Detecting and analysing genomic structural variants

Prepared with A. Tigano & M. Wellenreuther

RECAP: Forms of genetic variation

Sequence

1. Single base-pair changes – point mutations (SNPs)
2. Change in Copy Number Variants (CNVs)
   - Deletions
   - Duplications
3. Change in chromosomal location
   - Translocations
   - Fusions
4. Change in orientation
   - Inversions
5. Changes in chromosome number (e.g. aneuploidy)

Cytogenetics

Using sequencing to detect SV

- Massive parallel sequencing drastically reduced costs and enabled population-wide sequencing

- In 2020: many tools available with advantages and drawbacks

  - Short-reads (illumina)

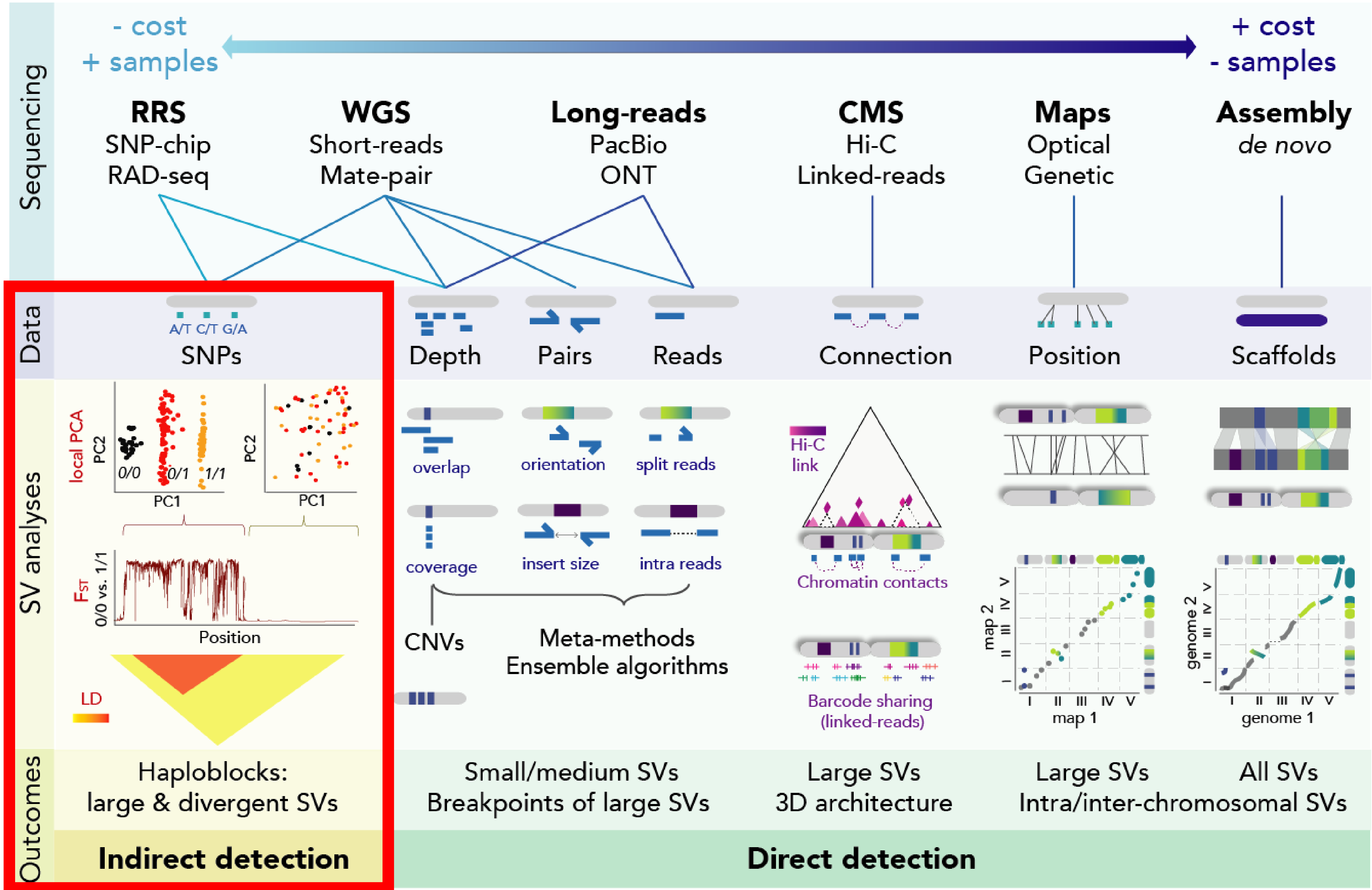
- high single-nucleotide accuracy & paired-end
- underrepresentation of high-GC regions

  - Long-reads (PacBio/Nanopore)

- Higher error rate (~15%) and single-end (but see PacBio Hi-Fi!)
- Longer sequences (~1-50kb)

  - Emerging technologies (Hi-C, 10x, optical mapping)

⇒ How can we exploit this amazing resource to detect SV?



Sequencing

- cost + samples

+ cost - samples

RRS SNP-chip RAD-seq

WGS Short-reads Mate-pair

Long-reads PacBio ONT

CMS Hi-C Linked-reads

Maps Optical Genetic

Assembly *de novo*

Data

SNPs · Depth · Pairs · Reads · Connection · Position · Scaffolds

SV analyses

CNVs

Meta-methods Ensemble algorithms

Outcomes

Haploblocks: large & divergent SVs

Small/medium SVs Breakpoints of large SVs

Large SVs 3D architecture

Large SVs Intra/inter-chromosomal SVs

All SVs

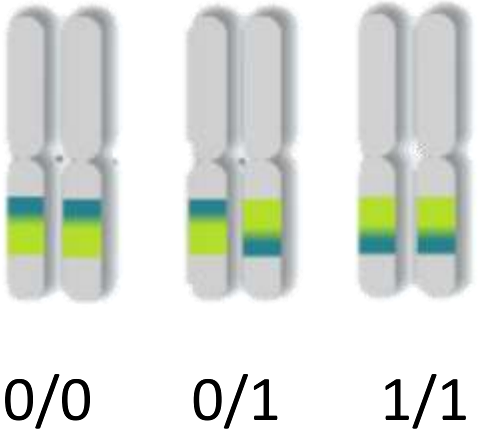
Indirect detection

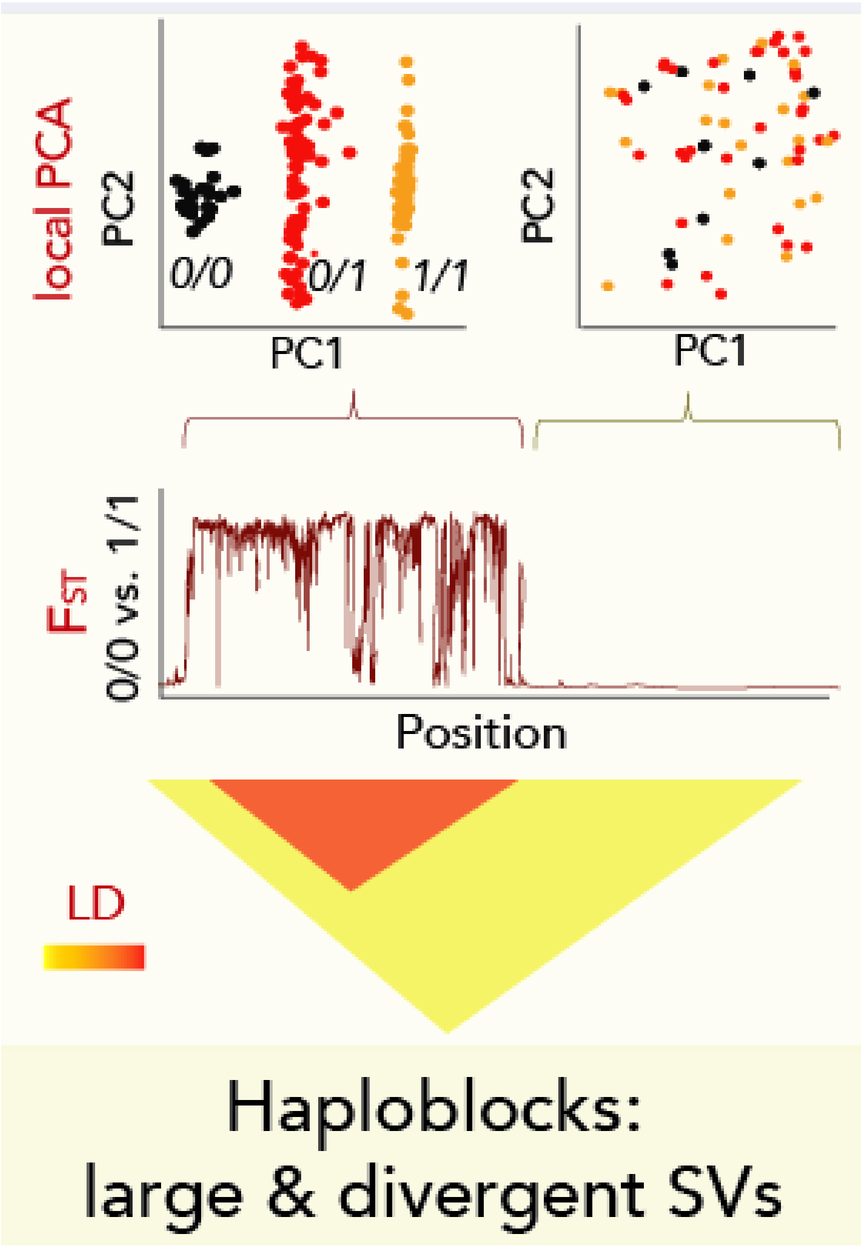
Direct detection

Indirect detection

It is based on the idea that large rearrangements (like an inversion) block recombination.

Hence when they are polymorphic in a species, they appear as large non-recombining haploblocks with two (or more) divergent haplotypes.





Indirect detection

- Using population genomics data:
  - Many samples
  - Many SNPs (from short-reads, SNPchip, RAD-seq….)

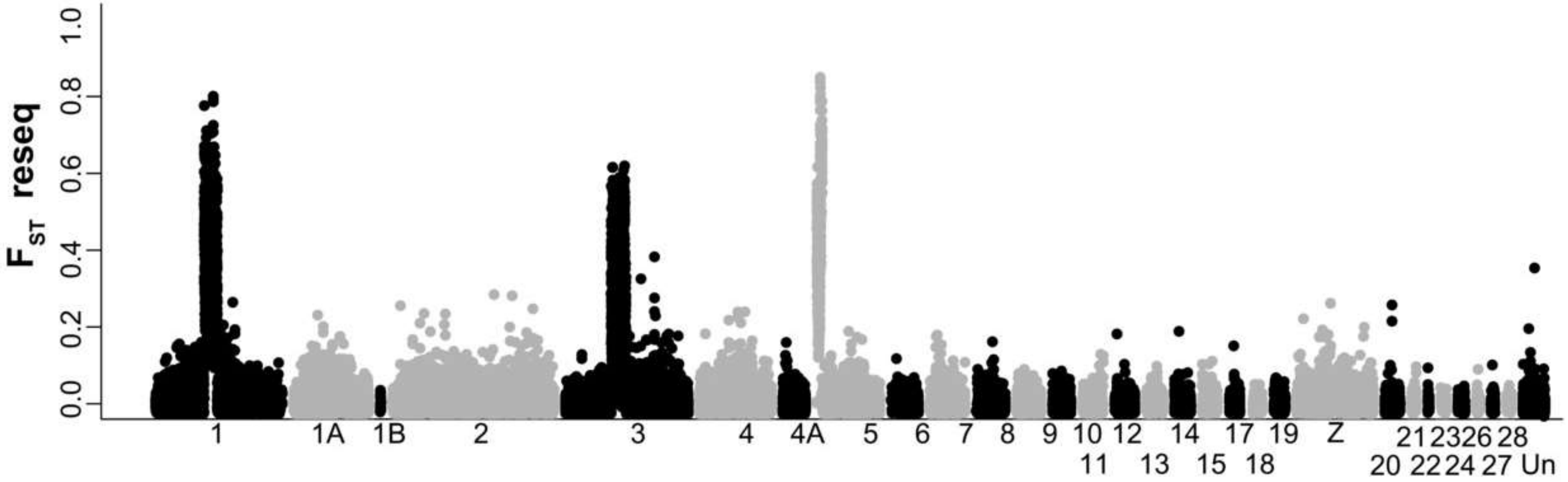
- Able to detect chromosomal rearrangements if they are:
  - Large (> 100 kb)
  - Polymorphic
  - Divergent

⇒ Typically good to detect large inversions (or fusions, large blocks without recombination)…

- Tools:
  - Fst - Linkage disequilibrium - PCA & clustering

Indirect detection : Fst/islands of divergence

Genetic differences between willow warbler migratory phenotypes are few and cluster in large haplotype blocks



-> chromosomal rearrangements preventing recombination?

-> linked selection? Hitch-hikng around specific loci?

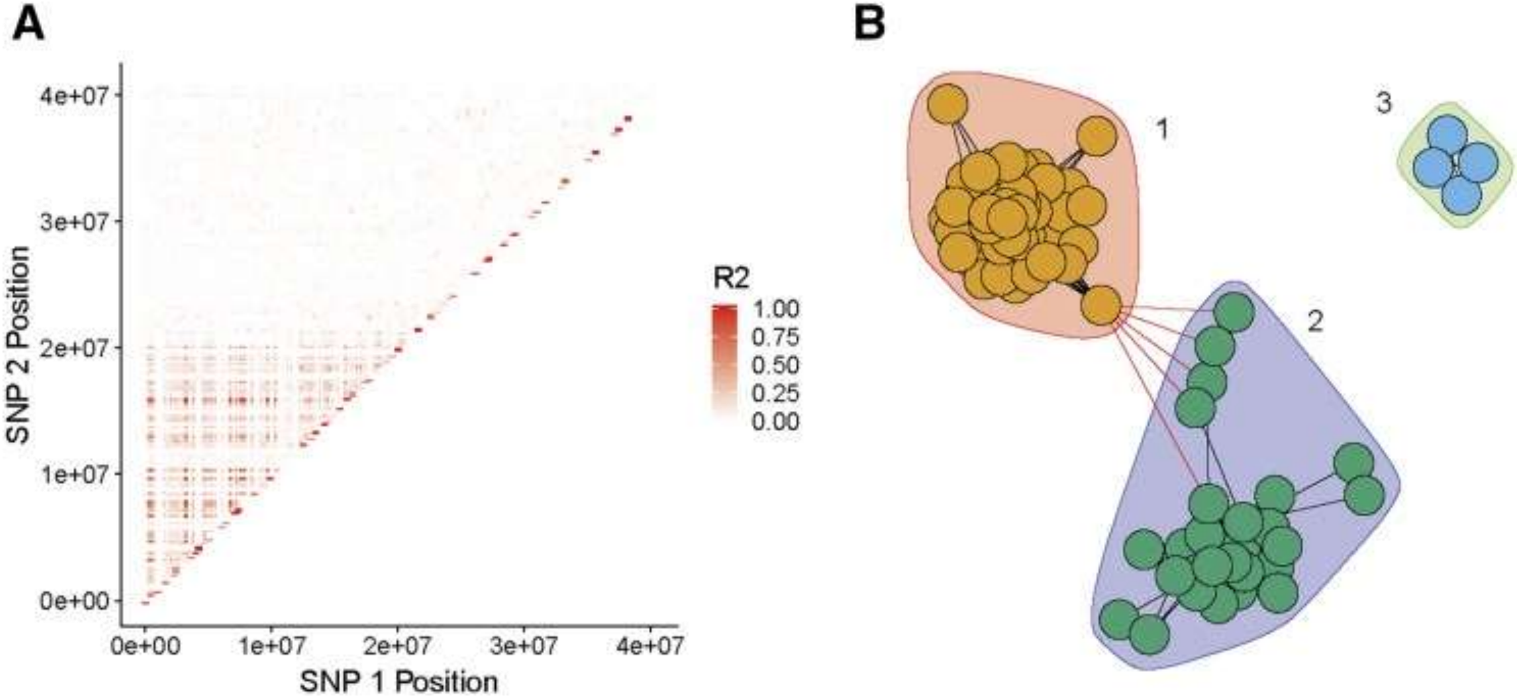
Lundberg et al. Evolution Letters 2017

Indirect detection : LD networks

SNPs within an inversion will be in high linkage disequilibrium and belong to one cluster of LD

-> can be applied without reference genome

-> any methods to get SNPs



McKinney et al 2020. *G3, 10*(5), 1553–1561. https://doi.org/10.1534/g3.119.400972

Ldna Package:

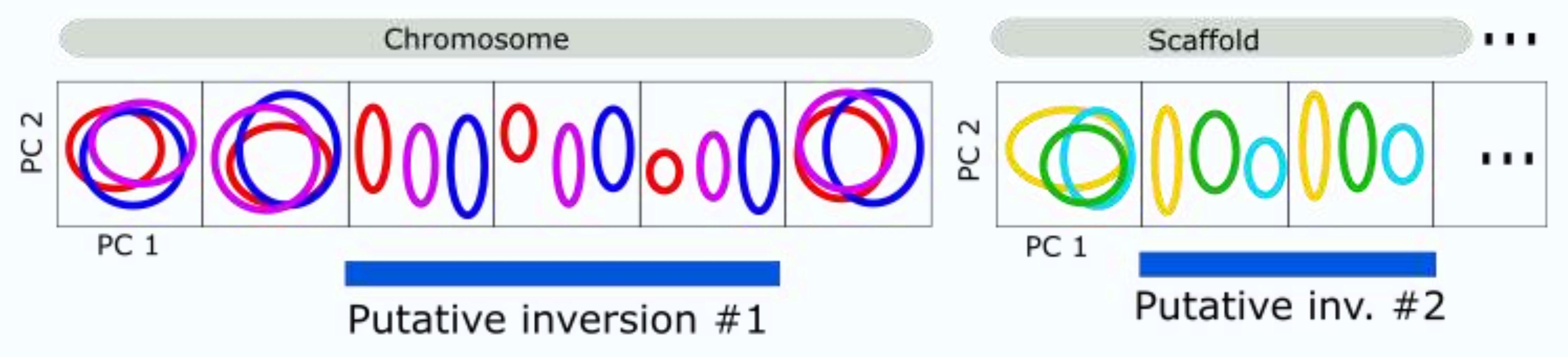
Kemppainen P, Knight CG, Sarma DK, et al. *Mol Ecol Resour*. 2015;15(5):1031-1045. https://doi.org/10.1111/1755-0998.12369

Detection of 17 inversions in Littorina:

Faria et al. *Mol Ecol*. 2019; 28: 1375– 1393. https://doi.org/10.1111/mec.14972

Indirect detection : Local PCA

A PCA performed on SNPs belonging to aninversion will usually display three clusters while PCA outside will show no clustering



Lostruct Package:
Li & Ralph. 2019 Genetics https://doi.org/10.1534/genetics.118.301747

Detection of 7 inversions in Helianthus with Rad-seq data:
Huang et al. *Mol Ecol*. 2020. https://doi.org/10.1111/mec.15428

Indirect detection :

Indirect methods typically identifies non-recombining blocks of haplotypes which may or may not be due to an inversion.

What else can haploblocks be?

- Recent introgression?
- Linked selection?

⇒ Breakpoints should start eroding with gene flow

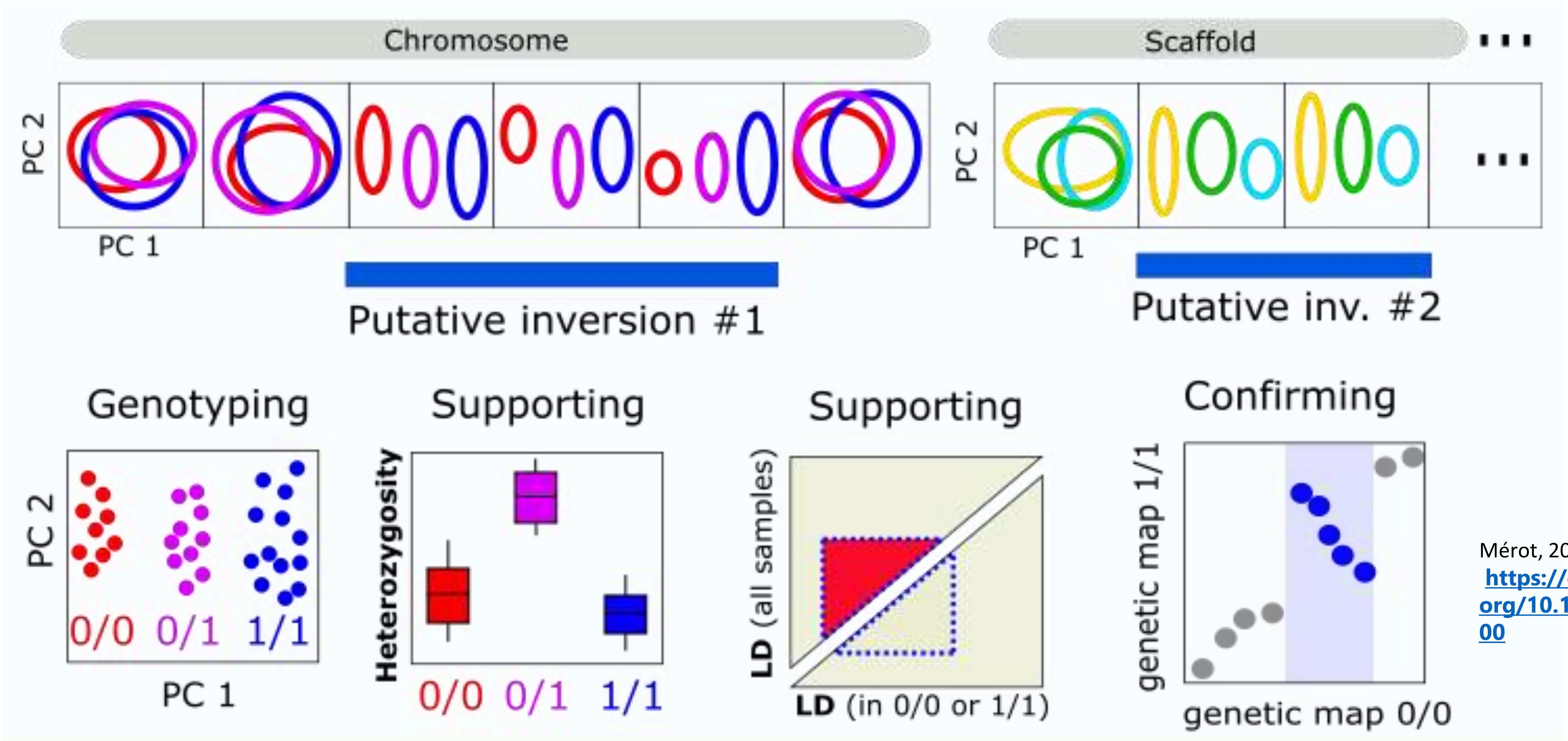
⇒ Perhaps less likely when blocks are very large (>1MB)

- Low-recombination regions?

⇒ LD should be observed in all clusters

Indirect detection :

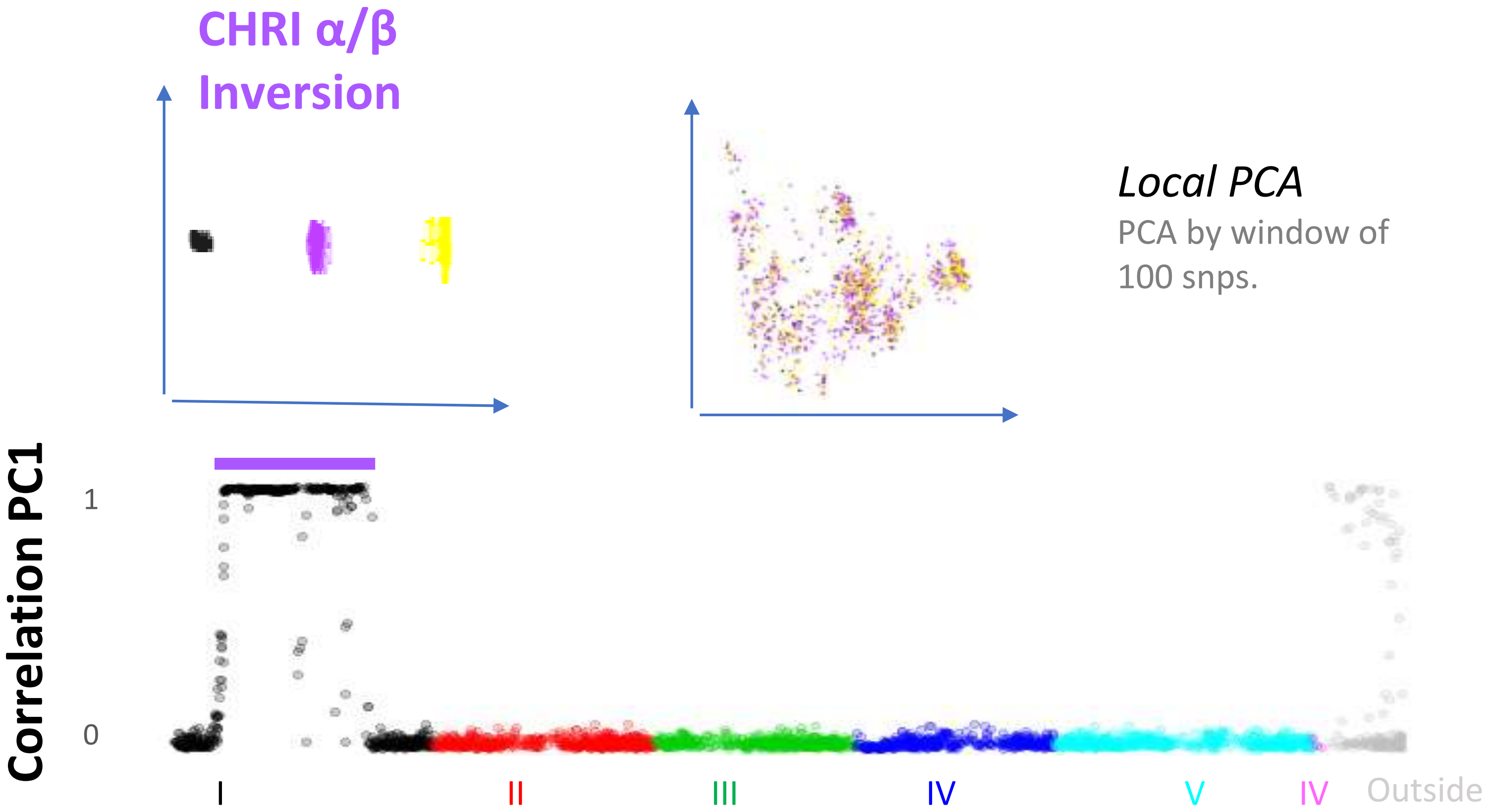
How can we support that an haploblock is an inversion?



Mérot, 2020, MolEcol
https://doi-org/10.1111/mec.15500

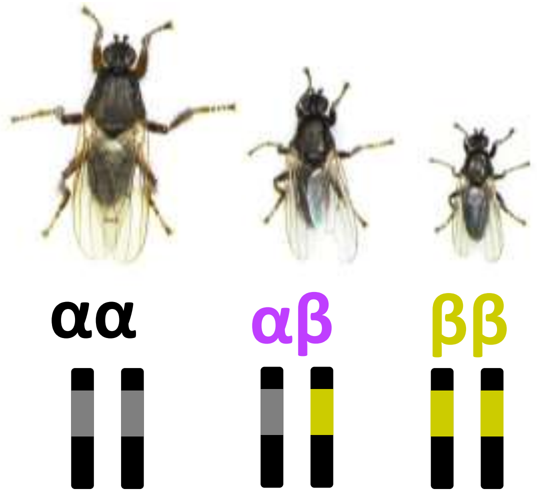
Indirect detection: Case study in the seaweed fly *Coelopa frigida*

- Whole-genome sequencing at low coverage for 1,446 flies



Local PCA

PCA by window of 100 snps.



CHR-I inversion

27Mb

11% genome

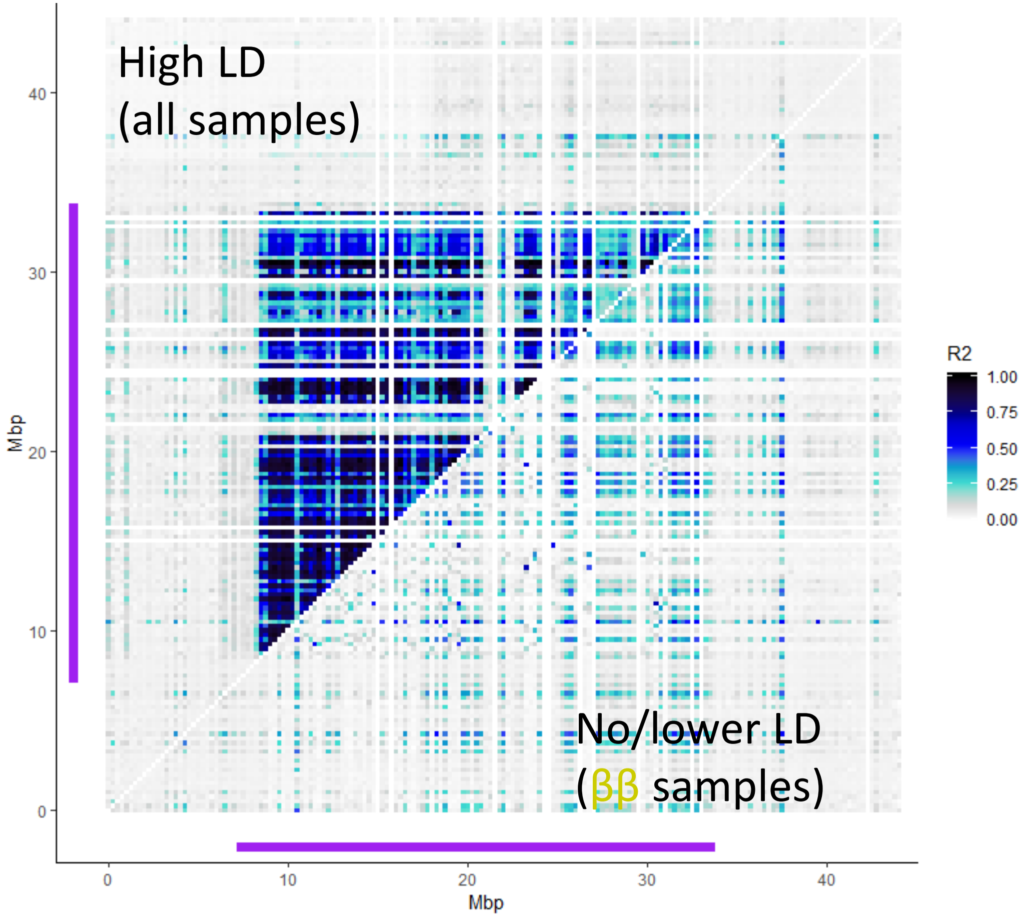
16,5% of SNPs

1500 genes

Indirect detection: Case study in the seaweed fly *Coelopa frigida*

Exploration of the haploblock/inversion

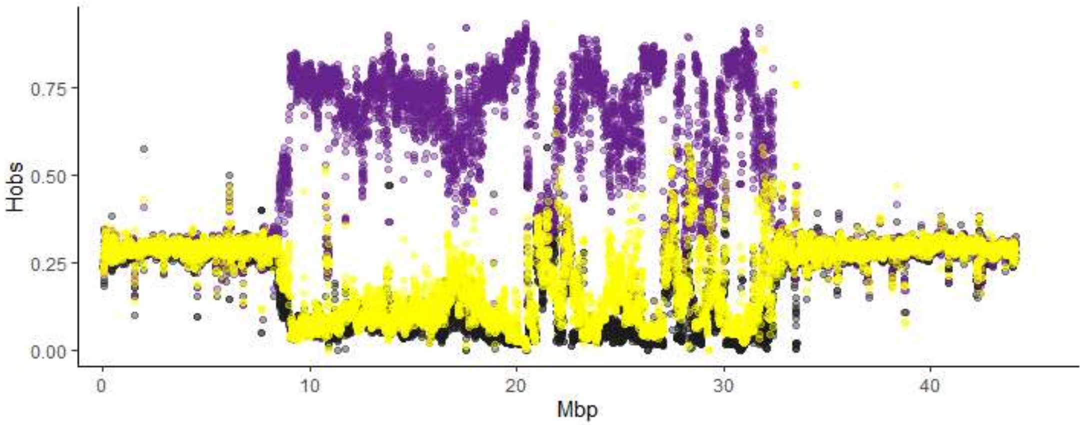
Chr I



High LD
(all samples)

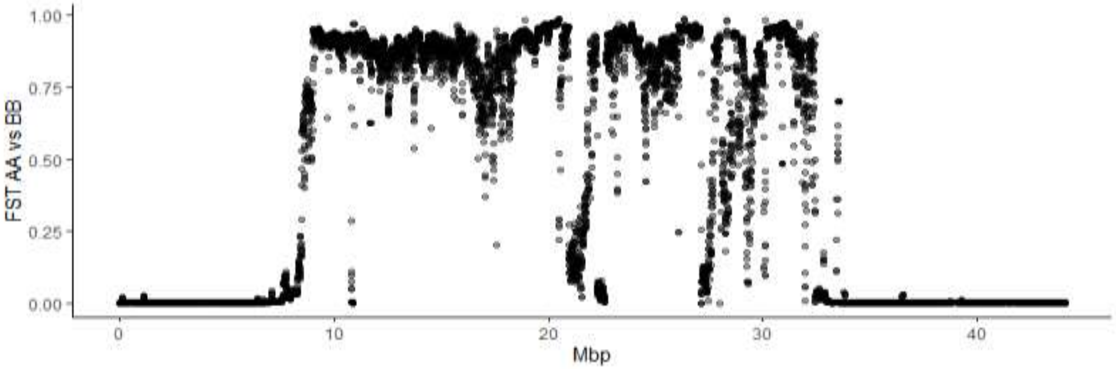
No/lower LD
(ββ samples)

Higher observed heterozygosity in αβ than in αα or ββ



Chr I

High FST differentiation between αα and ββ



Chr I

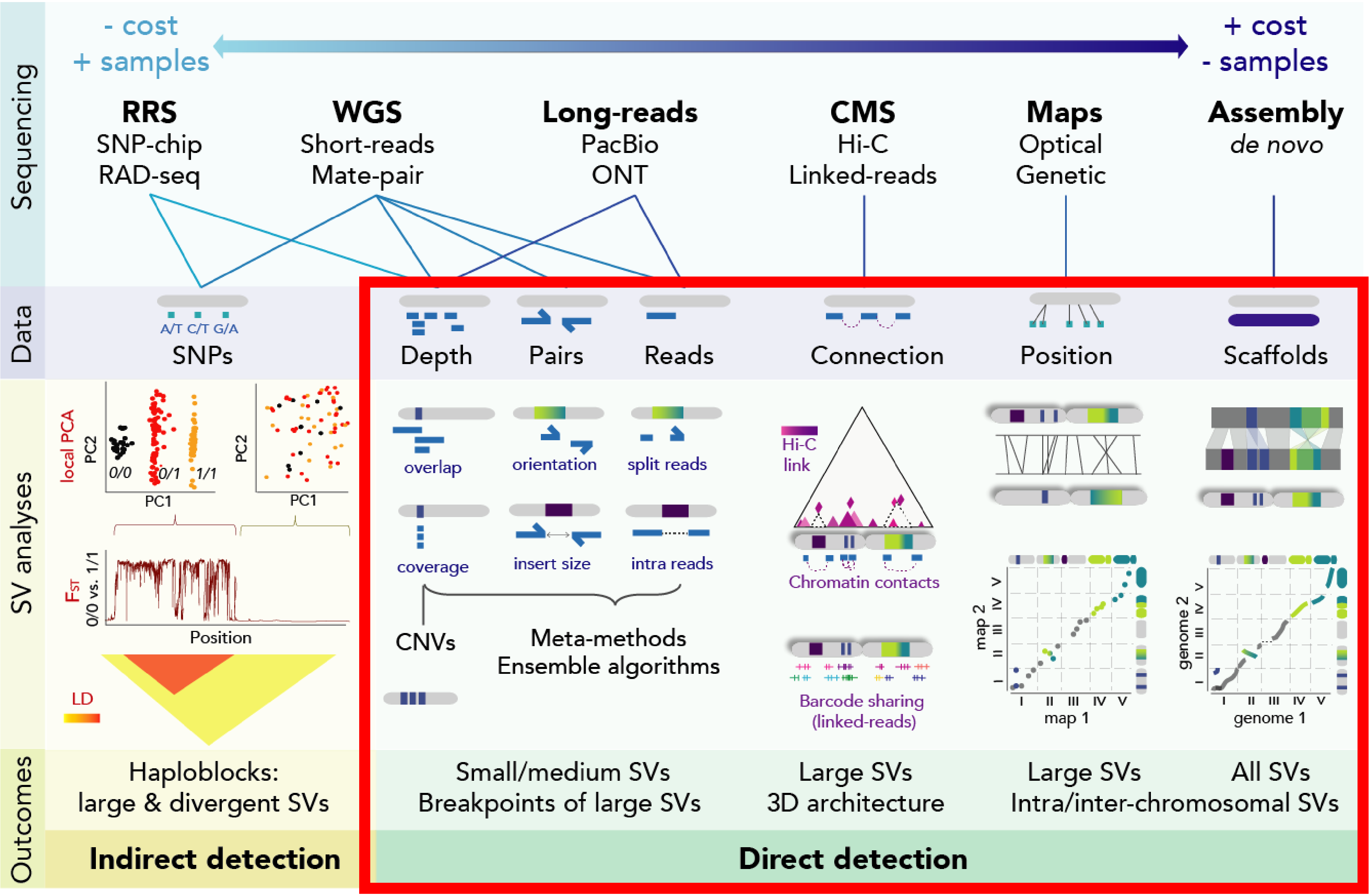
Indirect detection of SV :

Advantages:

- Same data as population genomics (even RAD-seq)
- Genotyping inversions accross large datasets

Drawbacks:

- Better confirmed with direct detection methods (cytogenetics or sequence analysis)
- Easier with a reference genome



Sequencing

- cost
+ samples

+ cost
- samples

RRS
SNP-chip
RAD-seq

WGS
Short-reads
Mate-pair

Long-reads
PacBio
ONT

CMS
Hi-C
Linked-reads

Maps
Optical
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Assembly
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Haploblocks:
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Breakpoints of large SVs

Large SVs
3D architecture

Large SVs
Intra/inter-chromosomal SVs

All SVs

Indirect detection

Direct detection

2nd generation sequencing : Short-reads (illumina)

- SVs are usually inferred indirectly from aberrant short-read alignments, such as an unexpected depth of coverage or inconsistent orientation or distance between the alignment of paired-end reads

- Low costs of short-reads allow population-wide sequencing

⇒ SV can be genotyped in many individuals

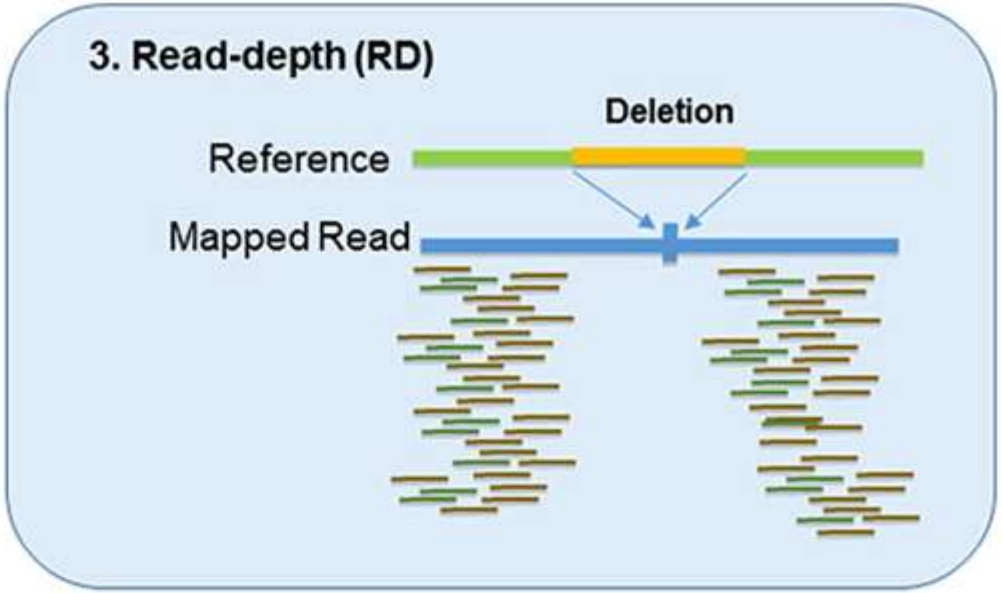
- Short-reads (100-150 bp) single or paired-end

⇒ Limited range of Sv that can be detected by this technology

Direct detection : with read depth

- Detect CNVs (duplications, indels)

- Applicable to SNP-chip, RAD-seq, WGS (short & long reads)



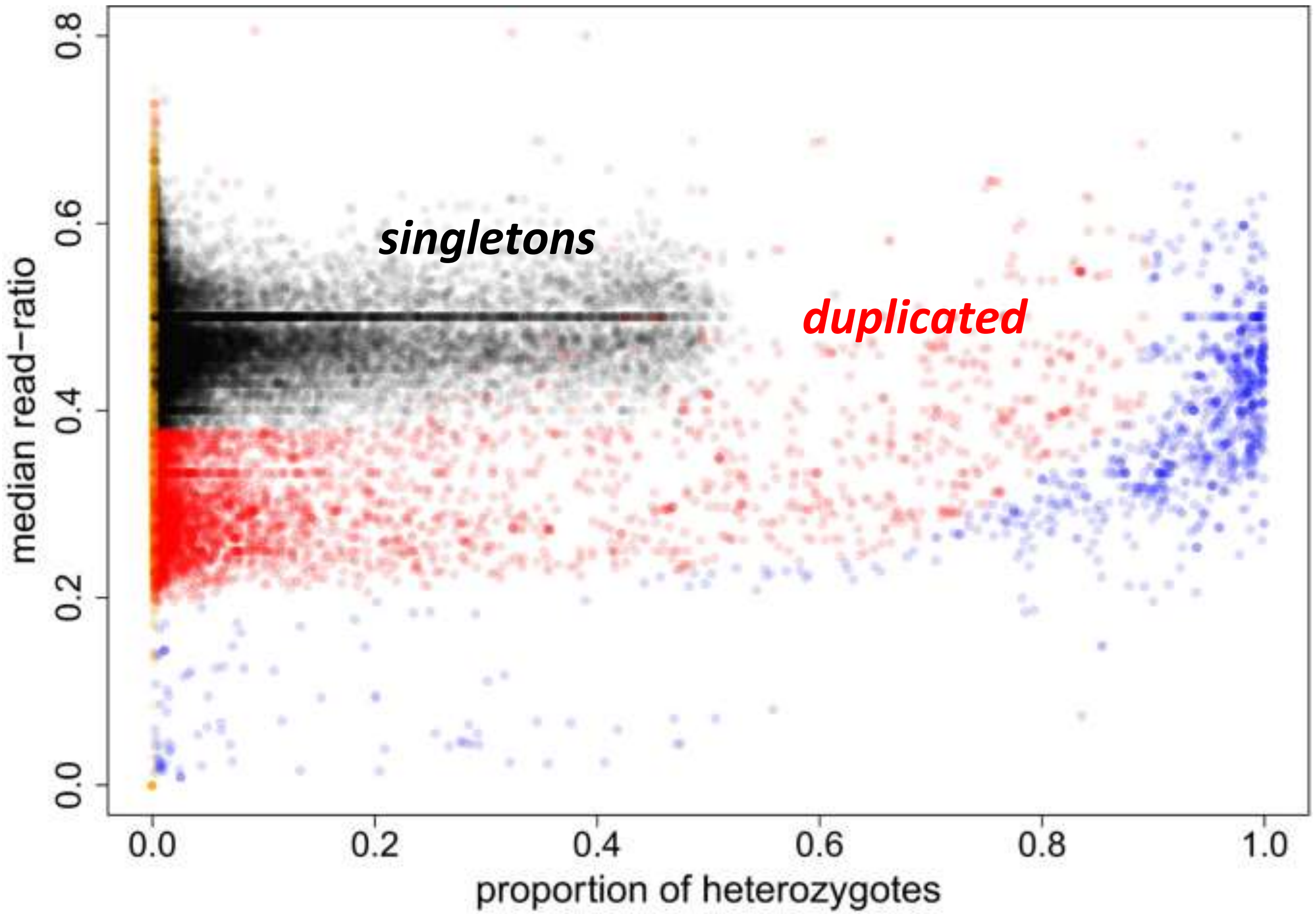
Direct detection : with read depth

Adding allelic information and heterozygote information…

⇒ Detect duplicated loci in RAD-seq

-> Filter them out for regular analysis

-> Keep them apart to analyse CNVs

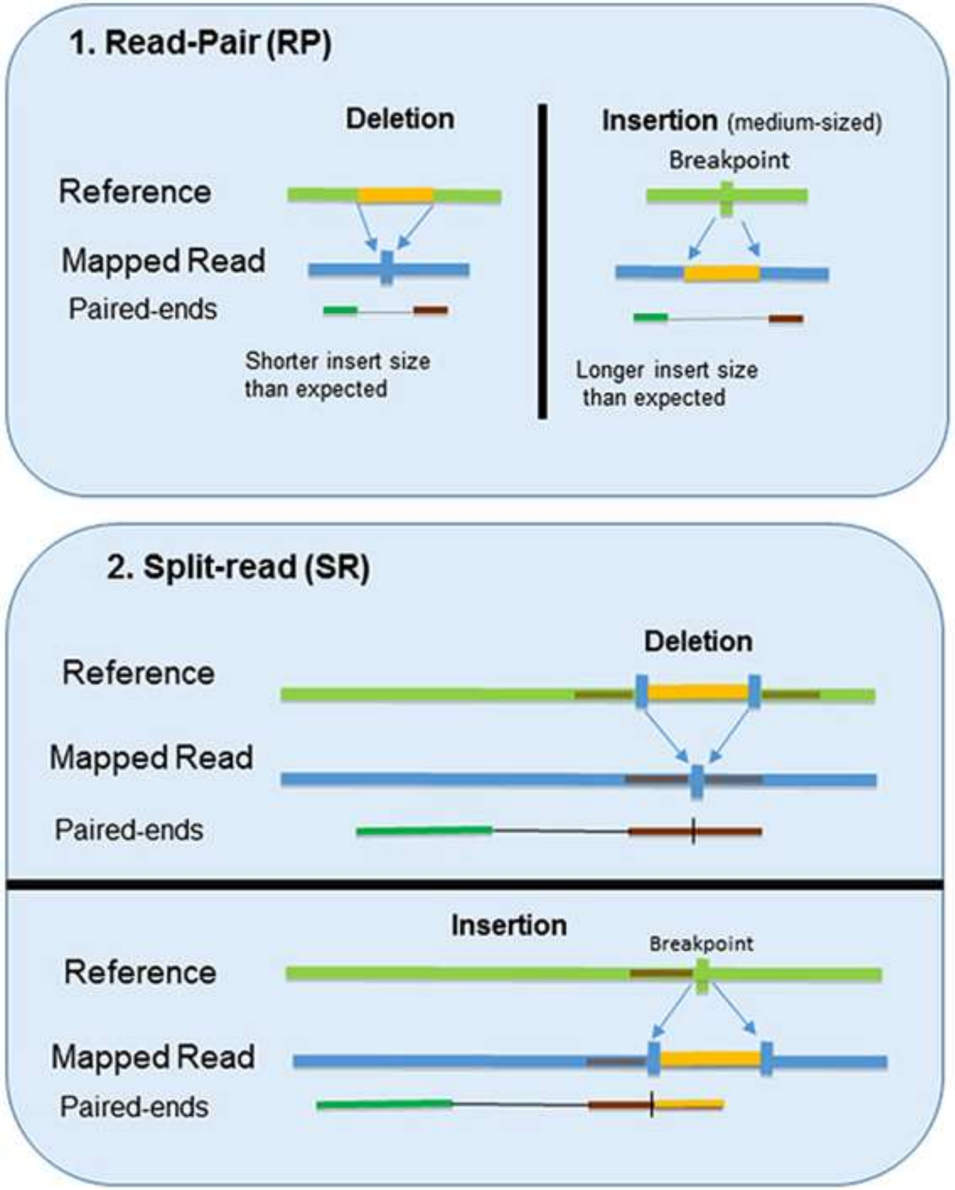


Dorant et al 2020. MolEcol https://doi.org/10.1111/mec.15565
McKinney,et al. 2017 MolEcol Ressources. https://doi.org/10.1111/1755-0998.12613

Direct detection : with paired-read orientation & split-reads

This will detect shorts indels and breakpoints of duplications, translocations or inversions

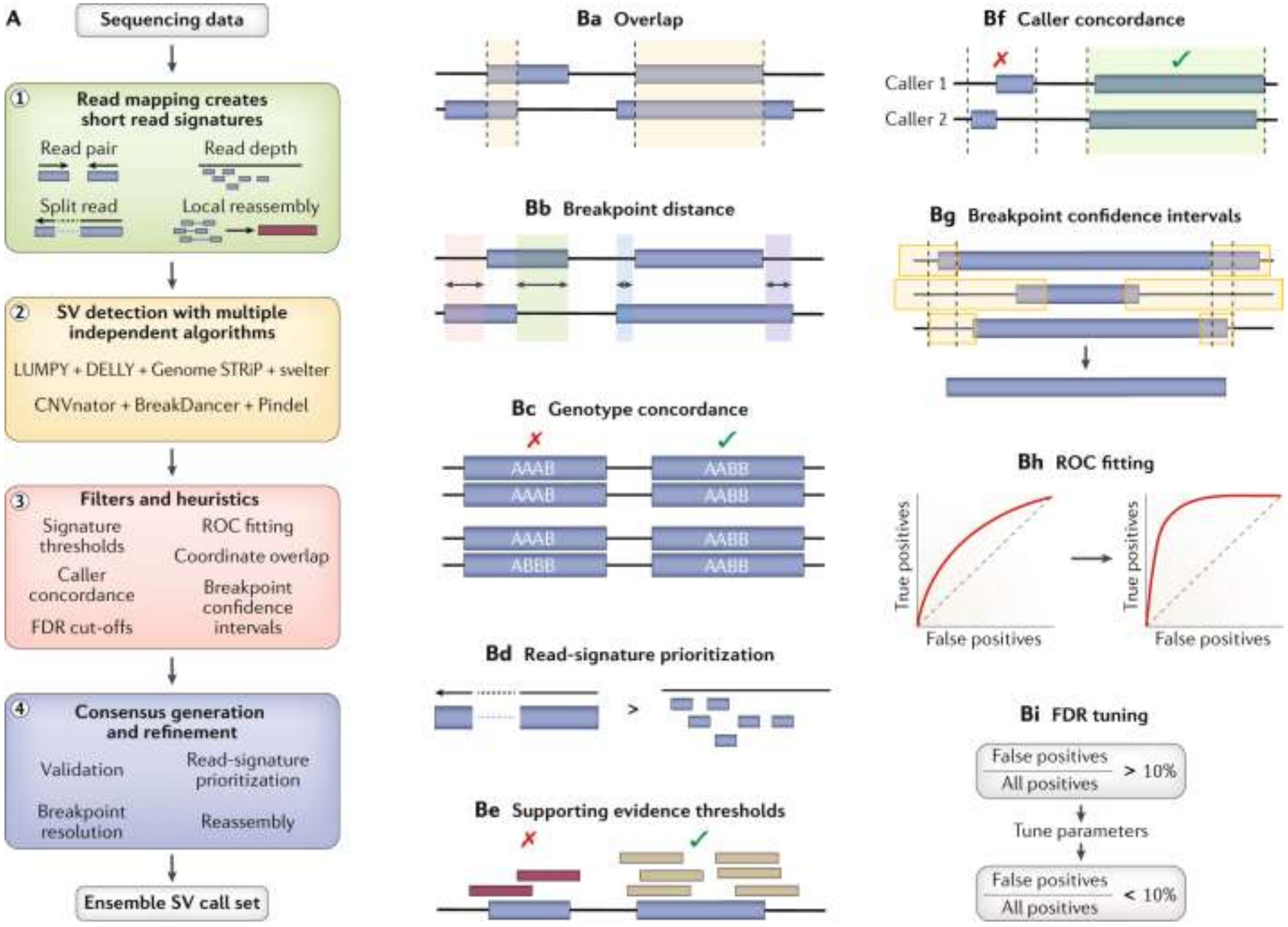
Most-used tools:
Delly, Manta, GRIDSS



Direct detection : Ensemble methods

- Combining
- read depth,
- paired-reads distance
- paired-end orientation
- split-reads.

- Merge the output of several tools to improve confidence



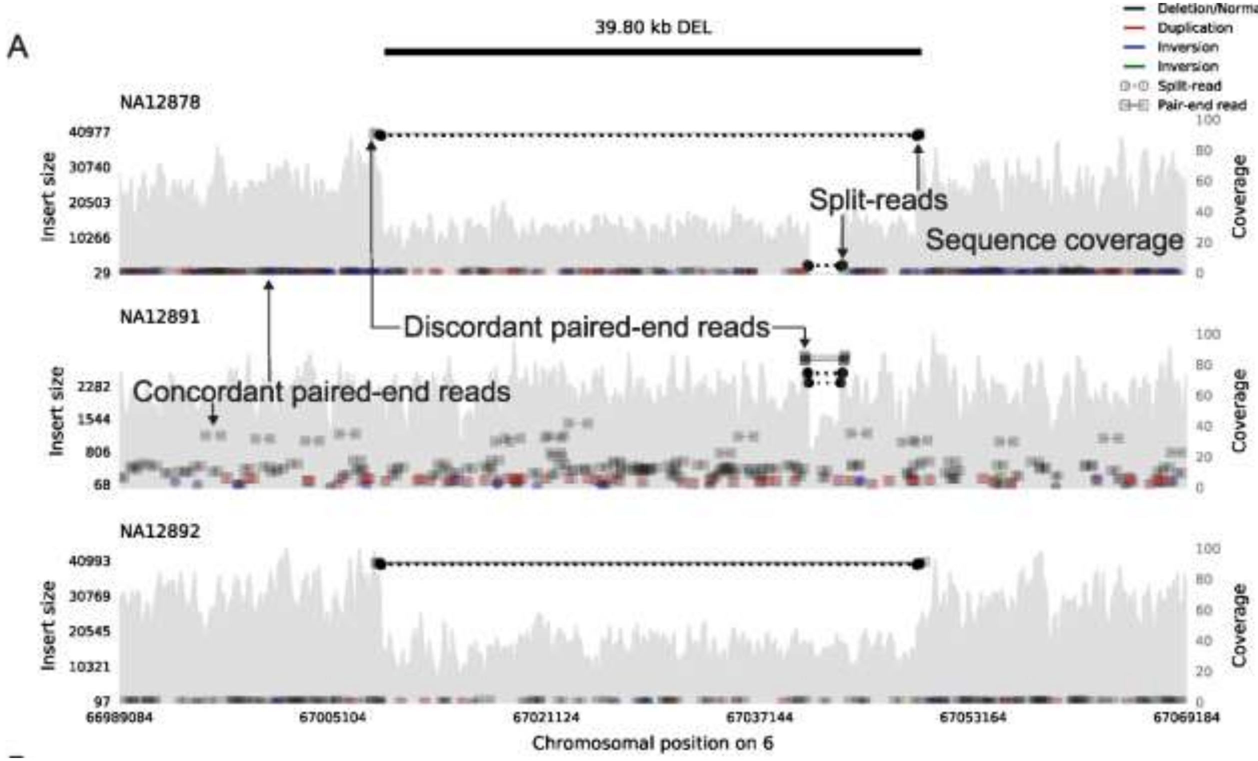
Ho, S.S., Urban, A.E. & Mills, R.E. Structural variation in the sequencing era. *Nat Rev Genet* **21,** 171–189 (2020). https://doi.org/10.1038/s41576-019-0180-9

Direct detection : based on short-reads

Lots of false positive!!

Manual curation with SV-plaudit in 492 Atlantic Salmon

*« The overall estimated false discovery rate was **0.91** with 149,491 out of 65,116 of calls which had low confidence »*
Bertolotti et al, 2020 BioRxiv https://doi.org/10.1101/2020.05.16.099614



Belyeu et al, 2018
GigaScience https://doi.org/10.1093/gigascience/giy064

Recent improvements:

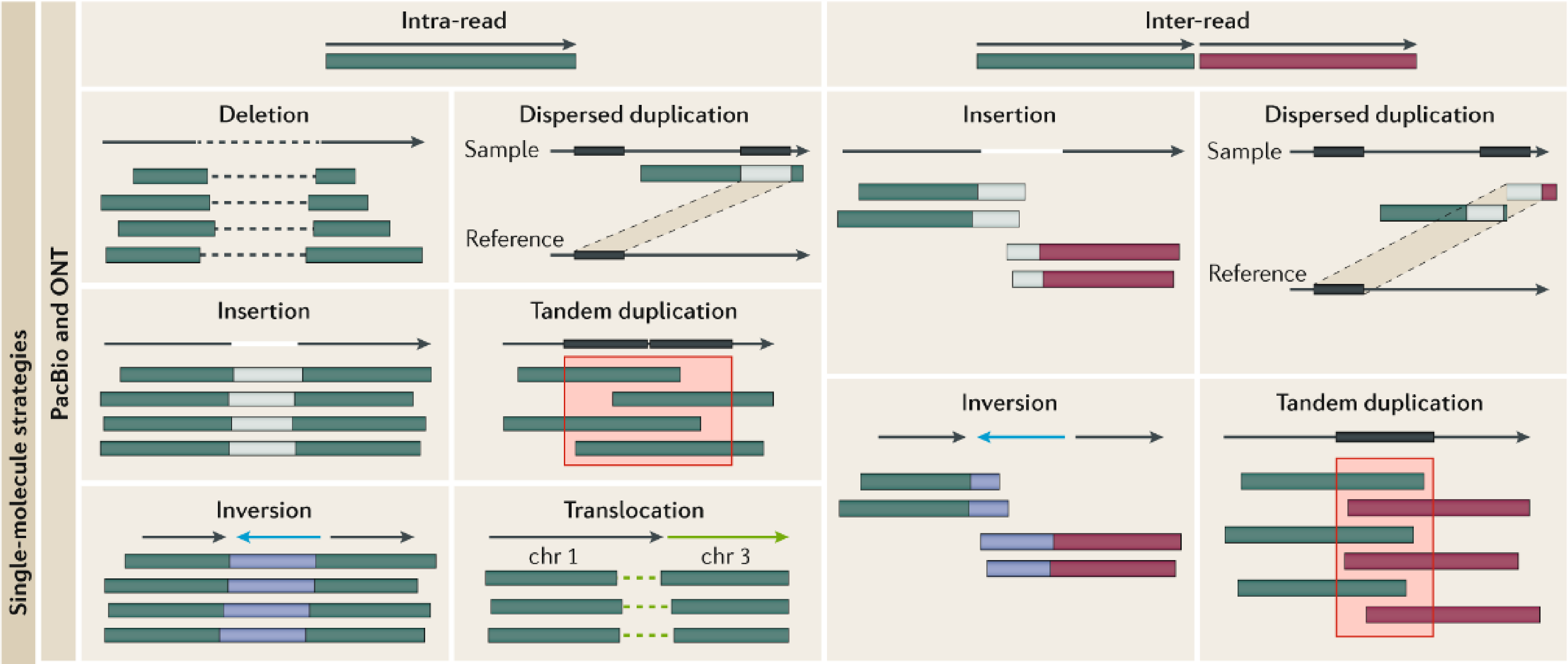
- graph-based approaches
- population-scale genotyping of SV

Eggertsson *et al. Nat Commun* **10,** 5402 (2019).
https://doi.org/10.1038/s41467-019-13341-9

Direct detection : using long-reads

- Long reads will allow to detect longer SV, will cover the highly-repetitive regions at breakpoints, etc.

- But they are expensive, we cannot genotype SV at population scale…



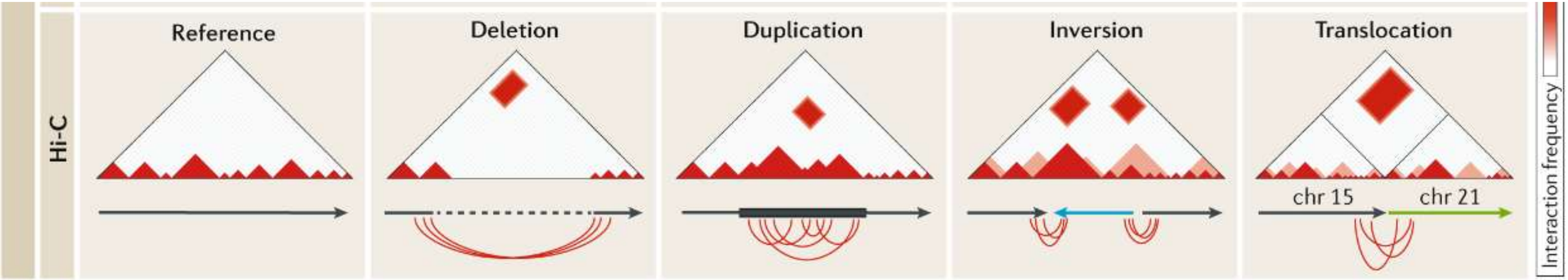
H et al . *Nat Rev Genet* (2020). https://doi.org/10.1038/s41576-019-0180-9

Direct detection: Connected-molecule strategies

Hi-C (DoveTail)

- Analyze the spatial organization of chromatin in a cell
- Output the interactions between fragments of DNA

⇒ Allows detecting medium to large rearrangements



H et al . *Nat Rev Genet* (2020). https://doi.org/10.1038/s41576-019-0180-9

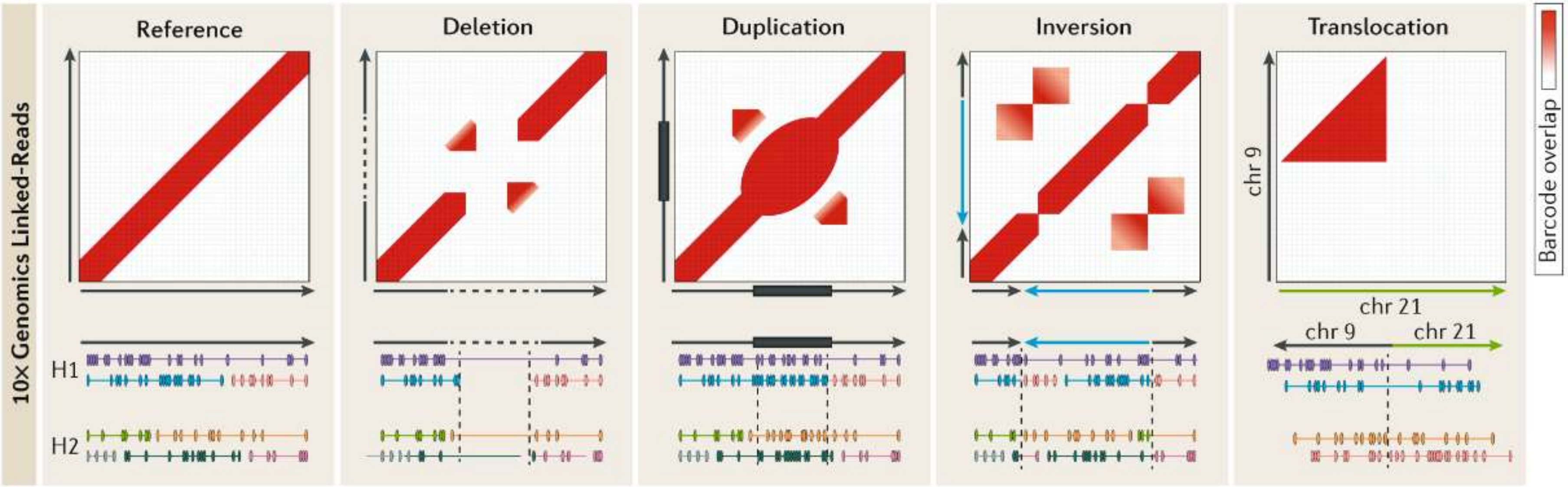
Direct detection: Connected-molecule strategies

Linked-reads
(10xGenomics, Emerging in-house haplotagging)

Meier et al bioRxiv 2020
https://doi.org/10.1101/2020.05.25.113688

- Long DNA fragments (50kb-100kb) are barcoded before short –reads sequencing

⇒ Sequences that are physically close share the same barcodes



H et al . *Nat Rev Genet* (2020). https://doi.org/10.1038/s41576-019-0180-9

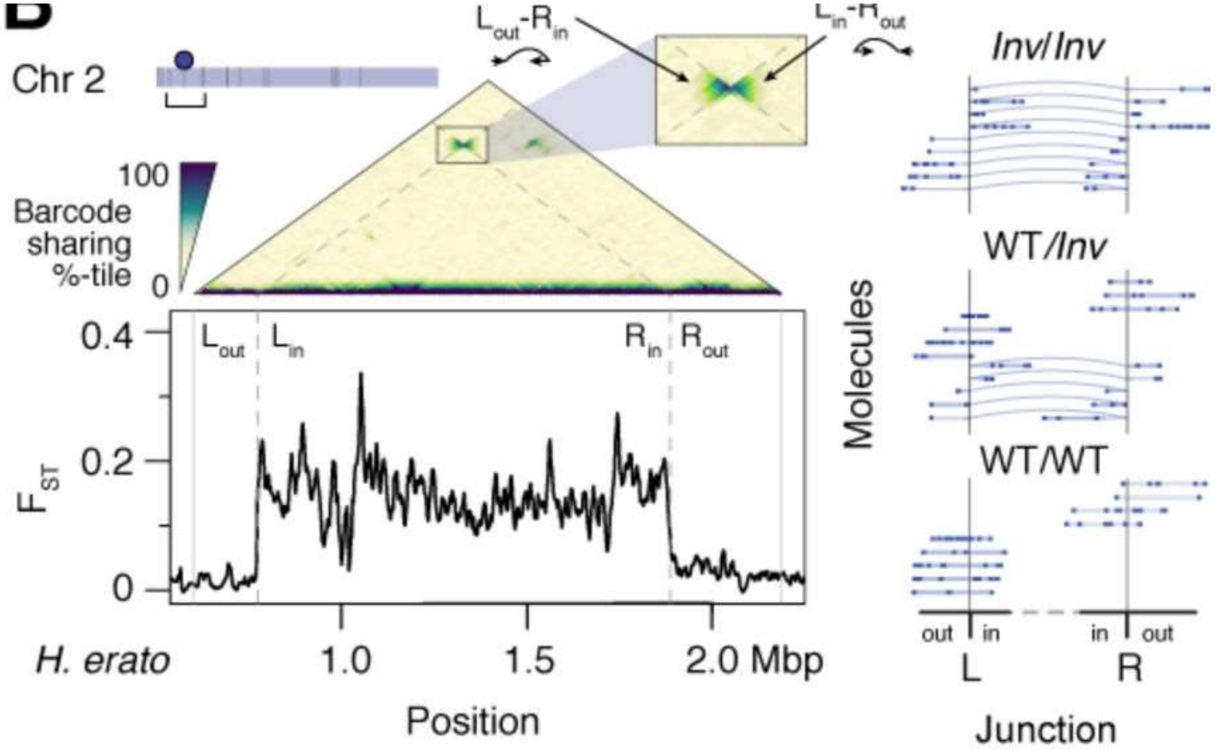
Direct detection: Connected-molecule strategies

Linked-reads

⇒ Medium and large inversions & indels

- Example:

Inversion detection in *Heliconius* butterflies



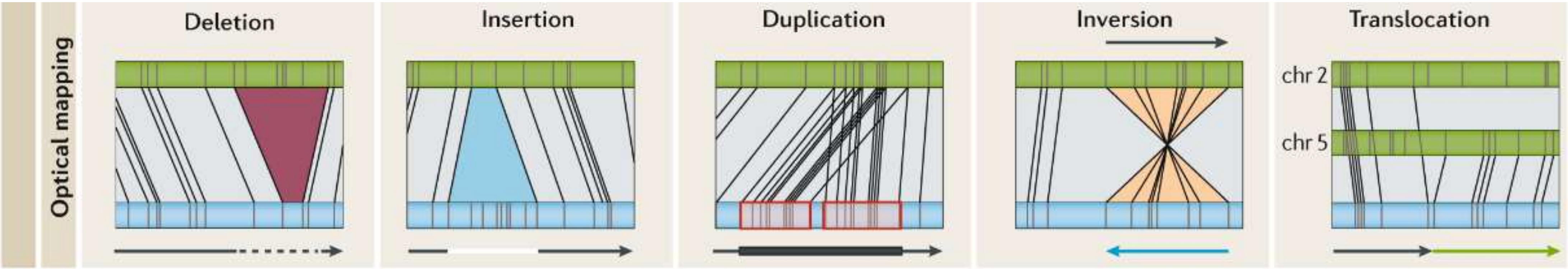
Meier et al bioRxiv 2020
https://doi.org/10.1101/2020.05.25.113688

Direct detection: genetic maps

Optical maps (BioNano)

- Maps the location of restriction enzyme sites along the chromosomes

⇒ Good for detecting large rearrangements encompassing several sites



H et al . *Nat Rev Genet* (2020). https://doi.org/10.1038/s41576-019-0180-9

Direct detection: genetic maps

Linkage maps (based on families)

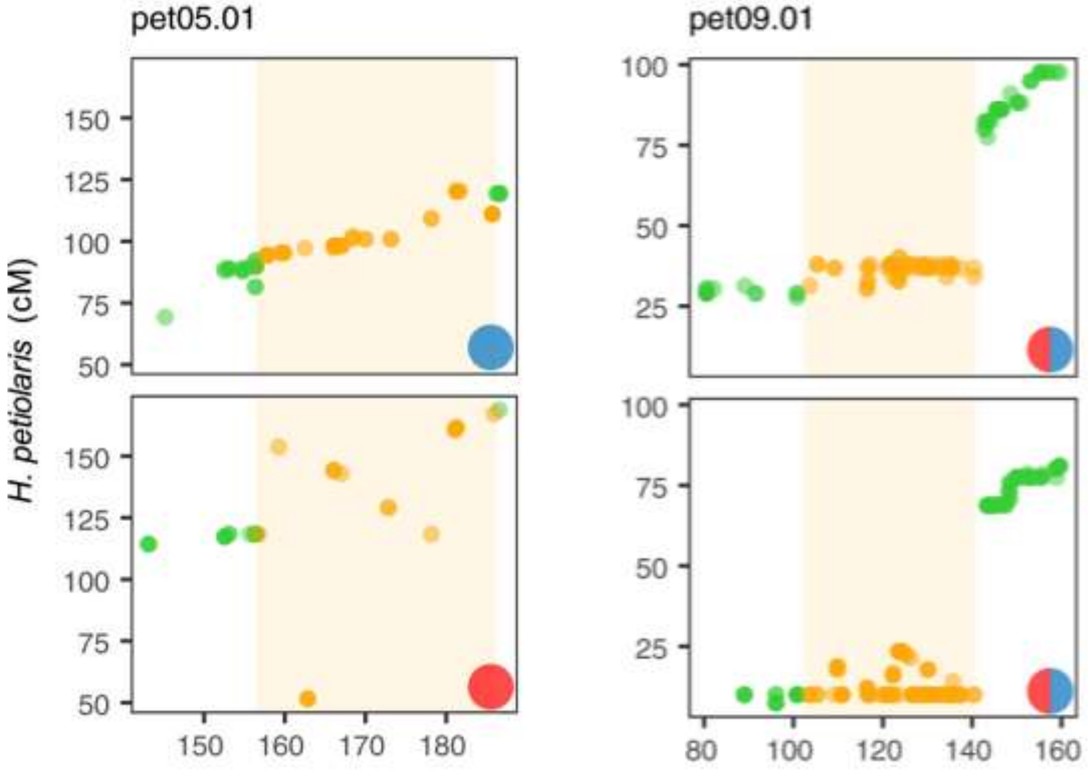
- compare marker position between families or between one family and reference genome

- Easy even on very divergent species

⇒ will detect large rearrangements, including inter-chromosomal fusion, translocation, etc

Homozygotypic parents
-> order is inversed

Heterozygotypic parents
-> no recombination



Huang et al 2020 MolEcol
https://doi-org/10.1111/mec.15428

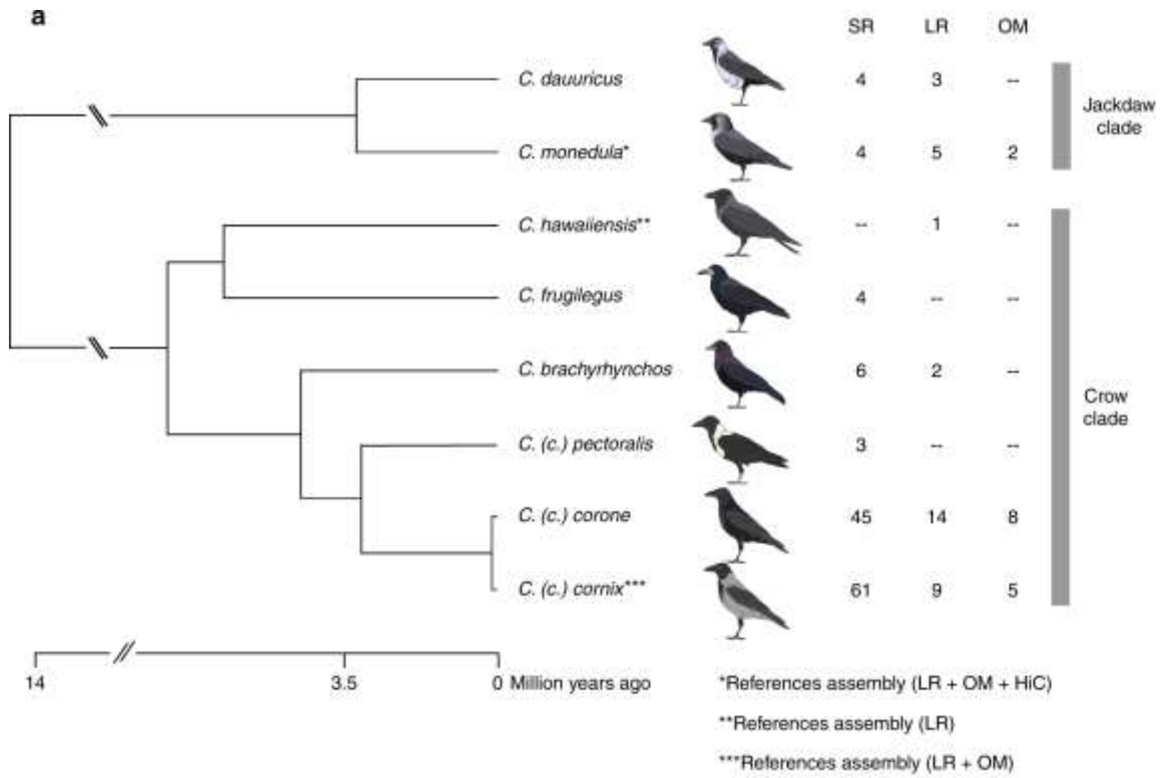
Direct detection: genome comparison

Except for highly repetitive regions, assembly-based SV identification is accurate but expensive due to the requirement of high sequence coverage.

⇒ Will typically be done only on a limited number of samples (for instance 1 sample per species)

Direct detection : combining platforms

Short-reads Long-reads Optical Mapping



	SR	LR	OM
C. dauuricus	4	3	--
*C. monedula**	4	5	2
*C. hawaiiensis***	--	1	--
C. frugilegus	4	--	--
C. brachyrhynchos	6	2	--
C. (c.) pectoralis	3	--	--
C. (c.) corone	45	14	8
*C. (c.) cornix****	61	9	5

*References assembly (LR + OM + HiC)
**References assembly (LR)
***References assembly (LR + OM)

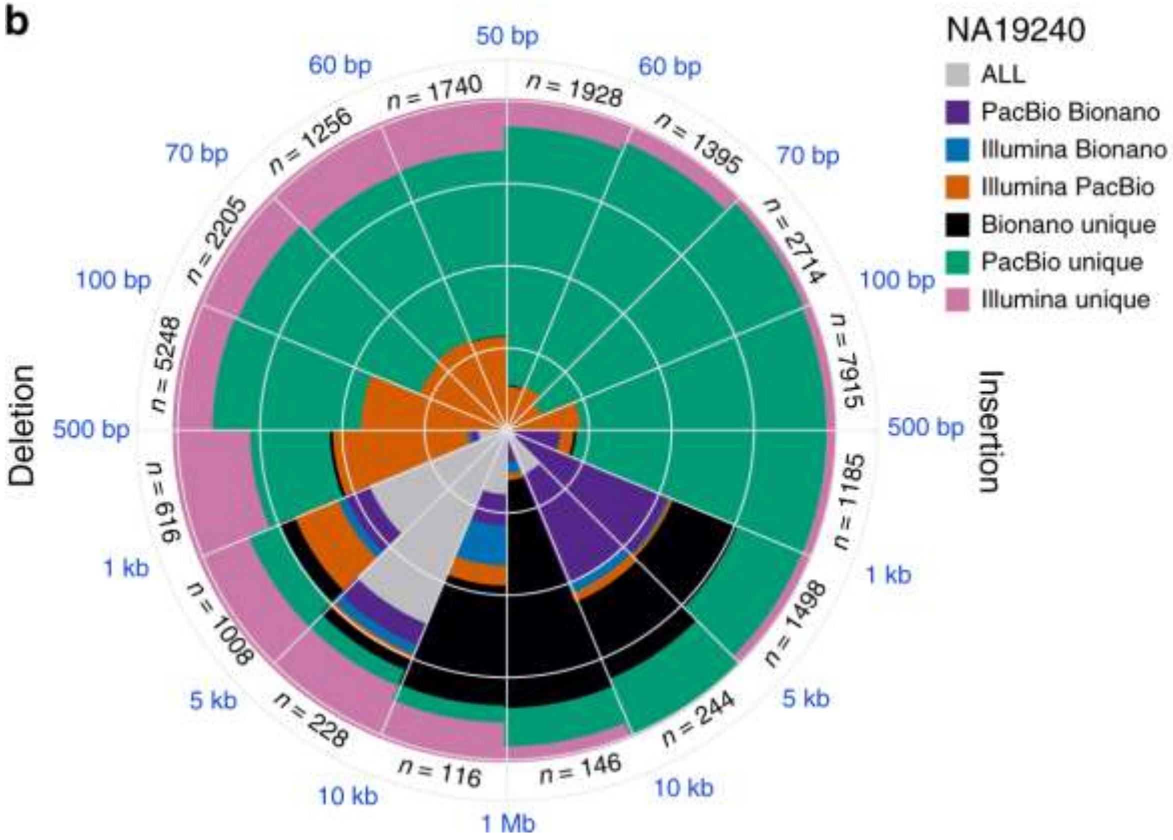
Long-reads/optical mapping
-> a few individuls per species

Short-reads
-> many individuals (pop genomics)

Weissensteiner et al *Nat Comm* https://doi.org/10.1038/s41467-020-17195-4

Direct detection : combining platforms

Different platforms detect indels of different sizes



In humans
Chaisson et al, 2019, Nat Comm
https://doi.org/10.1038/s41467-018-08148-z

10kb->1MB: Bionano

20bp -> 1kb illumina + PacBio

Short-reads only : just a fraction of Sv, more deletions tahn insertions

Direct detection : combining platforms

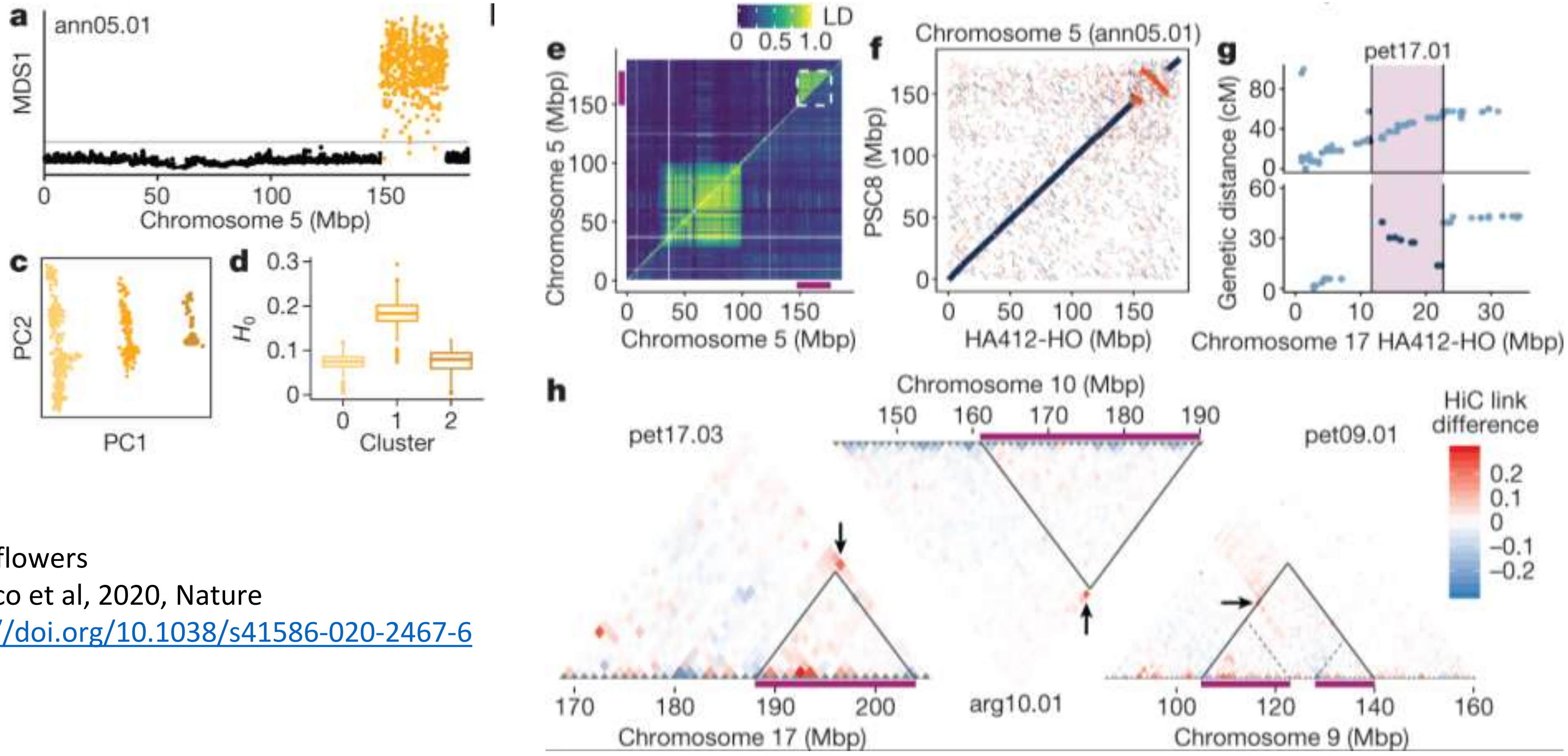
Indirect detection (local PCA)

Indirect detection (LD)

Direct detection (genome comparison)

Direct detection (genetic maps)

Direct detection (Hi-C)



In Sunflowers
Todesco et al, 2020, Nature
https://doi.org/10.1038/s41586-020-2467-6

Summary

- Structural variation has been systematically missed

- Previous technologies missed most of the SVs due to technical limitations.

- The majority of SVs are novel and rare variants, implicating that structural variation databases are not saturated yet

We can detect SV… now what?!
=> Why does it matter to understand adaptation?

- Avoid misinterpretation:
  - Large rearrangements can drive artefactual population structure
  - Not the same interpretation if an islands of divergence is an inversion or not…

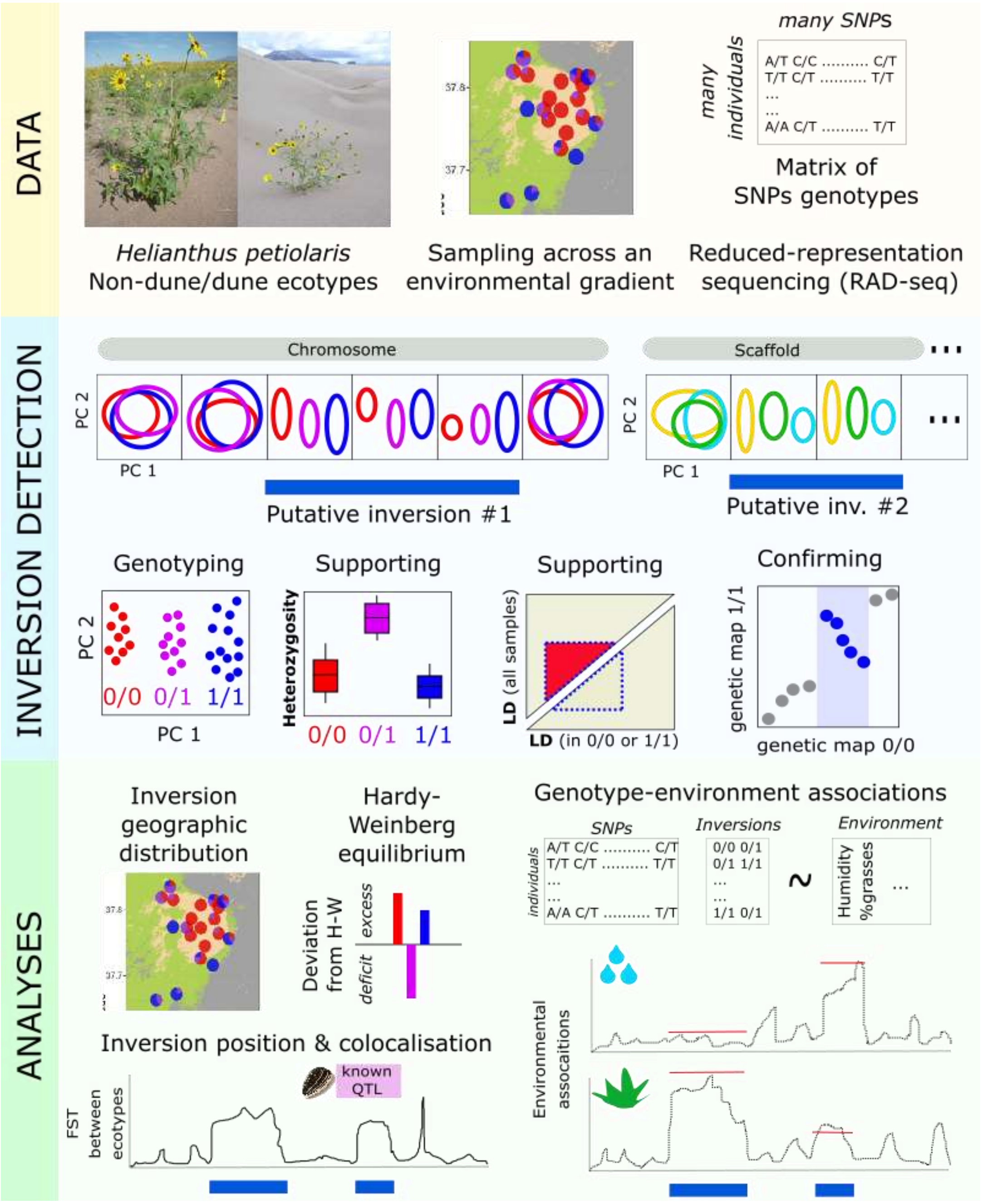
- Test the role of SV in adaptation
  - Evidence of adaptive SV are anecdotical…
  - Can we test which SV are putatively adaptive as we did on SNPs?

⇒ Need of methodological development

SV and adaptation genomics

Previously identified « islands of divergence »… are now identified as inversions

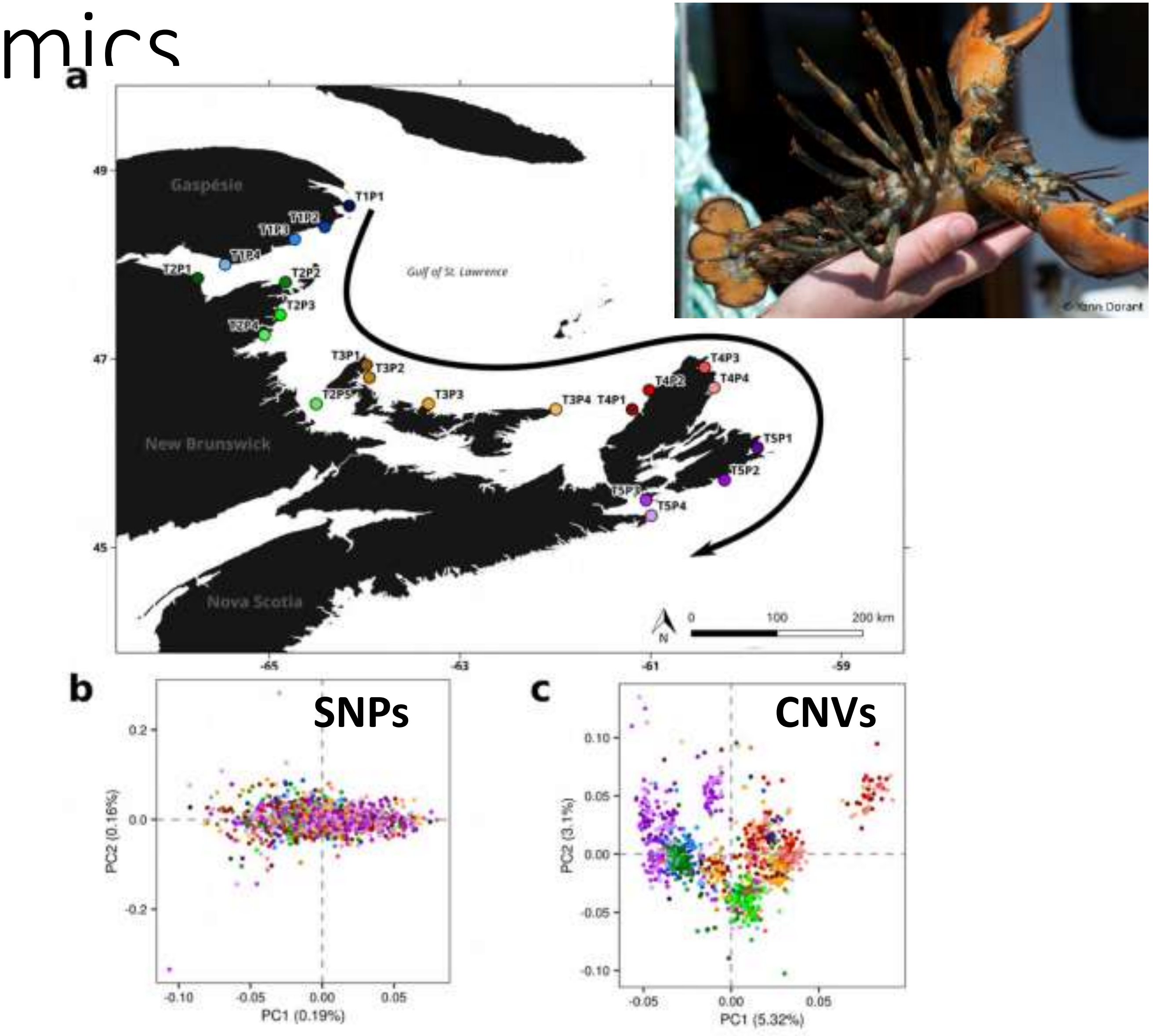
Analyse SV within population genomics or landscape genomics frameworks?



Mérot, 2020, Mol Ecol

Huang *et al* 2020, Mol Ecol

SV and adaptation genomics



Use SV as a different kind of markers?

In the American Lobster, fine-scale structure and adaptation are better described by CNVs than by SNPs

Dorant et al. (2020) Mol Ecol

Remaining challenges

- Large repetitive regions remain inaccessible due to constraints of read length and sequence composition

- Statistical tools for population genomics, adaptation genomics, ecological genomics are based on SNPs