping maize DNA sequences, and possibly may lead to the transfer of C4 traits to a C3 plant. 5) In attempting to clone a QTL for early flowering, it was discovered that the pertinent sequence was, unexpectedly, non-coding and probably interacted with an apetula-like gene 70 kb upstream. So, start your experiments on an important question but be sure to follow serendipitous leads as an effective way to make new discoveries.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture
(USDA


www.GenerallyAwesome.com

Your attention please!

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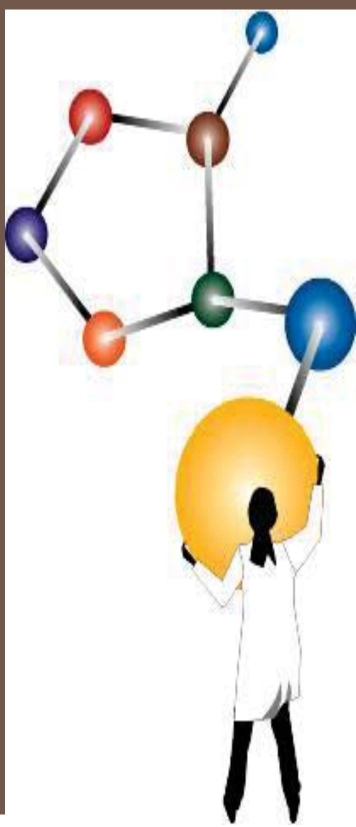


Ronald L. Phillips

Regents Professor Emeritus
McKnight Presidential Chair in Genomics
University of Minnesota, USA

Serendipitous defined

The word was not in my college dictionary (published circa 1950); an old word for Sri Lanka but first used in popular English literature sometime before 1757

Serendipitous defined

Lucky in making unexpected and fortunate discoveries

Serendipitous defined

Serendipity …"an apparent aptitude for making fortunate discoveries accidentally"

Webster's New Twentieth Century Dictionary (2nd edition)

Serendipitous defined

Phenomenon whereby an unsought-for discovery is more important than the one expected

Webster's Online Dictionary 2010

Louis Pasteur

- He said "In the fields of observation chance favors only the prepared mind"
  - Lecture, University of Lille, December 7, 1854

- He also said: "Let me tell you the secret that has led me to my goal. My strength lies solely in my tenacity"

“Serendipity ….

…rewards the prepared mind”

R. Karim, University of Minnesota-Duluth

Note in PNAS said...

(Marder, Kettenmann, and Grillner 2010 PNAS 107: 21233)

Even though prestigious journals are those that place the highest premium on perceived novelty and significance…..

remember….

"Many, if not most, important scientific findings come from serendipitous discovery"

Goal of this talk

To encourage especially the younger scientists to get started on an important question and then follow the leads that might come from serendipity

Some important questions

Will quantitative traits ever be fully defined genetically?

Will environmental effects on gene expression be understood, or predictable?

Is all variation in segregating populations due to polymorphisms in the parents?

Will plant manipulative techniques eliminate sexual crossing barriers?

Will apomixis be transferable via genetic engineering?

Will recombination systems allow the use of smaller populations?

Will phenotyping become more of a science?

What is the molecular genetic basis of heterosis?

Will human nutrition be the main basis of varietal selection?

Will plants be commonly bred as a source of medicine?

Will plants be modified to grow with lower amounts of nutrients and water?

Phillips, R.L. 2006 Crop Sci. 46: 2245-2252

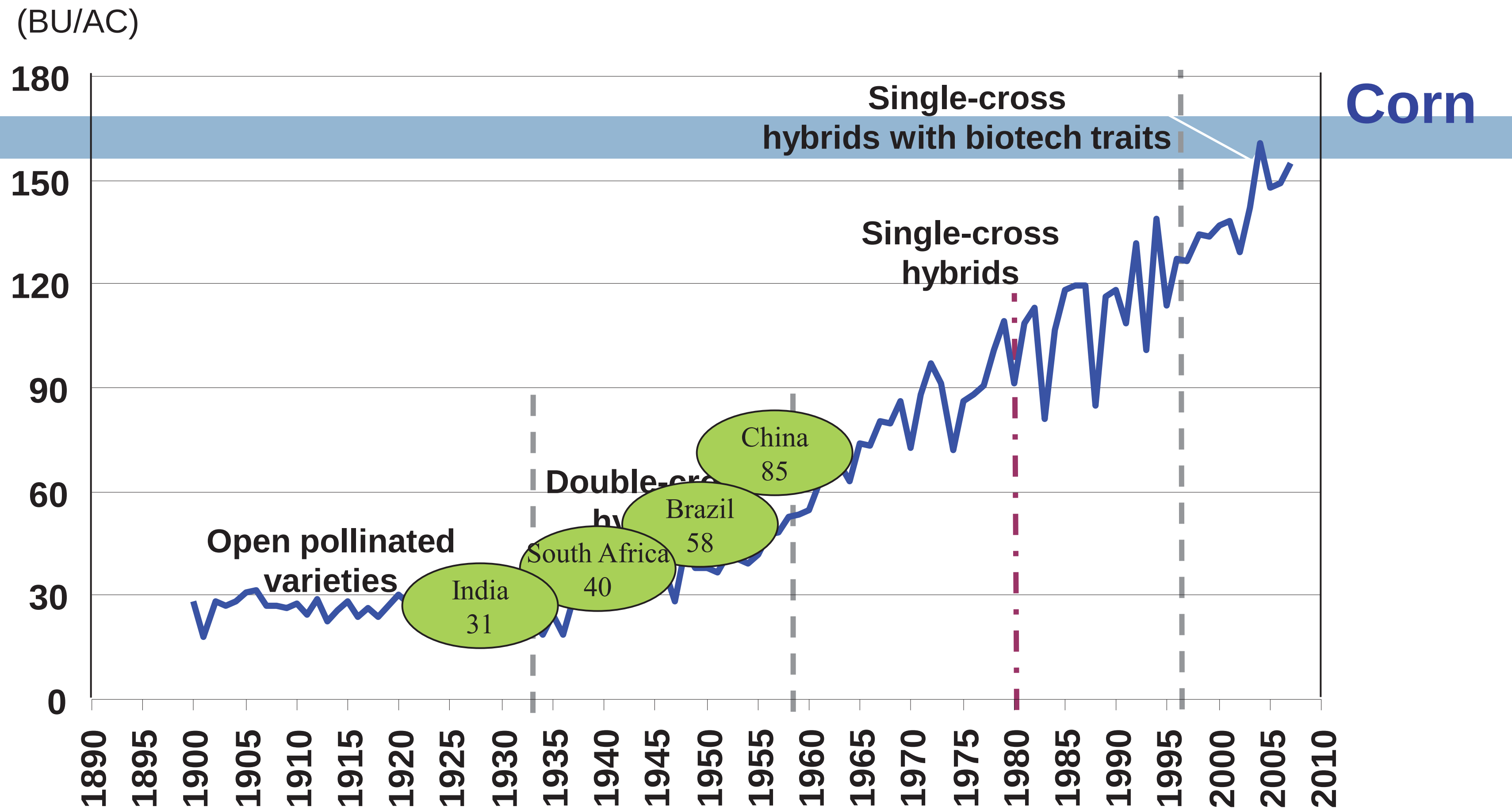
Biology-Based Solutions to Societal Problems

(New Biology for the 21st Century, Natl. Acad. Press 2009

Health Energy Environment Food

Genetics and breeding applies to all of these areas!

Historical U.S. Corn Yield Improvement



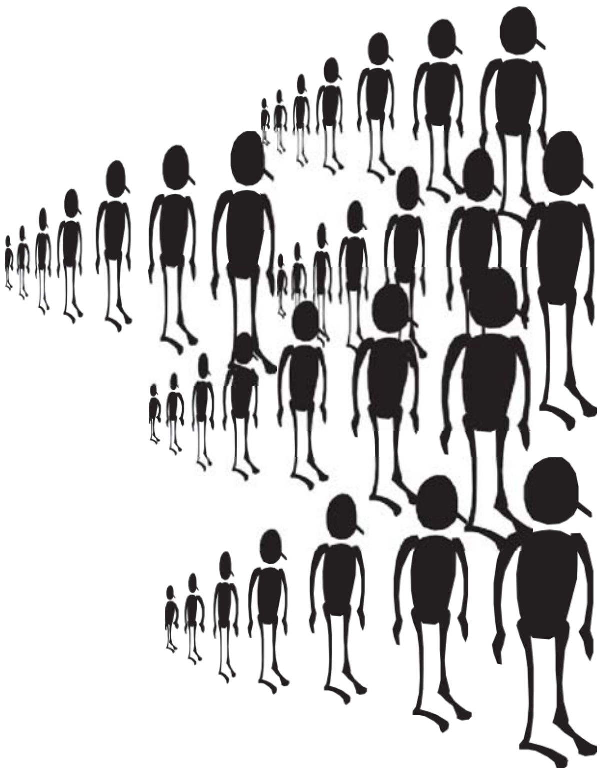
Source : USDA, IRRI and FAO

World Population (billions)

- 1940 2.30
- 1952 2.64
- 1964 3.28
- 1976 4.16
- 1988 5.11
- 2000 6.08
- 2012 7.00

Current rate of growth =

1 billion every 14 years



My university program has related to research and teaching in plant genetics applied to plant improvement with an attempt to bridge basic and applied aspects.

The goals have been to:

1) Enhance the efficiency of plant improvement through the understanding of important traits

2) Development of methods for the directed transfer of genes controlling these traits

3) Production of strains providing higher quality food, feed, fuel or otherwise enhance agricultural practices or human health

Advised 55 graduate students and 23 postdoctoral scientists

Ron Phillips' Retirement Symposium
Monday, May 24, 2010, 8:30 a.m. – 4:00 p.m.
Cargill Building for Microbial and Plant Genomics – St. Paul Campus

Left-to-Right - 1st Row: Ginger Peschke - Rick Jellen - Ron Phillips - Wayne Keim - Pat Buescher - Steve Thompson - Howard Rines 2nd Row: Mike Lee - Penny Kianian - Mary Ann Start - Candida Cabral - Roberto Tuberoso - Oscar Riera-Lizarazu - Olga Danilevskaya - Jeff Chen - Jason Walling - Scott Jackson - Dick Kowles - Mike McMullen Back Row: Paul Bullock - Mike Olsen - Nathan Springer - Shawn Kaeppler - Bill Rooney - Shahryar Kianian - Marc Albertsen - Peter Imle - Steve Fox - David Altman - Wayne Kennard - Alex Kahler - Ralf Kynast - John McLaughlin - Todd Krone - Matt Walch

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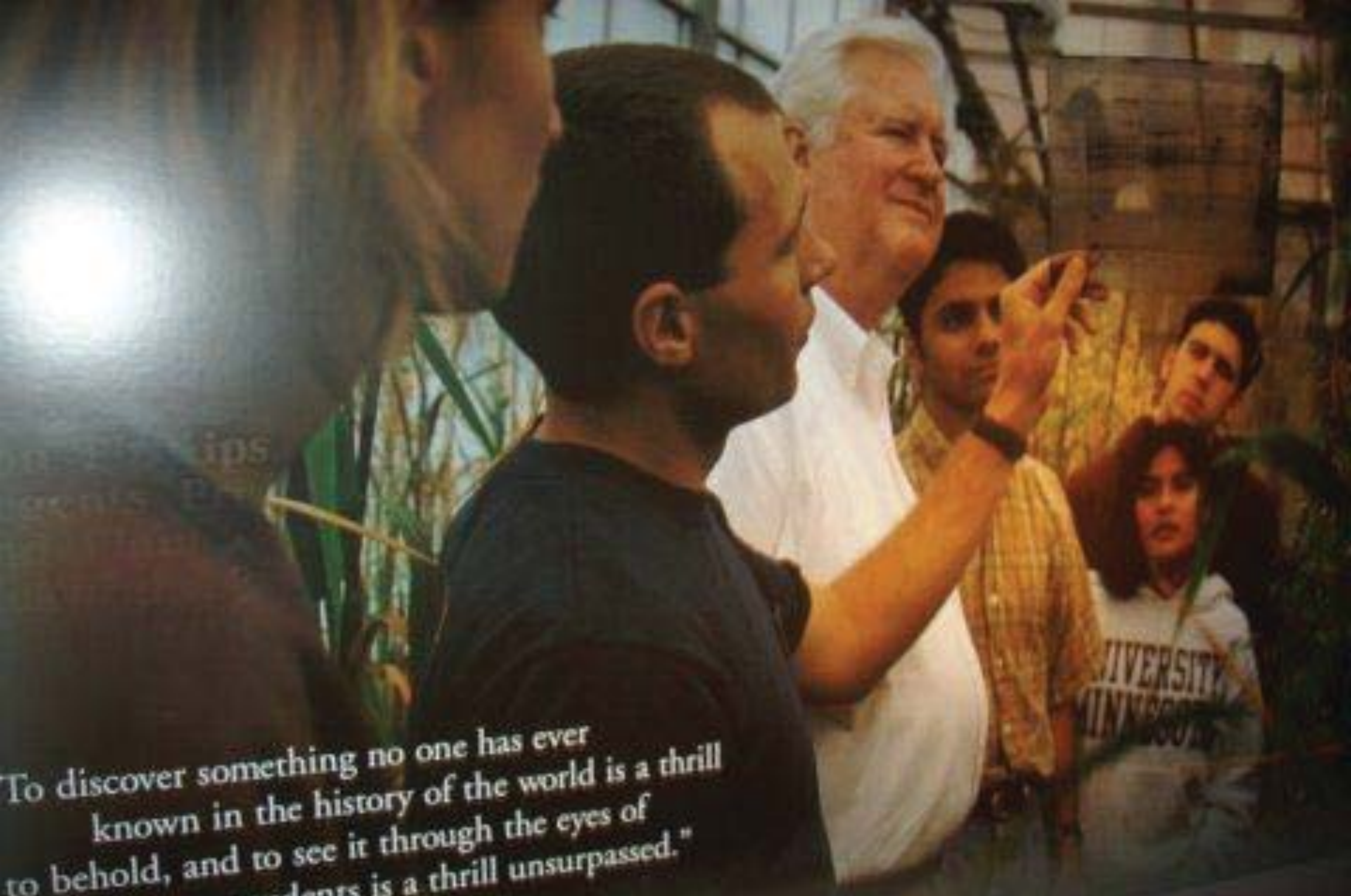
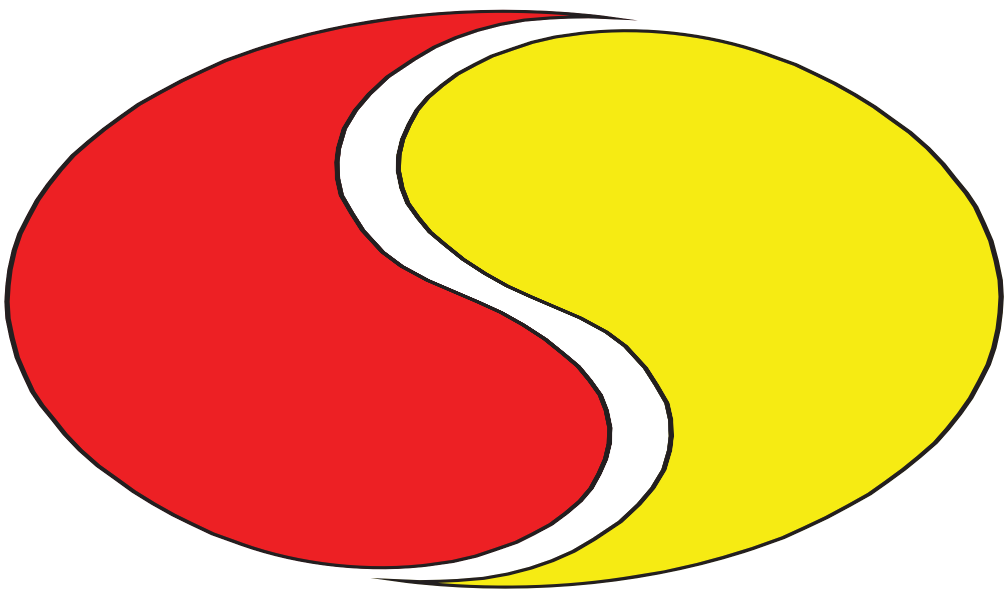


"To discover something no one has ever known in the history of the world is a thrill to behold, and to see it through the eyes of one of your students is a thrill unsurpassed."

onald Phillips, Regents Professor of Agronomy and Plant Genetics
Chairholder in Genomics

Genetics helps us understand life and the world around us



Plant genetics

Double challenge
Double reward

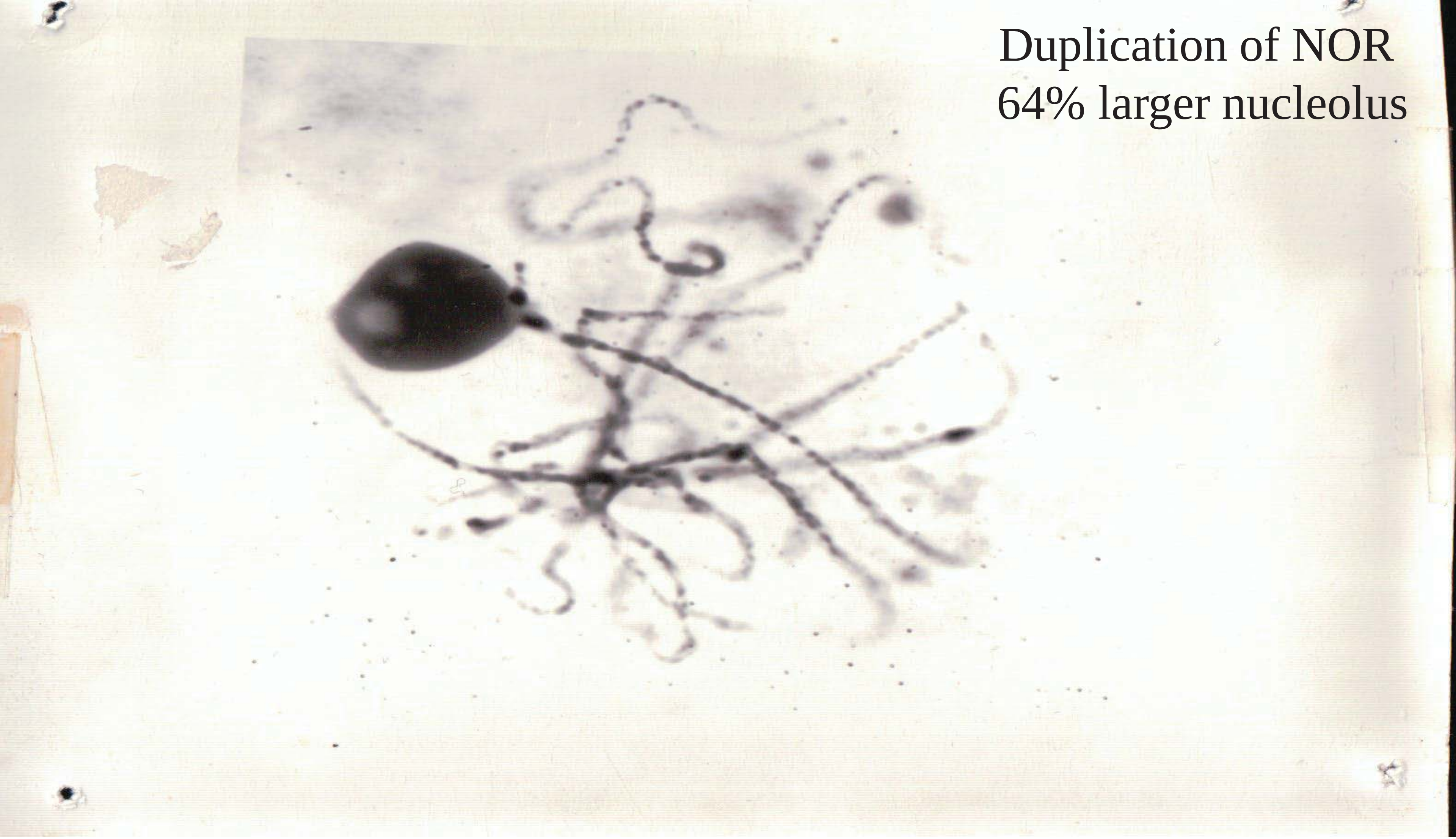
Approach today:

Retrace the major phases of my research over the years and show the involvement of serendipity in six examples

Developing maize stocks with various knobs

Example 1

Noticed stock with an apparent duplication of the nucleolus organizer region (NOR)

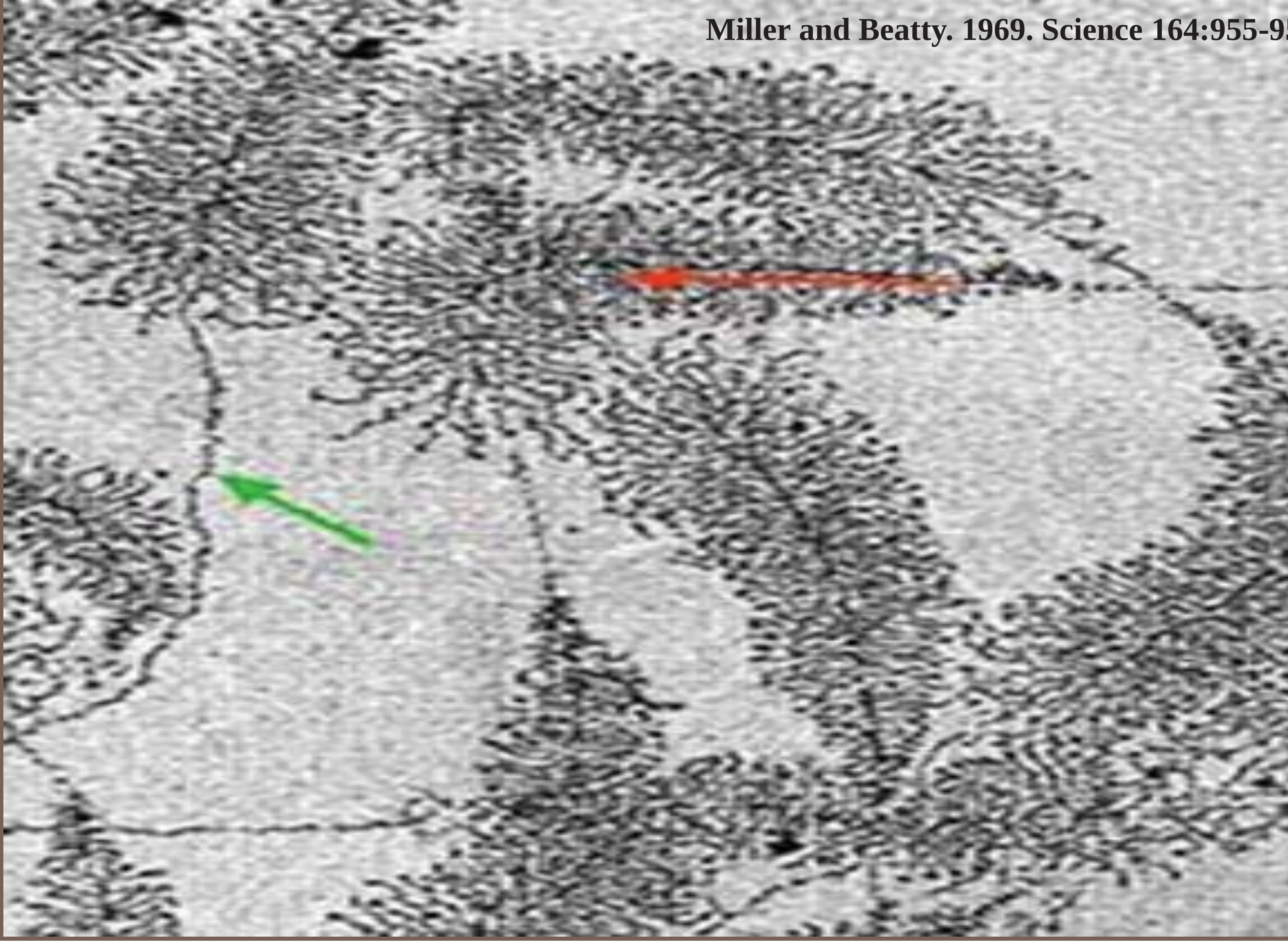


Phillips, Kleese, and Wang. 1971. Chromosoma 36:79-88

Determined the location of ribosomal RNA genes

Located the genes at a specific place (NOR) on chromosome 6 of corn

Found that the number of genes totaled 8000



Chromosome location of genes producing ribosomal RNA for ribosomes - involved in all protein synthesis

Example 2

Normal Brittle mosaic brittle

Brittle-1 Mutable

Rough texture kernel

Sometimes segregated bt-1

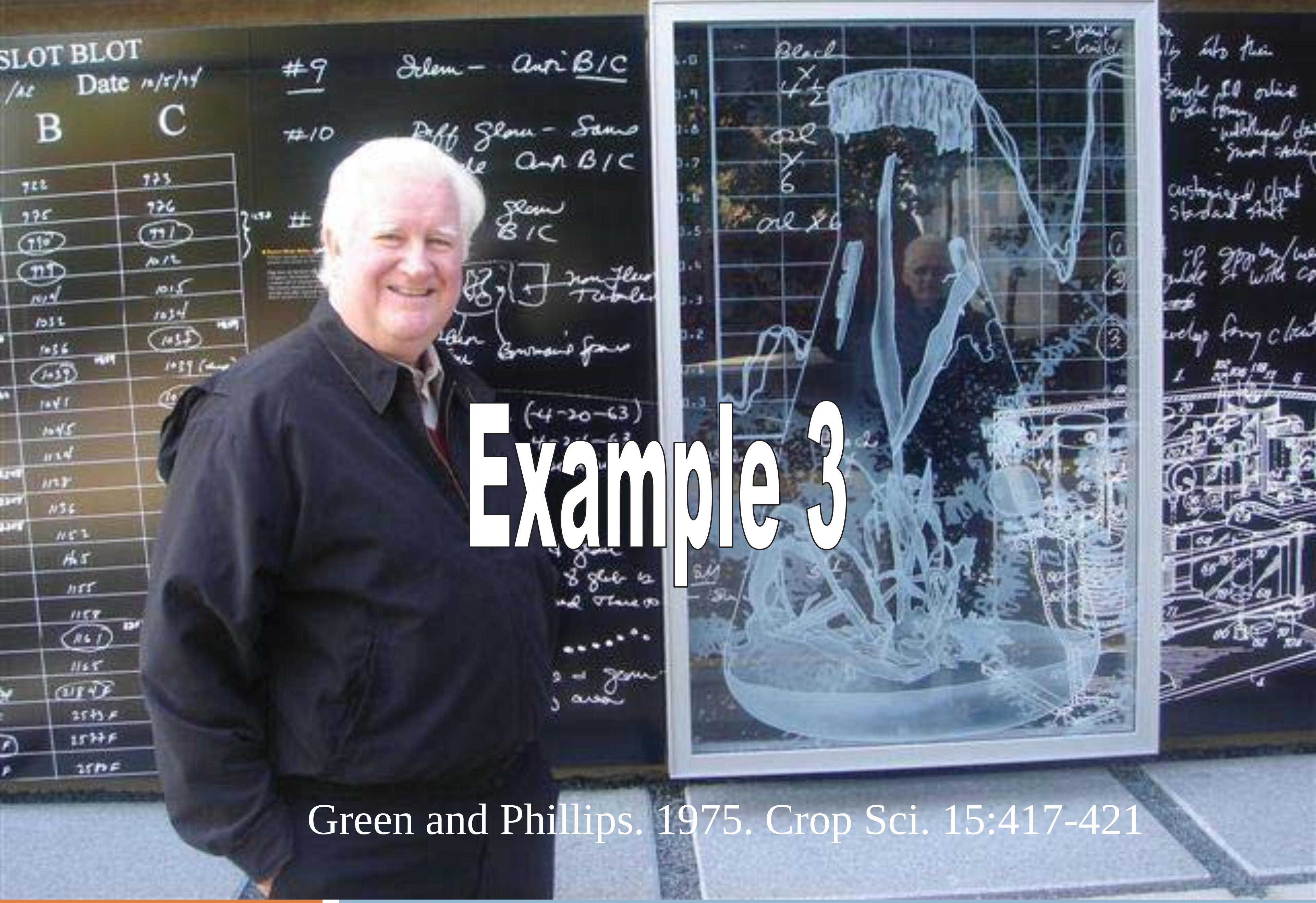
Idea: kernel was a mosaic for bt-1from reading McClintock's papers

McClintock and Peterson provided Spm and En testers

Conclusion: Phenotype due to bt-m controlled by En/Spm

Sullivan/Nelson used to clone bt-1

Sullivan, Strelow, Illingworth, Phillips, and Nelson. 1991. The Plant Cell 3:1337-11348.



Example 3

Green and Phillips. 1975. Crop Sci. 15:417-421

Regeneration of corn plants from tissue culture

Developed technology for the regeneration of whole corn plants from tissue culture

- Key was starting with the right tissue (immature embryos), the correct culture medium, and the proper genotype

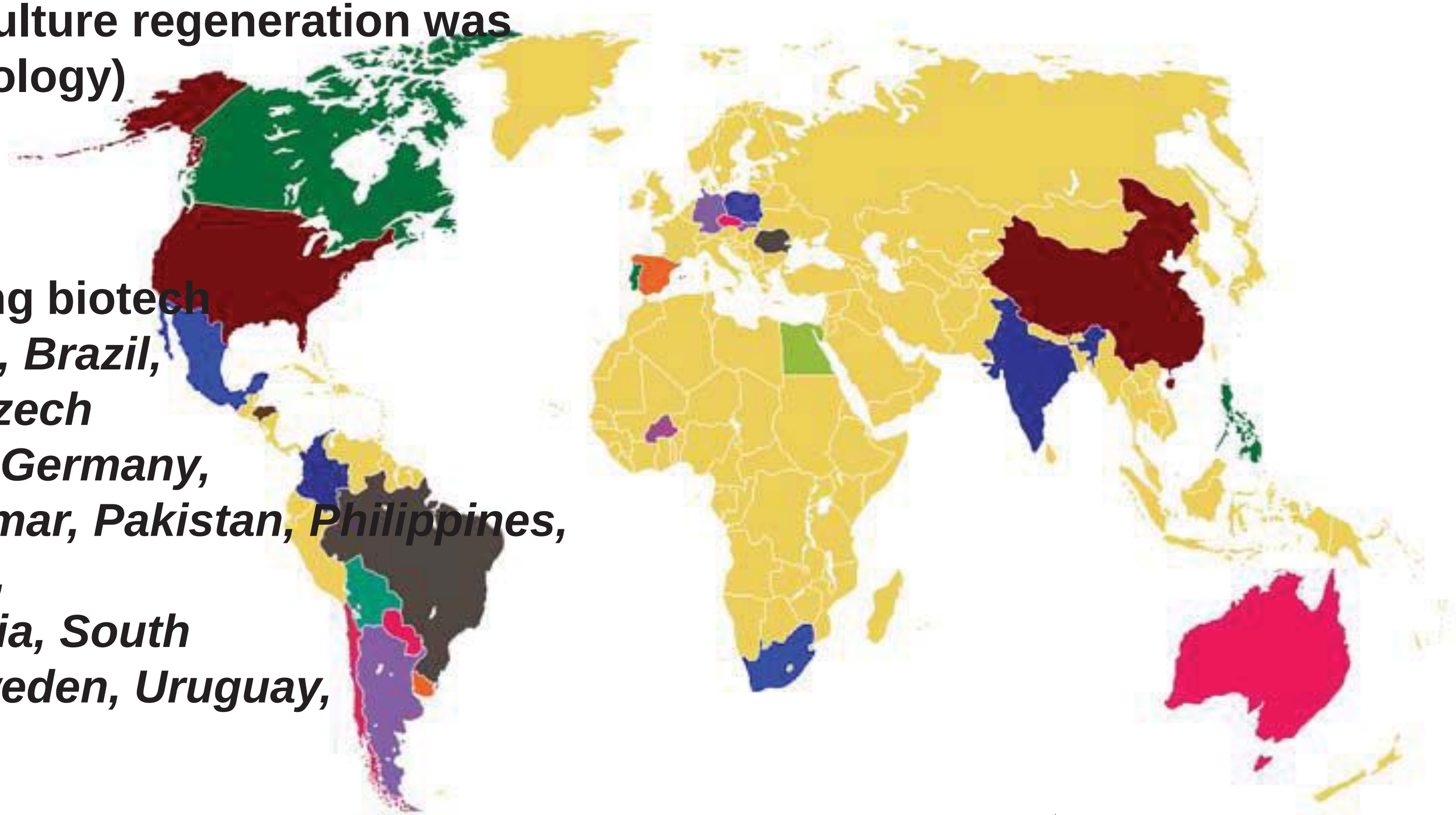
Peschke, Phillips, and Gengenbach. 1987. Science 238:804-807

Kaeppler and Phillips 1993. PNAS 90:8773-8776

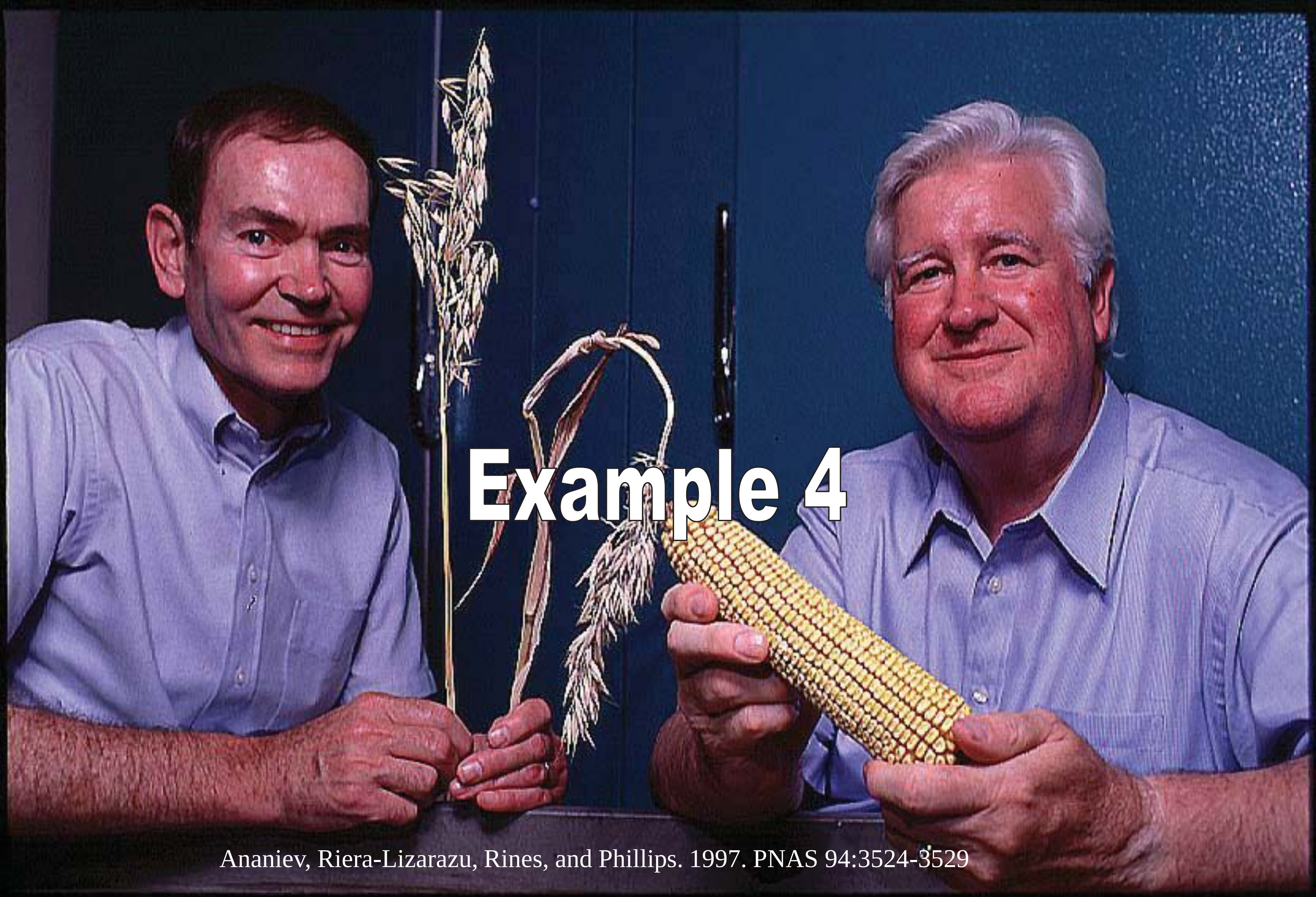
In vitro technology + prepared minds led the way to new technology in the form of genetic engineering

Genetically Engineered Maize

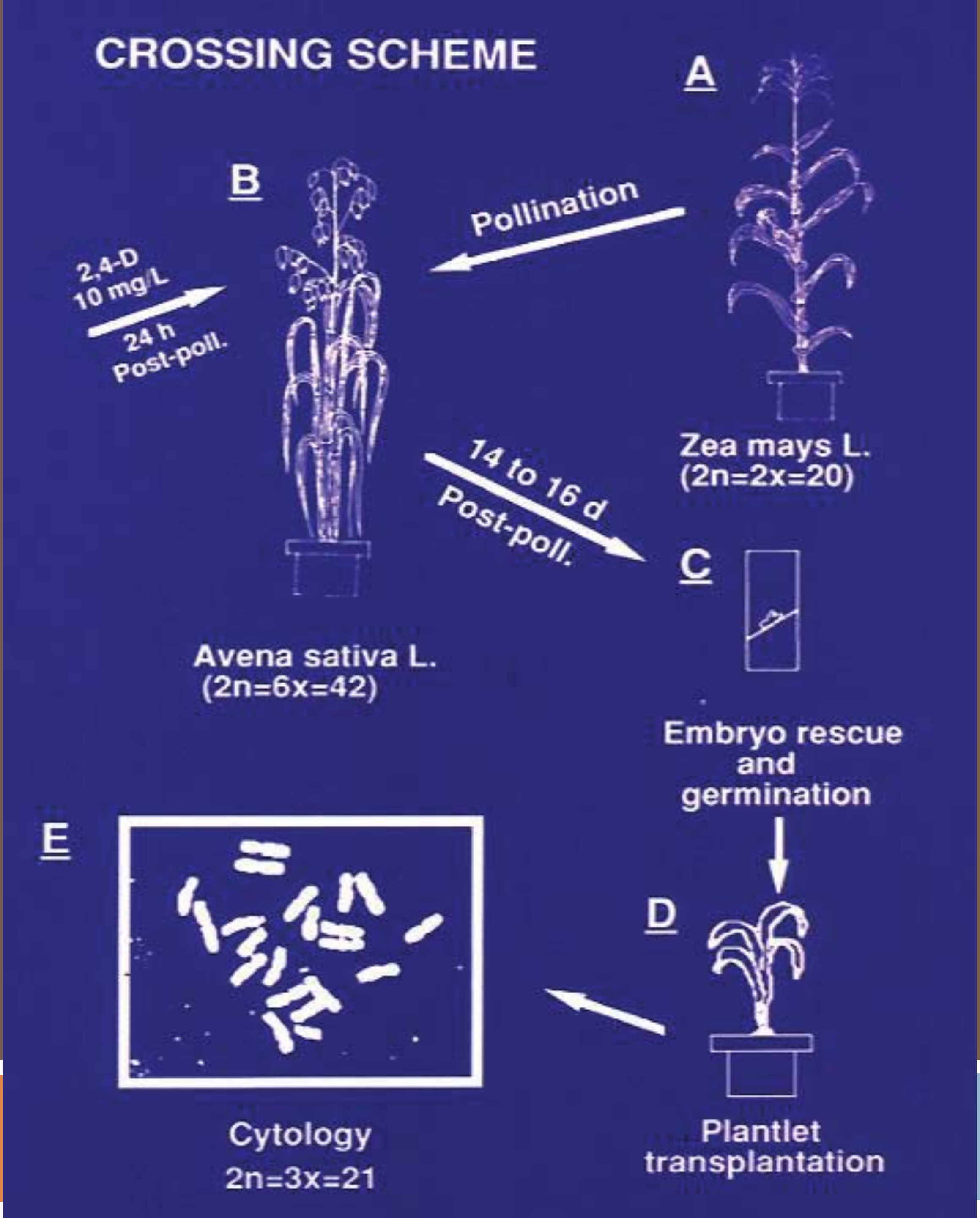
• **29% (46 million has.) of total global maize planted is biotech (tissue culture regeneration was part of the technology)**



Countries growing biotech maize: ***Argentina, Brazil, Canada, Chile, Czech Republic, Egypt, Germany, Honduras, Myanmar, Pakistan, Philippines, Poland, Portugal, Romania, Slovakia, South Africa, Spain, Sweden, Uruguay, and the USA.***



Method to map corn genes to chromosome and chromosome segment



CROSSING SCHEME
A
B
Pollination
2,4-D
10 mg/L
24 h
Post-poll.
Zea mays L.
(2n=2x=20)
14 to 16 d
Post-poll.
C
Avena sativa L.
(2n=6x=42)
Embryo rescue
and
germination
E
D
Cytology
2n=3x=21
Plantlet
transplantation

Oat x Maize Hybrid Zygote

Cell divisions

Maize chromosome elimination

Complete (66%)

Oat haploids (2n=21)

Partial (34%)

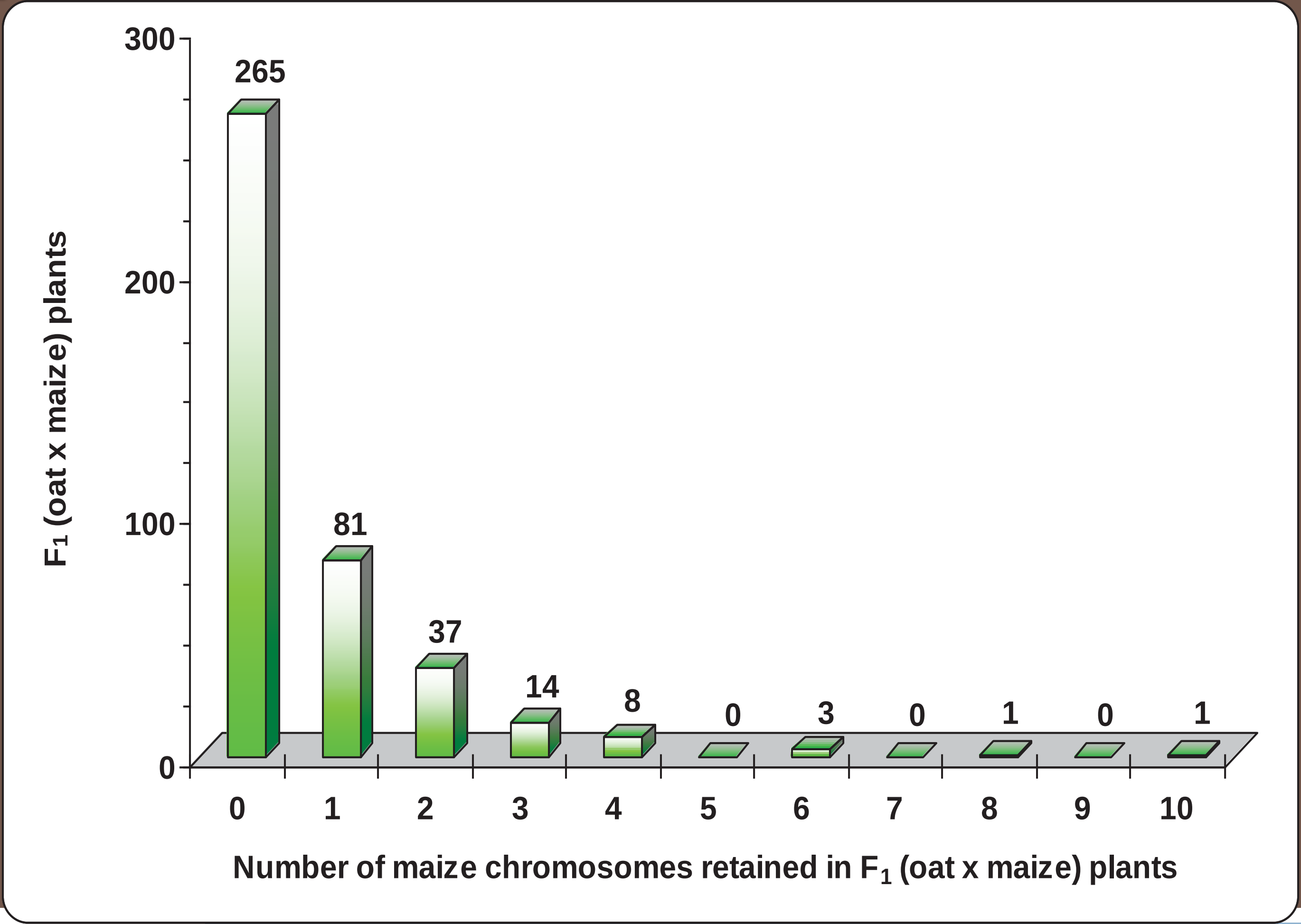
Oat haploids with maize chromosomes (2n=22-31)

Phillips and Rines 2009. Handbook of Maize (eds. Bennetzen and Hake) Vol. 2

ST1053-1   2n=23

21 oat + 2 maize

Maize Chromosome Retention in F_1 Plants

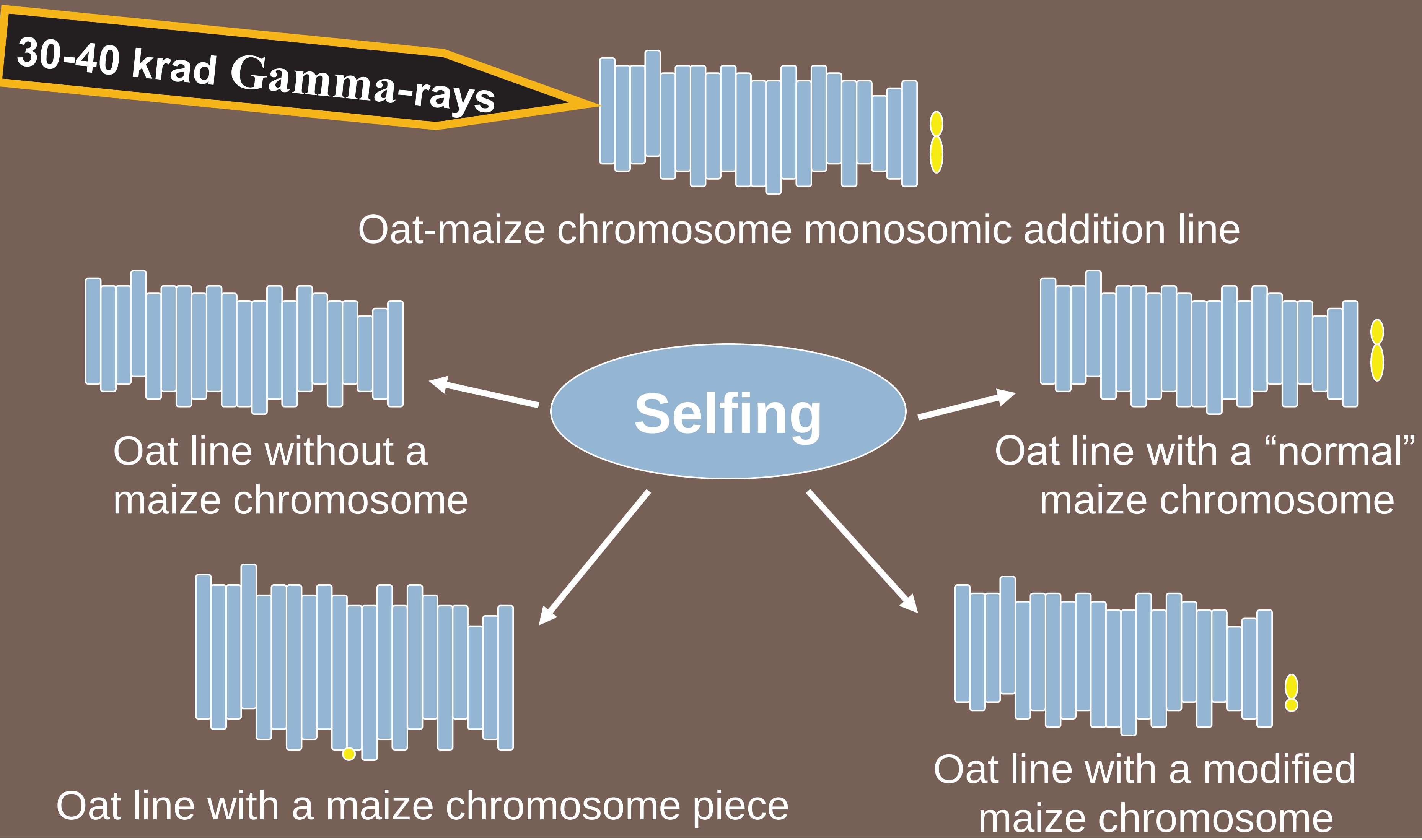


Available oat-maize addition lines

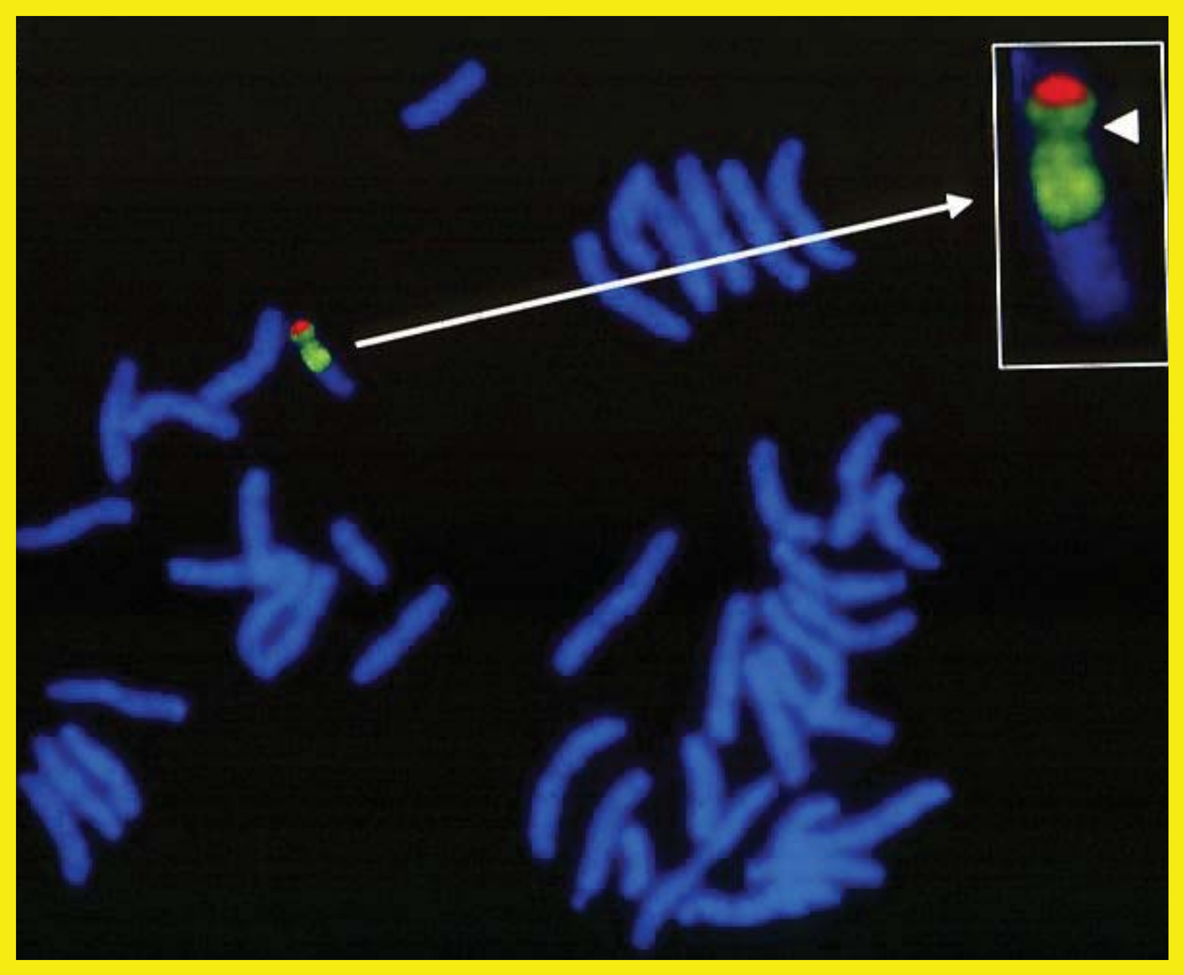
Maize Chromosome Donor	Oat-Maize Addition Line										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**B**
Seneca 60	1	10	2	6	3	3	3	1	7	1*	
B73	1	2		3	13	3		1	1	1	
Mo17		11	1*	1	8	4	1*	1		2	
A188				1							
bz1-mum9		1						1			
B73 w/Black Mexican Sweet B Chr											2

*DNA only

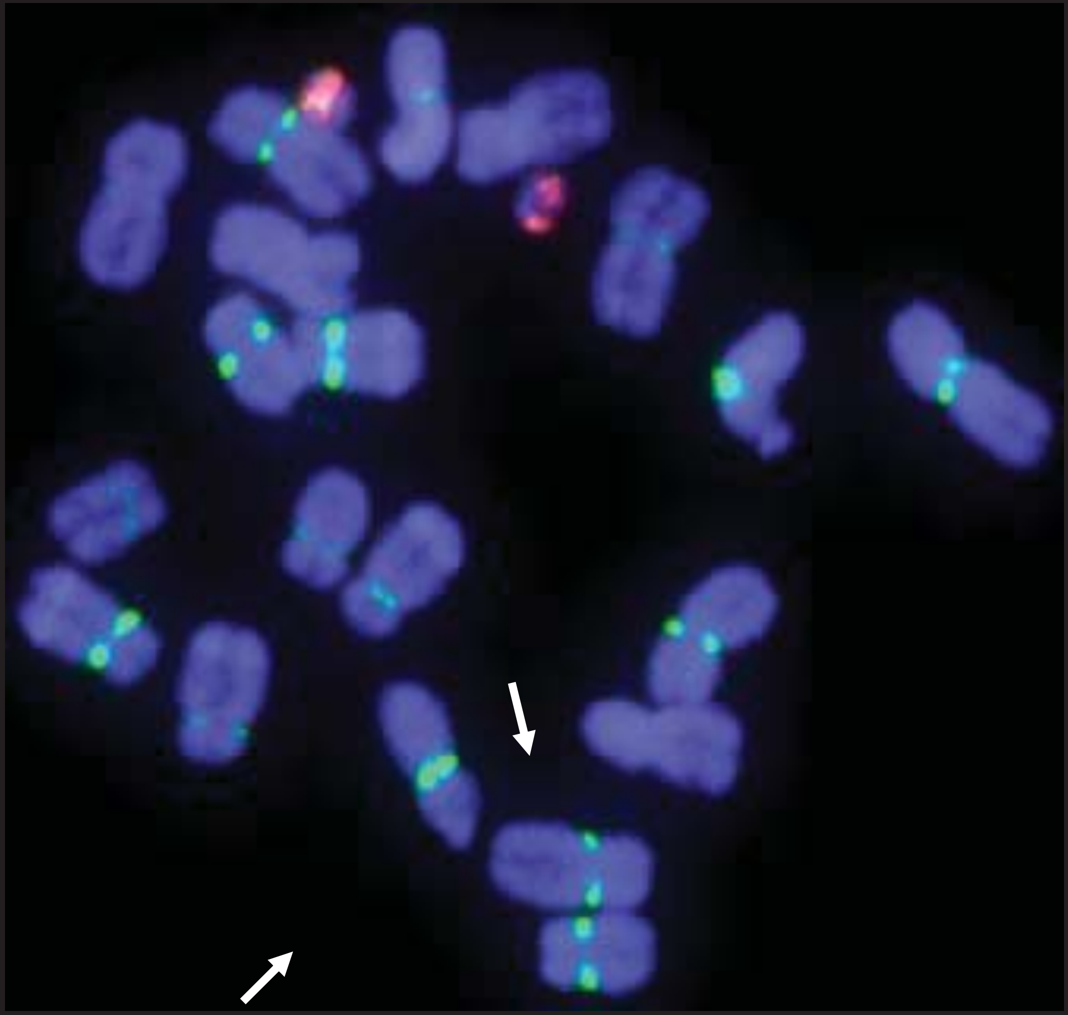
Irradiation of an Oat-Maize monosomic addition line

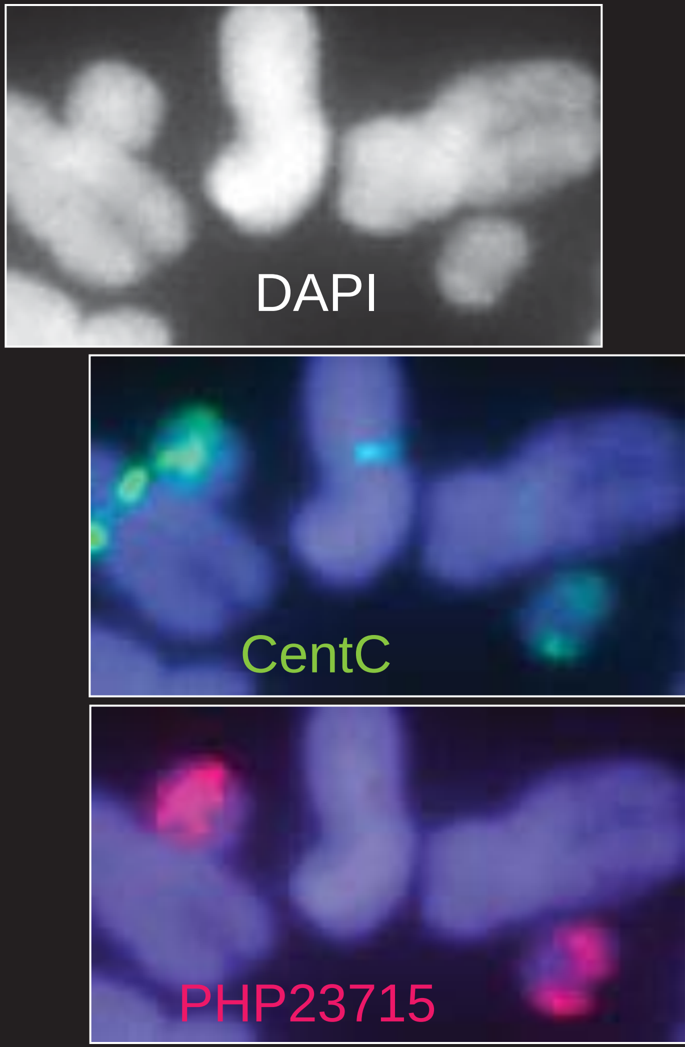


Radiation Hybrid Line 901
Chromosome 9



Maize artificial chromosomes





20 native + 2 artificial chromosomes

Provided by E. Ananiev, M. Chamberlin, and S. Svitashev

PIONEER. A DUPONT COMPANY

DUPONT

Uses of OMA and RH Lines

- **H**igh resolution mapping
- Mapping gene families
- Chromosome sorting
- Mapping transpositions of transposable elements
- Meiotic pairing
- Identification of new centromeric-specific elements
- Contributed to construction of transmitting maize mini-chromosomes
- Study the introgression of useful traits from maize to oat (C4 to C3 photosynthesis)

Example 5

Identifying and cloning a gene controlling flowering time in corn

Salvi, Sponza, Morgante, Fengler, Meeley, Ananiev, Svitashev, Li, Hainey, Rafalski, Tingey, Tomes, Miao, Phillips, and Tuberosa. 2007. PNAS 104:11376-11381

Identified and cloned a gene for early flowering

Gaspé Flint N28E N28

Silvio Salvi

Cloning a gene controlling flowering time in corn

- **Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize**

High Oil Corn as a Higher Value Commodity

Example 6

Phillips, Suresh, Stec, Springer, Ruan, Zhang, and Tiffany. 2010. Maize Genetics Conference. Abstract P239

http://www1.umn.edu/iree/e3/archive/archive_2010/E3_PhillipsTifanny.pdf

Developing high oil corn

3-4% Oil

- Dr. Kim Joo gave us a high oil line from North Korea
- This line of corn had 20% oil and could serve as a renewable source of biodiesel
- In Minnesota – plan to Increase the requirement for biodiesel blends from 2% to 20% by 2015

High oil corn has the potential for being a dual source of biofuel (ethanol and biodiesel) as well as for feed and food

- Seeds were soaked in water overnight to separate the endosperm from the embryo

Endosperm | Embryo

Oil in endo= 3.1%
vs. 3.2 % in normal corn

Oil in emb= 50.0 %
vs. 25% in normal corn

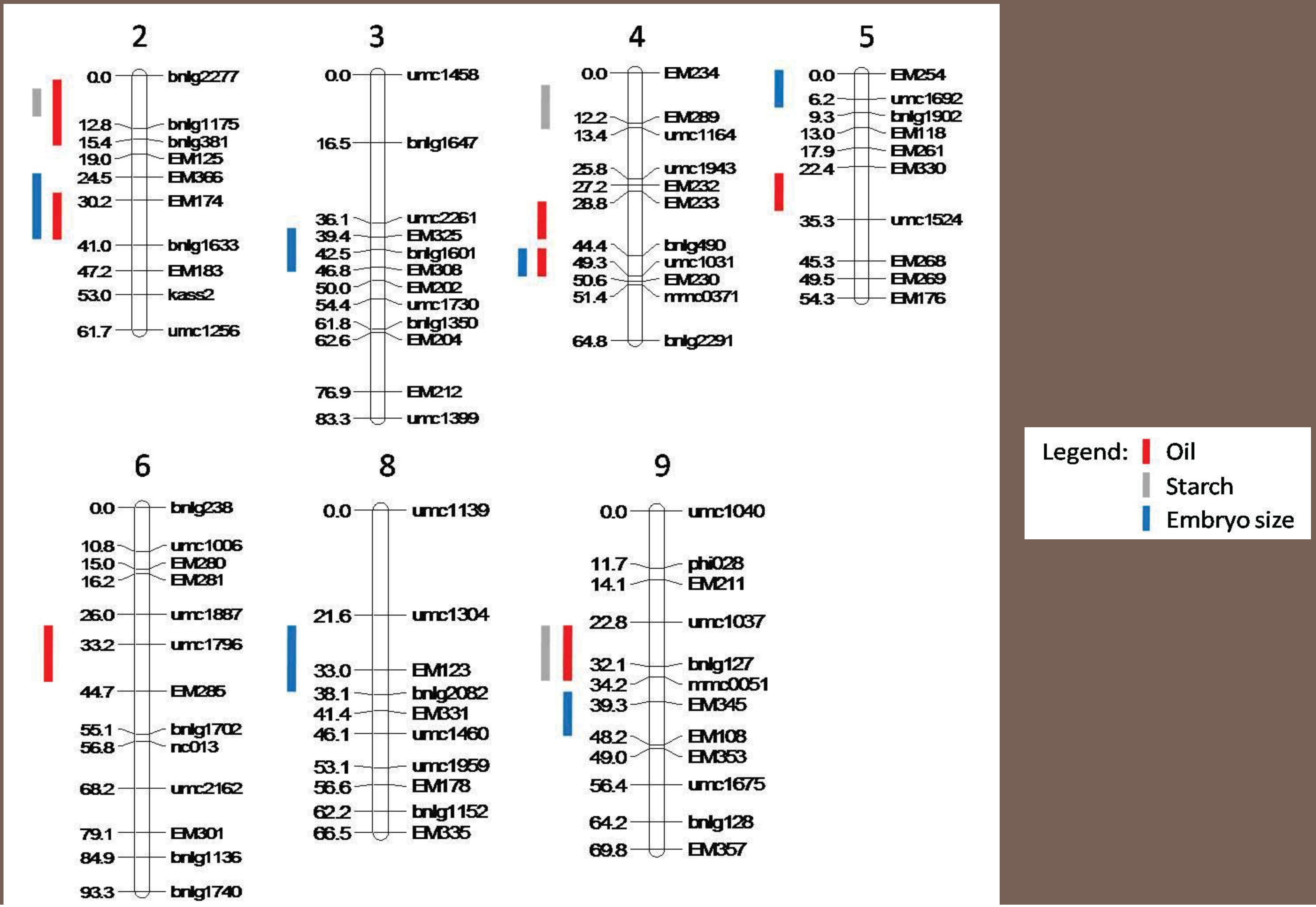
Embryo of KHO is 31% of seed by dry weight

Percent oil and starch in testcrosses with B73 and Mo17

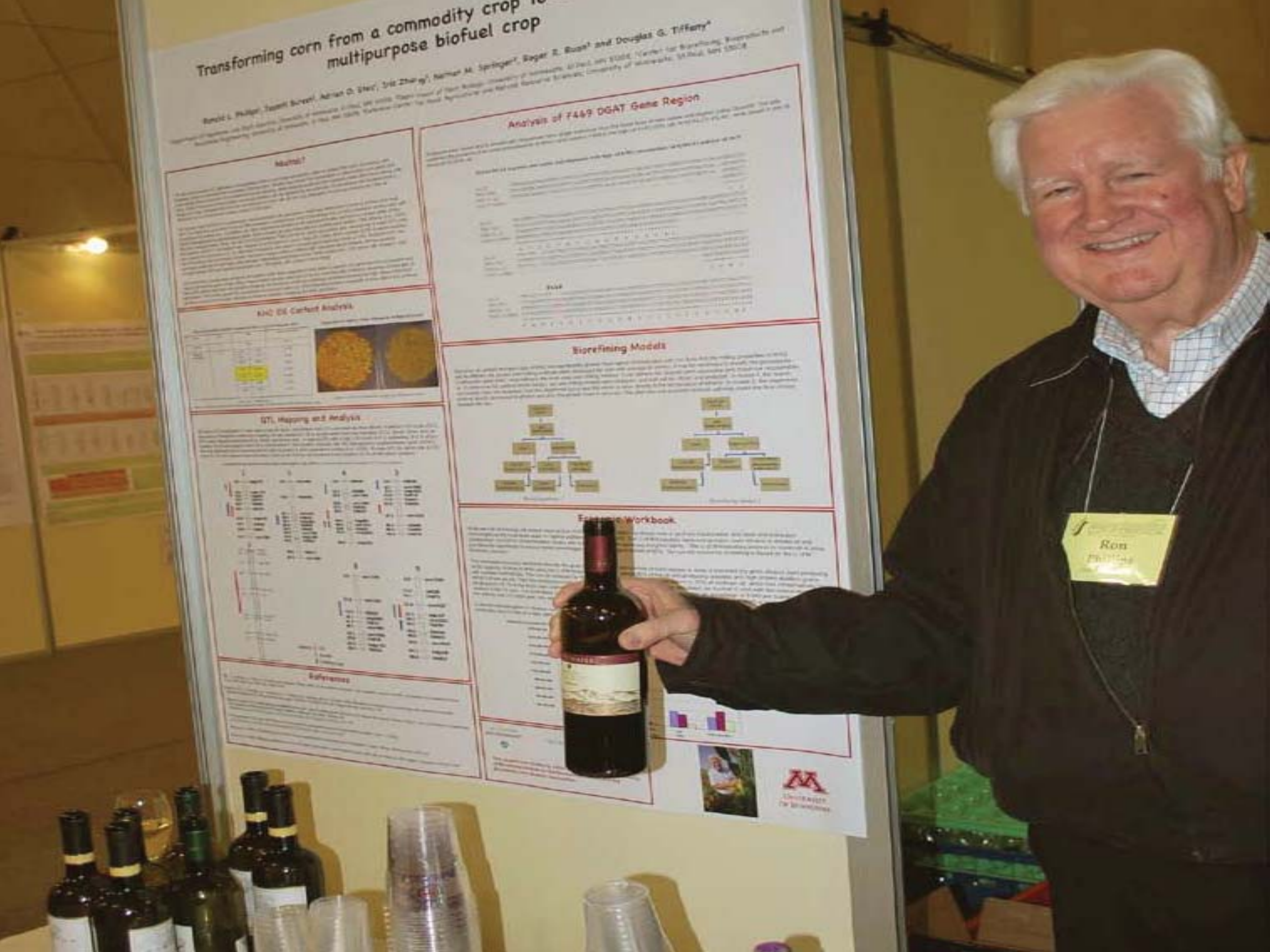
Corn Line	Oil (%)	Starch (%)
KHO	20.00	41.33
B73	3.10	65.90
KHO x B73 F1	12.10	55.50
KHO x B73 BC1	5.70	63.50
Mo17	3.10	64.40
KHO x Mo17 F1	11.20	57.10
KHO x Mo17 BC1	5.10	62.70

Field Yield Tests

Genotype	Grain Yield (bu/ac)	Grain Moisture %	% Oil (Projected)	Lbs oil/ac (Projected)
Three commercial hybrids	183	15.0	4	410
A632 x A619	154	18.1	4	348
B73 x Mo17	148	25.3	4	332
KHO x five dent lines	126	23.0	12	847

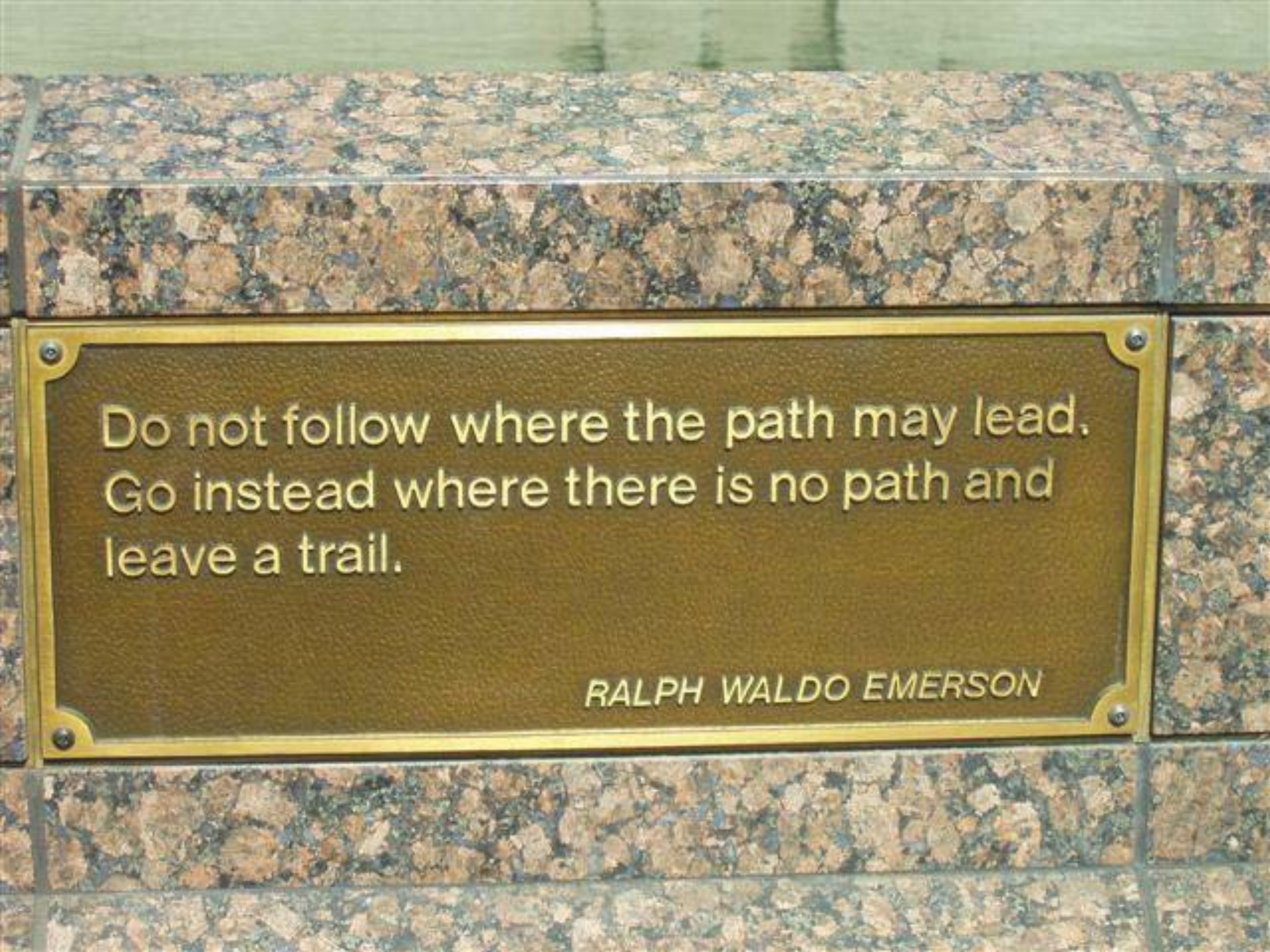


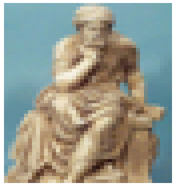
Locations of oil, starch, and embryo size QTLs


Transforming corn from a commodity crop
multipurpose biofuel crop
Analysis of F469 DGAT Gene Region
Biorefining Models
QTL Mapping and Analysis
References
Ron

Founder of the Noble Foundation said:

"As I look around at the strides that have been made in our research laboratories, as I look at the things undreamed of a few years ago…the only degree to which we have reached the end of the road of opportunity is the degree to which we have exhausted the imaginative capacity of the human mind."


Do not follow where the path may lead.
Go instead where there is no path and
leave a trail.
RALPH WALDO EMERSON



Personal Philosophy

- Believe in your data
- Look ahead – assume technology will improve
- Think about important research
- Maintain absolute integrity
- Gain an international perspective
- Recognize the importance of writing
- Enjoy the best job in the world

AND -----

Watch for those serendipitous opportunities!