Genetic diversity in *Brassica rapa.*

Domestication and morphological traits

6-12-2012, Guusje Bonnema

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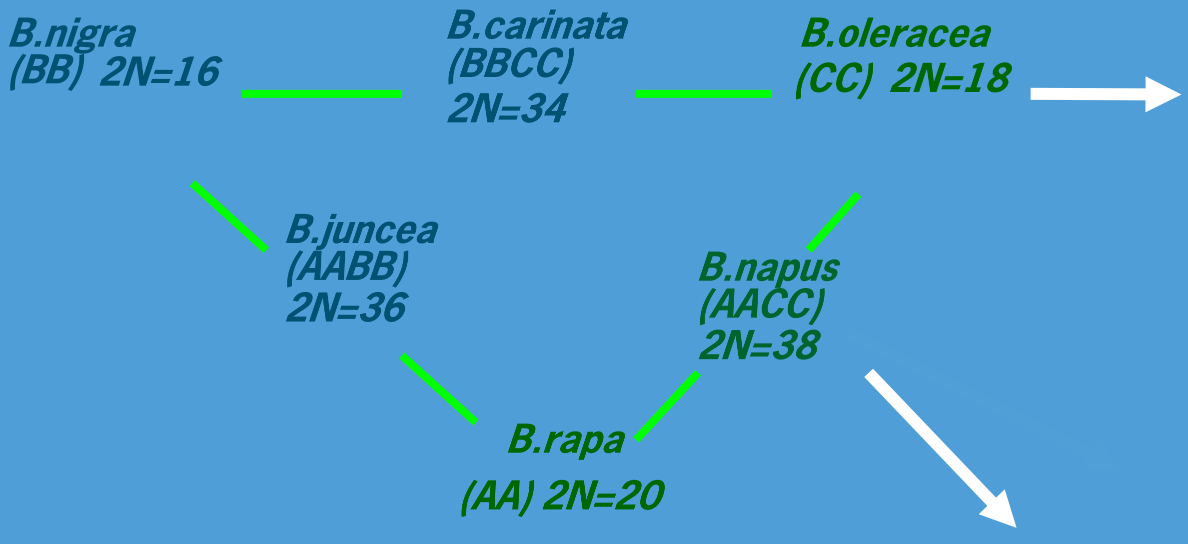
Brassica species: vegetable and oil crops; enormous variation



B.nigra
(BB) 2N=16

B.carinata
(BBCC)
2N=34

B.oleracea
(CC) 2N=18

B.juncea
(AABB)
2N=36

B.napus
(AACC)
2N=38

B.rapa
(AA) 2N=20

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Strategy: exploitation of genetic variation

Agronomic traits/ developmental biology

Transcript profiling

Metabolite profiling

Genome sequences

Arabidopsis

B. oleracea (CC)

B. napus (AACC)

core collection (DFFS)

Populations (DH, BILs, F2)

MBGP

Nutritionists

Product quality, chain

(a)biotic interactions

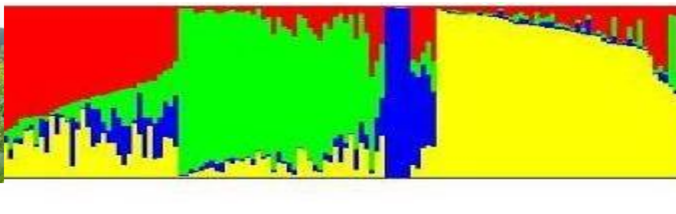
QTL mapping

Genetical genomics

Association mapping;

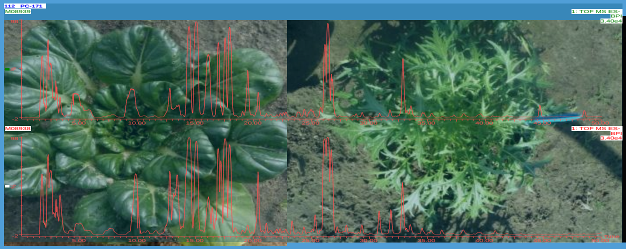
PSA program

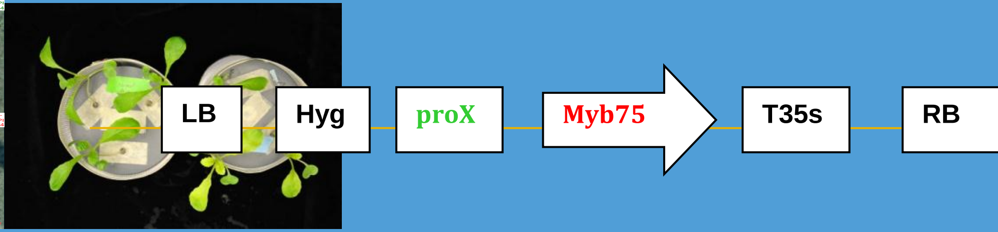
Material and Markers Genetic populations



Developmental Traits

Health/metabolites Food / Soil





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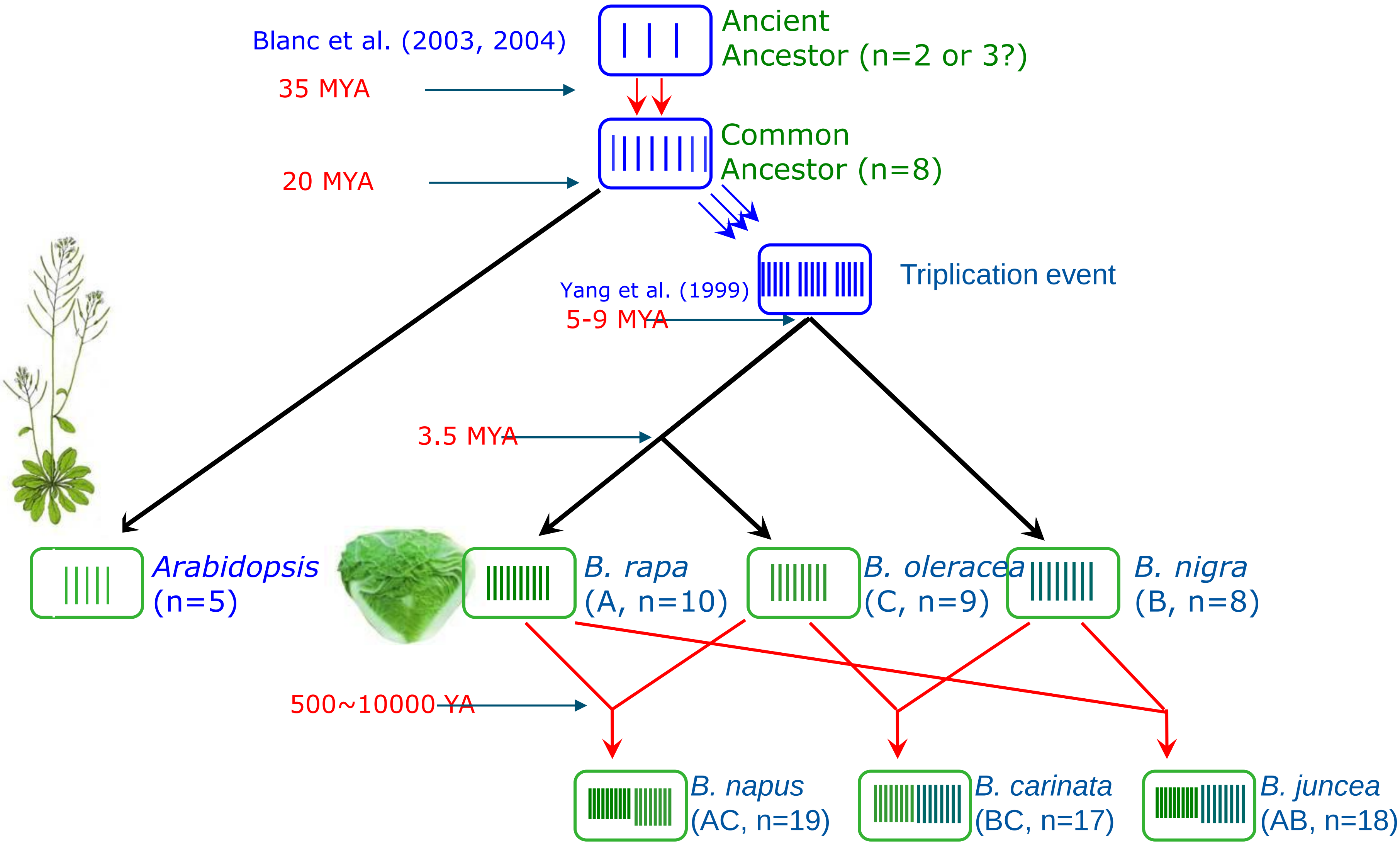
CAAS



Blanc et al. (2003, 2004)

35 MYA

Ancient Ancestor (n=2 or 3?)

20 MYA

Common Ancestor (n=8)

Triplication event

Yang et al. (1999)

5-9 MYA

3.5 MYA

Arabidopsis (n=5)

B. rapa (A, n=10)

B. oleracea (C, n=9)

B. nigra (B, n=8)

500~10000 YA

B. napus (AC, n=19)

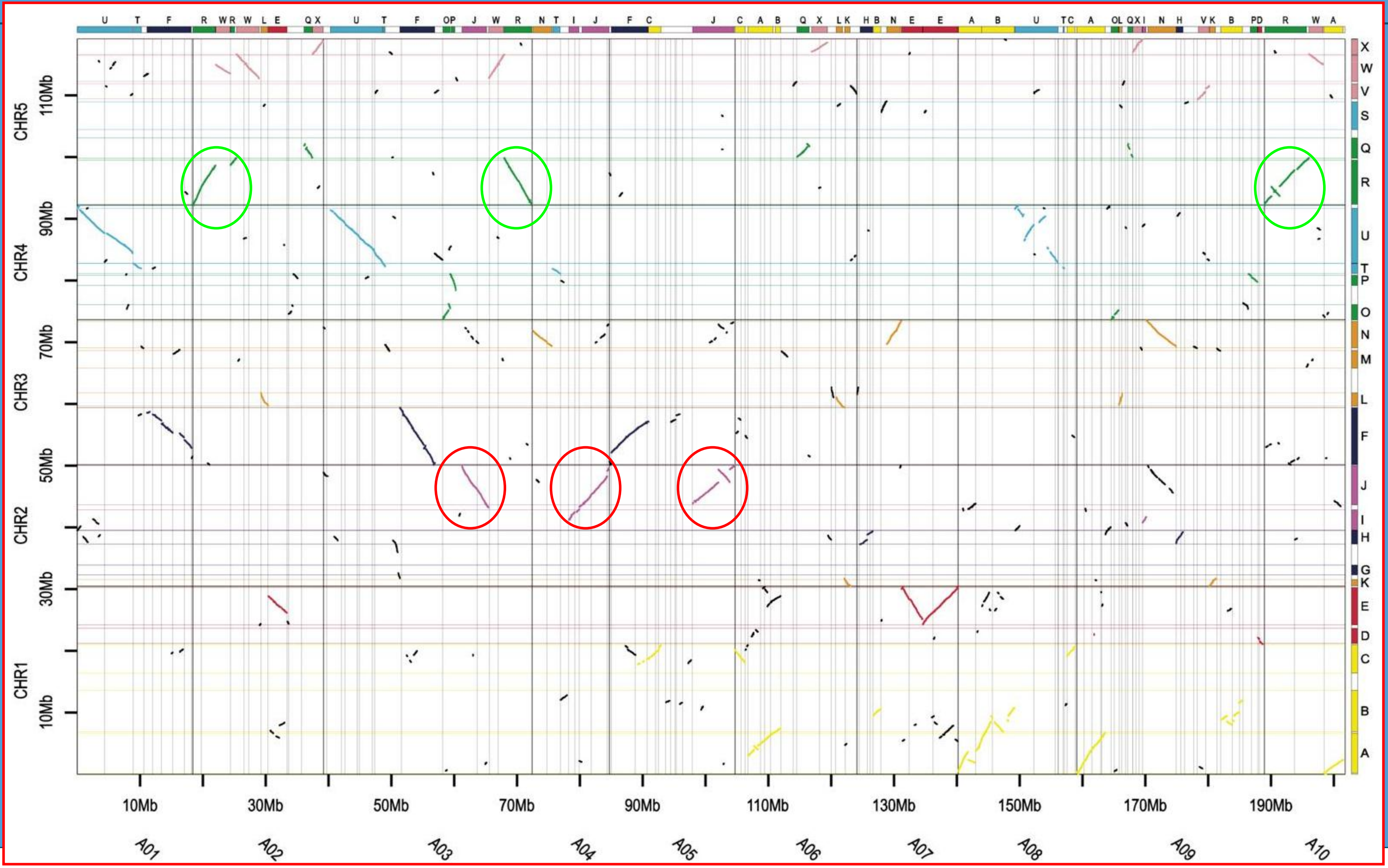
B. carinata (BC, n=17)

B. juncea (AB, n=18)

(from Lim and Wang)

B. rapa genome sequence: Triplicate blocks are identified

Arabidopsis



B. rapa

(WANG ET AL. 2011; NATURE GENETICS

Retention patterns of genes duplicated by polyploidy

- *A. thaliana* 30.000
    - *B. rapa* not 3 x 30000 genes, however genome fractionation by gene loss:
- *B. rapa*: 42000
    - 64 (0.4%) *A. thaliana* genes have more than 3 orthologs in *B. rapa*,
    - 578 (9.8%) have three syntenic orthologs.
    - 5,258 (33.1%) have two syntenic orthologs
    - 9,175 (56.7%) *A. thaliana* genes 1 syntenic ortholog in *B. rapa*,
- genome triplication may have expanded gene families that underlie environmental adaptability as observed in other polyploidy species.
- 214 flowering time genes in Arabidopsis
    - 422 paralogous in *B. rapa*
        - 56 (26%)≥3; 67 (31%) 2; 66 (31%) 1; 25 (12%) 0

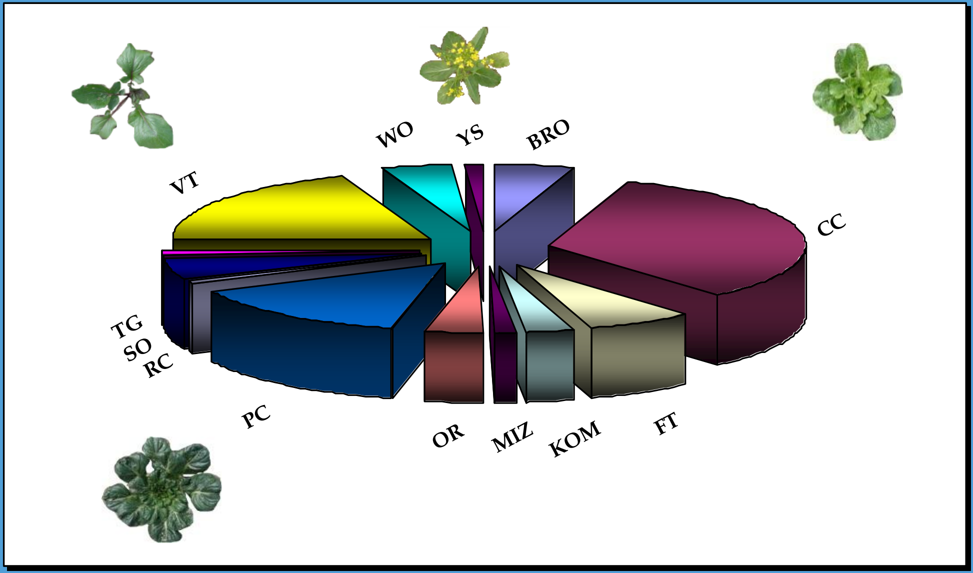
Wide variation in morphological traits

B. rapa core collection

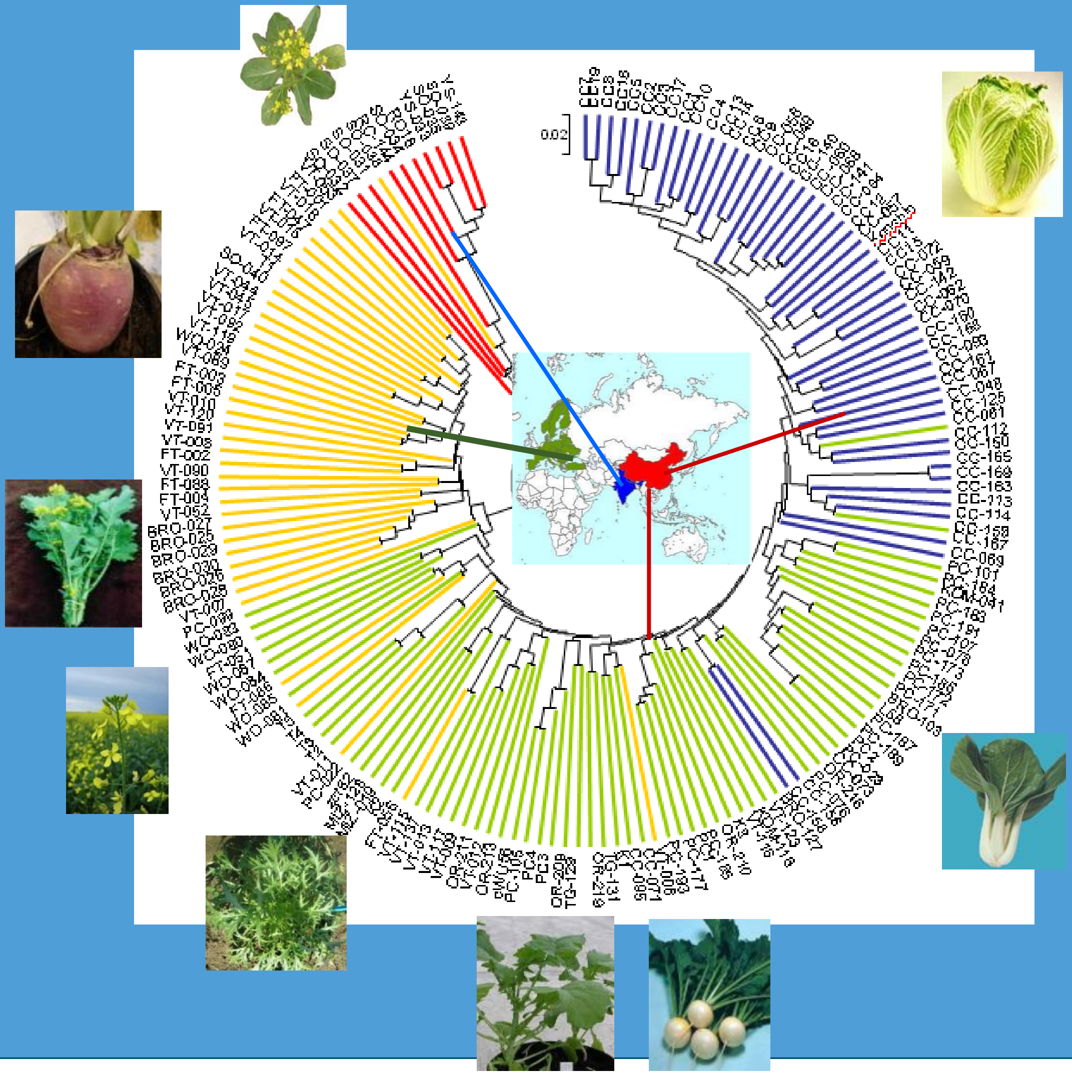
168 *Brassica rapa* accessions

137 public seed bank

31 lines from companies



Genetic diversity in *Brassica rapa*



- Genetic distance more related to geographical origin (East Asia vs. Europe) than to morphotypes

- Suggesting that different morphotypes evolved or domesticated independently in Asia and Europe

Zhao et al, 2005;2010
Pino Del Carpio et al, 2011

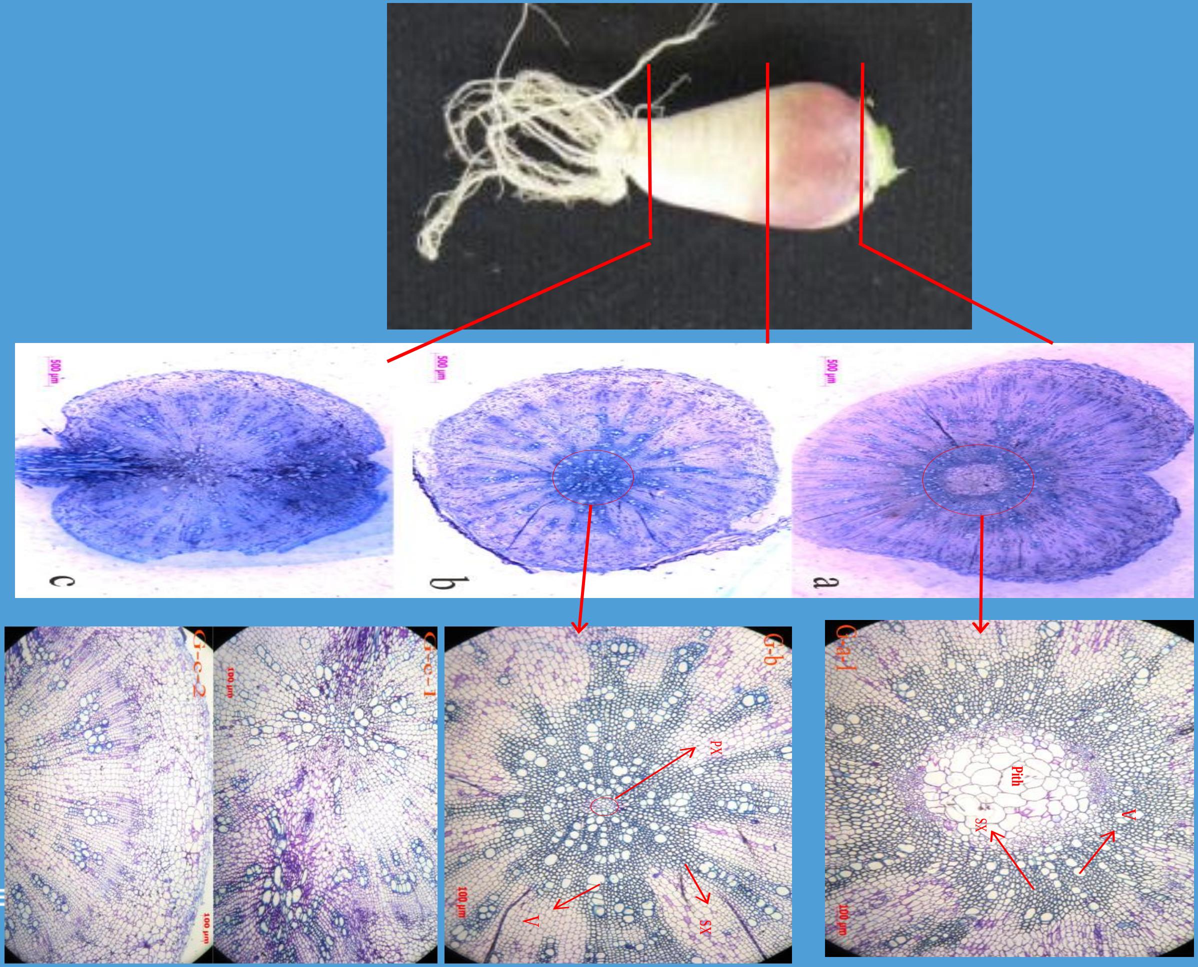
Why are there so many diverse morphotypes in Brassica species?

B. rapa turnip (equilvalents in *B. oleracea* kohlrabi and *B. napus* swede)
- Morphology: what is a turnip?
- Physiology (induction turnip formation in tissue culture)
- Quantitative genetics
- Resequencing strategy

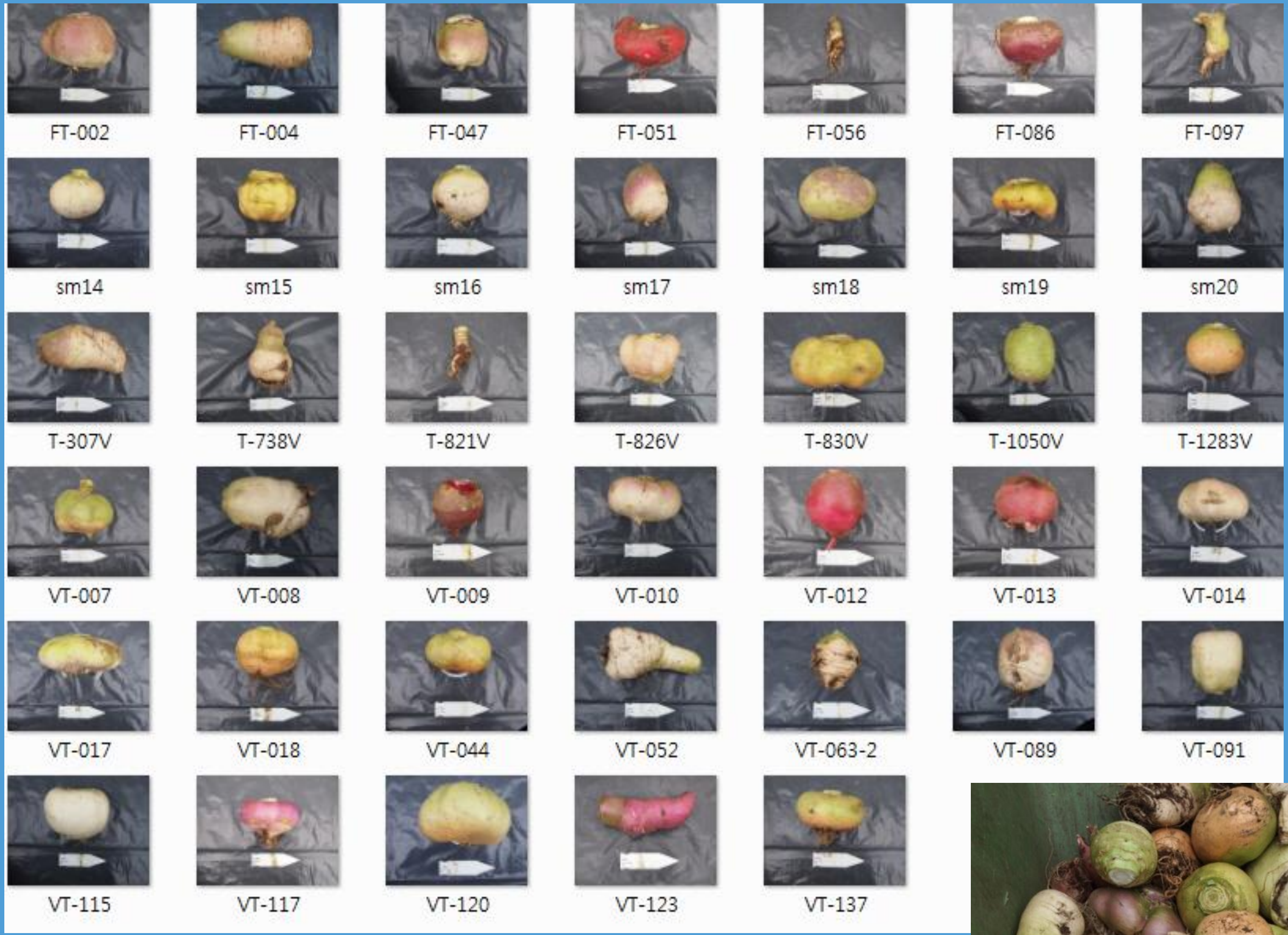
Chinese cabbage (equivalent in *B. oleracea* the cabbages)
- Leaf folding
- Quantitative genetic strategy
- Resequencing strategy (many accessions to low depth)

Turnip: genetic regulation??

- Turnip is a combination of thickened stem and root, which differs from well studied potato tubers (stem) and sugar beet (root), but may share quite some similarities



Diversity in turnip collection

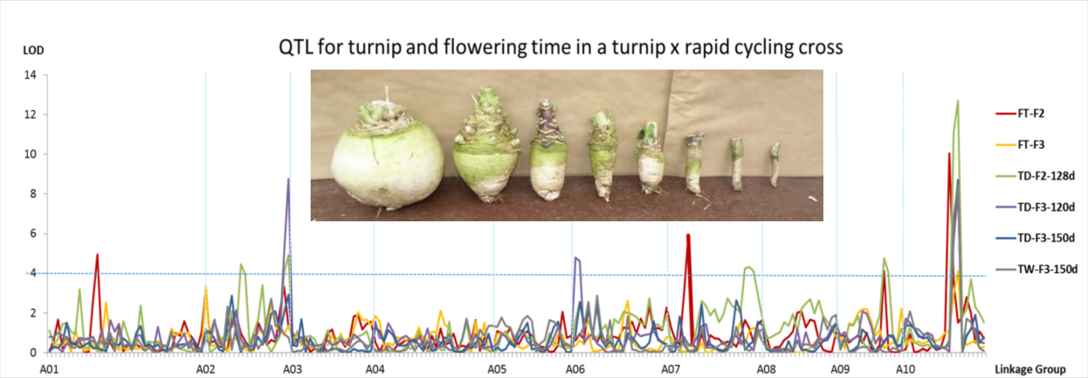


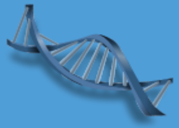
Different morphology

Different metabolic composition

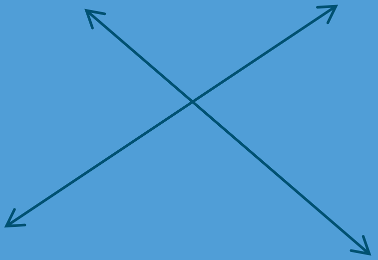
Different sugar/starch content

Genome re-sequence, transcriptome and gene targeting

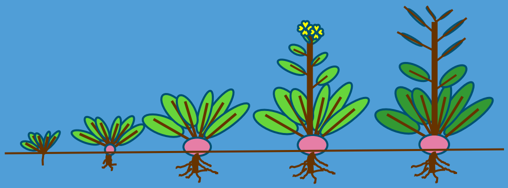




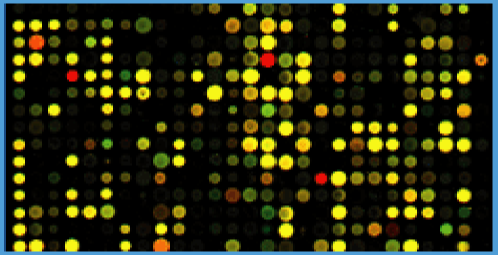
Validate gene annotation via RNA-seq



Genome comparison for turnip specific genes



Expression profiling via customized 60K microarray



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RIL (recombinant inbred lines) development

X

F1 ⓧ **…** ⓧ **F7 or F8**

F8 plants (148 lines)+ F7 (57 lines)

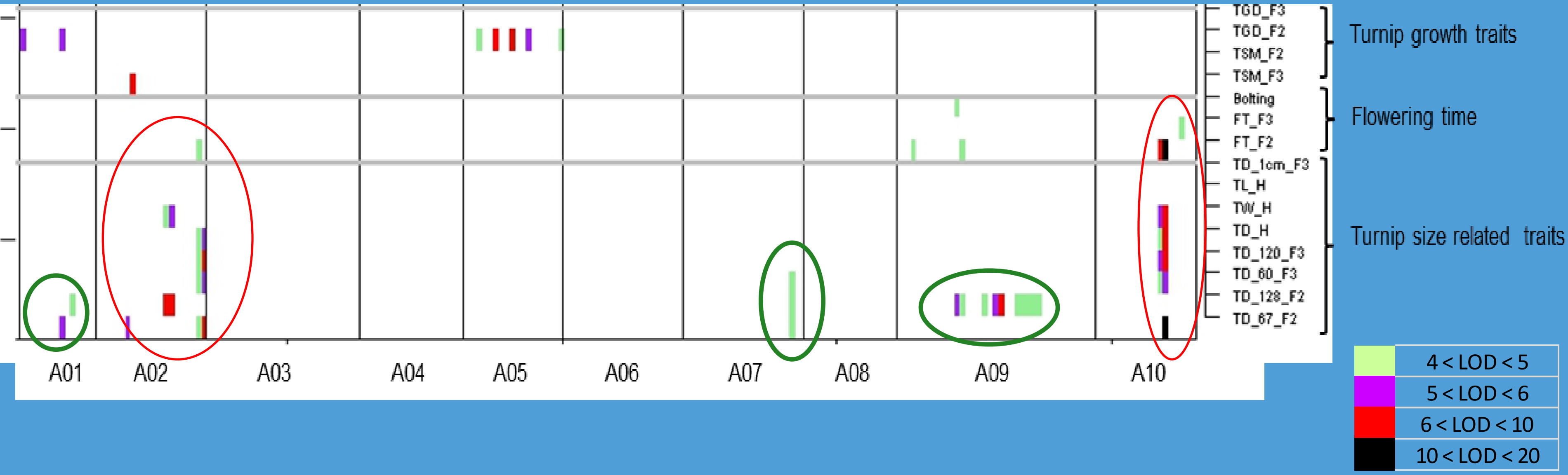
Homozygous lines &
Replicated phenotyping

X

→ **F1** ⓧ **…** ⓧ **F7 or F8**

F5 seeds (110 lines)

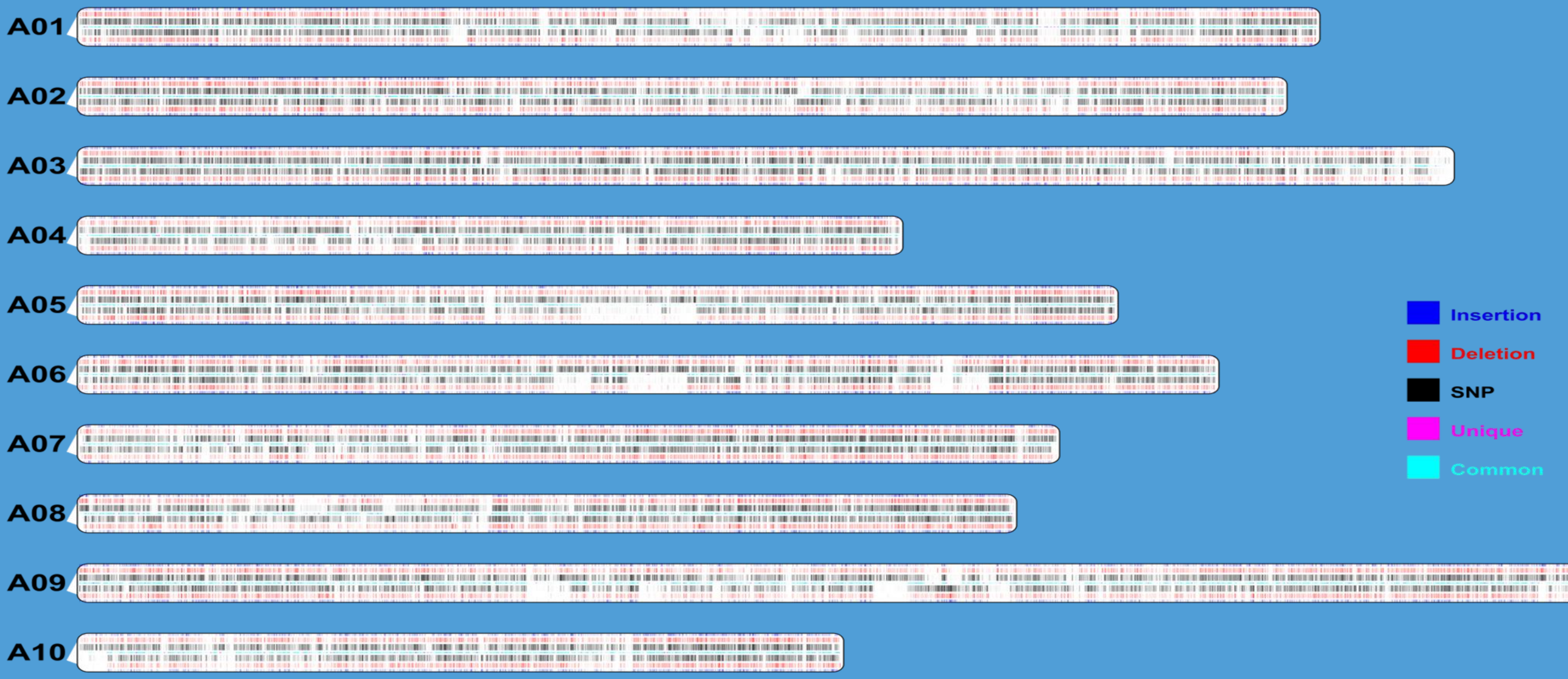
QTL mapping using VT-115 x RC-144



Whole genome comparison and functional annotation

- One Japanese Turnip and one Rapid Cycling was resequenced (27x)
- differences CC-RC > CC-T (1,137,859 and 1,309,157)
- Reconstructed reference genomes were reannotated
  - 1118, 1464 and 1090 unique genes in T, CC and RC
  - 38,186 genes present in all three genomes
- The divergence date among the *B. rapa* morphotypes estimated as 0.259 MYA
- much earlier than domestication (5-10.000 YA).

Sequence variation RC-CC; T-CC



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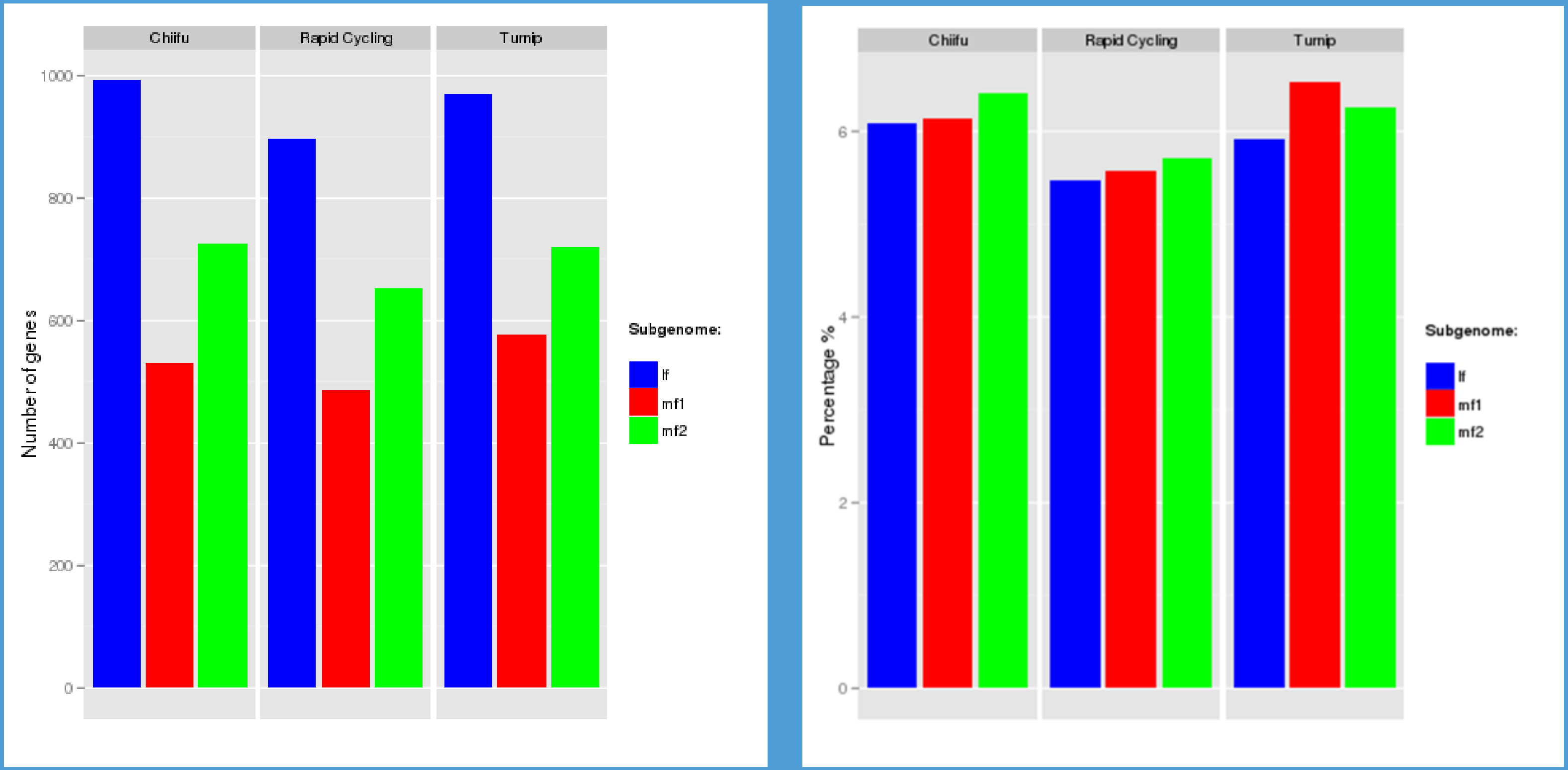
Gene loss



Dispensable and unique genes’ counted per Subgenome in CC, RC and T.
(a)Number of genes
(b)Frequency of gene counts
lf: least fractioned subgenome. mf1: medium fractioned subgenome1. mf2: most fractioned subgenome 2

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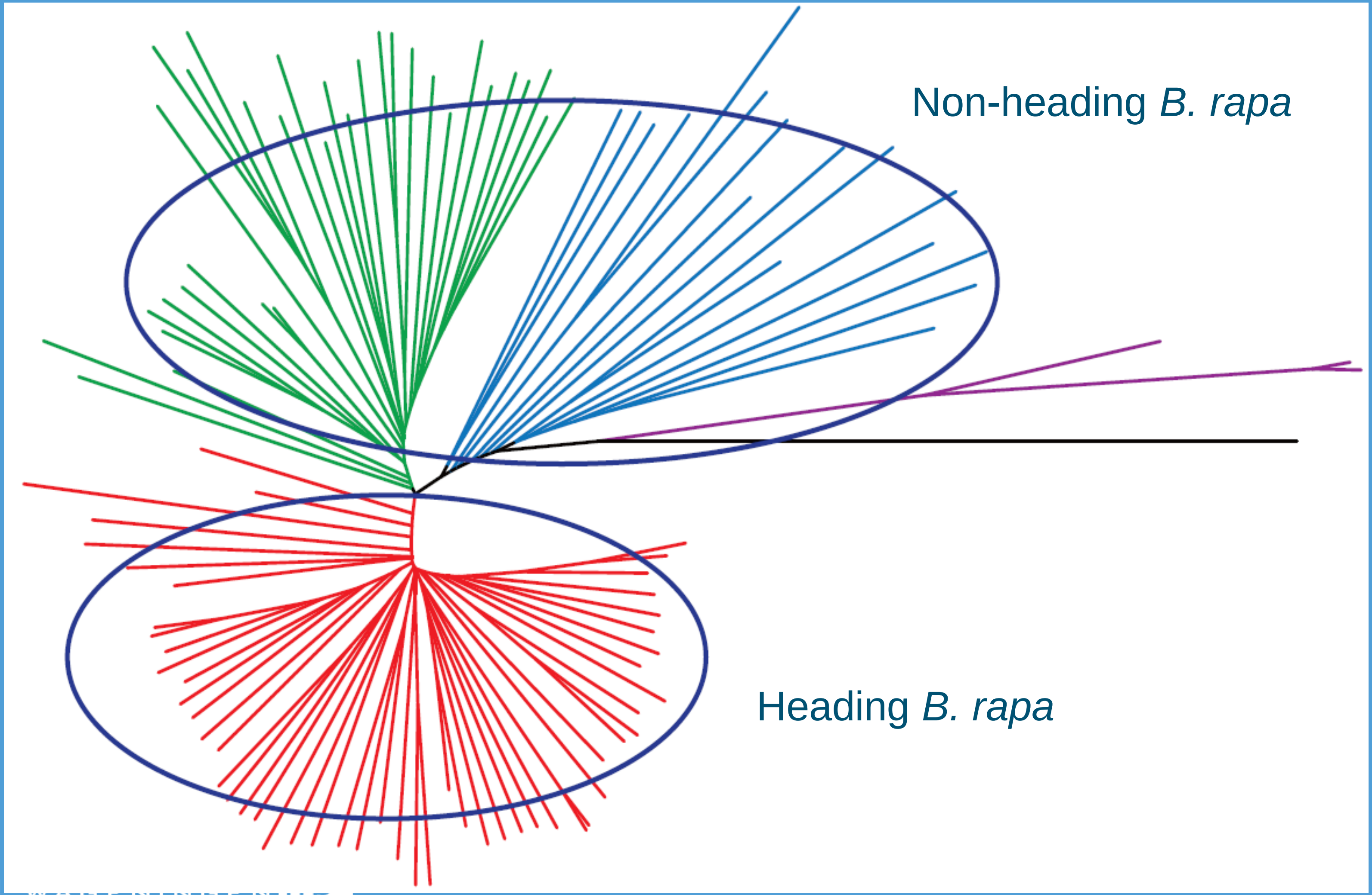
Can we pinpoint causal genes for turnip?

- GO term enrichment analysis can visualise which GO terms are specific to each genotype
- In turnip, the cellular component term “peroxisome” was over enriched compared to CC and RC (peroxidases)

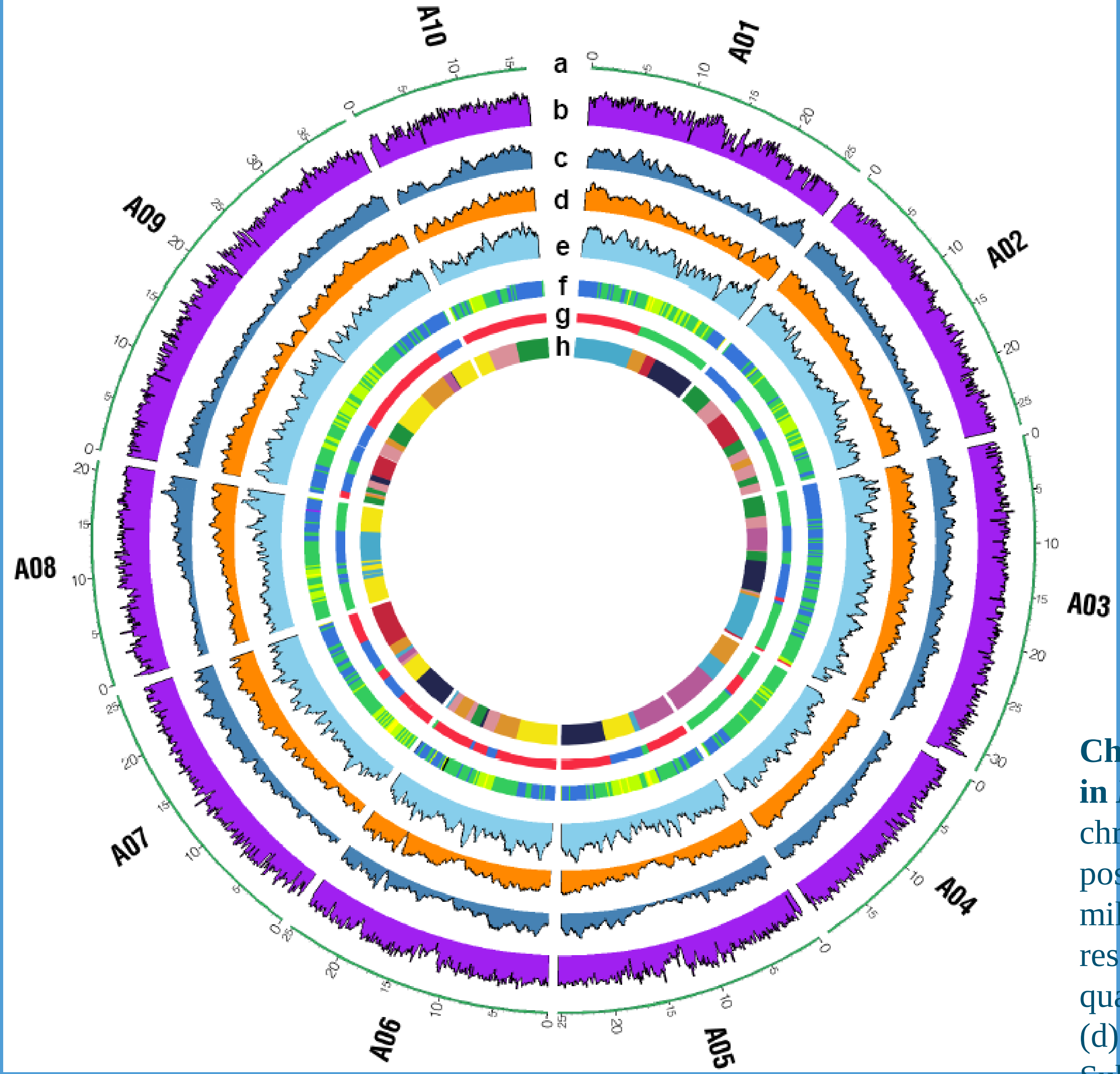
- We will combine reseq data with RNA seq data of diverse organs
- We will check whether turnip specific changes are common to other turnips (Asia and Europe)
- We will also look at peroxidases in potato and tomato.

Genetics of heading; Dr Wang and Dr Cheng, IVF-CAAS

Phylogeny tree



Phylogeny tree built based on the SNP data



Chromosomal distribution of the variations in *B. rapa* populations. (a) The 10 chromosomes of B. rapa, and the physical position along each chromosome in units of million bases; (b) The heterozygosity of the resequenced *B. rapa* population. Area charts quantify the functional SNPs/InDels (c), InDels (d), SNPs (e). (f) Heat map for gene density. (g) Subgenome partition in *B. rapa* genome, red, green, and blue corresponding to subgenomes LF, MF1, and MF2, respectively. (h) The triplicated 24 genomoc blocks in *B. rapa*, colors of blocks were defined elsewhere

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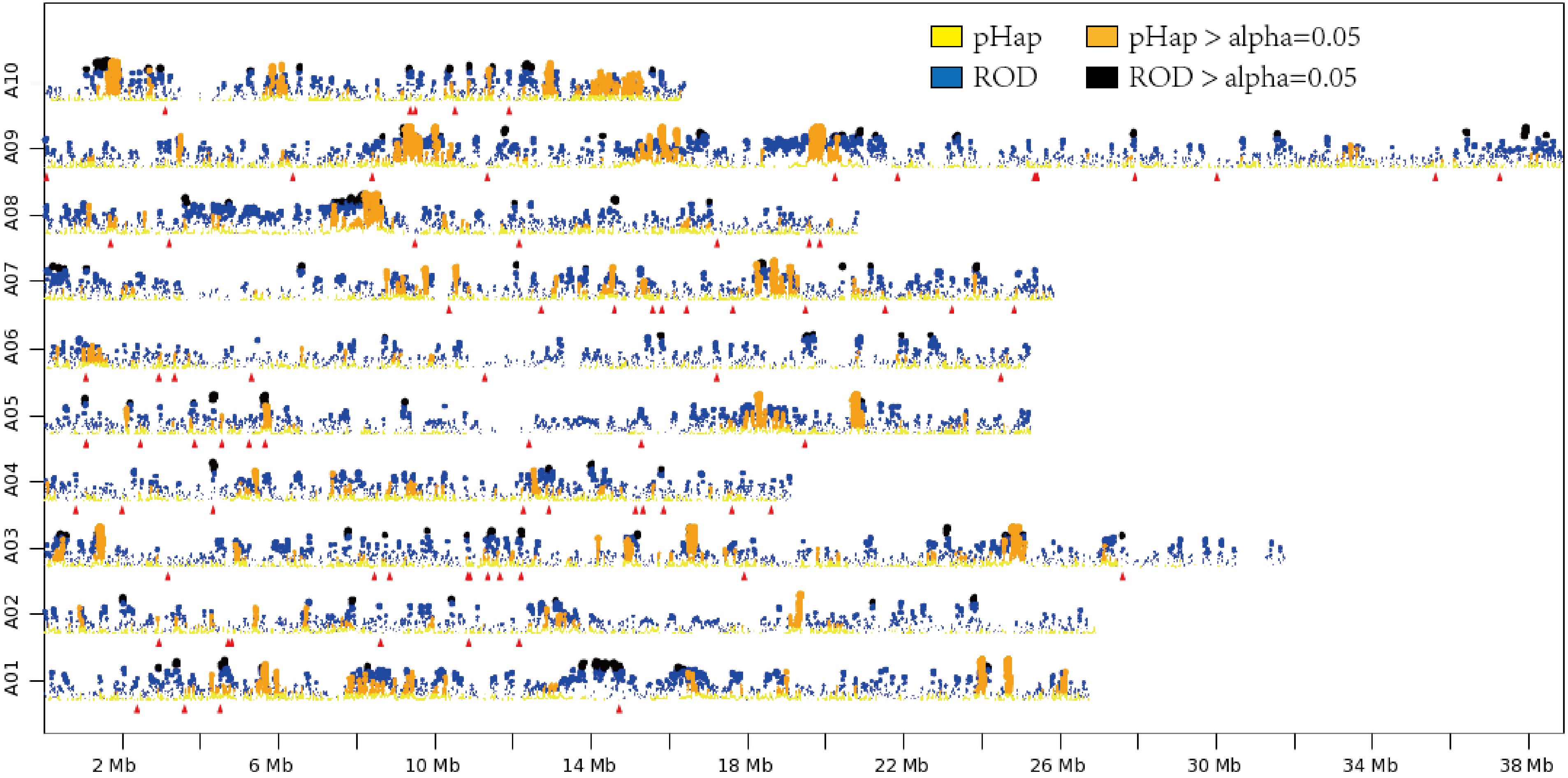
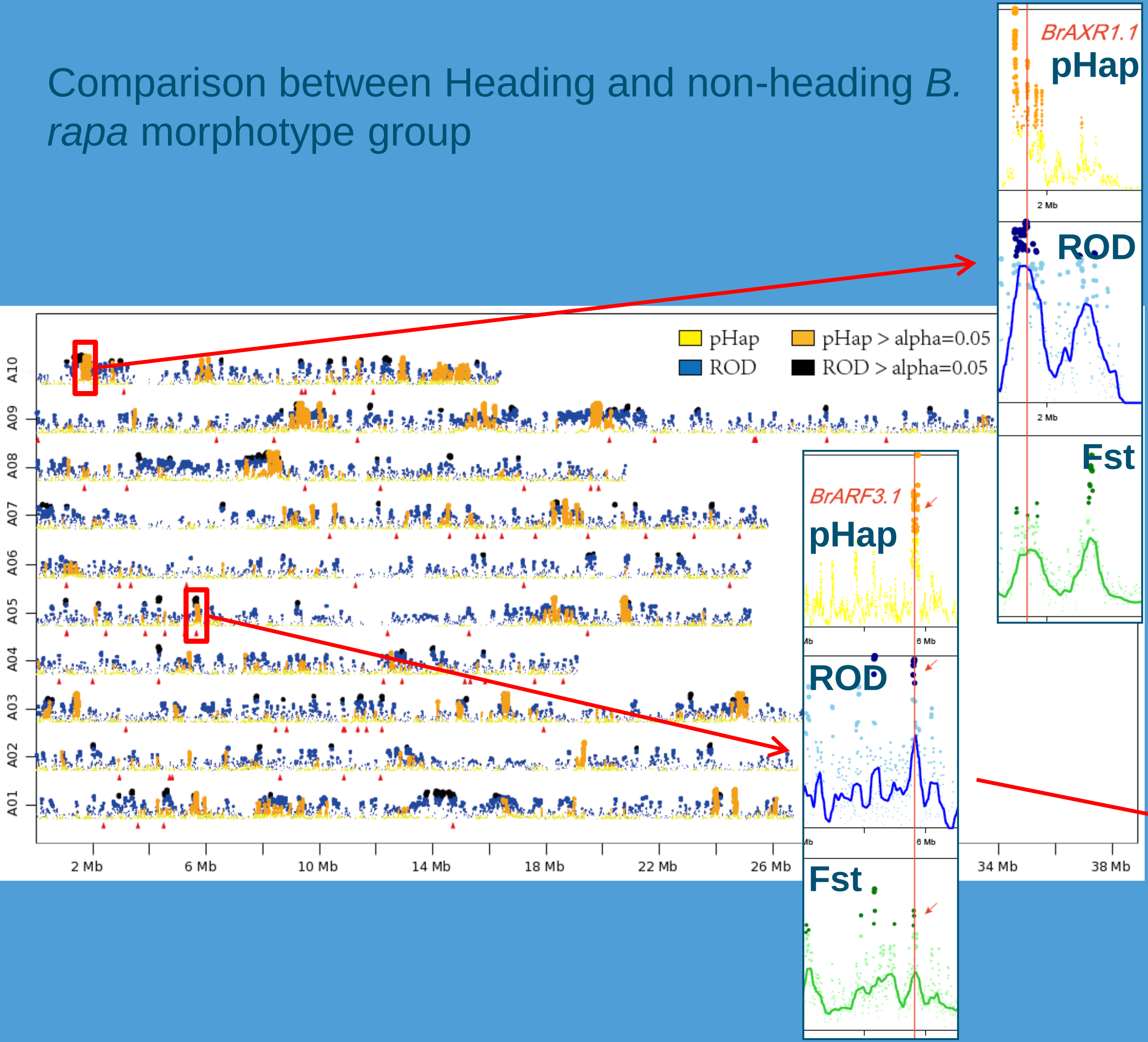
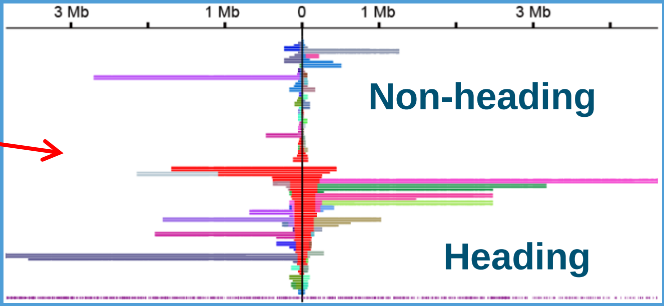


Selection signals detected by pHap, ROD across 10 chromosomes of *B. rapa*. Values of pHap, ROD were plotted in colors of yellow and skyblue, respectively. Outliers (empirical alpha=0.05) of pHap and ROD were plotted with colors of orange and darkblue. p-iHS was calculated for each core SNP across the chromosome, while ROD were calculated with a 30kb window and 10kb step sliding the whole chromosome.

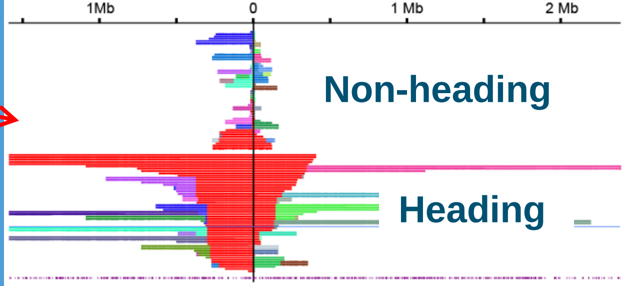
Comparison between Heading and non-heading *B. rapa* morphotype group



Haplotype extending



Haplotype extending



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Conclusions

- Genetic distance more related to geographical origin (East Asia vs. Europe) than to morphotypes
- Genetic variation within morphotypes larger (90%) compared to variation between types (19%)
- Resequencing led to identification of unique and dispensable genes in Turnip: additional experiments needed to select geness for further studies (QTL, transcriptomics)
- Shallow re-sequencing strategy of many Chinese cabbages and many non-Chinese cabbages led to identification of selective sweeps harboring genes involved in heading

- Divergence morphotypes (.25MYA) preceeds domestication

Acknowledgements

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Cheng Feng
Dr Jian Wu

IOP

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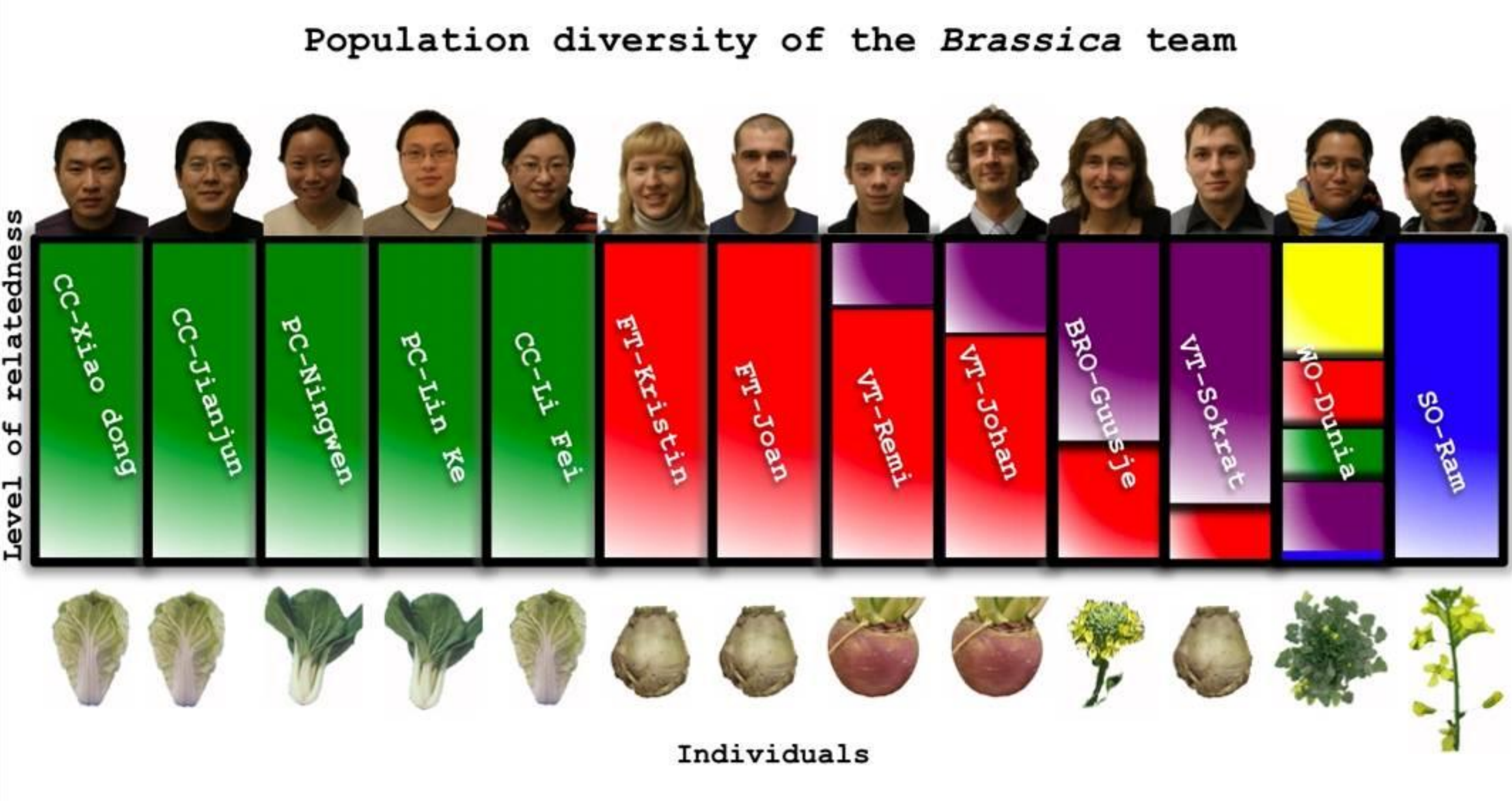
KNAW

Questions?



Population diversity of the *Brassica* team