

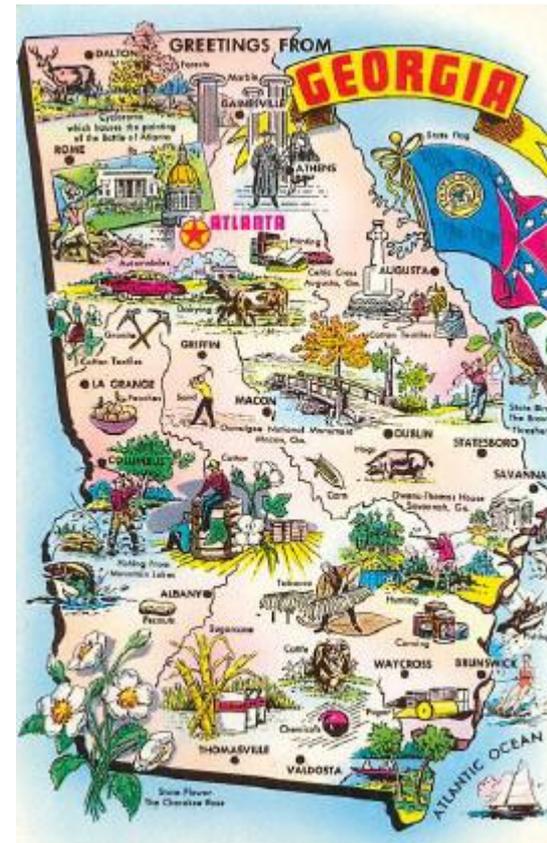
The biology of naturally occurring insertions

Justin Vaughn, PhD
University of Georgia, Athens

14 January 2014



The University of Georgia®

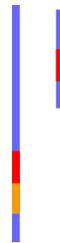


Sources of unintended effects associated with genetic engineering

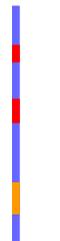
Unanticipated function of the transgene sequence



Structural rearrangement at the site of transgene insertion



Genome-wide structural rearrangements resulting from genetic transformation method and/or the molecular biology of insertion



What is the molecular nature of these effects?

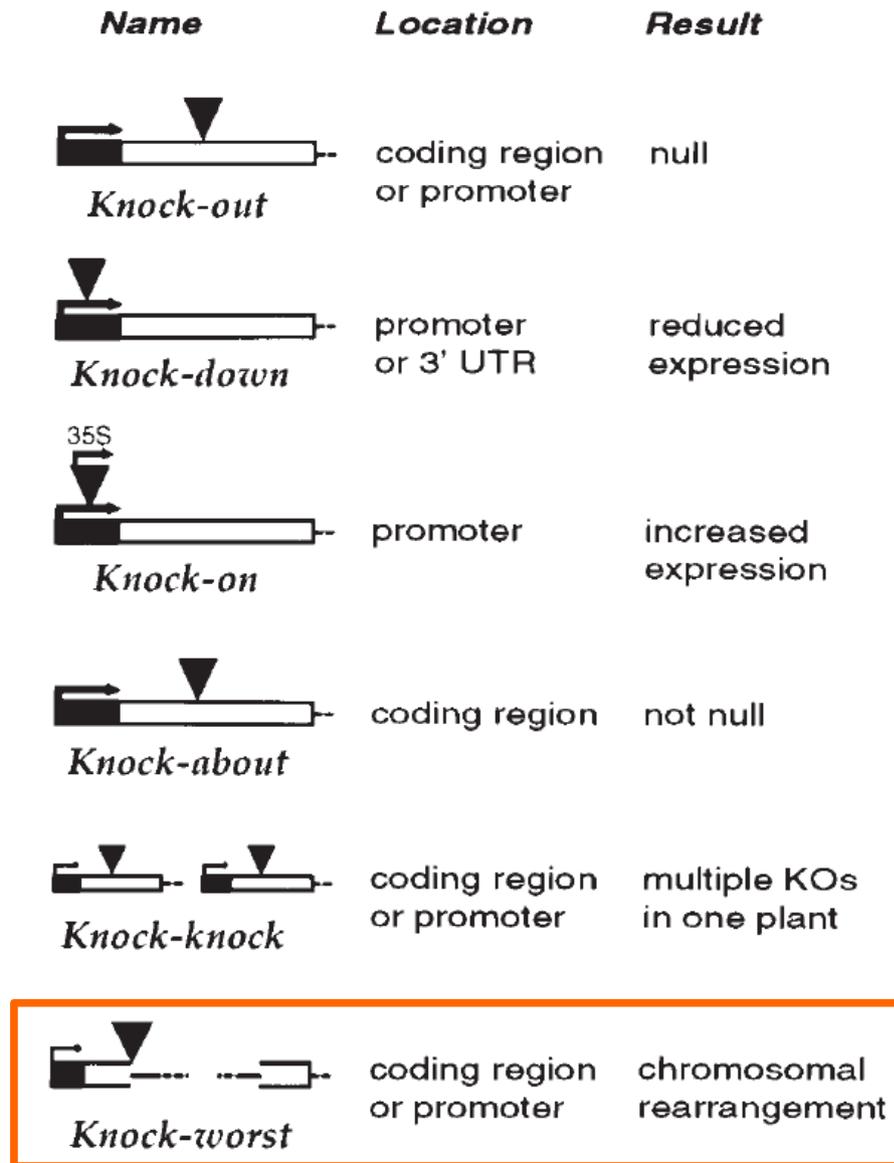
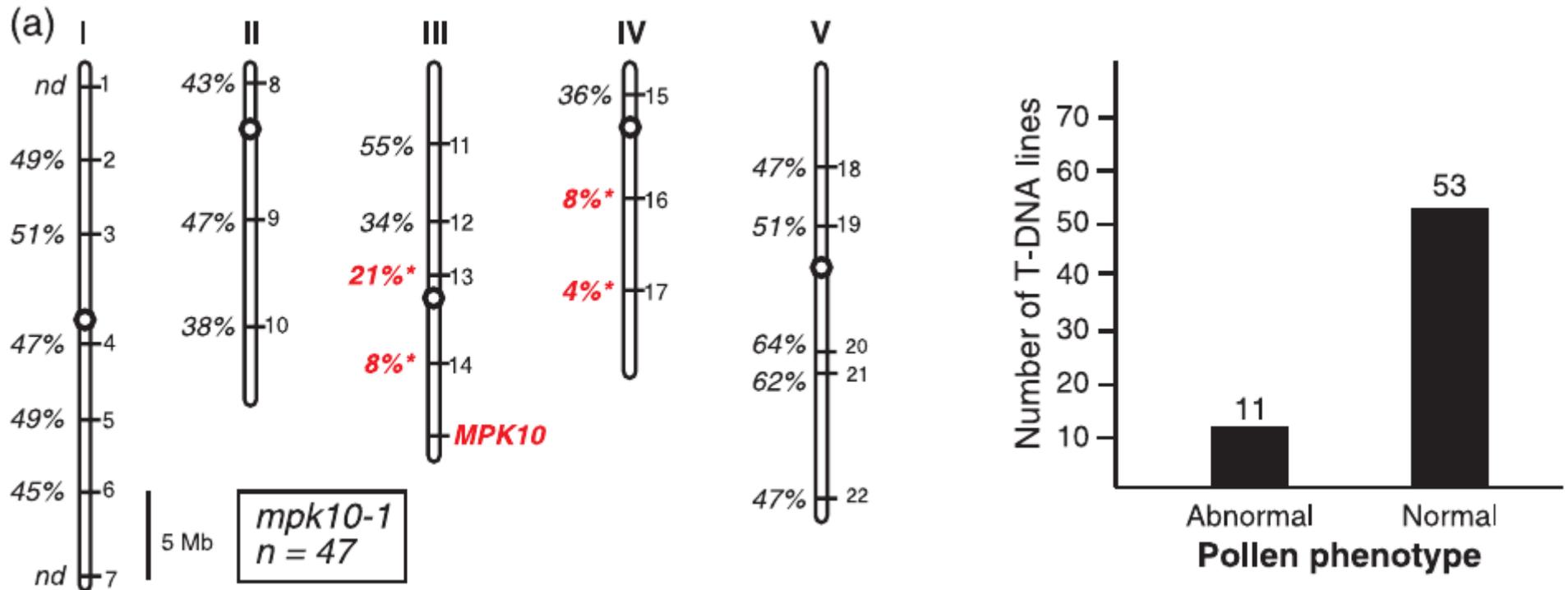


Figure 1. "Knockology."

Krysan et al., 1999

Chromosomal translocations are a common phenomenon in *Arabidopsis thaliana* T-DNA insertion lines

Katie A. Clark and Patrick J. Krysan*



12 translocations / 64 lines

Even with single T-DNA insertions, structural rearrangement still exists



Plant Molecular Biology **52**: 161–176, 2003.
© 2003 Kluwer Academic Publishers. Printed in the Netherlands.

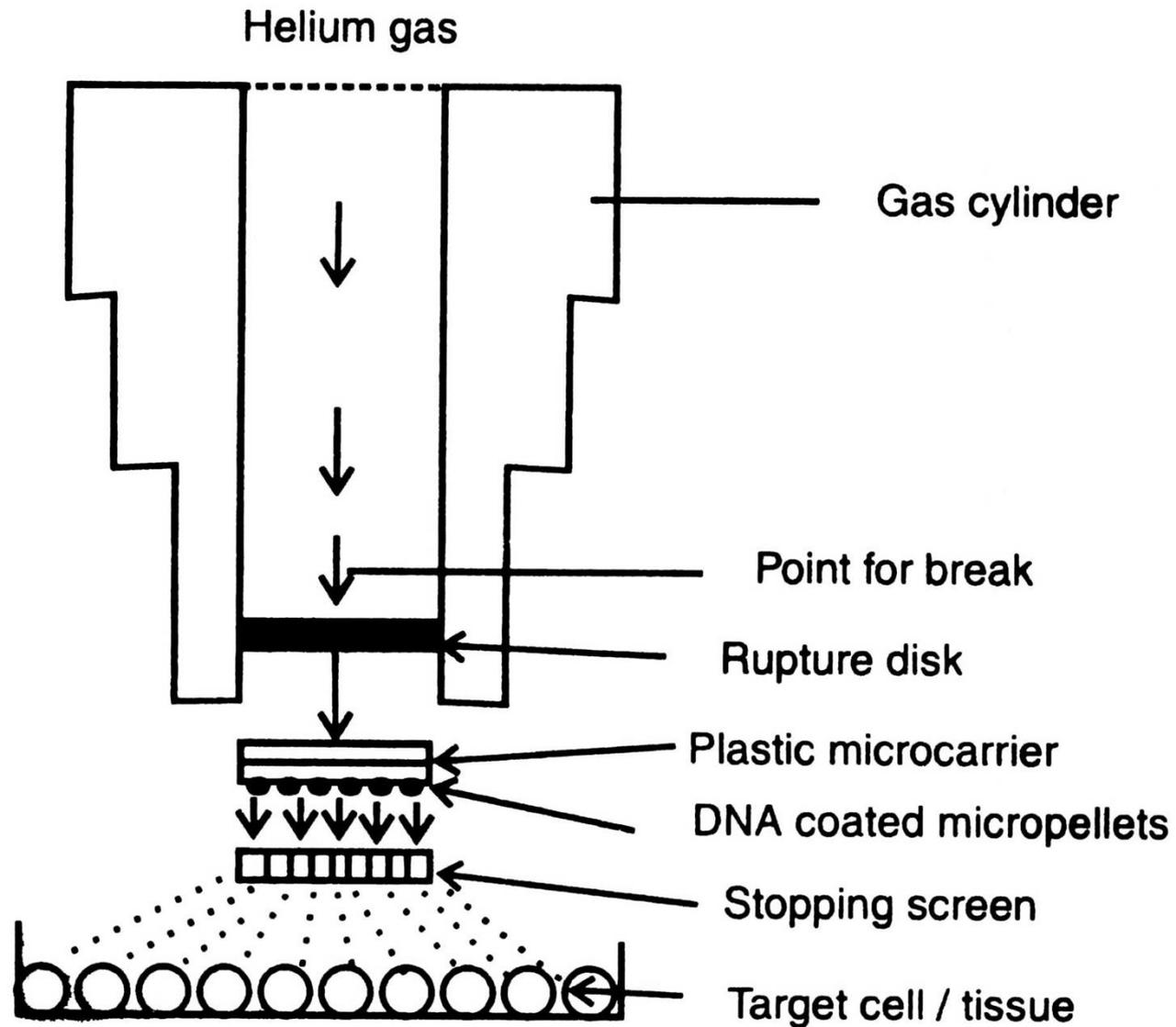
A comprehensive characterization of single-copy T-DNA insertions in the *Arabidopsis thaliana* genome

Alexandra Forsbach¹, Daniel Schubert, Berthold Lechtenberg, Mario Gils² and Renate Schmidt*

Table 2. Sequence analysis of junctions between T-DNA borders and genomic target sites.

		Number of LB junctions	Number of RB junctions
Identical nucleotides between target site and border	1–8 bp	39 (41.9%)	27 (28.7%)
Exact joining between target site and border		4 (4.3%)	8 (8.5%)
Filler nucleotides between target site and border		50 (53.8%)	59 (62.8%)
	1–25 bp	39 (41.9%)	43 (45.7%)
	26–100 bp	11 (11.8%)	13 (13.8%)
	>100 bp	0 (0%)	3 (3.2%)

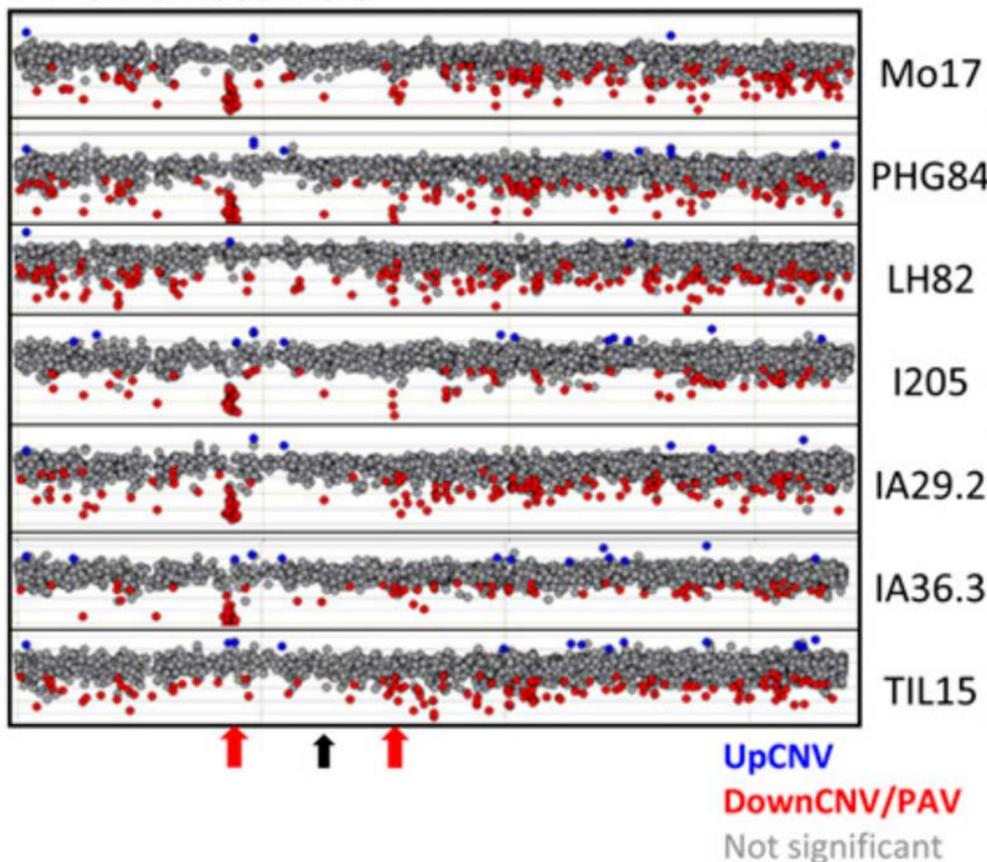
Biolytic transformation generally creates more complex insertions



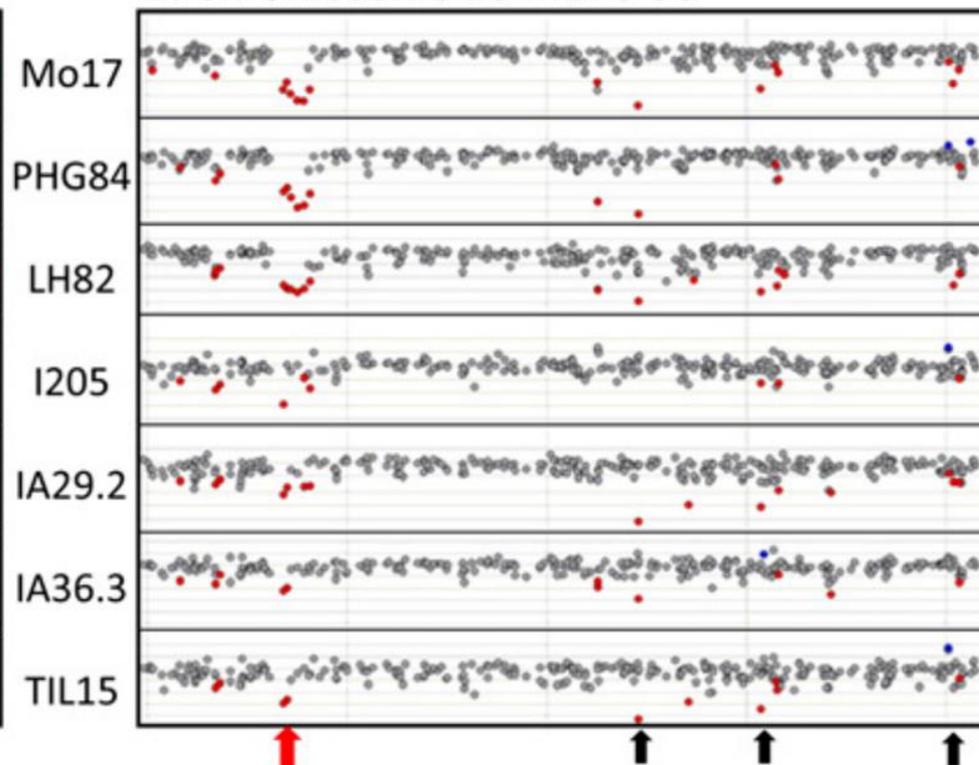
Pervasive gene content variation and copy number variation in maize and its undomesticated progenitor

Ruth A. Swanson-Wagner,^{1,5} Steven R. Eichten,^{1,5} Sunita Kumari,² Peter Tiffin,¹ Joshua C. Stein,² Doreen Ware,^{2,3} and Nathan M. Springer^{1,4,6}

A. Chromosome 6

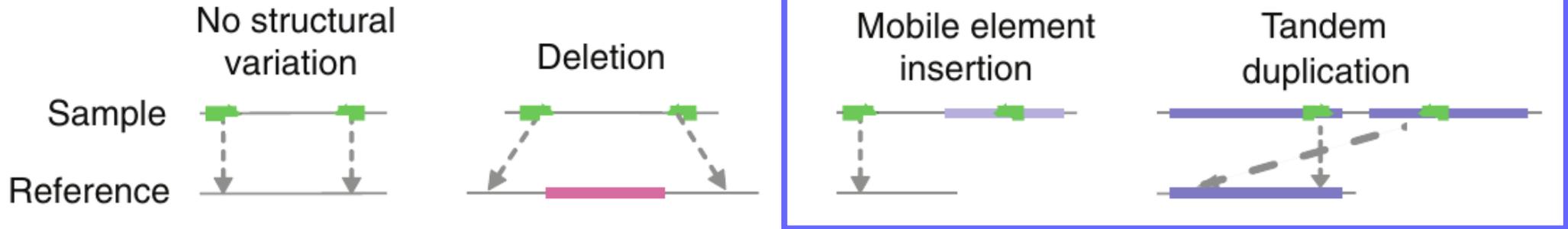


B. Chromosome 10 Mb70-90

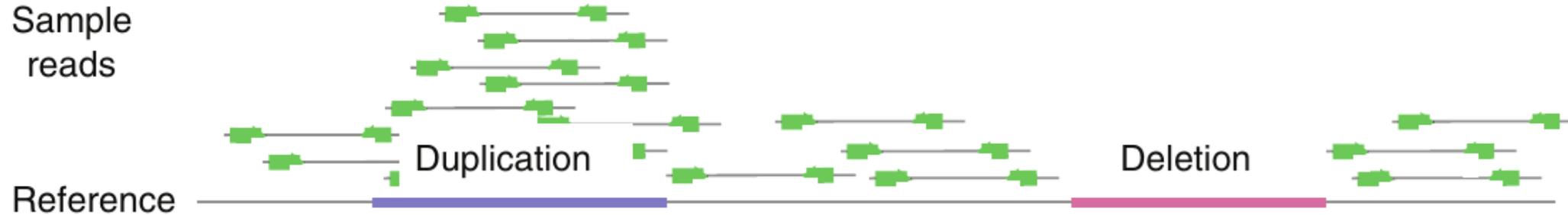


How do we find structural variants with next-generation sequencing data?

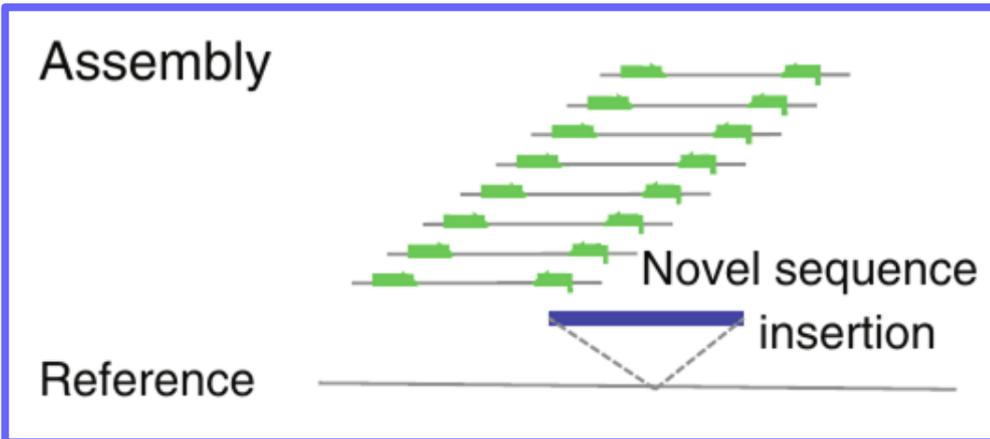
Read pairs



Read depth



Split reads



How do natural events compare with induced structural variation?

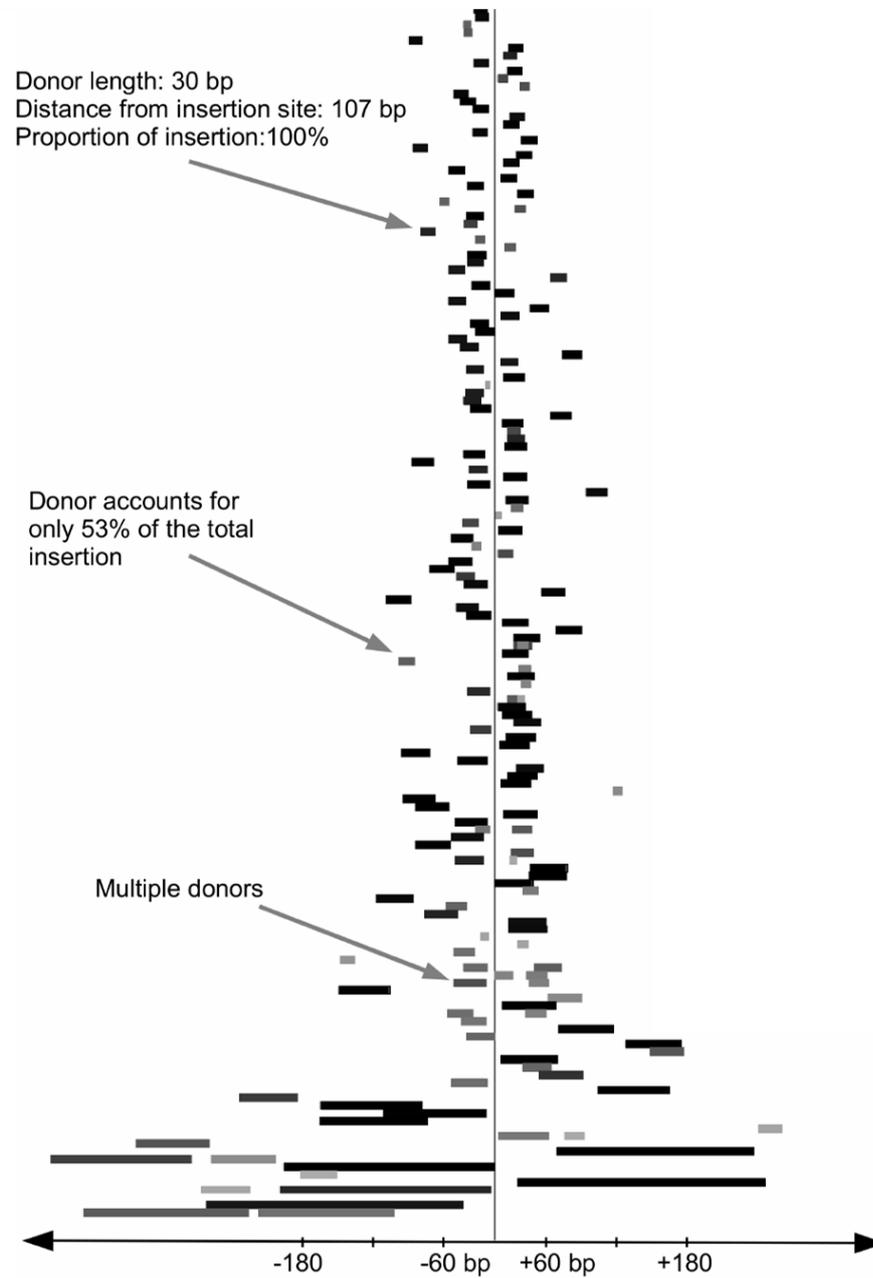
Chr.	Chr. size (Mb) ^a	Structural variations	Adjacent events ^b	Deletions	Insertions	Inferable events (%)
1	43.2	22278	53	4912	4390	42
3	36.4	14990	18	4285	3544	52
8	28.4	14757	9	3047	2711	39
10	23.1	13366	12	2907	2400	40
Total	131.1	65391	92	15151	13045	43

^afrom www.gramene.org

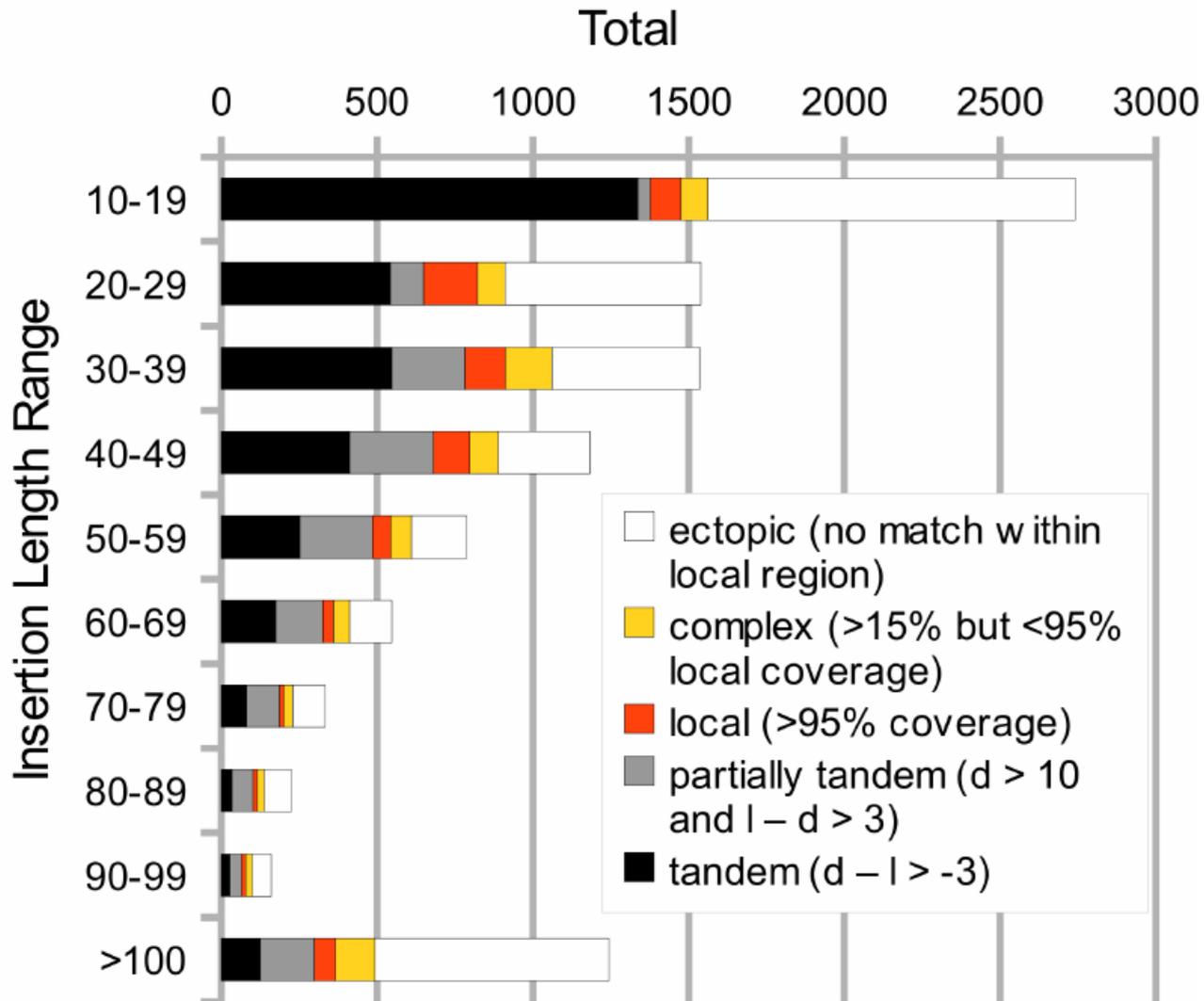
^bAdjacent events are defined as insertion polymorphisms directly adjacent to deletion polymorphisms.

Vaughn and Bennetzen, PNAS, accepted

Local duplications are commonly observed



Origins of inserted sequences



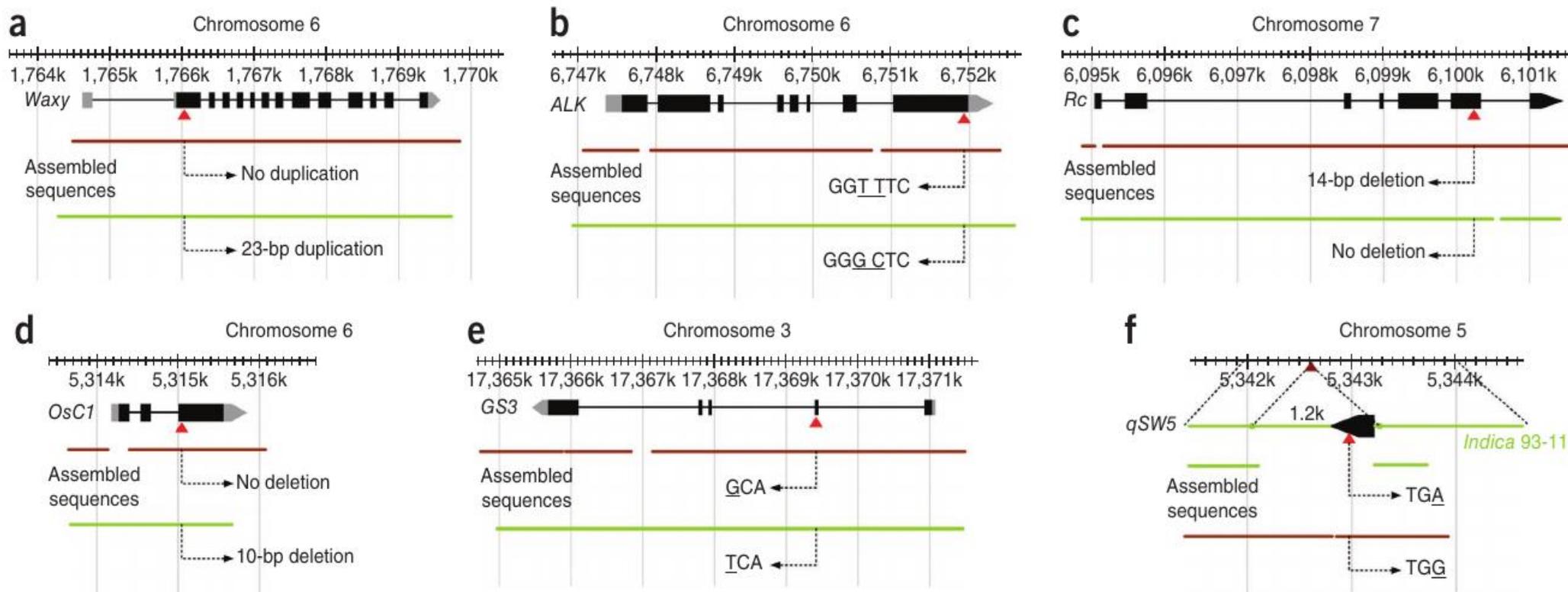
Vaughn and Bennetzen, PNAS, accepted

Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm

Xuehui Huang^{1,2,5}, Yan Zhao^{1,2,5}, Xinghua Wei^{3,5}, Canyang Li¹, Ahong Wang¹, Qiang Zhao¹, Wenjun Li¹,

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VOLUME 44 | NUMBER 1 | JANUARY 2012 NATURE GENETICS



Rapid recent growth and divergence of rice nuclear genomes

Jianxin Ma and Jeffrey L. Bennetzen*

Department of Genetics, University of Georgia, Athens, GA 30602

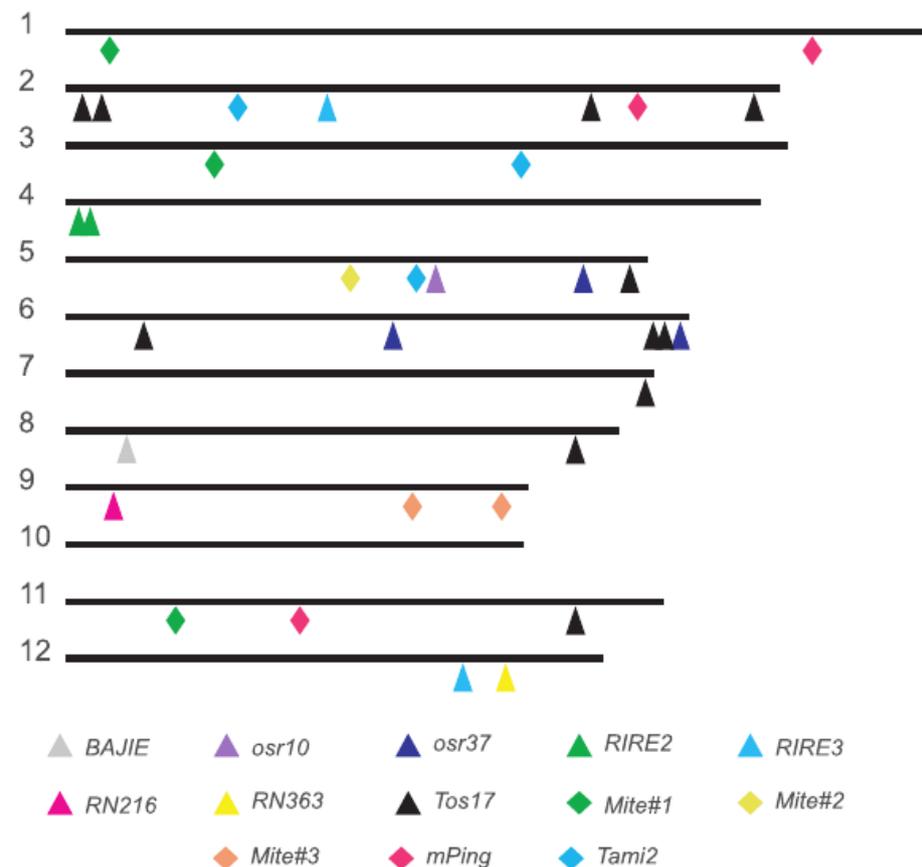
12404–12410 | PNAS | August 24, 2004 | vol. 101 | no. 34

Table 1. Amount of DNA involved in small DNA arrangements

Subspecies	Small DNA rearrangements*	Number	Total size, bp
<i>indica</i>	Insertions [†]	41	81,202
	Insertions of LTR-retrotransposons	13	56,775
	Insertions of MITEs	16	4,353
	Insertions of other DNA transposons	2	15,627
	Insertions (unknown)	10	4,447
	Deletions	51	19814 [‡]
	Unclear indels [§]	92	38,735
<i>japonica</i>	Tandem duplications	8	298
	Amplification of SSRs	9	563
	Insertions [†]	42	111,127
	Insertions of LTR-retrotransposons	15	95,065
	Insertions of MITEs	16	4,085
	Insertions of other DNA transposons	1	11,480
	Insertions (unknown)	10	4,431
Deletions	38	2487	
Unclear indels [¶]	84	40,885	
Tandem duplications	9	309	
Amplification of SSRs	19	611	

Transpositional landscape of the rice genome revealed by paired-end mapping of high-throughput re-sequencing data

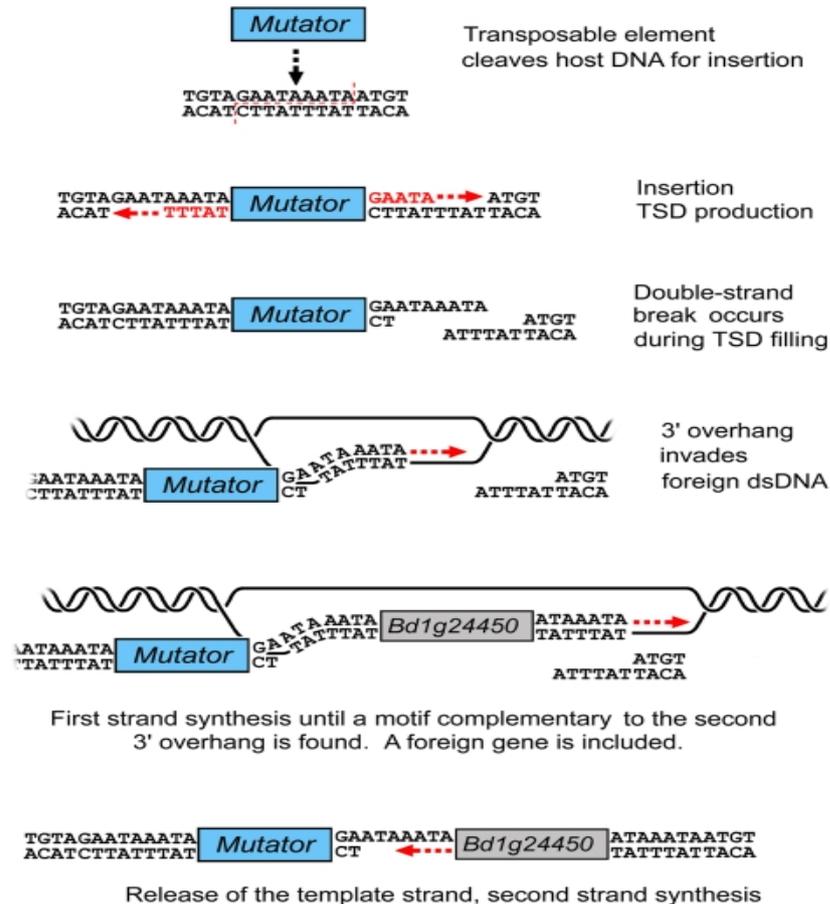
François Sabot^{1,†}, Nathalie Picault^{1,†}, Moaine El-Baidouri¹, Christel Llauro¹, Cristian Chaparro¹, Benoit Piegu¹, Anne Roulin¹, Emmanuel Guiderdoni², Mélissa Delabastide³, Richard McCombie³ and Olivier Panaud^{1,*}



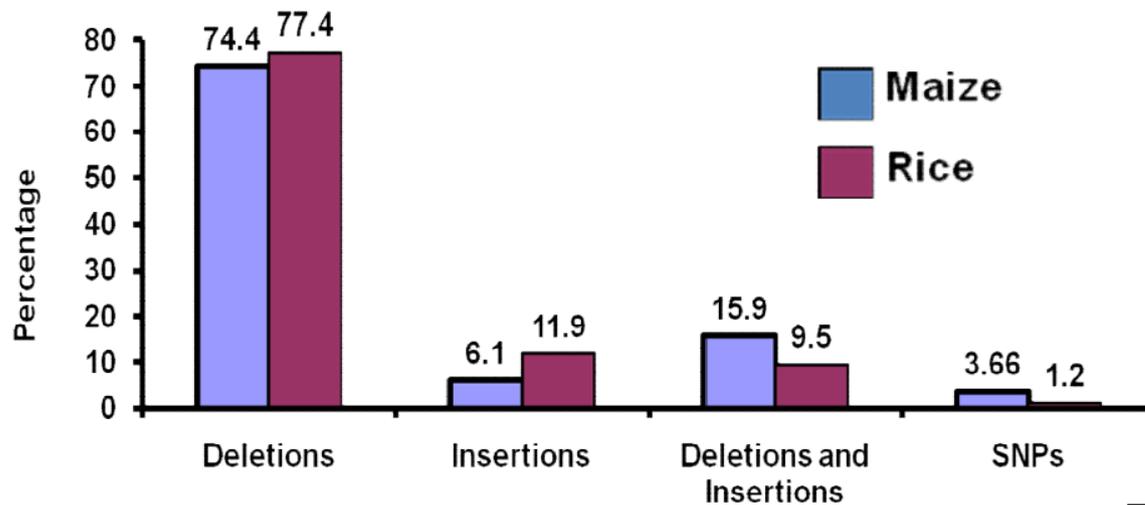
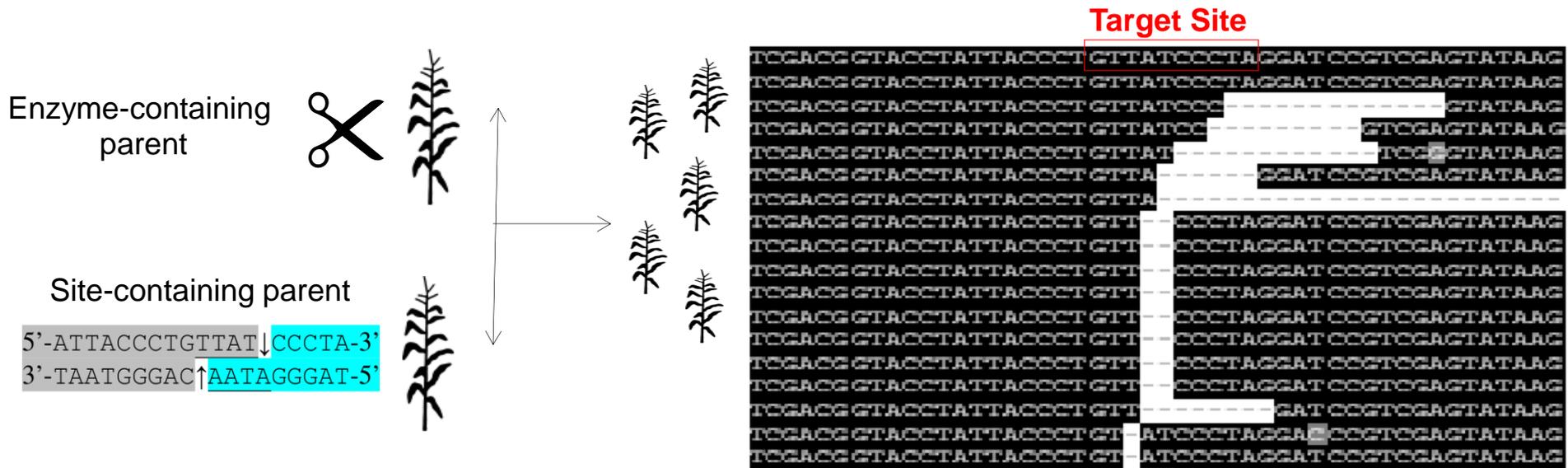
Patching gaps in plant genomes results in gene movement and erosion of colinearity

Thomas Wicker, Jan P. Buchmann and Beat Keller

Genome Res. 2010 20: 1229-1237 originally published online June 7, 2010

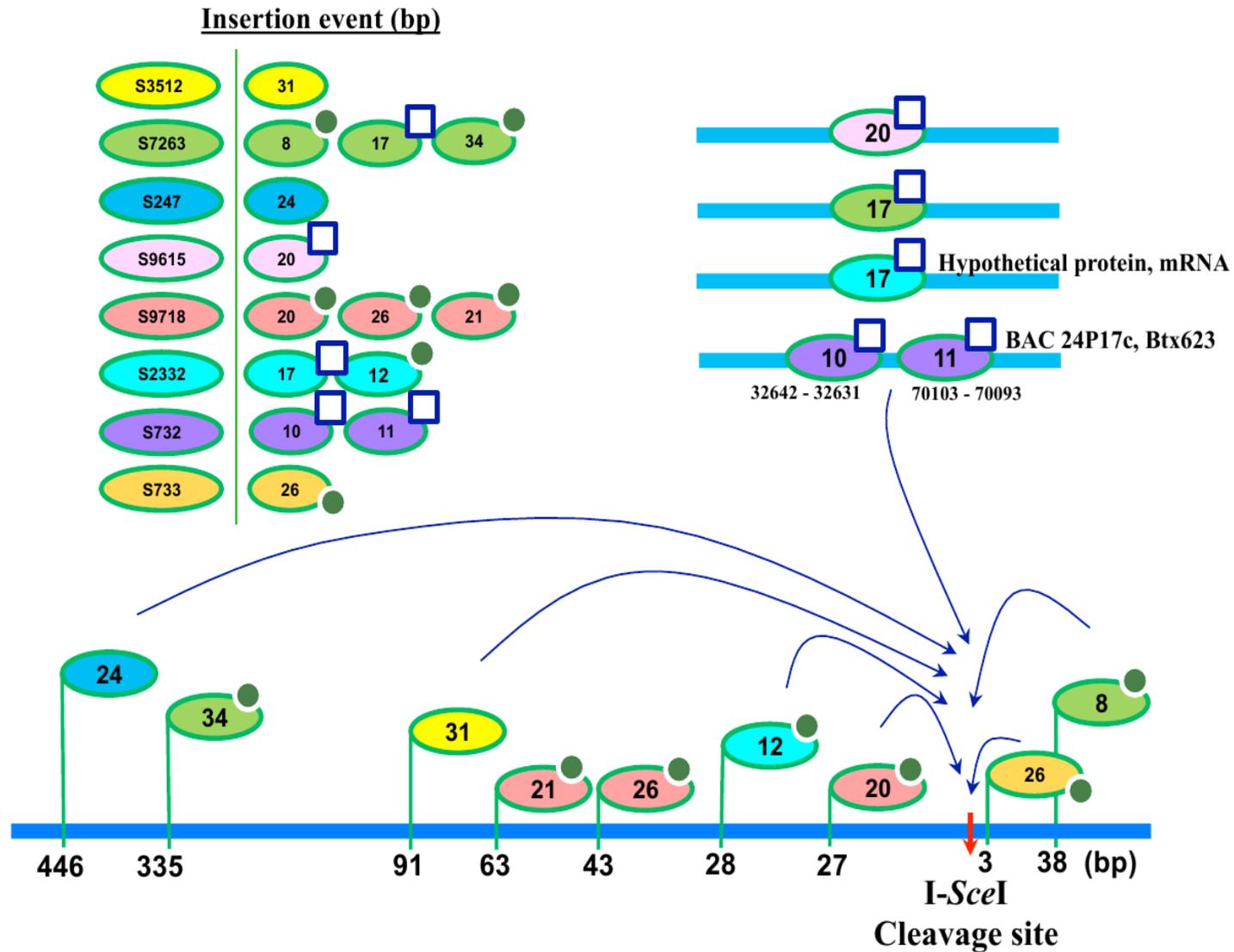


Scel homing endonuclease induces precise double strand breaks (DSB)



Fang Lu, unpublished

Induced double strand break repair creates complex insertion patterns very similar to those observed in natural populations



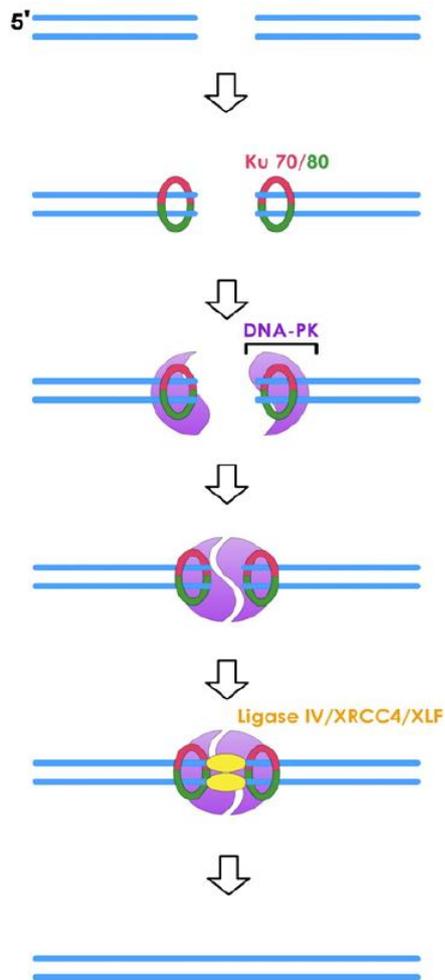
Double-strand breaks can create large insertions but do not appear to activate transposable elements

Line	Deletion (bp)	Length of filler (bp)	Origin of filler sequence
B9-86	1	90	T92 gene (auxin-binding prot.)
C15-36 ^a	0	200	sub-telomeric repeats
B9-44	2	330	low repetitive tobacco sequence
C19-1	11	359	high repetitive tobacco sequence
B9-82	1	582	polygalacturonase-like sequence
B9-1	4	795	retrotransposon Tnt1-like sequence

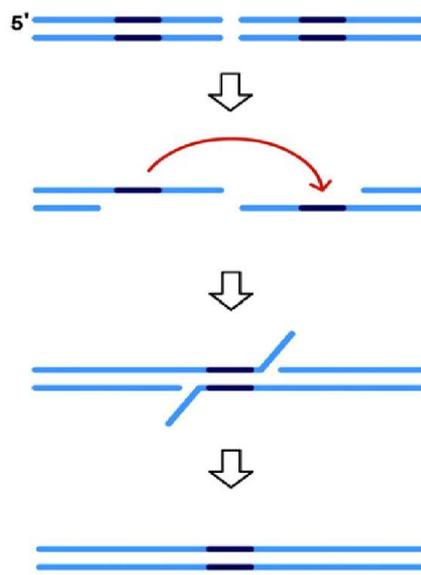
Salomon and Puchta, The EMBO Journal, 1998 (modified)

Double-strand break repair mechanisms

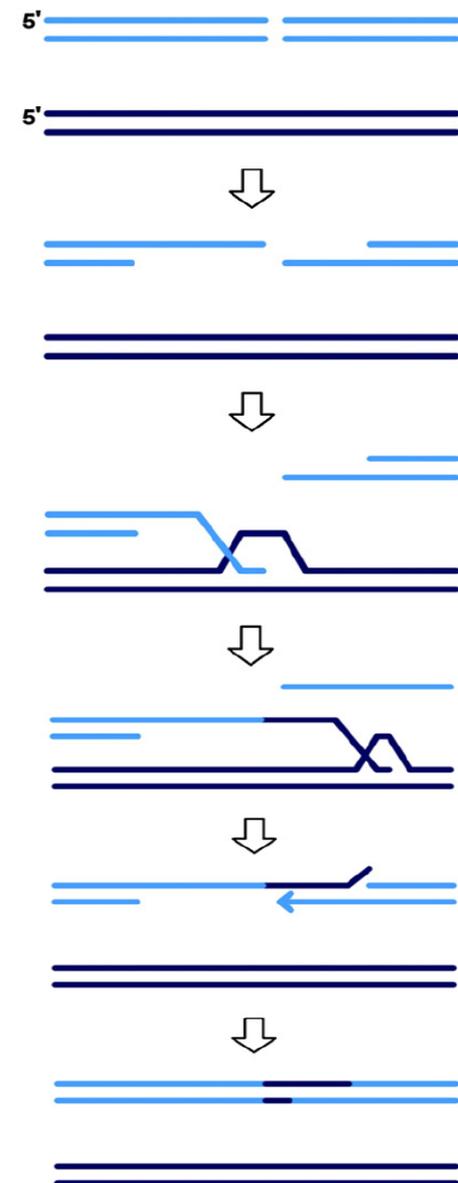
Nonhomologous end-joining (~65%)



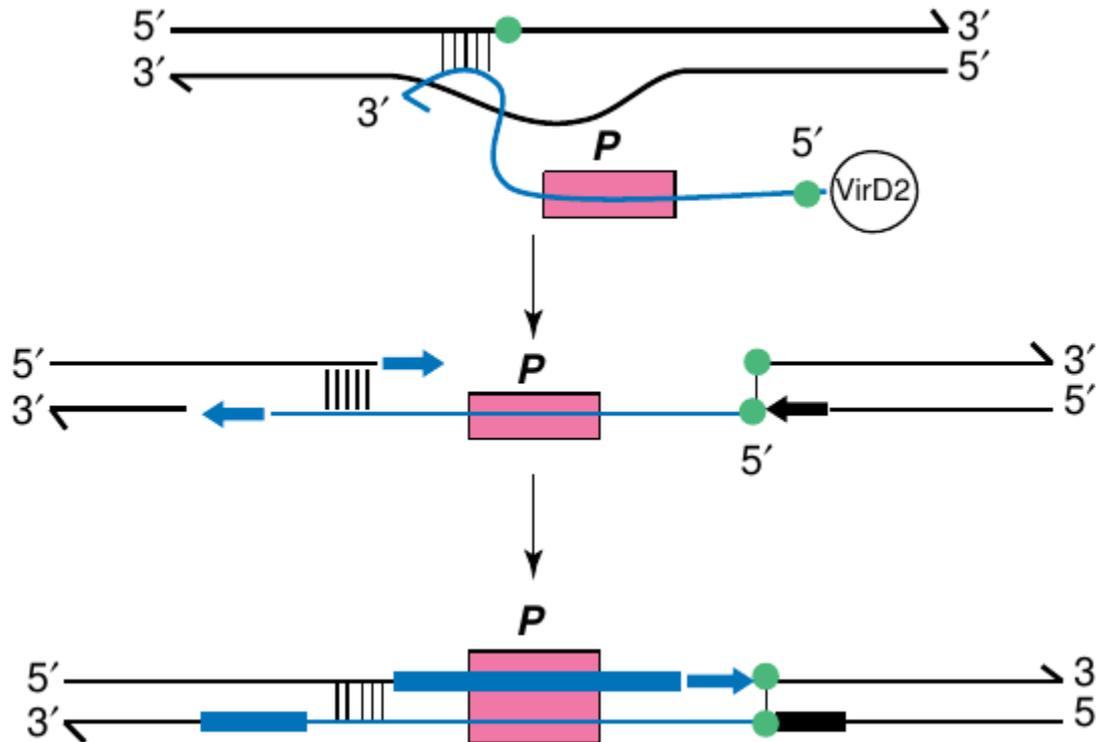
Single strand annealing (~30%)



Synthesis-dependant strand annealing (~5-0.001%)



T-DNA-induced rearrangement has a pattern of mutation similar to double strand break repair



Iida and Terada, Current Opinion in Biotechnology, 2004

General observations

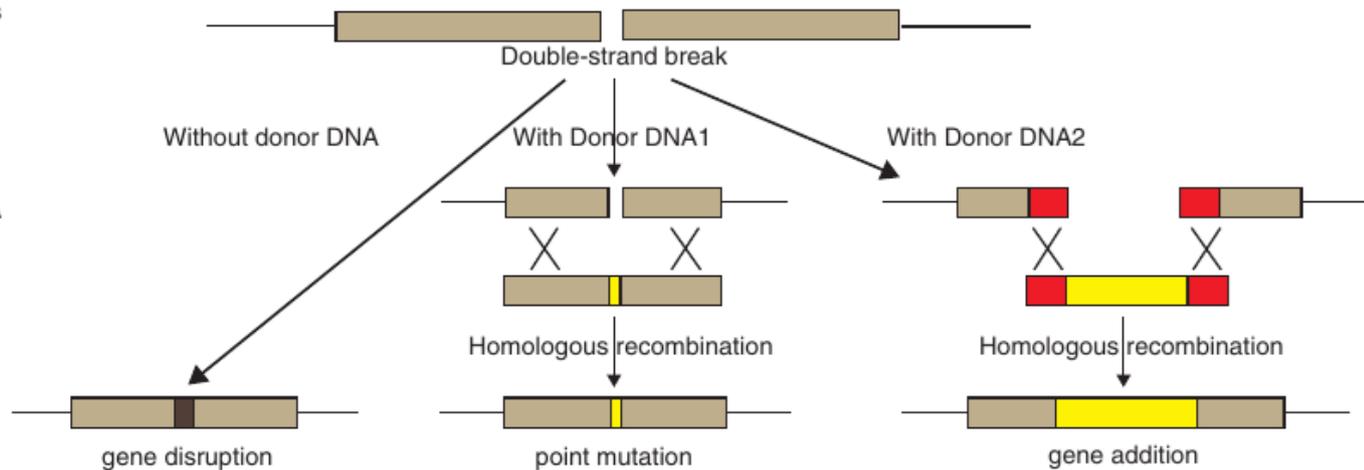
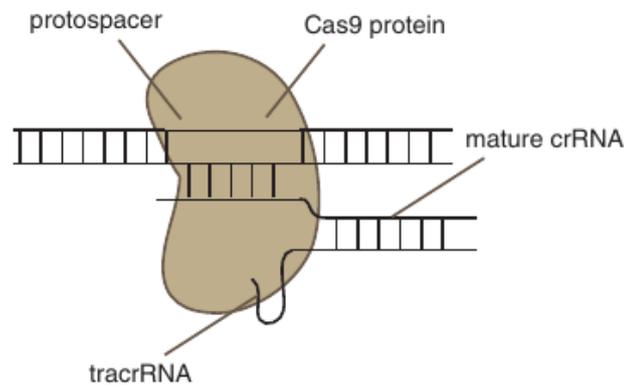
Structural rearrangements are not unique to transgenic plants

Double strand breaks and transposon activity result in structural variation in essentially every new plant variety

T-DNA-induced rearrangement has a pattern of mutation similar to double strand break repair though it is likely to be less disruptive than transposon movement

A new generation of transgenics

Clustered regularly interspaced short palindromic repeats
(CRISPR)/CRISPR-associated(Cas) system



Thanks



“Keep crops the way God intended”



The way man intended



“The way Monsanto intended”





A Lonely Quest for Facts on Genetically Modified Crops

By AMY HARMON JAN. 4, 2014



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SAVE



MORE

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Grand Budapest
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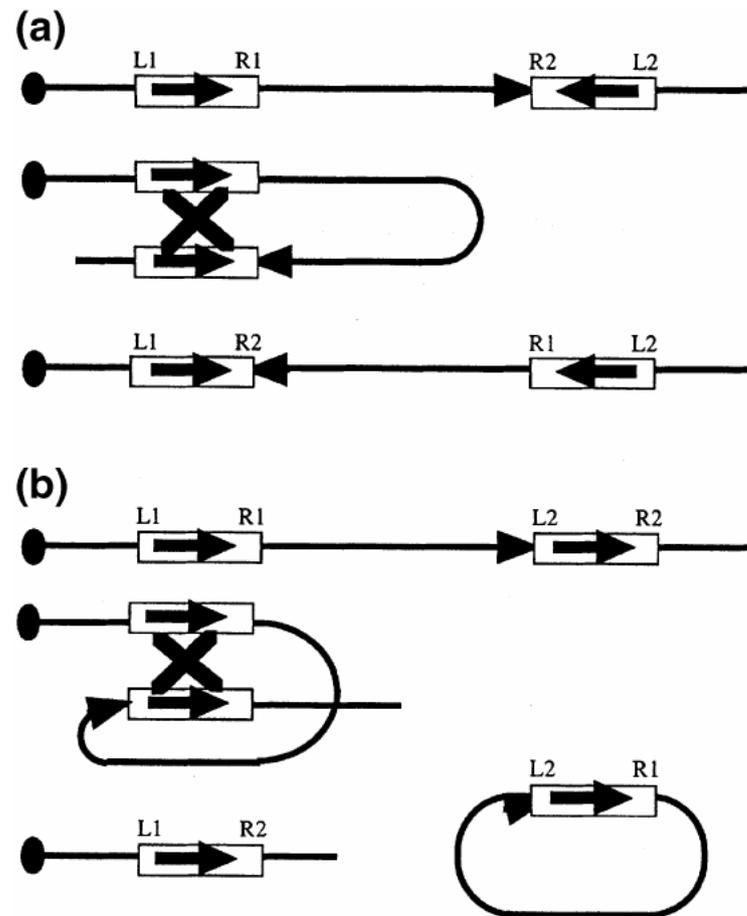
KONA, Hawaii — From the moment the bill to ban [genetically engineered crops](#) on the island of Hawaii was introduced in May 2013, it garnered more vocal support than any the County Council here had ever considered, even the perennially popular bids to decriminalize marijuana.

Public hearings were dominated by recitations of the ills often attributed to genetically modified organisms, or G.M.O.s: cancer in rats, a rise in childhood allergies, out-of-control superweeds, genetic contamination, overuse of [pesticides](#), the disappearance of butterflies and bees.



A chromosomal paracentric inversion associated with T-DNA integration in *Arabidopsis*

Patrick Laufs^{*,†}, Daphne Autran and Jan Traas



T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum

1.4 inserts per
transformant

Kenneth A. Feldmann*

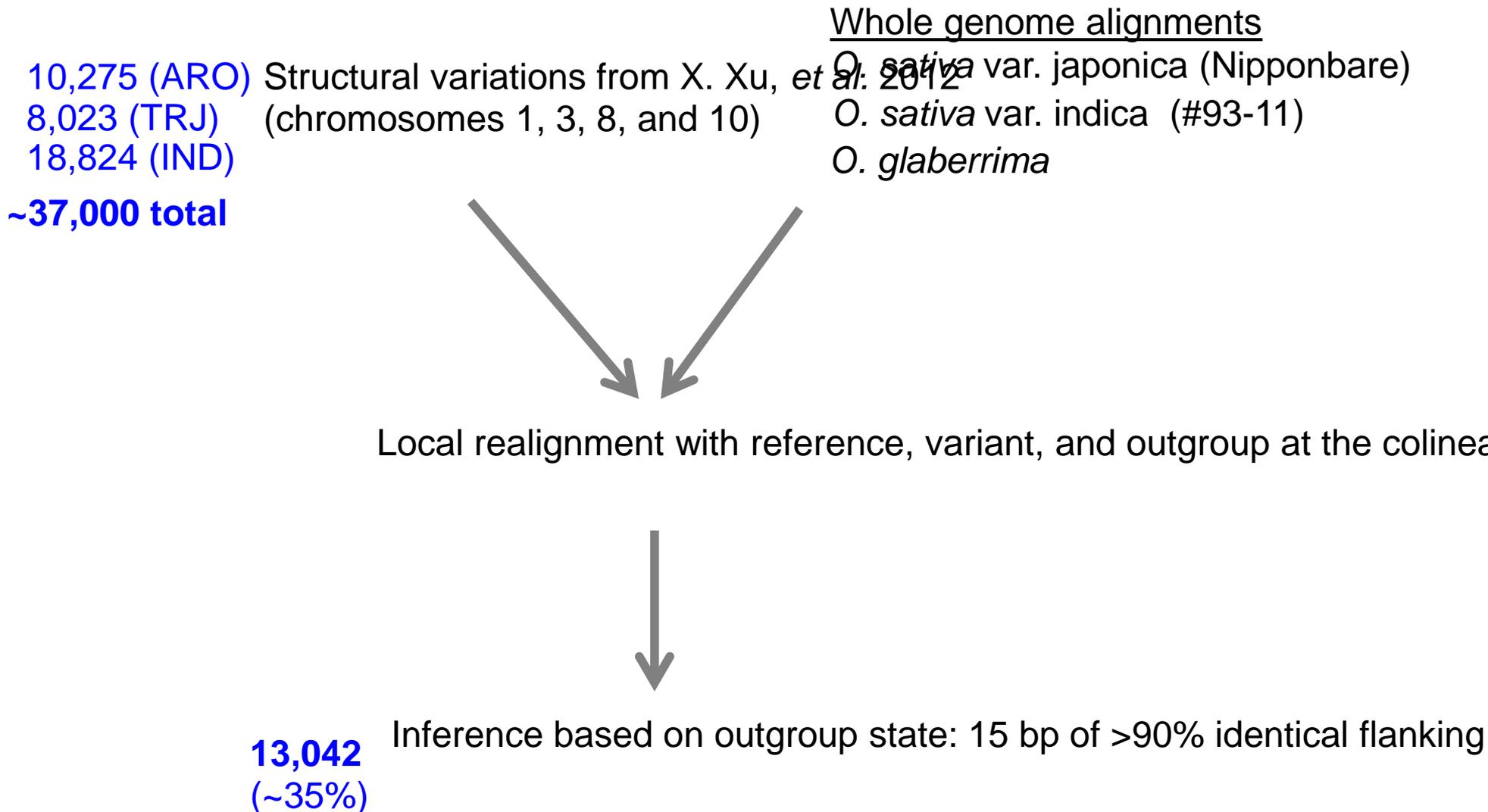
The Du Pont Company, Wilmington, DE 19880, and

†Department of Plant Sciences, The University of Arizona,
Tucson, AZ 85721, USA

Table 3. Frequency of various phenotypic classes observed from the screen of 8000 transformants of *Arabidopsis*

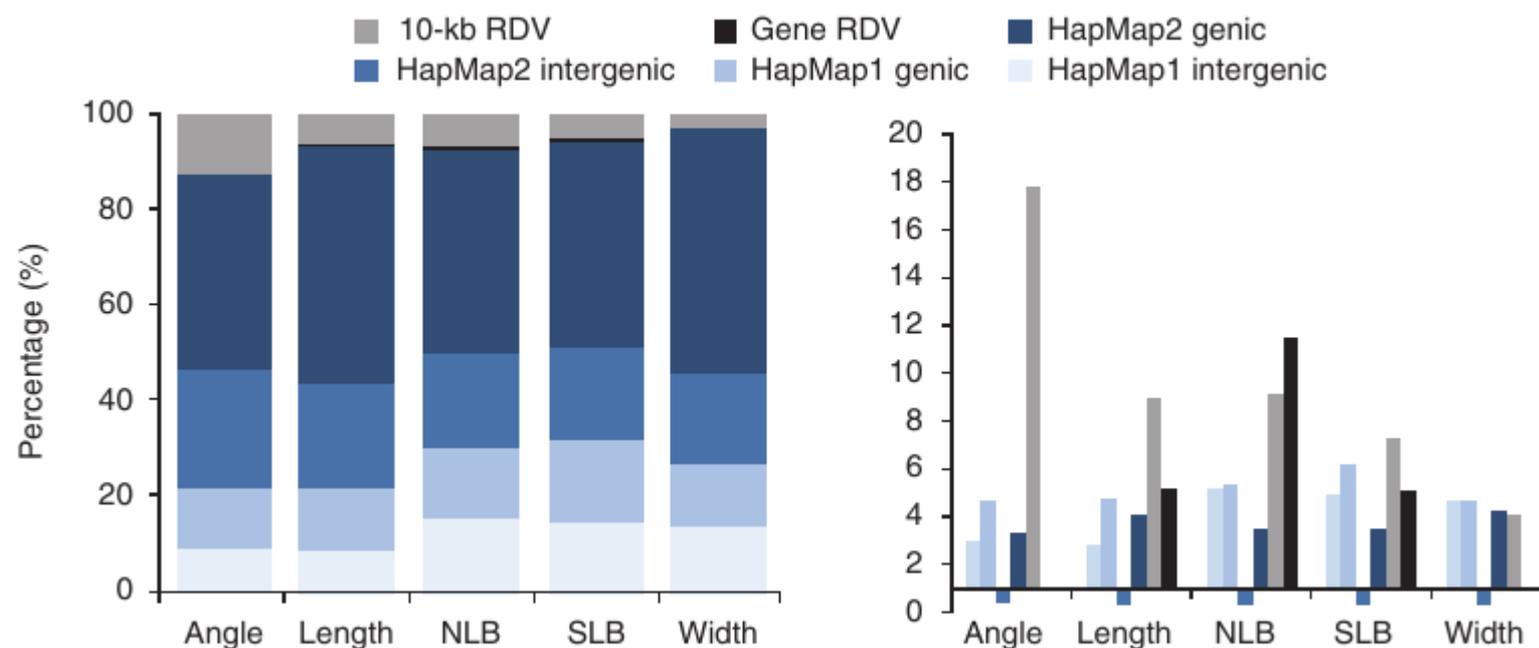
Phenotype	Percentage
Seedling-lethal	3–5 ^a
Size variant	3–5 ^b
Embryo-defective	2.5–3.5
Reduced-fertility	1–2
steriles	0.2
Pigment mutants	2–3 ^c
<i>fusca</i>	0.1
albino	1.2
yellow–green	1.3
dark green	0.2
Dramatic mutants	2.5–3
flower	0.5–0.6
root	0.1–0.3 ^d
root hair	0.35
trichomes	0.25
dwarfs	0.15
Physiological mutants	1–?
flowering time	0.5
<i>eceriferum</i>	0.1
high florescence	0.1
Total	15–26

Inference of insertions based on *Oryza glaberrima* genome



Maize HapMap2 identifies extant variation from a genome in flux

Jer-Ming Chia^{1,24}, Chi Song^{2,24}, Peter J Bradbury^{3,4}, Denise Costich^{3,4}, Natalia de Leon^{5,6}, John Doebley⁷,

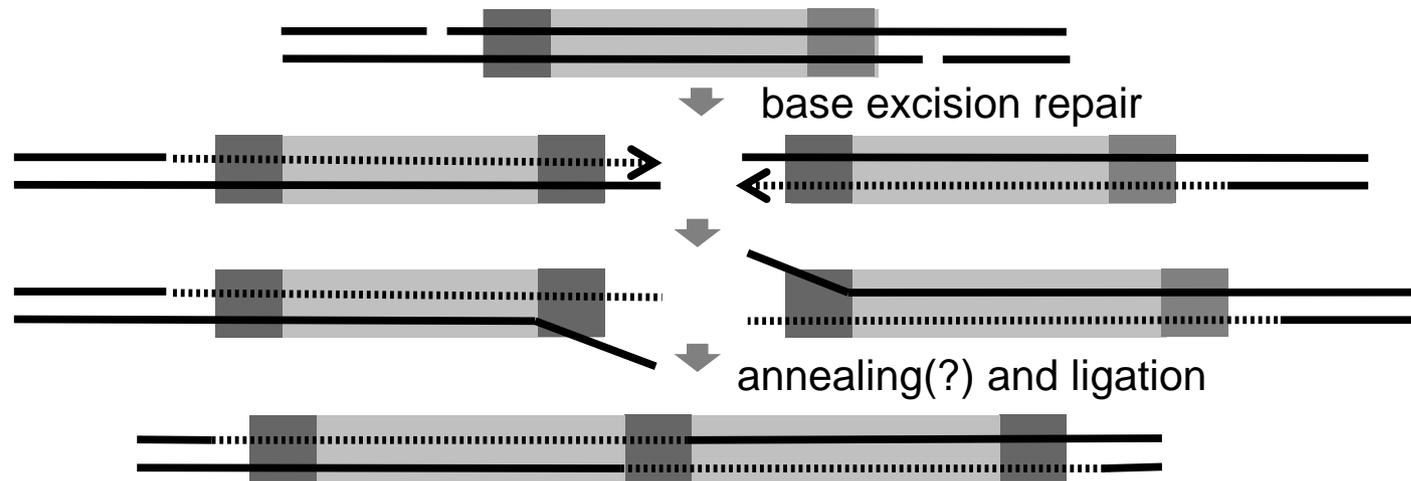


~20% of transgenic plants will have an unintended effect

Inversely proportional to genome size

but represents a lower limit

Patch-mediated model for the origin of tandem duplications



THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 275, No. 35, Issue of September 1, pp. 27386–27392, 2000
Printed in U.S.A.

DNA Repair Patch-mediated Double Strand DNA Break Formation in Human Cells*

Received for publication, April 12, 2000, and in revised form, May 19, 2000
Published, JBC Papers in Press, May 25, 2000, DOI 10.1074/jbc.M003126200

Stéphane Vispé and Masahiko S. Satoh‡

From the DNA Repair Group, Health and Environment Unit, Laval University Medical Research Center, CHUQ, Faculty of Medicine, Laval University, 2705 Boulevard Laurier, Sainte-Foy, Quebec G1V 4G2, Canada

Jorge Castro 20.1k 59 266 476
Alex 411 3 12 28

Welcome to Ask Ubuntu! Please define your question. For more details on best practices consider reading the [FAQ on asking questions](#). Regards - [Ringtail](#) Mar 30 '12 at 22:24

what do i need to add? - [Alex](#) Mar 30 '12 at 22:25

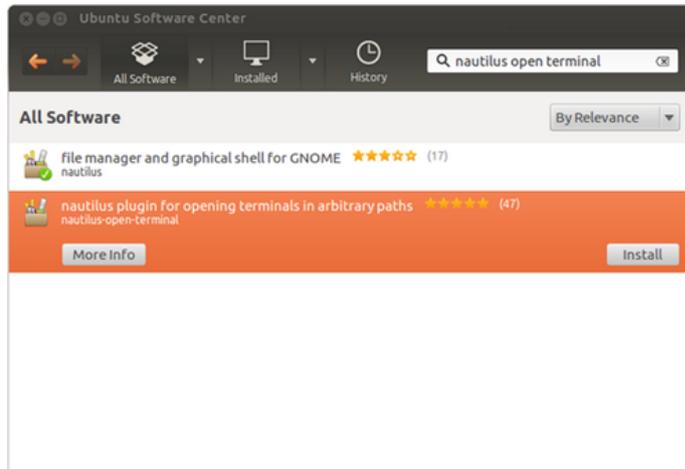
add comment

1 Answer

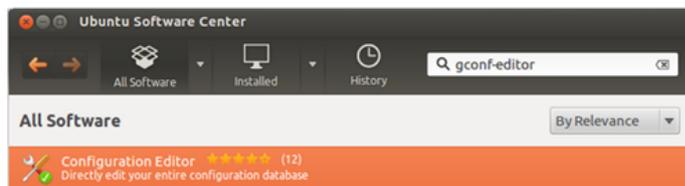
ACTIVE OLDEST VOTES

You first need to install the package `nautilus-open-terminal` from Software Center or Synaptic Package Manager

20



Then you need to install `gconf-editor` similarly from Software Center or Synaptic Package Manager



asked 1 year ago
viewed 13143 times
active 10 months ago



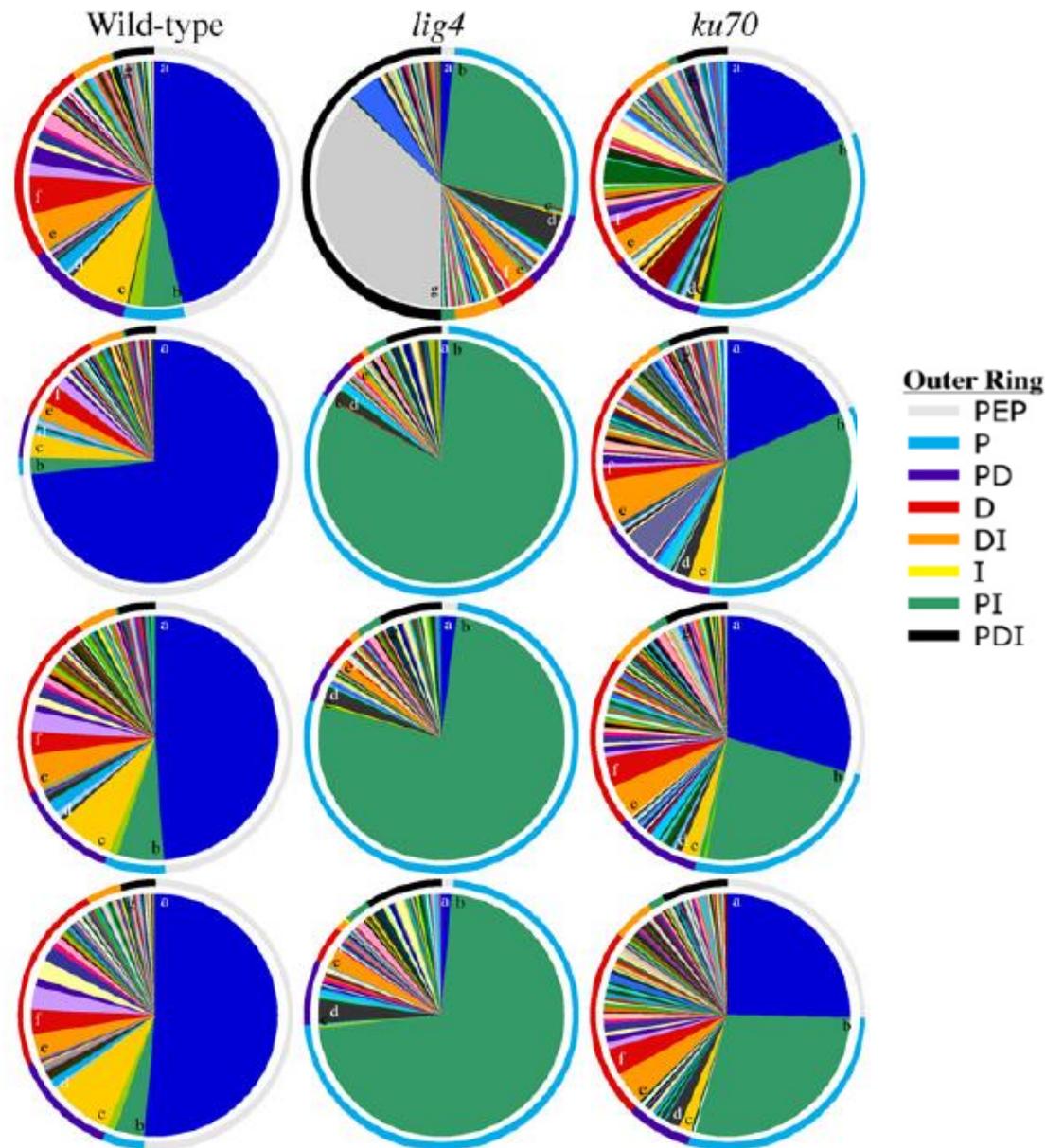
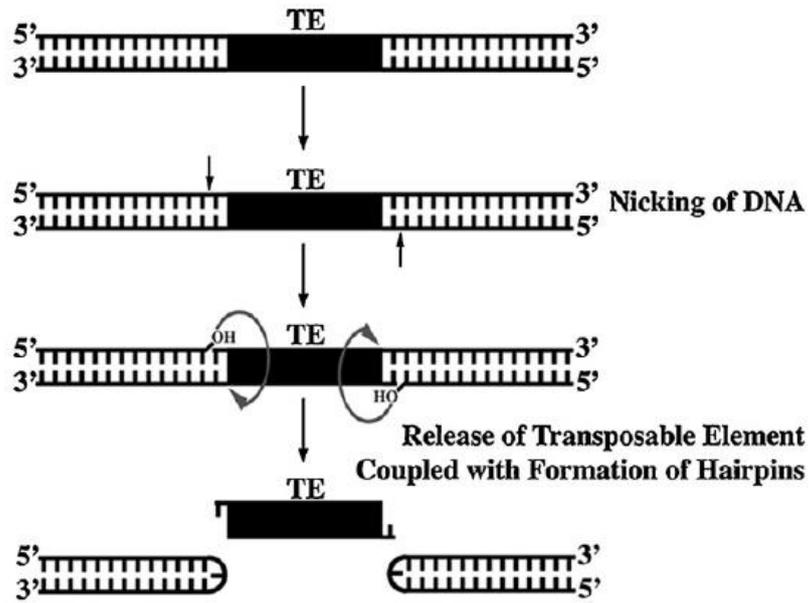
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Hot Network Questions

- Produce the number 2014 without any numbers in your source code

Deep sequencing DSB repair sites



Do structural variants explain a disproportionate amount of phenotypic variation?

There is theoretical justification for the hypothesis that even a small structural variant has a