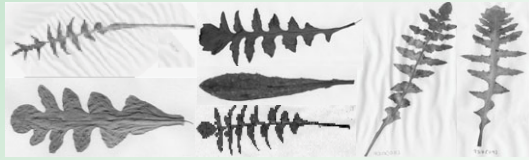


Molecular biology and ecology of flowering-time variants of *Capsella*



WISHART, J., BEGG, G., YOUNG, M., HACKETT, C., SQUIRE, G.R., IANNETTA, P.P.M. *
 Agroecology, Environment Plant Interactions, SCRI, Invergowrie, Dundee DD2 5DA.

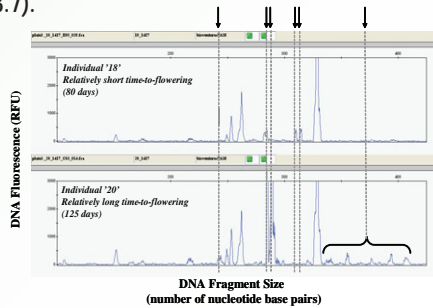
Historically, *Capsella* leaf shape variants are used to imply functional differences



- We isolated 157 accessions (from 53 lines), from 34 arable fields across the UK.
- Site parameters and cultivation history were noted.
- Phenotypic traits were measured under controlled (glasshouse) conditions.
- Molecular genotyping of the accessions was carried out.
- The relationship between site-of-origin, phenotypic and molecular parameters were tested.

A novel robust high-throughput I-SSR genotyping method is developed

- FAM-labelled anchored microsatellite primers generated PCR products.
- Single stranded PCR products were run on an ABI (Applied Biosystems), automated '3730 DNA-Analyser'.
- Analysis was carried out using ABI GeneMapper® Software (v3.7).
- This demonstrated excellent QA between samples and runs.
- The number of bands *per* individual is conserved.

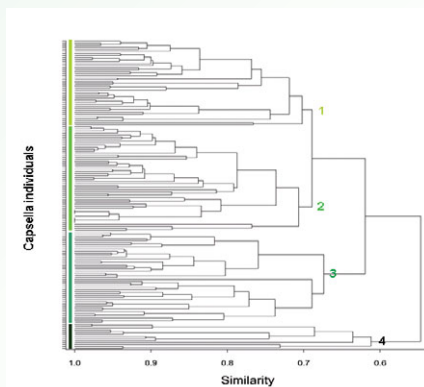


The profile of fluorescent I-SSR PCR products from two functional variants are exemplified in the graph opposite. Distinguishing peaks are arrowed.

Primer			Bands			
Code	Sequence	T _a	Total N ^a (50-1000b)	Common	Unique	Individual ¹ (Avg ± SD)
*UBC 880	6FAM-VHV (GT) ₇	53.4	79	4	75	22 ± 3
*UBC 886	6FAM-VBV (AT) ₇	53.4	93	12	81	25 ± 4
*UBC 888	6-FAM-VBV (CT) ₇	60	43	7	36	13 ± 2
*UBC 889	6-FAM-DBD(CA) ₇	60	58	6	52	13 ± 3
*SCRI 1417	6-FAM-BDT(CA) ₇	57.2	55	6	49	16 ± 3
*SCRI 1424	6-FAM-BDB(CAC) ₅	57.2	52	7	45	20 ± 3

* = Sourced from University of British Columbia; # = Sourced from SCRI; T_a = Annealing Temperature.

Cluster analysis identified four distinct functional groups



SITE DATA		Functional Group				Statistical Significance		
Trait		1	2	3	4	Chi ² (d.f)	p	
Discontinuous (N _c)	Previous Crop	Beet				313.21 (18)	< 0.001	
		Spring Rape		6	3			
		Wheat			6			
		ZL	2	5	7			
	Soil Texture	ZC	6		7	95.79 (24)	< 0.001	
		ZL						
Continuous		Mean	6.4a	6.5a	6.2b	6.2b	SE ²	F Statistic
	Soil pH		0a	31.245b	66.854c	1.44621d	396.85	< 0.001***
	Latitude		23.7a	21.3a	26.8b	23.3a	1.4	< 0.001***
	Diversity		25.5a	11.3b	25.2a	30.5a	5.3	< 0.001***
	N _c							

a = lowest
b = low
c = intermediate
d = highest

N = Number of species Site¹; 1 = N_c = Number of Capsella Individuals Site¹; 2 = of difference; 3 = degrees, relative from most Southerly = 0
Blank entries = 0

ISSR GENOTYPING (the diagnostic bands below are highly significant with high intensity (i.e. relative fluorescence > 5%).							
Primer Code	Number of Diagnostic Bands	Significance (Avg)	Functional Group Identified	Principle Coordinate Analysis % of variation	Linked to the following parameters		
886	1	< 0.001	2 < 1, 3 < 4	16.7	latitude	Soil pH	Previous Crop
	3	< 0.004	3 > rest				TTF
888	2	< 0.001	3 > rest	20	latitude	Soil pH	TTF
	1	0.002	2, 3 > 1, 4				
	1	< 0.001	3 > 1, 4				
889	3	0.003	4 > rest				
1417	2	< 0.001	3 > rest	16.7	latitude	Soil pH	Previous Crop
							TTF
1424	3	0.005	3 > rest	19.7	latitude	Soil pH	TTF

PLANT GROWTH & REPRODUCTION		Functional Group				Statistical Significance		
Traits	Leaf Shape	1	2	3	4	Chi ² (d.f)	p	
Discontinuous	1		52			83.97 (12)	< 0.001	
	2	31		45				
	3				9			
	4				3			
	5	2						
	6	7						
	7	2						
Continuous		Mean	460a	466a	491b	520c	SE	F
	Rosette Diameter (mm)					17.4	0.012*	
	Number Leaves		84.4a	52.27b	79.1a	85.6a	11.6	0.002**
	Flowering Stem Number		11a	10a	11a	6b	1.16	0.002**
	Time to Flowering (days)		74a	70a	84b	129c	3.7	< 0.001***
	Date of Flowering		0a	0a	+15b	+42c	4.5	< 0.001***
	Reproductive Duration (days)		83a	78b	74b	65c	4.3	0.005*
	Date to Seed Harvest		0	0	0	+33		
	Total Seed Number		330a	310a	310a	138b	24.21	< 0.001***
	Total Seed Weight (g)		13a	12a	11a	3b	1.5	< 0.001***
Date of Germination		0a	+6b	+6b	+3b	1.7	< 0.001***	

Conclusions

- Ecologically important traits (especially, time to flowering (TTF), are pleiotropic with other attributes.
- Strong statistical correlations exist between TTF-variants and site characteristics.
- We have proven the utility of a model approach to monitor environmental change.
- Future work should focus upon testing this model in blind-trials.

References

- Hawes C., Begg G.S., Squire G.R., Iannetta P.P.M. (2005) *Oikos* 109, 521-534.
- Iannetta P.P.M., Begg, G.S., Hawes, C., Young M., Russell, J., Squire, G.R. (2007) *Physiologia Plantarum* 129, 542-554.

* Contact: Pete.Iannetta@scri.ac.uk