

Exploring resilience to environmental stress in the wider *Brassica* C genome



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Brassica oleracea is an economically and nutritionally important species, for example more than 20 million tonnes of cauliflower and broccoli alone were estimated to be produced globally in 2011 (FAO-STAT, 2013). Future production rates will increase to accommodate expanding global food requirements. This intensification needs to be sustainable, with reductions in inputs and reduced wastage across the supply chain. To address these challenges, we need to breed resilient *Brassica* vegetable crops that maintain normal vegetative growth when faced with both biotic and abiotic perturbations. We are exploring the use of *Brassica* crop wild relatives as a source of allelic variants that confer resilience to abiotic stress; these variants and the alleles with which they interact may be adapted to improve our domesticated vegetables.

Wide variation in crop wild relatives

Crop domestication and subsequent breeding efforts has resulted in modern crops with a restricted genetic base, sometimes referred to as 'allelic canalisation' (Fig. 1).

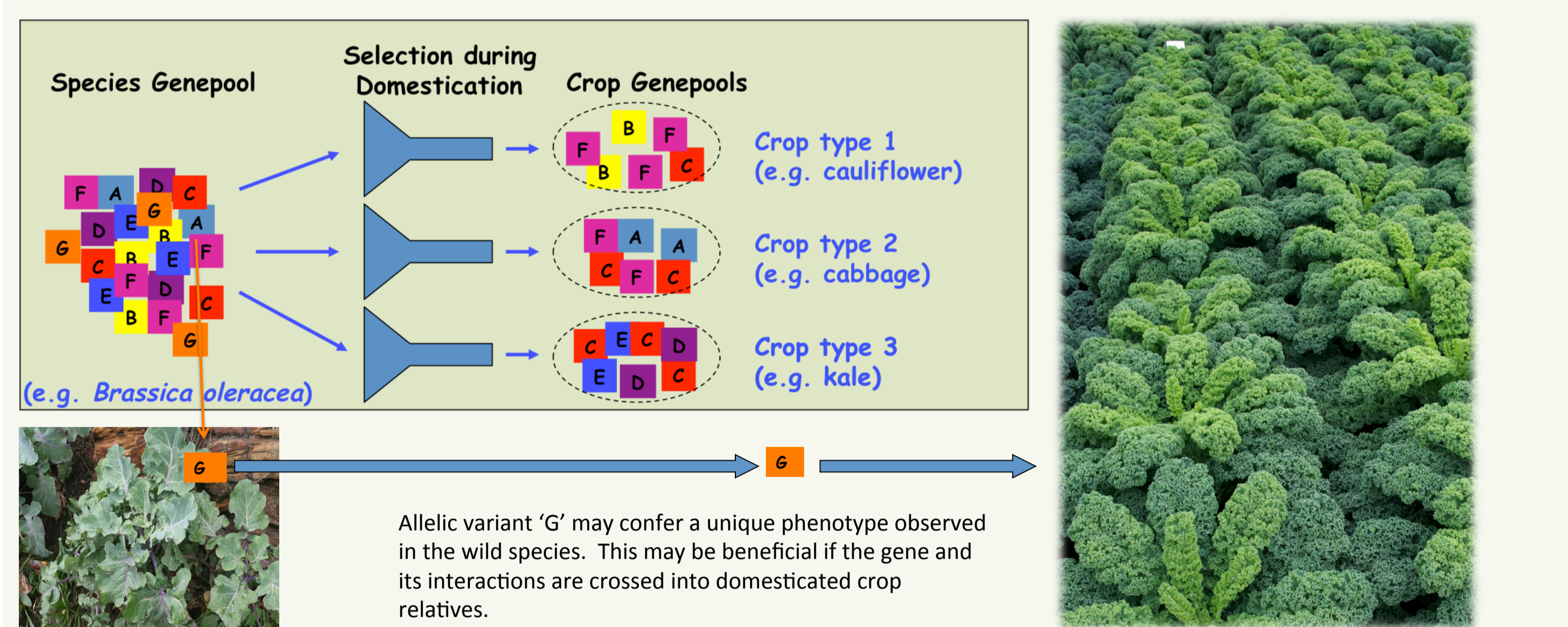


Figure 1. The concept of utilising allelic variation from crop wild relatives. In this example the allelic variant "G" coloured orange, was not take through the domestication bottle-neck. This allele may contribute towards traits such as insect resistance, or tolerance to growth in saline conditions. Once identified this allelic variant can be crossed into our domesticated crop types.

From the large diverse Brassica collection, a smaller core diversity set has been assembled. These lines were crossed to a cultivated type and fixed using microspore culture (Fig. 2).

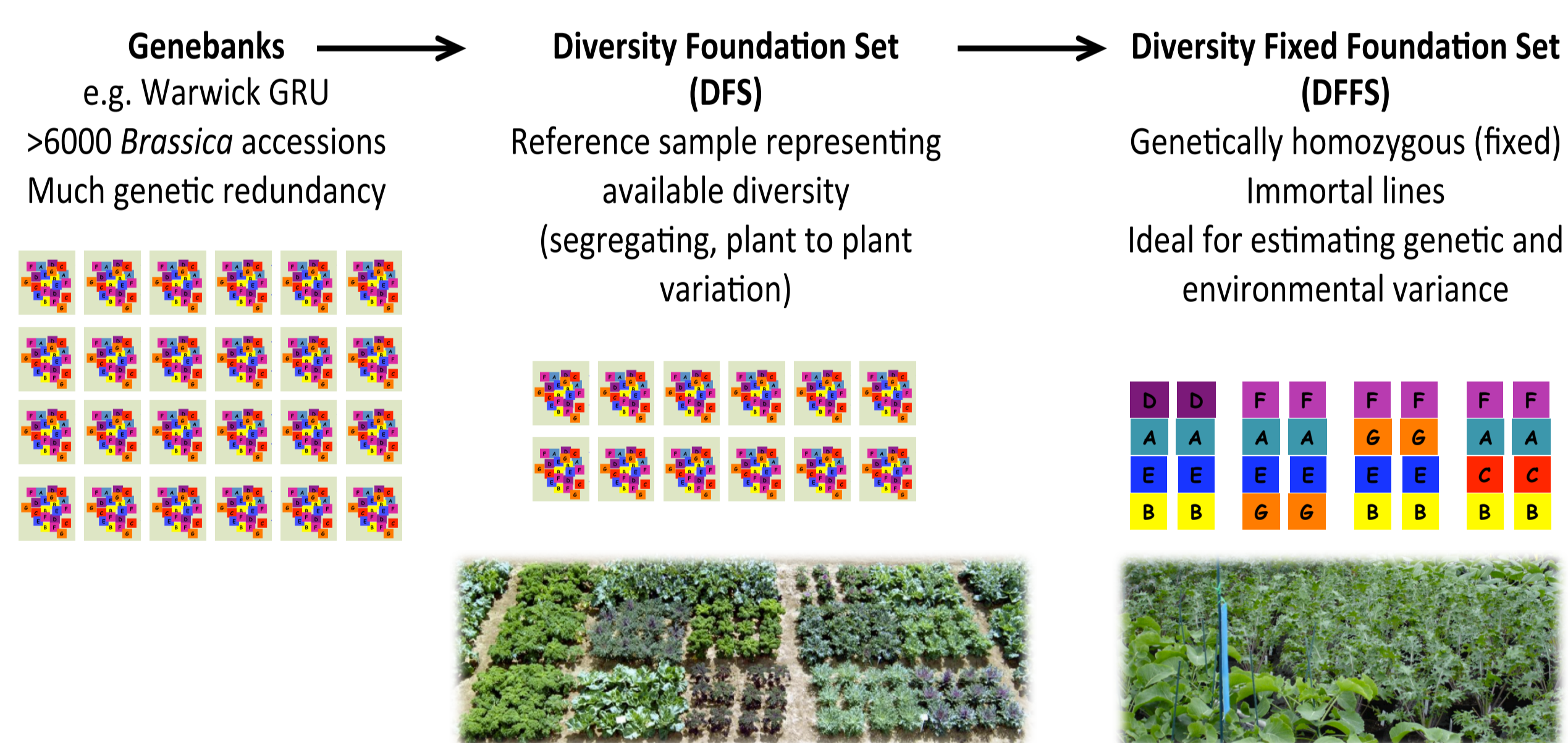


Figure 2. Schematic illustrating the creation of *Brassica* C genome DFS and DFFS.

The Vegetable Genetic Improvement Network (VeGIN) developed a panel of *Brassica* SNP KASPar markers; these were used to genotype both the founder DFS and the fixed DFFS. These data have been used to determine population sub-structure (Fig. 2) and phylogenetic relationships between the wider *Brassica* C genome species.

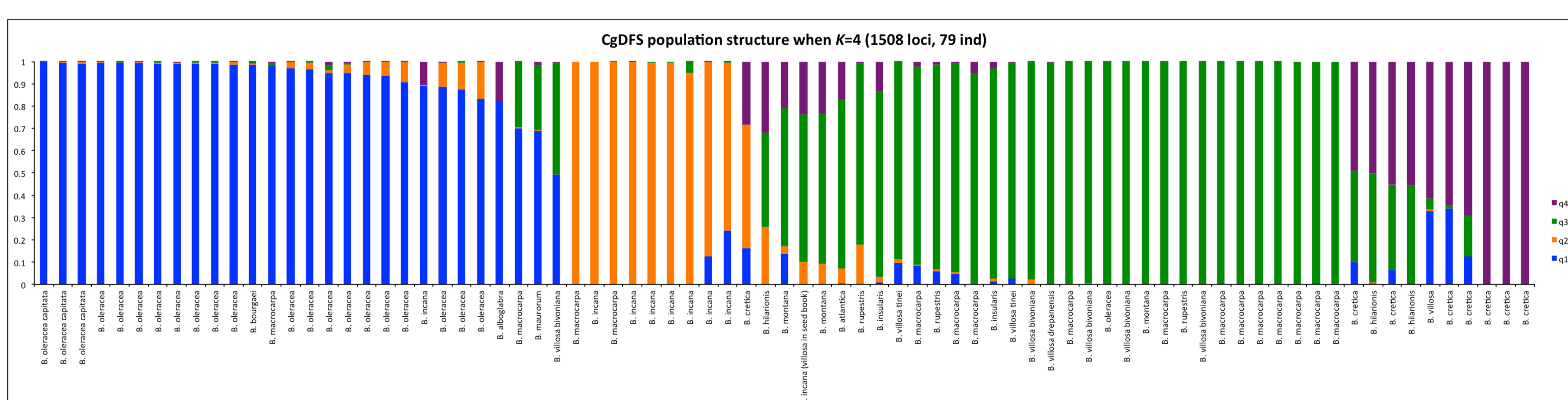


Figure 2. Population sub-structure in the C genome DFS.

Phenotype data – abiotic stress

Temperature stress

- 50 accessions representing the diversity in the *Brassica* C genome
- 3 replicates each grown at 20 °C (control) and 35 °C (heat stress)
 - Both growth rooms 20 °C 16L:8D for 2 weeks
 - Stress room - Ramp 5 °C over 3 days to 35 °C
 - Keep at 35 °C for 3 days
- Harvest leaf 7 from control and stressed = 300 samples



Table 1. Pre-screen of phenolic compounds in 5 *Brassica* spp.

Peak No.	Peaks matched to LC-MS data	<i>B. eratica</i>	<i>B. macrocarpa</i>	<i>B. montana</i>	<i>B. oleracea</i>	<i>B. incana</i>	Stress indicator?
1		-1	-1	-1	-1	-1	
2		0	-1	-1	-1	-1	
3		-1	-1	-1	-1	-1	
4		-1	-1	-1	0	0	Increase
5		-1	-1	-1	-1	-1	
6		-1	-1	-1	-1	-1	
7		-1	-1	-1	-1	-1	
8		-1	-1	-1	-1	-1	
9		-1	-1	-1	-1	-1	
10		0	1	1	0	0	
11		-1	-1	-1	-1	-1	
12		-1	-1	-1	0	1	
13		-1	-1	-1	-1	-1	
14	p-coumaric acid	-1	-1	-1	0	0	possible
15		-1	-1	-1	-1	-1	
16		-1	-1	-1	-1	-1	
17		0	0	-1	-1	-1	
18		-1	-1	-1	0	1	
19		-1	-1	-1	0	0	
20		-1	-1	-1	-1	-1	possible
21	unknown 3	-1	-1	-1	-1	-1	Decrease
22	unknown 4	-1	-1	-1	-1	-1	
23	unknown 5	0	0	0	1	1	
24	kaempferol	-1	-1	-1	-1	-1	Decrease
25	unknown 6	0	0	0	1	1	
26		-1	-1	-1	-1	-1	
27	unknown 1	-1	-1	-1	-1	-1	Increase
28	unknown 2	-1	-1	-1	-1	-1	
29	coumaroylquinic acid	-1	-1	-1	-1	-1	Increase

- Secondary metabolite analyses



- Flavonoids and phenolics (The flavour centre – University of Reading)

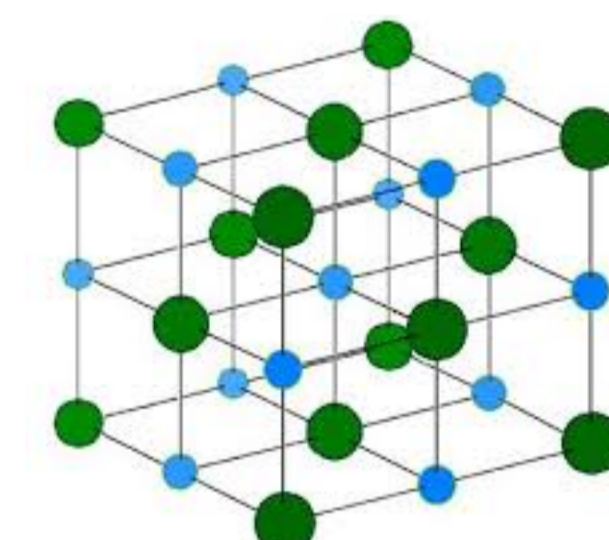
LC-MS pre-screen (Table 1) then HPLC



- Glucosinolates (Clinical Sciences Research Laboratories – Warwick Medical School)

LC-MS/MS

Saline stress



- 50 accessions representing the diversity in the *Brassica* C genome
- 3 replicates grown at 20 °C
- Stress: 250 mM NaCl
- Harvest leaf 7 from control and stressed = 300 samples
- The expression patterns of genes previously determined to be up-regulated during saline stress will be quantified.
- Phenotype data will be used for association analyses to explore the presence of genes that may confer resilience to saline stress.

These data will direct the selection of fixed DFFS lines for phenotypic assessment. Genes and KASPar markers will then be used during breeding and selection. Quality attributes and pleiotropic interactions will be determined.

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