

Replication of *ToxB* in Tan Spot of Wheat

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Tan spot of wheat

- *Pyrenophora tritici-repentis* (Ptr) is a fungal pathogen
- Foliar disease of wheat (sometimes other cereals)
- Worldwide occurrence: ~5 to 15% global losses¹
- Three primary effectors: ToxA, ToxB/toxb, ToxC
 - inactive (pointing to ToxB/toxb)
 - multi-copy (pointing to ToxB/toxb)
- Little work done on ToxB
- *ToxB* isolates primarily found in wheat center of origin²
- Homologs found in other species³

<i>ToxA</i>	✦ <i>ToxB</i> ✦	<i>ToxC</i>
Necrosis	Chlorosis B	Chlorosis C
		

Aboukhaddour Lab

¹Savary et al., 2019. *Nat Ecol Evol* 3(3):430–9; ²Kamel et al., 2019. *Front Plant Sci* 10: 1562; ³Hafez et al., 2023. *MPMI*: ja

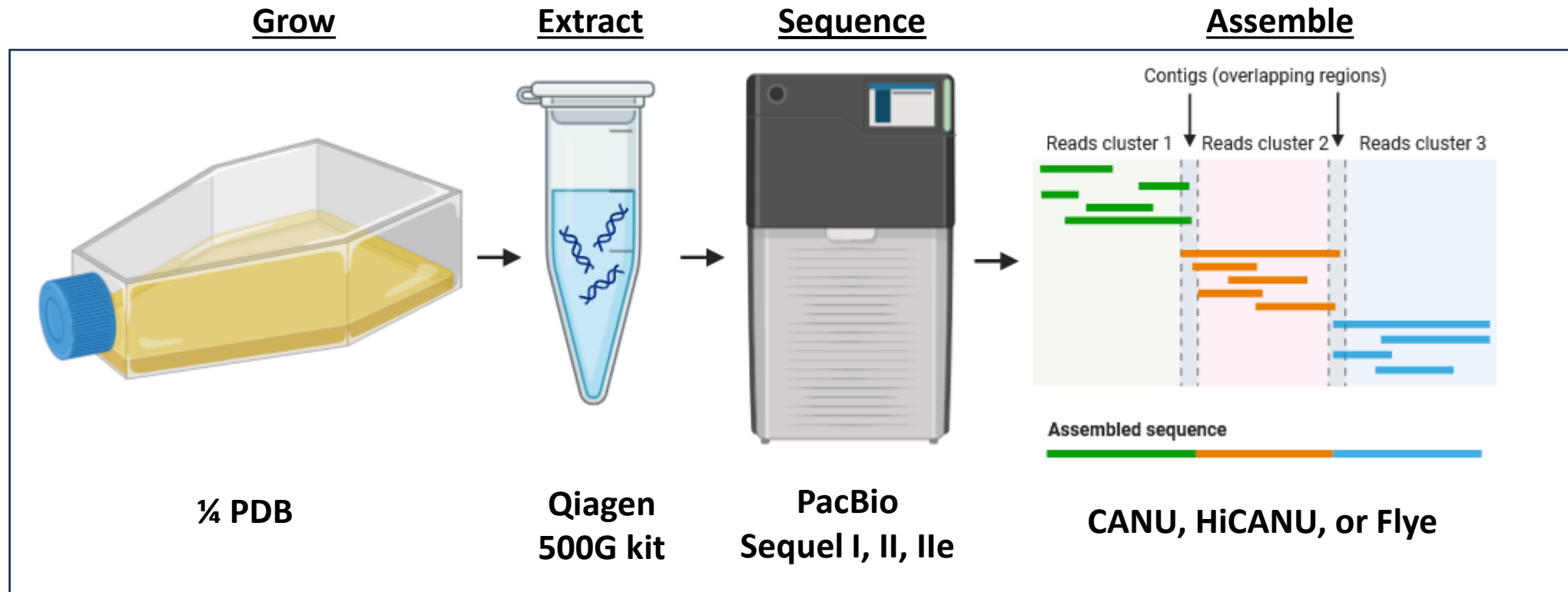
Why explore *ToxB* replication?

- Copy number variation is a driving force of evolution¹
 - *ToxB* copy number linked to increased symptoms^{2,3}
- A large portion of Canadian germplasm susceptible to *ToxB* (~30%)⁴
- Excess copies of *ToxB* may evolve into new effectors in the future
- Is *ToxB* mobile?
 - Is the mechanism associated with horizontal transfer?
i.e. could *ToxB* be passed to other pathogens?



¹Lauer et al., 2019. *Curr Genet* 65(2):1287-1295; ²Strelkov et al., 2002. *Can J Plant Pathol* 24:29–35; ³Martinez et al., 2004. *MPMI* 17:467–474; ⁴Tran et al., 2017. *Can J Plant Pathol* 39:149–162

Workflow



Aligners

geneious

CIRCOS

mauve
Multiple Genome Alignment

MAFFT

Databases

giri
REPBASE

UniProt

NCBI



Common mechanisms of duplication

✦ Unequal crossing over ✦

- Template-switching
- Break-induced replication (BIR)
- Breakage-fusion-bridge cycles
- Transposons

- Retrotransposon

✦ DNA transposon ✦

- Subclass I
- Subclass II
- Composite transposon

-Many unique features
-Several overlapping features

Recommended Reviews

Mechanisms of Gene Duplication and Amplification

Cold Spring Harb Perspect Biol 2015;7:a016592
Andrew B. Reams¹ and John R. Roth²

Mechanisms of change in gene copy number

[P. J. Hastings](#) ✉, [James R. Lupski](#), [Susan M. Rosenberg](#) & [Grzegorz Ira](#)

[Nature Reviews Genetics](#) **10**, 551–564 (2009) | [Cite this article](#)

Transposable elements a.k.a transposons

- Mobile genetic sequences
- Own evolutionary units
- Confer advantages, disease, or inviability
 - antibiotic resistance - bacteria
 - porphyria - humans
 - spore killer (*Spok*)- fungi



Barbara McClintock; Nobel Prize 1983

Recommended Reviews

A unified classification system for eukaryotic transposable elements

Thomas Wicker, François Sabot, Aurélie Hua-Van, Jeffrey L. Bennetzen, Pierre Capy, Boulos Chalhoub, Andrew Flavell, Philippe Leroy, Michele Morgante, Olivier Panaud, Etienne Paux, Phillip SanMiguel and Alan H. Schulman

[Nature Reviews Genetics](#) **8**, 973–982 (2007)

Annual Review of Genetics

A Field Guide to Eukaryotic Transposable Elements

Jonathan N. Wells and Cédric Feschotte

Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14850; email: jnw72@cornell.edu, cf458@cornell.edu

Annu. Rev. Genet. 2020. 54:539–61

Genomes

Red = active *ToxB*
 Blue = inactive *tox**b*

7 multi-copy isolates

10 single copy

7 zero copy

Total 24 genomes

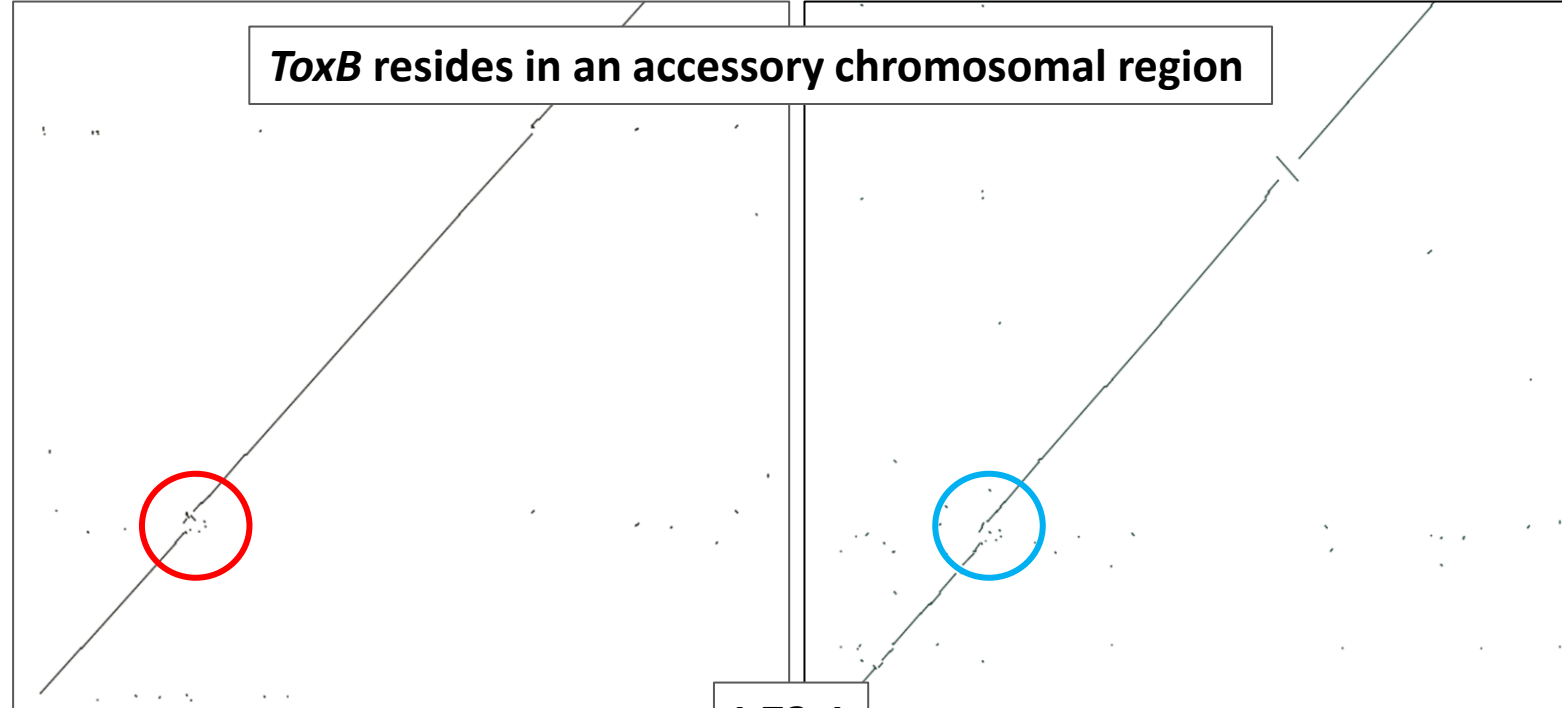
Isolate	ToxB copies	Assembler	Size (Mb)	Contigs		N50 (Kbp)
				Total	>1Mb	
Alg3-24	≥5	Hi-Canu	42.4	119	11	3,402
AlgH1	5	Hi-Canu	42.5	103	12	3,698
I-73-1	4	Flye+Pilon	39.9	39	11	3,647
T103-1	4	CANU	39.3	236	5	570
T128-1	4	CANU	39.8	553	11	3,243
I-34-1	3	Hi-Canu	43.3	150	12	3,253
92-171	2	Hi-Canu	44.7	36	14	2,880
107224	1	Hi-Canu	37.5	35	18	2,075
331-2	1	Hi-Canu	43.7	123	12	3,470
90-2	1	CANU	36.2	310	12	2,960
Alg4x-1	1	CANU	53.9	1,024	0	156
D308	1	Flye+Pilon	39.6	70	11	3,667
SC22-2	1	Hi-Canu	44.6	160	12	3,161
SC29-1	1	CANU	41.5	490	6	468
SC29-8	1	Hi-Canu	43.2	175	13	2,933
SW21-5	1	Hi-Canu	42.1	96	13	2,768
Tptr3-1	1	Hi-Canu	42.8	126	13	3,604
Den17	0	Hi-Canu	47.2	97	12	3,465
G9-7	0	Hi-Canu	43.3	135	11	3,552
I-33-16	0	Hi-Canu	41.3	75	13	2,705
K11	0	Hi-Canu	41.2	150	12	3,271
K6	0	Hi-Canu	40.0	135	11	3,071
K9	0	Hi-Canu	40.5	130	12	2,812
T126-1	0	Hi-Canu	42.5	175	12	3,241



North America
North Africa
Europe
Japan
Fertile Crescent

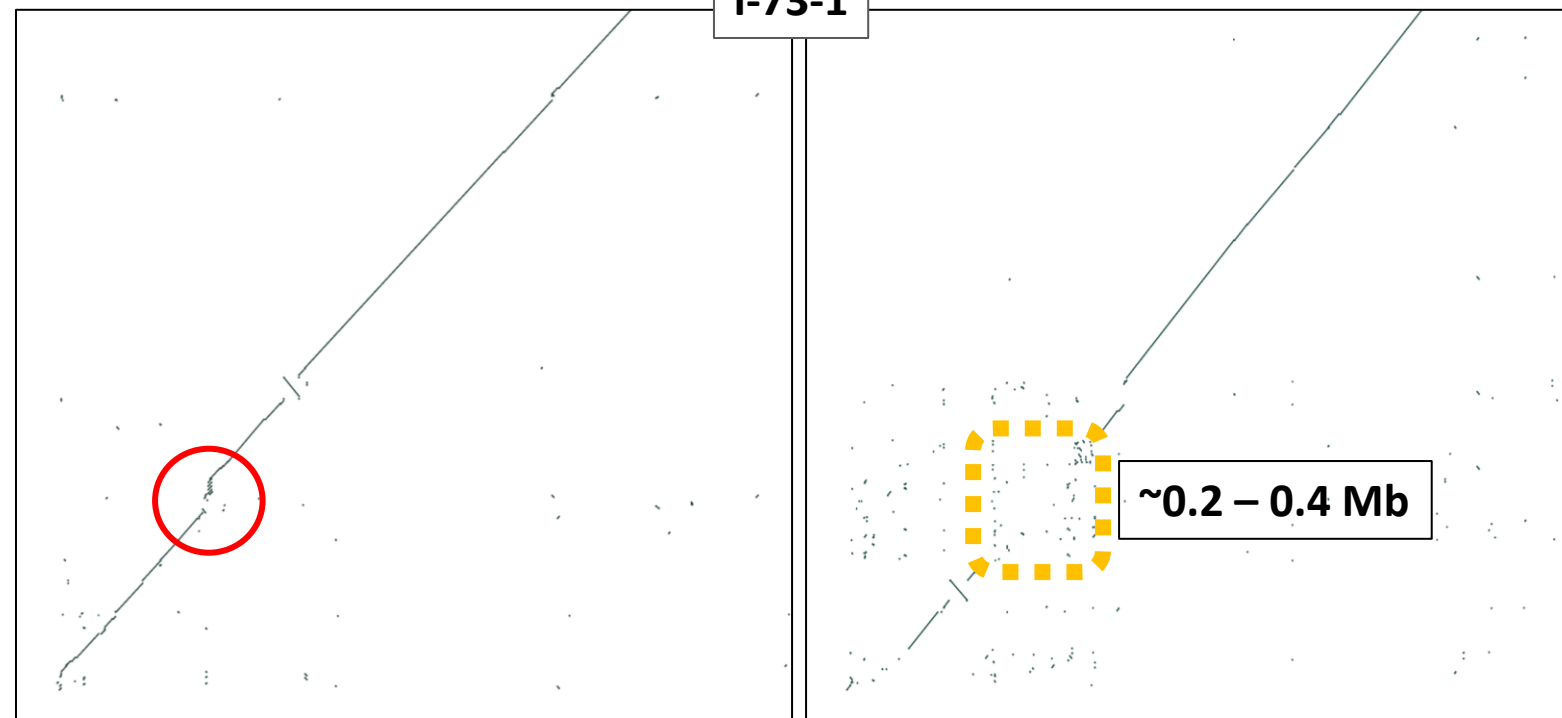
Red = active *ToxB*
Blue = inactive *tox**b*

Single vs Multi
Tpnr3-1



ToxB* vs *tox**b
D308

Multi vs Multi
AlgH1

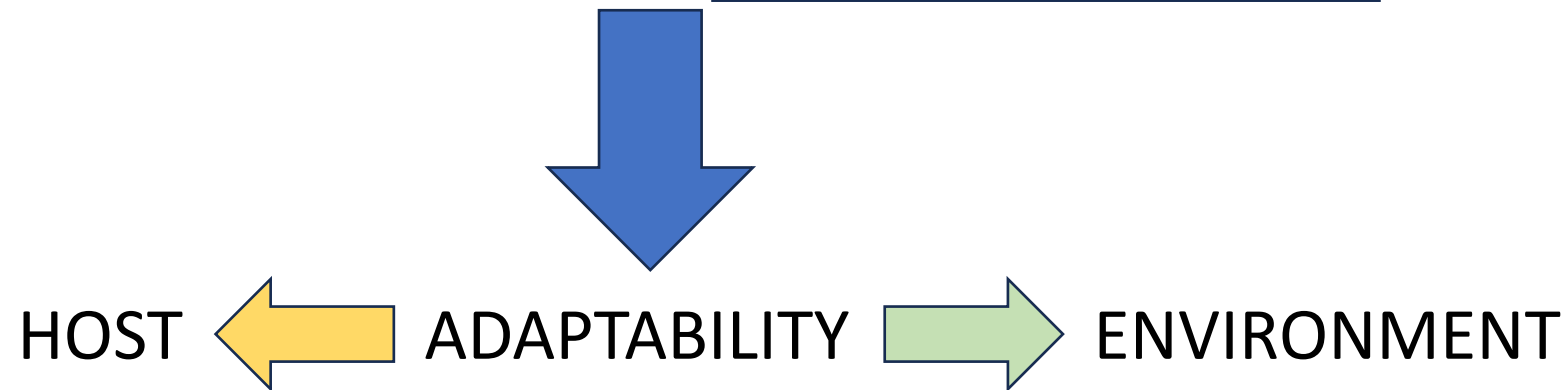


***ToxB* vs none**
K11

~0.2 – 0.4 Mb

What are accessory regions?

- Whole chromosomes or sections of chromosomes
(e.g. mini-chromosomes, plasmids, chromosome arms, etc.)
- Not necessary to maintain base metabolism or reproduction
- Often contain **virulence factors** and **secondary metabolite** synthesis clusters



MPMI Vol. 31, No. 8, 2018, pp. 779–788. <https://doi.org/10.1094/MPMI-06-17-0135-F1>

CURRENT REVIEW

Accessories Make the Outfit: Accessory Chromosomes and Other Dispensable DNA Regions in Plant-Pathogenic Fungi

Stefania Bertazzoni,¹ Angela H. Williams,¹ Darcy A. Jones,¹ Robert A. Syme,¹ Kar-Chun Tan,¹ and James K. Hane^{1,2,†}

Variability of chromosome structure in pathogenic fungi — of ‘ends and odds’

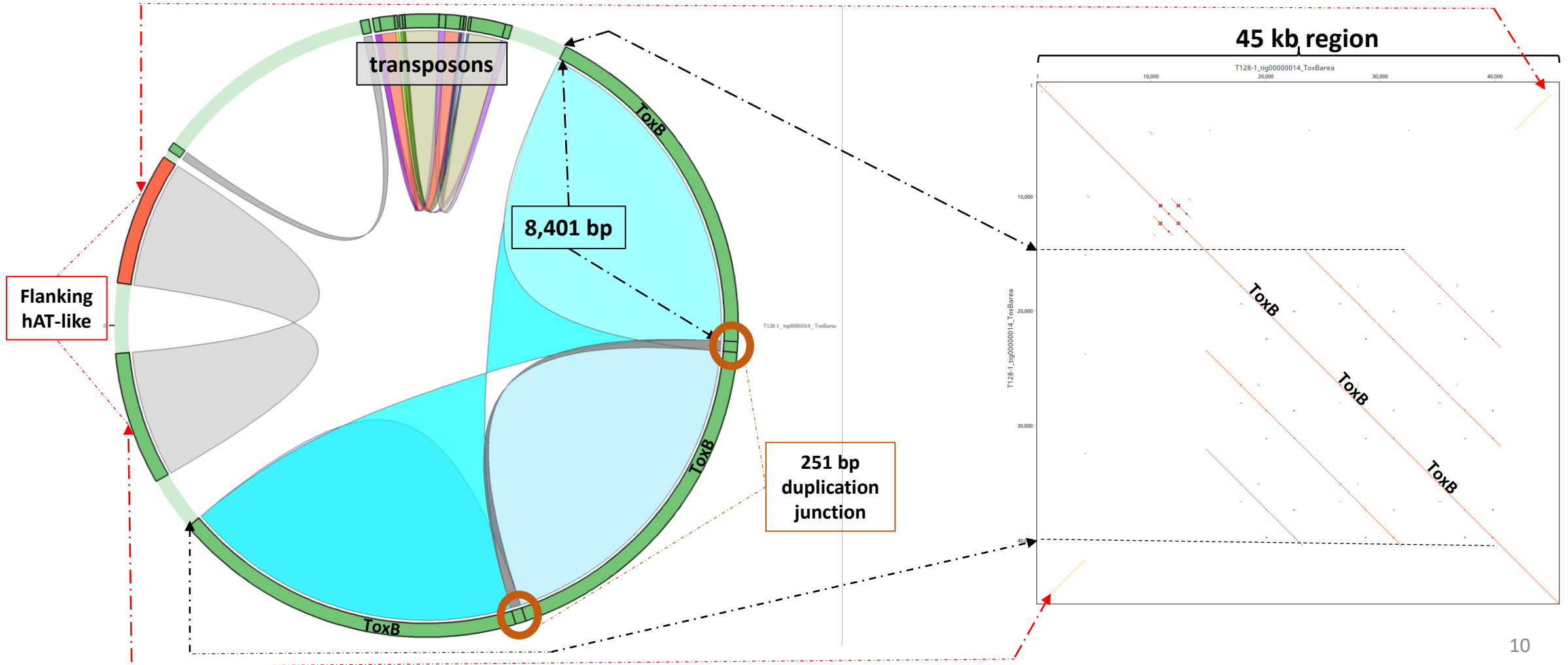
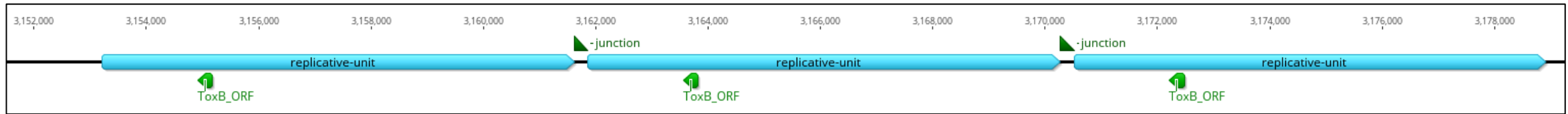
Jonathan M Galazka and Michael Freitag

Current Opinion in Microbiology

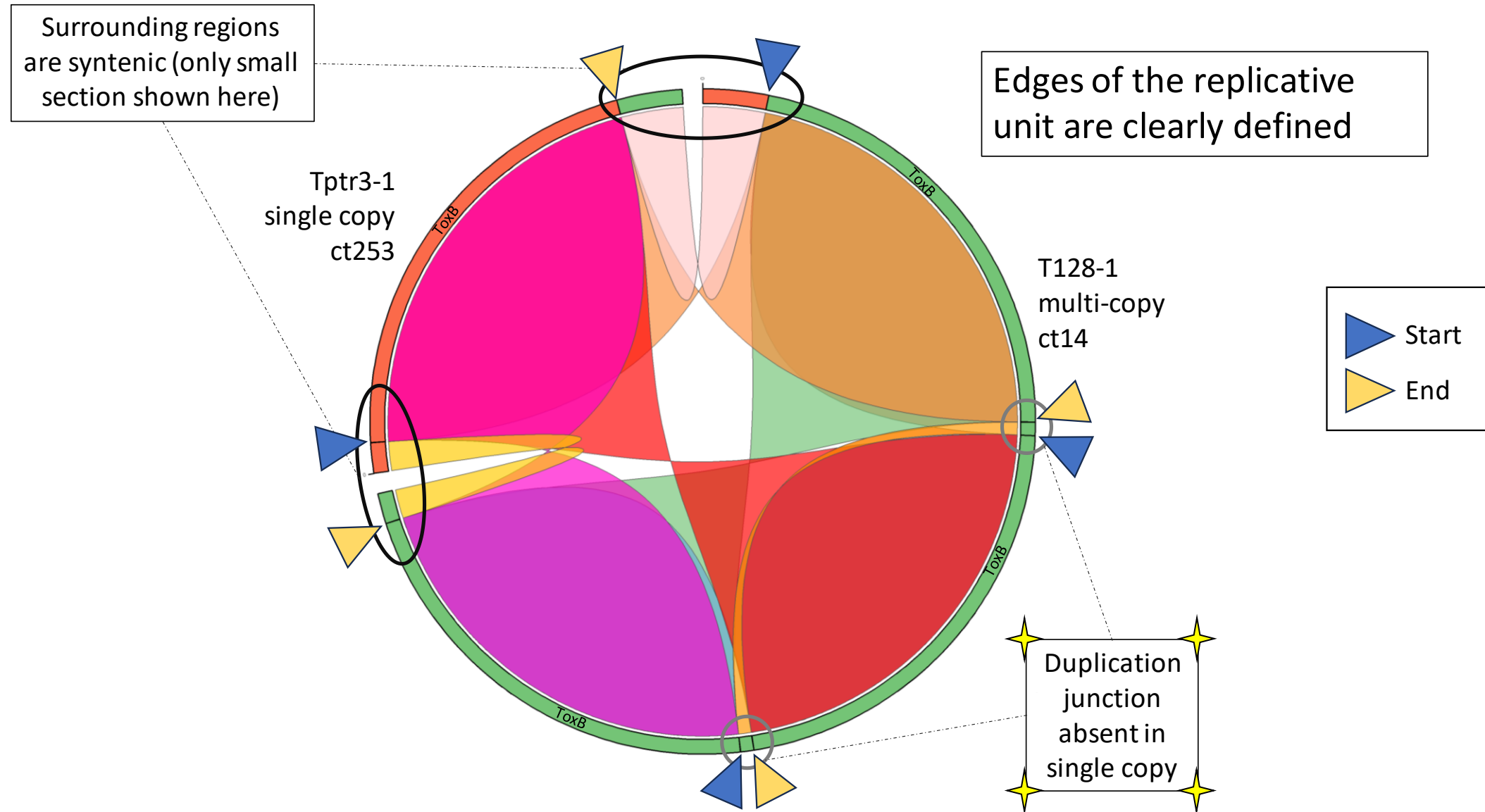
Volume 20, August 2014, Pages 19-26

Recommended Reviews

Self-alignment of *ToxB* region

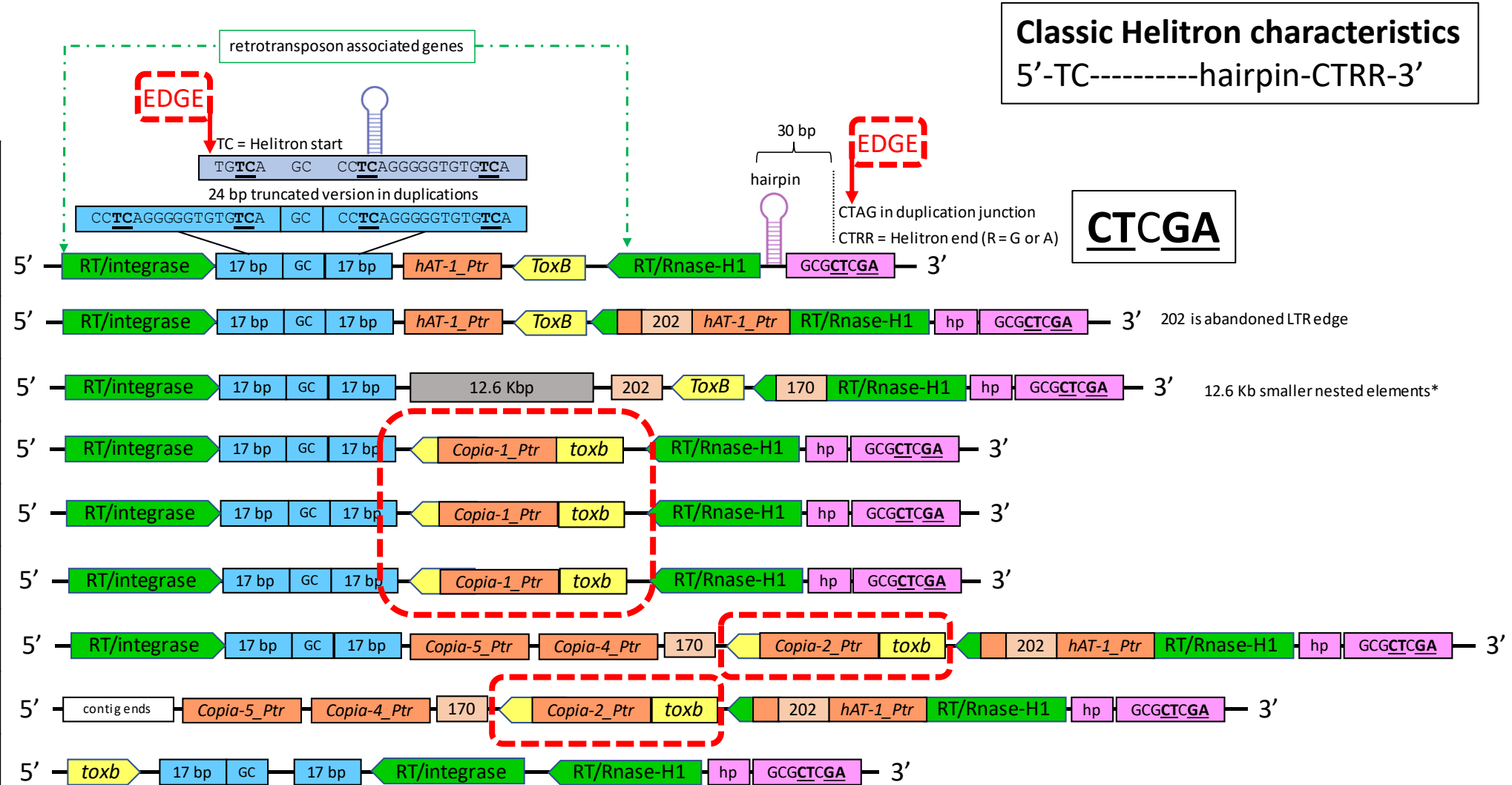


Single copy vs multi-copy

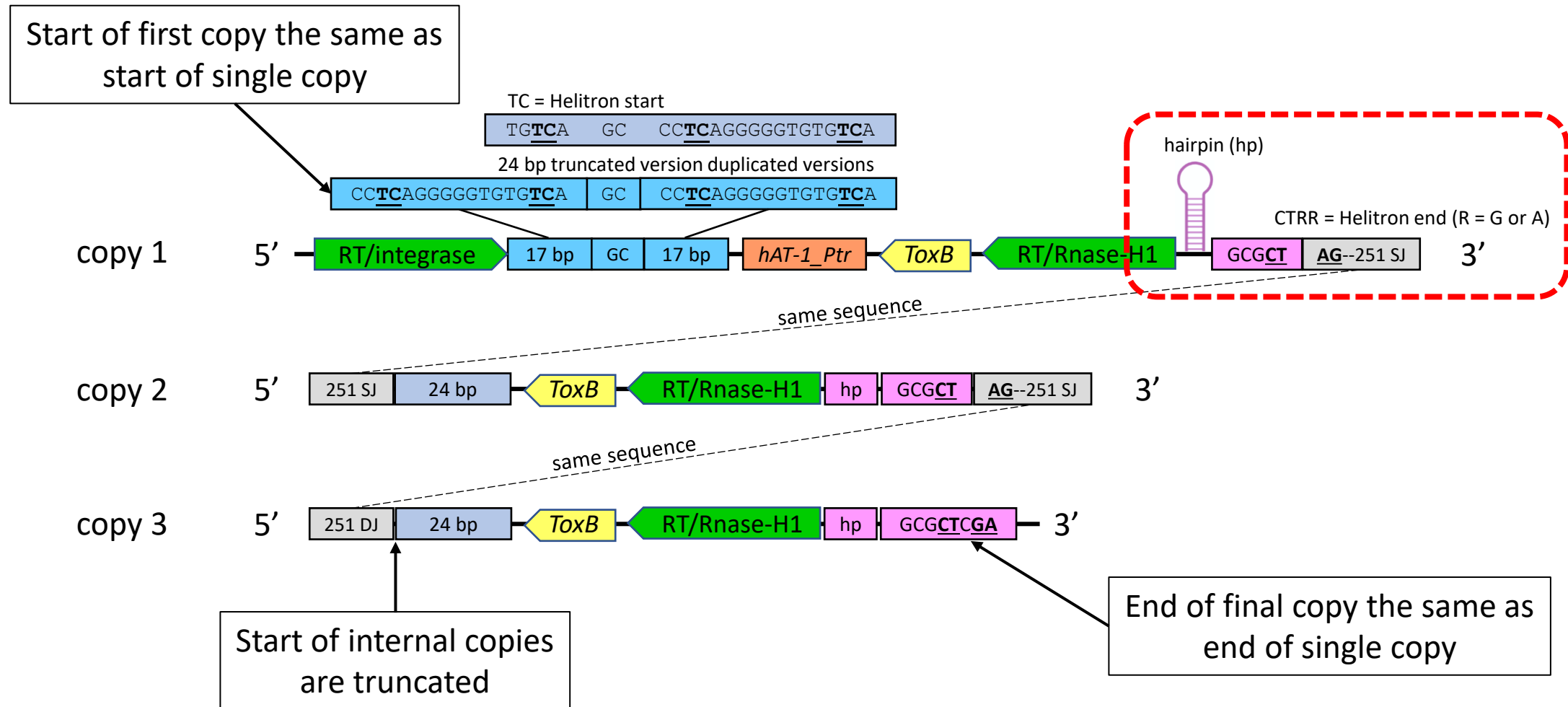


Anatomy of the *ToxB/toxb* replicative unit – single copies

Isolate	contig	~length (bp)
Tptr3-1	253	8,404
Alg4x-1	296	15,158
L1-2	137	18,761
D308	5	11,459
SC22-2	160	11,559
SW21-5	39	11,459
SC29-8	184	24,701
SC29-1	457	18,831
90-2	344	-

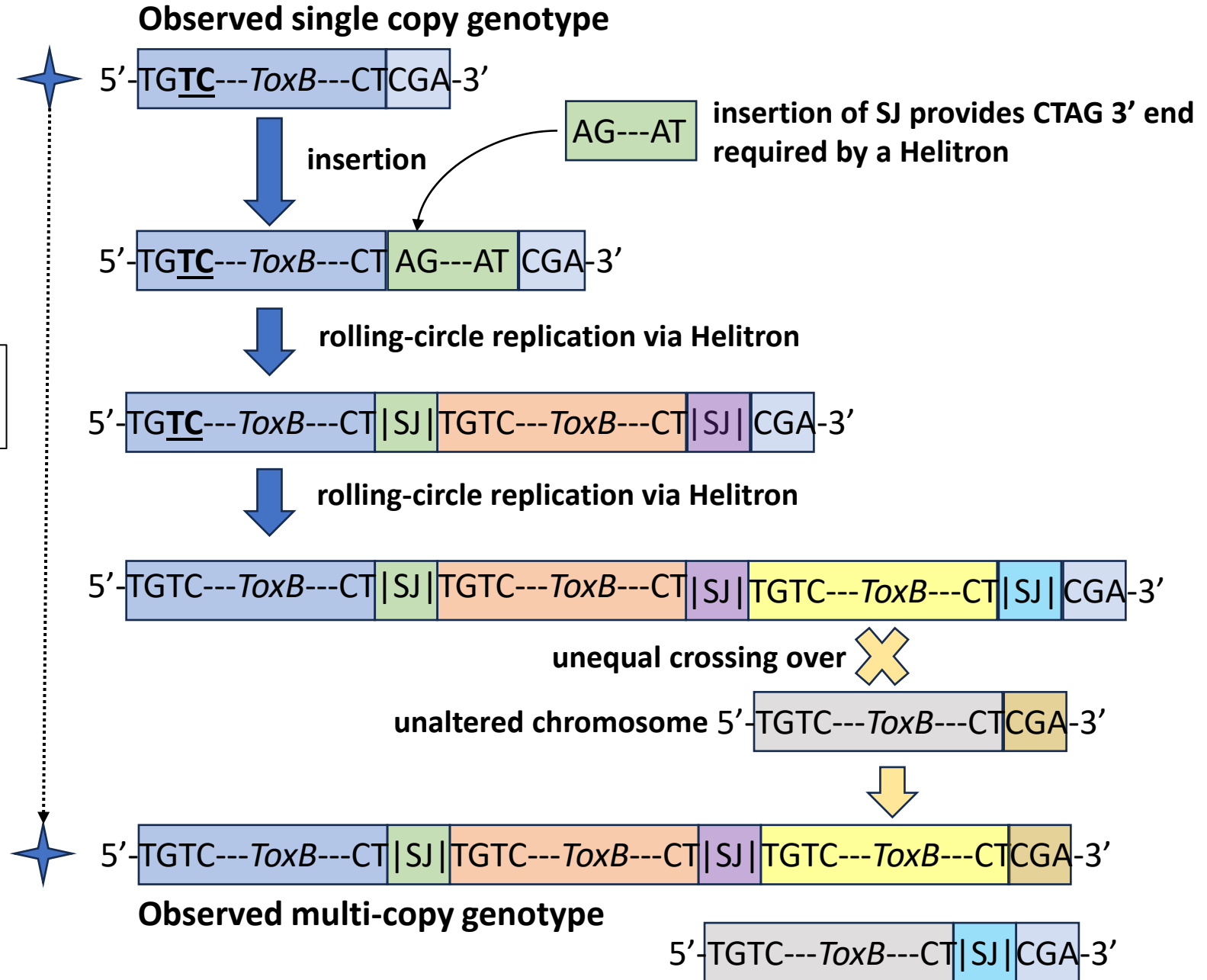


Anatomy of the *ToxB/toxb* replicative unit – multi-copy



Potential replication model


How to do we get from here to here?



Helitrons are capable of horizontal transfers

Fungi

Highly expressed captured genes and cross-kingdom domains present in Helitrons create novel diversity in *Pleurotus ostreatus* and other fungi

[Raúl Castanera](#), [Gúmer Pérez](#), [Leticia López](#), [Rubén Sancho](#), [Francisco Santoyo](#), [Manuel Alfaro](#), [Toni Gabaldón](#), [Antonio G Pisabarro](#), [José A Oguiza](#) & [Lucía Ramírez](#) 

[BMC Genomics](#) **15**, Article number: 1071 (2014) | [Cite this article](#)

Bacteria



➤ [Mol Biol Evol.](#) 2022 Jan 7;39(1):msab334. doi: 10.1093/molbev/msab334.

Pif1 Helicases and the Evidence for a Prokaryotic Origin of Helitrons

[Pedro Heringer](#) ¹, [Gustavo C S Kuhn](#) ¹

Animals

The limited distribution of *Helitrons* to vesper bats supports horizontal transfer

[Jainy Thomas](#) ^a, [Mehran Sorourian](#) ^{b 1}, [David Ray](#) ^c, [Robert J. Baker](#) ^d, [Ellen J. Pritham](#) ^a  

Gene

Volume 474, Issues 1–2, 15 March 2011, Pages 52–58

Insects

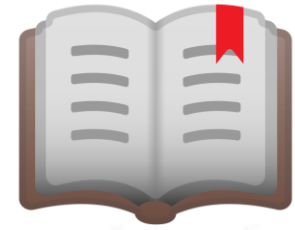
Horizontal transfer of a non-autonomous *Helitron* among insect and viral genomes

[Brad S Coates](#) 

[BMC Genomics](#) **16**, Article number: 137 (2015) | [Cite this article](#)

Conclusions

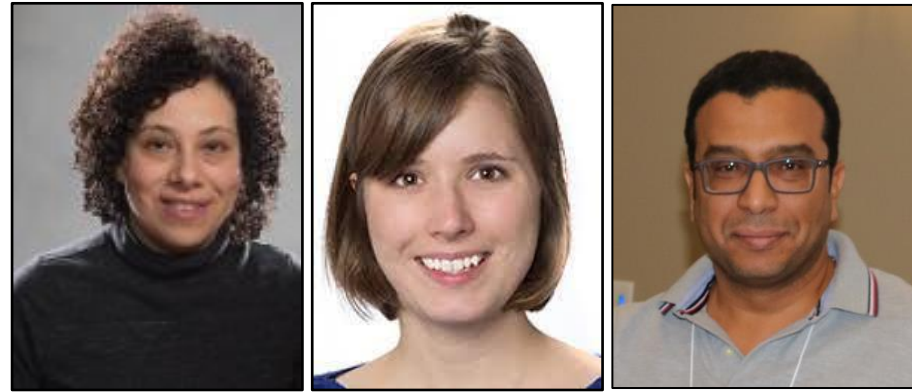
- 24 fully annotated genomes for the pathology community
- Provide framework for understanding replication
 - insertion → Helitron → unequal crossing over
- Disruption of *ToxB* ORF by multiple retrotransposons
- *ToxB* is potentially mobile and resides in a dynamic accessory region



Acknowledgements

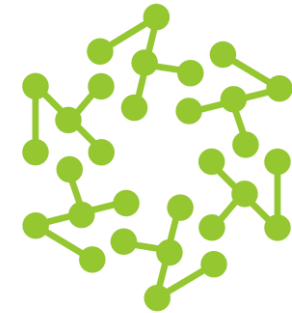
Collaborators

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Megan McDonald
Mohamed Hafez



Support

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