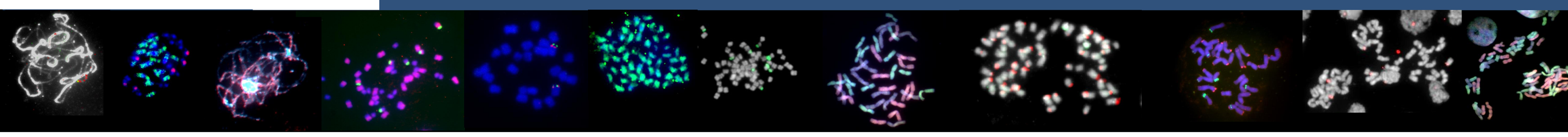
PLATE-FORME DE CYTOGENETIQUE MOLECULAIRE VEGETALE

Des outils au service de l'analyse des génomes



UMR 1349 IGEPP INRAe Centre Bretagne-Normandie 35653 LE RHEU CEDEX

http://www6.rennes.inrae.fr/igepp/L-IGEPP/Plateformes/Cytogenetique-moleculaire
http://www.biogenouest.org/contenu/plates-formes/bio-imagerie/cytogenetique

OBJECTIFS ET MISSIONS DE LA PLATE-FORME

L'objectif de cette plate-forme est de participer au développement chez les plantes supérieures des programmes d'études de génomes faisant appel à l'hybridation *in situ* Fluorescente (FISH) pour accroître :

- la caractérisation cytogénétique du matériel végétal impliquant les hybrides interspécifiques afin de valoriser les gènes d'intérêt présents au sein des espèces apparentées, notamment les gènes de résistance,
- la compréhension de la structure des génomes chez des espèces polyploïdes.

Cette plate-forme a également une mission de formation et d'information des chercheurs et techniciens pour un transfert de technologie.

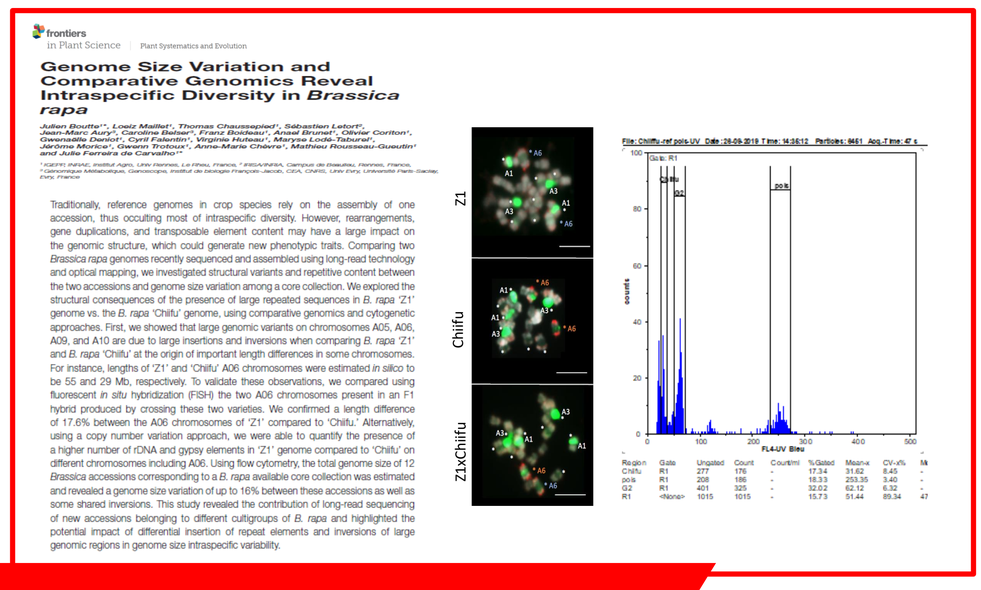
ACCESSIBILITÉ

- ❑ La plateforme est accessible à la communauté scientifique publique et privé.
- ❑ Réalisation de différentes techniques de cytogénétique dans le cadre de collaborations de recherche ou prestations.
- ❑ Développements méthodologiques en lien avec vos besoins.
- ❑ Organisation de formations à la demande.

ÉQUIPEMENTS

- 2 stations de microscopie Zeiss équipé en fluorescence
- Système d'imagerie : Caméra refroidie Photometrics Cool SNAP HQ
- Logiciel d'acquisition METAVUE (Universal Imaging) et Zen (ZEISS)
- Cytomètre de flux - Cyflow Partec

EXEMPLES DE PROJETS


frontiers in Plant Science | Plant Systematics and Evolution

Genome Size Variation and Comparative Genomics Reveal Intraspecific Diversity in *Brassica rapa*

Julien Boutte[1*], Loeiz Maillet[1], Thomas Chaussepied[1], Sébastien Letort[2], Jean-Marc Aury[3], Caroline Belser[3], Franz Boideau[1], Ansel Brunet[1], Olivier Coriton[1], Gwenaëlle Deniot[1], Cyril Falentin[1], Virginie Huteau[1], Maryse Lodé-Taburel[1], Jérôme Morice[1], Gwenn Trotoux[1], Anne-Marie Chèvre[1], Mathieu Rousseau-Gueutin[1] and Julie Ferreira de Carvalho[1*]

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Traditionally, reference genomes in crop species rely on the assembly of one accession, thus occulting most of intraspecific diversity. However, rearrangements, gene duplications, and transposable element content may have a large impact on the genomic structure, which could generate new phenotypic traits. Comparing two *Brassica rapa* genomes recently sequenced and assembled using long-read technology and optical mapping, we investigated structural variants and repetitive content between the two accessions and genome size variation among a core collection. We explored the structural consequences of the presence of large repeated sequences in *B. rapa* 'Z1' genome vs. the *B. rapa* 'Chiifu' genome, using comparative genomics and cytogenetic approaches. First, we showed that large genomic variants on chromosomes A05, A06, A09, and A10 are due to large insertions and inversions when comparing *B. rapa* 'Z1' and *B. rapa* 'Chiifu' at the origin of important length differences in some chromosomes. For instance, lengths of 'Z1' and 'Chiifu' A06 chromosomes were estimated *in silico* to be 55 and 29 Mb, respectively. To validate these observations, we compared using fluorescent *in situ* hybridization (FISH) the two A06 chromosomes present in an F1 hybrid produced by crossing these two varieties. We confirmed a length difference of 17.6% between the A06 chromosomes of 'Z1' compared to 'Chiifu.' Alternatively, using a copy number variation approach, we were able to quantify the presence of a higher number of rDNA and gypsy elements in 'Z1' genome compared to 'Chiifu' on different chromosomes including A06. Using flow cytometry, the total genome size of 12 *Brassica* accessions corresponding to a *B. rapa* available core collection was estimated and revealed a genome size variation of up to 16% between these accessions as well as some shared inversions. This study revealed the contribution of long-read sequencing of new accessions belonging to different cultigroups of *B. rapa* and highlighted the potential impact of differential insertion of repeat elements and inversions of large genomic regions in genome size intraspecific variability.

Z1 / Chiifu / Z1xChiifu

Comptage chromosomique et cytométrie en flux


G3 Genes | Genomes | Genetics

Information for Authors Editorial Board Submit a Manuscript

G3 (Bethesda). 2017 Jul; 7(7): 2185–2193. PMCID: PMC5499127
Published online 2017 May 25. doi: 10.1534/g3.117.041509

Assessment of Gene Flow Between *Gossypium hirsutum* and *G. herbaceum*: Evidence of Unreduced Gametes in the Diploid Progenitor

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Abstract

In the framework of gene flow assessment, we investigated the natural hybridization rate between *G. hirsutum* and *G. herbaceum*, the latter species, a diploid progenitor of *G. hirsutum* being spontaneously present in South Africa. Reciprocal crosses were performed without emasculation between *G. herbaceum* used as female or male and *G. hirsutum*. Neither examination of the morphological characteristics nor flow cytometry analysis of the 335 plants resulting from the cross *G. hirsutum* x *G. herbaceum* showed any to be hybrid plants. Of the 148 plants produced from the cross *G. herbaceum* x *G. hirsutum*, three showed a hybrid phenotype, this hybrid status was confirmed by SSRs markers. Analysis of DNA content by flow cytometry and morphological traits clearly showed that two of these plants were triploid (AAD). The third plant exhibited a value in flow cytometry slightly higher than *G. hirsutum*. In addition, some morphological characteristics (plant morphology, presence and size of petal spots, leaf shape) led us to conclude that this plant was AAAD and was the result of a fecundation with unreduced gamete AA from the female *G. herbaceum* parent. Fluorescent *In Situ* Hybridization (FISH) and meiotic behaviour confirmed this hypothesis. The occurrence of such gametes which, to our knowledge, is the first description in the species *G. herbaceum*, opens new avenues in breeding programmes and this plant material could also provide a useful tool for the study of the expression of genes duplicated in the A and D cotton genome.

Caractérisation cytogénétique de materiel végétal

Plant Science
An international journal of experimental plant biology

Evolutionary 1 dynamics of transposable elements and satellite DNAs 2 in polyploid *Spartina* species

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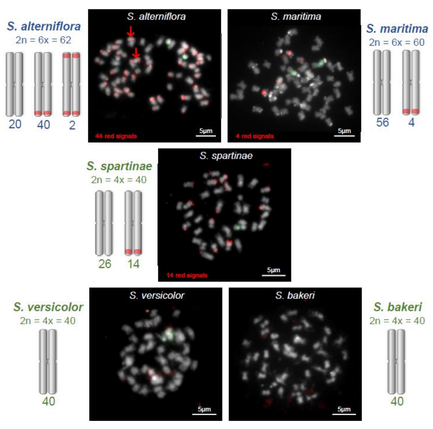
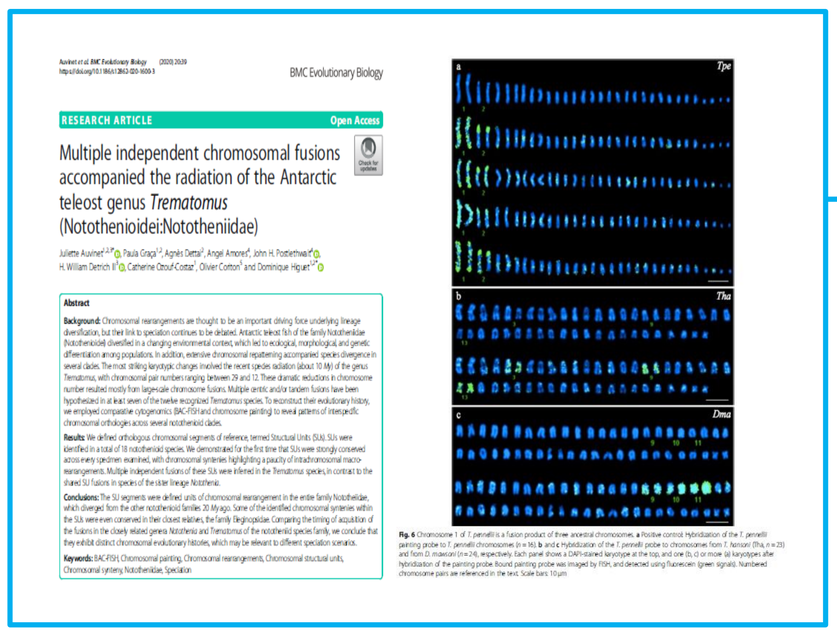
Abstract:

Repeated sequences and polyploidy play a central role in plant genome dynamics. Here, we analyze the evolutionary dynamics of repeats in tetraploid and hexaploid *Spartina* species that diverged during the last 10 million years within the Chloridoideae, one of the poorest investigated grass lineages. From high-throughput genome sequencing, we annotated *Spartina* repeats and determined what sequence types account for the genomesize variation among species. We examined whether differential genome size evolution correlated with ploidy levels and phylogenetic relationships. We also examined the tempo of repeat sequence dynamics associated with allopatric speciation over the last 3-6 million years between hexaploid species that diverged on the American and European Atlantic coasts and tetraploid species from North and South America. The tetraploid *S. spartinae*, whose phylogenetic placement has been debated, exhibits a similar repeat content as hexaploidy species, suggesting common ancestry. Genome expansion or contraction resulting from repeat dynamics seems to be explained mostly by the contrasting divergence times between species, rather than by genome changes triggered by ploidy level change *per se*. One 370 bp satellite may be exhibiting 'meiotic drive' and driving chromosome evolution in *S. alterniflora*. Our results provide crucial insights for investigating the genetic and epigenetic consequences of such differential repeat dynamics on the ecology and distribution of the meso- and neopolyploid *Spartina* species.



Evolution structural des génomes chez les espèces polyploïdes


Auvinet et al. BMC Evolutionary Biology (2020) 20:39
https://doi.org/10.1186/s12862-020-1600-3

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

Multiple independent chromosomal fusions accompanied the radiation of the Antarctic teleost genus *Trematomus* (Notothenioidei:Notothenidae)

Juliette Auvinet[2,3*], Paula Gragl[1,2], Agnès Dettaï[2], Angel Amores[3], John H. Postlethwait[3], H. William Detrich II[3], Catherine Ozouf-Costaz[2], Olivier Coriton[1] and Dominique Higuet[1,2*]

Abstract

Background: Chromosomal rearrangements are thought to be an important driving force underlying lineage diversification, but their link to speciation continues to be debated. Antarctic teleost fish of the family Nototheniidae Notothenioidei diversified in a changing environmental context, which led to ecological, morphological, and genetic differentiation among populations. In addition, extensive chromosomal repatterning accompanied species divergence in several clades. The most striking karyotypic changes involved the recent species radiation (about 10 My) of the genus *Trematomus*, with chromosomal pair numbers ranging between 29 and 12. These dramatic reductions in chromosome number resulted mostly from large-scale chromosome fusions. Multiple centric and/or tandem fusions have been hypothesized in at least seven of the twelve recognized *Trematomus* species. To reconstruct their evolutionary history, we employed comparative cytogenomics (BAC-FISH) and chromosome painting to reveal patterns of interspecific chromosomal orthologies across several notothenioid clades.

Results: We defined orthologous chromosomal segments of reference, termed Structural Units (SUs). SUs were identified in a total of 18 notothenioid species. We demonstrated for the first time that SUs were strongly conserved across every specimen examined, with chromosomal synteries highlighting a paucity of intrachromosomal macro-rearrangements. Multiple independent fusions of these SUs were inferred in the *Trematomus* species, in contrast to the shared SU fusions in species of the sister lineage Notothenia.

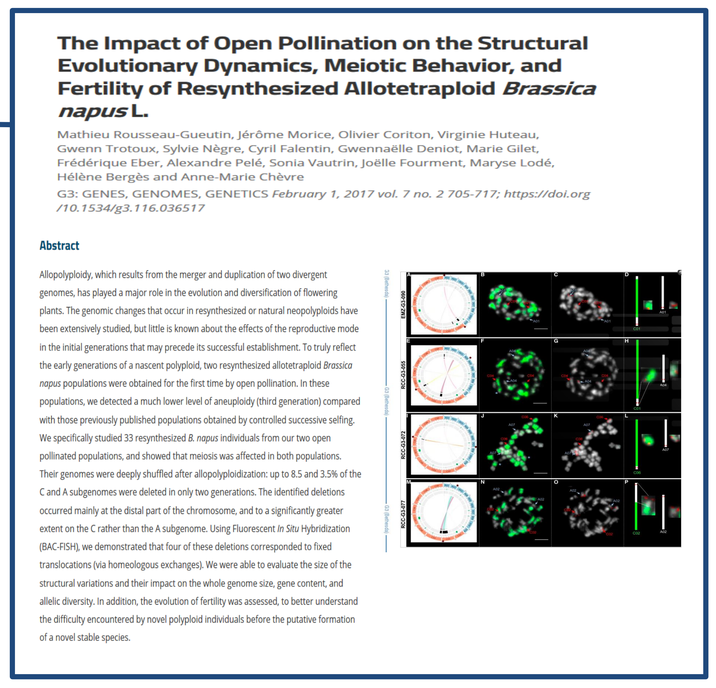
Conclusions: The SU segments were defined units of chromosomal rearrangement in the entire family Nototheniidae, which diverged from the other notothenioid families 20 Mya ago. Some of the identified chromosomal synteries within the SUs were even conserved in their closest relatives, the family Eleginopsidae. Comparing the timing of acquisition of the fusions in the closely related genera *Notothenia* and *Trematomus* of the notothenioid species family, we conclude that they exhibit distinct chromosomal evolutionary histories, which may be relevant to different speciation scenarios.

Keywords: BAC-FISH, Chromosomal painting, Chromosomal rearrangements, Chromosomal structual units, Chromosomal synteny, Nototheniidae, Speciation

Fig. 6 Chromosome 1 of *T. pennellii* is a fusion product of three ancestral chromosomes. **a** Positive control. Hybridization of the *T. pennellii* painting probe to *T. pennellii* chromosomes (n = 16). **b** and **c** Hybridization of the *T. pennellii* probe to chromosomes from *T. hansoni* (Tha, n = 23) and from *D. mawsoni* (n = 24), respectively. Each panel shows a DAPI-stained karyotype at the top, and one (b, c) or more (a) karyotypes after hybridization of the painting probe. Bound painting probe was imaged by FISH, and detected using fluorescein (green signals). Numbered chromosome pairs are referenced in the text. Scale bars: 10 μm

Caractérisation et identification de translocations chromosomiques

Cartographie physique sur chromosomes


The Impact of Open Pollination on the Structural Evolutionary Dynamics, Meiotic Behavior, and Fertility of Resynthesized Allotetraploid *Brassica napus* L.

Mathieu Rousseau-Gueutin, Jérôme Morice, Olivier Coriton, Virginie Huteau, Gwenn Trotoux, Sylvie Nègre, Cyril Falentin, Gwennaëlle Deniot, Marie Gilet, Frédérique Eber, Alexandre Pelé, Sonia Vautrin, Joëlle Fourment, Maryse Lodé, Hélène Bergès and Anne-Marie Chèvre

G3: GENES, GENOMES, GENETICS *February 1, 2017 vol. 7 no. 2 705-717; https://doi.org /10.1534/g3.116.036517*

Abstract

Allopolyploidy, which results from the merger and duplication of two divergent genomes, has played a major role in the evolution and diversification of flowering plants. The genomic changes that occur in resynthesized or natural neopolyploids have been extensively studied, but little is known about the effects of the reproductive mode in the initial generations that may precede its successful establishment. To truly reflect the early generations of a nascent polyploid, two resynthesized allotetraploid *Brassica napus* populations were obtained for the first time by open pollination. In these populations, we detected a much lower level of aneuploidy (third generation) compared with those previously published populations obtained by controlled successive selfing. We specifically studied 33 resynthesized *B. napus* individuals from our two open pollinated populations, and showed that meiosis was affected in both populations. Their genomes were deeply shuffled after allopolyploidization: up to 8.5 and 3.5% of the C and A subgenomes were deleted in only two generations. The identified deletions occurred mainly at the distal part of the chromosome, and to a significantly greater extent on the C rather than the A subgenome. Using Fluorescent *In Situ* Hybridization (BAC-FISH), we demonstrated that four of these deletions corresponded to fixed translocations (via homeologous exchanges). We were able to evaluate the size of the structural variations and their impact on the whole genome size, gene content, and allelic diversity. In addition, the evolution of fertility was assessed, to better understand the difficulty encountered by novel polyploid individuals before the putative formation of a novel stable species.

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