



Genotypic analysis and crossing of selected hybrid complexes

Tom Wood

Genotyping interspecifics

- Genotyping with DArTSeq platform
- Comparing heterozygosity, confirming hybrid parentage
- F1 crossing program – efficiency, viability

Genotyping

- Genotyped hybrid parents, siblings, some f1 crosses (131)
- DArTSeq: SNPs, presence/absence, £30 (\$40) per line; 50,000 markers
- Re-analysed with three historic datasets
 - SNP3:1584 *Phaseolus* accessions

Many non-polymorphic, 36388; 30 k called on average

Genomic, plastid markers

Majority SNPs homozygous, usually only a single allele observed for most markers – hybrids selfed

Lower call rate in *Pc*, *Pd*; ~60% call rate vs ~80-90% in *Pv*

Heterozygosity

Investigate levels of genetic diversity, how much potential variation?

No. polymorphic alleles, segregating?

Freq. heterozygous 71-1600 in hybrids/parents (F1s>);

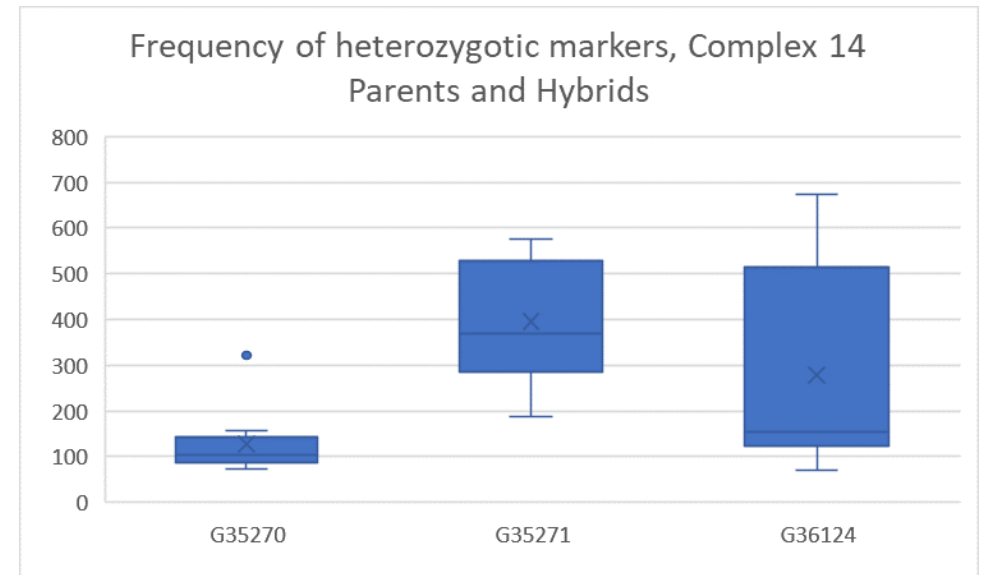
< G35988 (*Pc*); > G24764D (feral *Pv*)

Complex 14, low call rate 60% (~20 K SNPs)

-Genetic distance

-Spp. markers, track hybrid introgressions

Complex 14	<i>P. dumosus</i> G35270	<i>P. coccineus</i> G35271	Hybrids G36124
Homozygous_0	15223.4	15807.7	15709.2
Homozygous_1	5825.6	5620.1	5655.4
Heterozygous	127.6	395.5	278.3



Confirming hybrid parentage

- Putative pedigree based on collectors observations, co-location of germplasm
- Complex 14: G35270 (*P. dumosus*) x G35271 (*P. coccineus*)
- Plastid marker sequences, align to identify polymorphisms, compare the marker profiles in the progeny
- Mitochondrial: 395 kbp (Bi *et al.*, 2020), Chloroplast: 156 k bp (Meng *et al.* 2018)



G35270



G35271

=



G36124

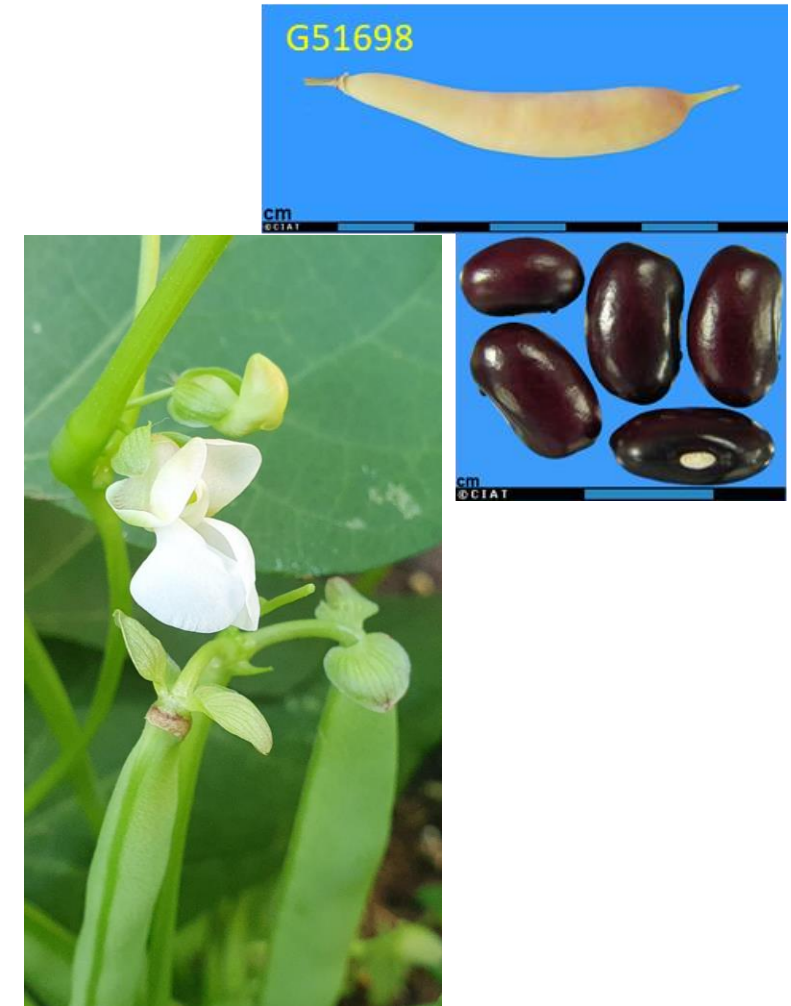
Confirming hybrid parentage

- 5 SNPs vary between parents
- Progeny exhibit *P. coccineus* haplotype
- *P. dumosus* pollen donor
- Web blight resistance from *P. dumosus*
- Same process to confirm other hybrids

Marker	SNP1	SNP2	SNP3	SNP4	SNP5	
CloneID	8199152	8196600	3375140	61148544	8212278	
Origin	Mito	Mito	Chloro	Chloro	Mito	
Line	50:A>C	65:T>C	28:T>G	28:T>G	20:T>C	
G36124_1	Hybrid	0	0	1	1	0
G36124_10	Hybrid	0	0	1	1	0
G36124_2	Hybrid	0	0	1	-	0
G36124_3	Hybrid	0	0	-	-	0
G36124_4	Hybrid	0	0	-	1	0
G36124_5	Hybrid	0	0	1	-	0
G36124_6	Hybrid	0	0	1	1	0
G36124_7	Hybrid	0	0	1	-	0
G36124_8	Hybrid	0	0	1	-	0
G36124_9	Hybrid	0	0	1	-	0
G35270_1	Parent	1	1	0	-	1
G35270_10	Parent	1	1	0	0	1
G35270_2	Parent	1	1	-	-	1
G35270_3	Parent	1	1	-	0	1
G35270_4	Parent	1	1	0	-	1
G35270_5	Parent	1	1	0	0	1
G35270_6	Parent	1	1	0	-	1
G35270_7	Parent	1	1	0	-	1
G35270_8	Parent	1	1	-	-	1
G35270_9	Parent	1	1	0	0	1
G35271_1	Parent	0	0	-	1	0
G35271_10	Parent	0	0	1	1	0
G35271_2	Parent	0	-	1	-	0
G35271_3	Parent	0	0	1	1	2
G35271_4	Parent	0	0	1	1	0
G35271_5	Parent	0	0	1	-	0
G35271_6	Parent	0	0	1	1	0
G35271_7	Parent	0	0	-	-	0
G35271_8	Parent	0	0	-	1	0
G35271_9	Parent	0	0	1	1	0

F1 Crossing program

- Incompatibility can be a barrier for
- Assess viability of hybrids for cross target disease resistant lines
- Ser-16 (G51698) as maternal line resistant
- Resistant accessions from Case 10
 - Anthracnose (G52443-2, G512741-
 - Sclerotinia (G52443-6)
 - Web blight (G52443-3, G36124)



F1 Crossing program

- Most hybrids relatively easy to cross with, high success rates
- Complex 19 not successful
- Pollen viability with potassium iodide, inspect anthers
- Selfs - heterozygosity similar to Ser-16, marker profile identical;
- F1 lines suitable for generating mapping resources
- Effective transfer of resistance?

Back Cross	Disease resistance	Complex	Seeds harvested
(Ser-16xG50785YI-12 red)	sclerotinia	7	29
(Ser-16xG50785YI-12 white)	sclerotinia	7	12
(Ser-16xG50879x4-12)	sclerotinia	8	19
(Ser-16xG5124I-7)	sclerotinia	11	20
(Ser-16xG523860H-13)	web blight	19	0
(Ser-16xG523860H-3)	web blight	19	0
(Ser-16xG52443-2)	sclerotinia	10	16
(Ser-16xG52443-3)	web blight	10	18
(Ser-16xG52443-6)	sclerotinia	10	11
(Ser-16xG52443-8)	web blight	10	5

	Ser-16	Ser 16 x G50879x4	Ser 16 x G50879x4	Ser 16 x G52443-8	Ser16 62 x G523860H-13	Ser 16 A15 x G523860H-3
Markers	30656	32291	32425	31271	31366	30859
Call rate	0.842476	0.88740794	0.89109047	0.85937672	0.86198747	0.8480543
Homo_A	24555	23861	23943	25032	25019	24693
Homo_B	6004	5679	5699	6158	6193	6057
Hets		97	2751	2783	81	154
				109		



Accessing project data and resources

Tom Wood

Variety information

The screenshot shows a web browser window with two tabs: "Gene Flow in Phaseolus Beans an..." and "Genesys PGR". The address bar displays "https://www.genesys-pgr.org". The website header includes the Genesys logo, navigation links for "Accession data", "Directory", "Resources", and "My List", and a "Login" button. A search bar is present with the text "Search Genesys...". Below the search bar, a banner states: "Genesys is an online platform where you can find information about Plant Genetic Resources for Food and Agriculture (PGRFA) conserved in genebanks worldwide." Three statistics are displayed: "4,298,787 Browse accession records", "289 Explore subsets", and "454 Explore C&E Datasets". The "Recent activity" section features three news items: "Unleashing Traits through Dynamic Visualization" (dated 25 May 2023), "Genesys: a gateway into the world's gene banks" (dated 18 May 2023), and "Genesys rolls out the Subsetting Tool" (dated 8 May 2023). Each item includes a brief description and a "Read more" link.

Gene Flow in Phaseolus Beans an... x Genesys PGR x +

https://www.genesys-pgr.org

Genesys Accession data > Directory > Resources > My List @ Login

Search Genesys...

Genesys is an online platform where you can find information about Plant Genetic Resources for Food and Agriculture (PGRFA) conserved in genebanks worldwide.

4,298,787 Browse accession records

289 Explore subsets

454 Explore C&E Datasets

Recent activity

Unleashing Traits through Dynamic Visualization
Genesys now unpacks raw characterization and evaluation data from genebanks.
25 May 2023
[Read more >](#)

Genesys: a gateway into the world's gene banks
Read about Genesys through the lens of DivSeek.
18 May 2023
[Read more >](#)

Genesys rolls out the Subsetting Tool
You can now create your own subsets of accessions by using the climate and soil variables of your choice.
8 May 2023
[Read more >](#)

FILTER ACCESSIONS

APPLY FILTERS

Reset

HISTORICAL RECORDS

- Yes
- No

TEXT SEARCH

HOLDING INSTITUTE

ACCESSION NUMBER

DATE SEARCH

CROP

TAXONOMY

ORIGIN OF MATERIAL

COLLECTING DATA

BIOLOGICAL STATUS OF ACCESSION

TYPE OF GERMPLOSM STORAGE

STATUS

REFERENCED ACCESSIONS

CLIMATE AT ORIGIN

Accession browser

1 accession Text: G52433 Excluding Historical

OVERVIEW ACCESSIONS MAP IMAGES SUBSETTING TOOL

Table view DOWNLOAD MCPD DOWNLOAD ZIP

1. G52433 • Phaseolus x(P. vulgaris x P. acutifolius) • Advanced/improved cultivar • Colombia
COL003 • Centro Internacional de Agricultura Tropical, Colombia • DOI: 10.18730/PJ13

+ Add to My list

Accession details

Passport data and everything else

G52433

SHOW CHANGES SIMILAR Add G52433 to my list

DOI	10.18730/PGJ13
Accession number	G52433
Holding institute	Centro Internacional de Agricultura Tropical — Colombia
Institute code	COL003
Data provider	CIAT Centro Internacional de Agricultura Tropical
Acquisition Date	8 May 2014
Provenance of material	Colombia
Biological status of accession	Advanced/improved cultivar
Ancestral information (pedigree)	ICA PIJAOx(G40001x(ICA PIJAOx(G40001x(ICA PIJAOx(ICA PIJAOxG40001))))))
Type of germplasm storage	Seed collection
Availability for distribution	Available for distribution
ITPGRFA MLS	Accession is part of the Multi-lateral system of ITPGRFA
Donor institute	CIAT
Safety duplication institute	MEX002 NOR051
Accession URL	http://genebank.ciat.cgiar.org/genebank/baccession.do?acc=G52433



G52433 pod.jpg



G52433 seed.jpg

Genotypic data

The screenshot shows the EVA website homepage. The browser tabs include 'Gene Flow in Phaseolus Beans an...' and 'The European Bioinformatics Inst...'. The address bar shows 'https://www.ebi.ac.uk/eva/'. The navigation menu includes 'Home', 'Submit Data', 'Study Browser', 'Variant Browser', 'GA4GH', 'API', 'RS Release', 'Help', and 'Feedback'. The main heading is 'European Variation Archive'. Below the heading, there is a search bar for SNPs with the text 'Search for SNPs' and a search button. The search bar contains the text 'ex: rs123 or ss567 or comma/space separated RS or SS IDs'. Below the search bar, there is a note: 'The RS ID release 4 is available in our FTP or through our API. See release page for details.' On the right side, there is a 'News' section with three tweets from EVA @evarchive. The first tweet is dated Jun 2 and mentions 'Data available from Shenyang Agricultural University: Liaoning Provincial Department of Education Project for Youth Scientists - buff.ly/3IUdZc #zeamays #maize'. The second tweet is dated May 31 and mentions 'The Affiliated Tumor Hospital Variant data now available to download - buff.ly/43fhDQK'. The third tweet is dated May 26 and mentions 'Variant calling in milk samples from Spanish Assaf ewes using Whole-Genome Bisulfite Sequencing data - buff.ly/3BO7kHk @unileon'.

Statistics

Short genetic variants studies (<50bp)



Structural variants studies (>50bp)



European Variation Archive

Variant Browser

Search the EVA variant warehouse using any combination of the filtering options on the left hand-side.

Search results can be exported in CSV format and individual variants can be further investigated using the in-depth Variant Data tabs found below the main results table.

Filter

Reset Search

Genome Assembly

Organism / Assembly: Horse / EquCab2.0

Position

Filter By: Chromosomal Location

1:3000000-3100000

Annotation

Select Version: VEP version 89 - Cache version 89

Consequence Type

Minor Allele Frequency

Protein Substitution Score

Studies Mapped To Assembly

search

Name ↑

- A genome-wide association analysis for show-jumping performance in Hanoverian stallions (PRJEB24624)
- Body size in horses (PRJEB24630)
- BROAD_GENOME - BROAD_EQUCAB2.0:2008.08.08 (BROAD_EQUCAB2.0:2008.08.08)
- eQTL Discovery and their Association with Severe Equine Asthma in European Warmblood Horses (PRJEB23301)

Variants found

Page 1 of 48 Variants 1 - 10 of 473

Chr	Position	Variant ID	Alleles	Class	Most Severe Consequence Type	Most Severe Protein Substitution Score		View
						PolyPhen2	Sift	
1	3000829	-	C/T	SNV	intergenic_variant	-	-	dbSNP
1	3001637	-	C/T	SNV	intergenic_variant	-	-	dbSNP
1	3001683	-	A/C	SNV	intergenic_variant	-	-	dbSNP
1	3002480	-	T/C	SNV	intergenic_variant	-	-	dbSNP
1	3002750	-	G/A	SNV	intergenic_variant	-	-	dbSNP
1	3002807	-	A/G	SNV	intergenic_variant	-	-	dbSNP
1	3003058	-	A/C	SNV	intergenic_variant	-	-	dbSNP
1	3003059	-	A/T	SNV	intergenic_variant	-	-	dbSNP
1	3003105	-	G/A	SNV	intergenic_variant	-	-	dbSNP
1	3004246	-	G/A	SNV	intergenic_variant	-	-	dbSNP

Results per Page: 10 Export as CSV Export as VCF (beta)

Variant Data

Annotations

Page 1 of 1 Transcripts 1 - 1 of 1

Ensembl Gene ID	Ensembl Gene Symbol	Ensembl Transcript ID	Ensembl Transcript Biotype	SO Term(s)	Codon	cDna Position	AA Change	PolyPhen	Sift
-	-	-	-	intergenic_variant	-	-	-	-	-

Phenotypic data

The screenshot shows the Zenodo website interface. At the top, there is a navigation bar with the Zenodo logo, a search bar, and links for 'Upload' and 'Communities'. On the right side of the navigation bar, there are 'Log in' and 'Sign up' buttons. Below the navigation bar, the main content area features a list of phenotypic data categories on the left and a large Zenodo logo with the text 'Celebrating our 10th Anniversary' on the right. Below this, there is a section titled 'Featured communities' which highlights the 'Coronavirus Disease Research Community - COVID-19'. This section includes a 3D model of a coronavirus particle, a description of the community's purpose, and a 'Curated by' field listing 'Covid19_Team_OpenAIRE'. There are also 'Browse' and 'New upload' buttons for this community. A link for 'Need help uploading? Contact us' is visible in the top right of the featured community section.

- Stomatal counts, images
- Root angles, images
- Disease screens, differential tests
- Pathogen isolate data
- Metadata

zenodo
Search [] [Q] Upload Communities Log in Sign up

zenodo
Celebrating our 10th Anniversary

Featured communities

Need help uploading? Contact us

Coronavirus Disease Research Community - COVID-19

This community collects research outputs that may be relevant to the Coronavirus Disease (COVID-19) or the SARS-CoV-2. Scientists are encouraged to upload their outcome in this collection to facilitate sharing and discovery of information. Although Open Access articles and datasets are...

Curated by: Covid19_Team_OpenAIRE

Browse New upload



Upload Communities

Delete

Save

Publish

New upload

Transform to Open Science

Instructions: (i) Upload minimum one file and fill-in required fields (marked with a red star). (ii) Press "Save" to save your upload for editing later. (iii) When ready, press "Publish" to finalize and make your upload public.

Files

Filename (1 files)	Size	Progress	Delete
Stomatal count raw data.xlsx md5:14bbc1a35b53455665cc562764f4311b	250 kB	✓	

Note: File addition, removal or modification are not allowed after you have published your upload. This is because a Digital Object Identifier (DOI) is registered with [DataCite](#) for each upload.

(minimum 1 file required, max 50 GB per dataset - [contact us](#) for larger datasets)

If you're experiencing issues with uploading larger files, read our [FAQ section](#) on file upload issues.

Communities

Specify communities which you wish your upload to appear in. The owner of the community will be notified, and can either accept or reject your request. Please make sure your record complies with the content policy of the communities you add; reported abuse will be followed by account inactivation.

Stomatal count raw data - Excel

File Home Insert Page Layout Formulas Data Review View PDFescape Desktop Creator Tell me what you want to do... Tom Wood Share

Clipboard: Paste, Cut, Copy, Format Painter

Font: Calibri, 11, Bold, Italic, Underline, Text Color, Background Color

Alignment: Wrap Text, Merge & Center

Number: General, Percentage, Decimals

Styles: Conditional Formatting, Format as Table, Cell Styles

Cells: Insert, Delete, Format

Editing: AutoSum, Fill, Clear, Sort & Find & Filter, Select

F11 fx 35

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
		ACCESSION	leaf number	Location	Side	stomata count 1	epidermal cell count 1	SI 1	stomata count 2	epidermal cell count 2	SI 2	stomata count 3	epidermal cell count 3	SI 3	stomata count 4	epidermal cell count 4	SI 4	stomata count 5	epidermal cell count 5
1																			
2	plot1	G52443-2	1	T	1	50	138	26.6	48	136	26.1	51	145	26.0	50	146	25.5	48	133
3		G52443-2	1	T	2	53	155	25.5	48	149	24.4	48	134	26.4	51	145	26.0	55	149
4		G52443-2	1	B	1	24	76	24.0	25	76	24.8	40	124	24.4	24	77	23.8	33	101
5		G52443-2	1	B	2	41	127	24.4	38	117	24.5	40	122	24.7	33	98	25.2	23	70
6		G52443-2	2	T	1	44	123	26.3	42	123	25.5	46	129	26.3	51	142	26.4	56	156
7		G52443-2	2	T	2	42	112	27.3	49	123	28.5	48	132	26.7	42	120	25.9	38	103
8		G52443-2	2	B	1	17	55	23.6	20	62	24.4	19	62	23.5	21	63	25.0	24	73
9		G52443-2	2	B	2	18	58	23.7	19	60	24.1	21	67	23.9	20	66	23.3	20	58
10		G52443-2	3	T	1	38	114	25.0	32	108	22.9	36	107	25.2	37	116	24.2	37	117
11		G52443-2	3	T	2	35	105	25.0	37	119	23.7	41	116	26.1	35	102	25.5	29	101
12		G52443-2	3	B	1	24	80	23.1	22	78	22.0	22	78	22.0	24	83	22.4	25	88
13		G52443-2	3	B	2	24	83	22.4	24	87	21.6	27	88	23.5	24	84	22.2	25	83
14		G52443-2	4	T	1	37	105	26.1	34	101	25.2	36	107	25.2	43	122	26.1	37	112
15		G52443-2	4	T	2	41	118	25.8	40	113	26.1	33	104	24.1	39	122	24.2	39	112
16		G52443-2	4	B	1	19	64	22.9	17	58	22.7	18	59	23.4	18	61	22.8	17	58
17		G52443-2	4	B	2	18	56	24.3	15	52	22.4	15	51	22.7	13	45	22.4	19	56
18		G52443-2	5	T	1	44	123	26.3	44	120	26.8	44	124	26.2	48	131	26.8	47	131

Batch 1 & 2 raw Batch 3 raw

-Stomatal frequencies, root angle, whorl number, root and stomata images, disease screening

Questions?