

United States Department of Agriculture National Institute of Food and Agriculture



Solanaceae Coordinated Agricultural Project



Downstream analysis with SNP markers Part I: Introduction to computer software for data analysis

Sung-Chur Sim The Ohio State University, OARDC SolCAP workshop

Outline

Ort I: Introduction to computer software

- MicroSatellite Analyzer (MSA)
- Graphical GenoType (GTT)
- STRUCTURE

 \checkmark What can you do using the software?

✓ Where can you download the software?

 \checkmark How can you format input data ?

Part II: The use of STRUCTURE for association mapping

• Detail steps to generate a Q-matrix using STRUCTURE

MicroSatellite Analyzer: MSA

- On independent analysis tool for large data sets (Dieringer and Schlötterer 2003)
 - Descriptive statistics per population and locus (e.g. allelic richness, heterozygosity, and Shannon index of diversity)
 - F_{ST} , F_{IS} , and F_{IT} based on the Weir and Cockerham method
 - F_{ST} per locus and population pair ; P-value for F_{ST} determined by permuting genotypes among groups
 - Genetic distance including Nei's standard genetic distance
 - Converts your data into the formats of GENEPOP, STRUCTURE, ARLEQUIN, etc.
- Version 4.05 available for Windows, Linux, and Mac: (<u>http://i122server.vu-wien.ac.at/MSA/MSA_download.html</u>)





http://i122server.vu-wien.ac.at/MSA/MSA_download.html

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One or two column format

- Specify **one (1) or two (2)** column format in the cell A1
- Enter name of population in the first column (no empty cell)
- Specify **inbred (h) or outbred (d)** for your species in the second column (no empty cell)
- Enter group number of population (no empty cell)
- SNP data converted from letter codes to numerical coding
- Missing data cam be indicated by -1, nd, dot(.), or empty cell
- Save your data in the format **"TAB DELIMITED"**

Identify loci that distinguish populations









 $F_{ST} = 0$: an allele of a gene is fixed or the gene is under balancing selection

 $F_{ST} = 1$: a gene under diversifying selection

Graphical GenoType: GGT

- A tool for representing molecular marker data by graphical representation and color coding of chromosomes
 - Useful for evaluation of plant material and selection of a desired genotype
- Advanced genetic analyses
 - Marker-trait association
 - Genetic distance
 - Linkage disequilibrium
- Version 2.0 available for Windows (<u>http://www.plantbreeding.wur.nl/UK/software_ggt.html</u>)



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Input format

- Two data files derived from locus and map data
- 👏 Locus file
 - Contains data on marker alleles using the MapMaker or JoinMap type of coding
 - A plain text file

Locus file

```
; This file was used as input for the JOINMAP mapping software
; use the BUIL GGT FILE option to merge '.loc' and '.map' files into a
'.ggt' file
; Fri, 10 Jan 1997, 11:54
; grouping file: mylvuniq.grp
; original file: mylvunig.loc
; linkage group: 1
name = lvuniq-1
popt = RI9
nloc = 72
nind = 103
E42M32-231#1
  aaaaa -ca-a caccc aacca cacac caaca aaaca cccca accaa accca
  caaac aaaaa accaa acccc accaa cccac acaaa caccc caaaa ccaac
  CCC
E33M61-740
  aaaaa aaaca caccc aacca aacac caaca caaca acaaa acacc accaa
  cacaa acaaa accca ccccc accaa accac accaa caaca caaaa caaac
```

Input format

- Two data files derived from locus and map data
- 👏 Locus file
 - Contains data on marker alleles using the MapMaker or JoinMap type of coding
 - A plain text file
- 🐡 Map file
 - Specifies marker positions on a linkage map
 - A plain text file

Map file

; Genetic map file of a Barley RIL population ; chromosome 1

chrom 1

E33M55-508	0.0
ЕЗ9М61-574	1.8
Е35м48-228	4.0
ЕЗЗМ61-740	14.6
E35M54-93	14.6
Е41м40-112	20.7
Е42м51-267	23.3
E42M32-231#1	26.5
Е42м40-287	28.5
ЕЗЗМ61-120	29.2
F37M32-00	38 O

Input format

- Two data files derived from locus and map data
- 👏 Locus file
 - Contains data on marker alleles using the MapMaker or JoinMap type of coding
 - A plain text file
- 🐡 Map file
 - Specifies marker positions on a linkage map
 - A plain text file
- Build a GGT file by merging the locus and map files using the 'Build GGT-file' option
- The GGT file can also be prepared from an Excel spreadsheet

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4	nind	5							
5	popt	F2				(<u></u>		
6	,locus	pos	alleles			<u> </u>]	
7									
8	name=group1								
9	marker1	0	A	В	A	A	В		
10	marker2	25.8	A	U	Α	H	B		
11	marker3	76.9	Н	В	A	H	H		
12	marker4	101.3	U	В	Н	Α	A	i i	
13									
14	name=group2					1			
15	marker21	15.5	Н	В	Α	Н	Α		
16	marker22	43.7	Н	В	A	H	В		2
17	marker23	81	Α	U	Α	Н	В		
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STRUCTURE

☆ A model-based clustering method (Pritchard et al. 2000)

- Inferring population structure using multi-locus genotype data
- Generating a Q-matrix to correct for population subdivision during marker-trait association analysis in complex populations (e.g. breeding populations)
- Identifying migrants and admixed individuals
- Version 2.3.3 available for Windows, Linux, and Mac: (<u>http://pritch.bsd.uchicago.edu/structure.html</u>)







http://pritch.bsd.uchicago.edu/structure.html

Input format

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7		Fla	754	7	1	I		12		12	12		13
8		Fla	777	1	1	1		14		12	12		13
9		Fla	777	1	-	I		14		12	12		13
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12		Fla	760	0	1	I		14		12	13		13
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16	N	IC23	E-2	(93)	1	1		14		12	13		13
17	N	C23	E-2	(93)	1	1		14		12	13		13
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21		NC	841)	73	1	I		12		13	12		13
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23		NC	9824	48	1	1		12		12	13		13
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25	I	NC 9	947	1-3	1	l .		12		12	13		13
26		NC	EBR	2	1	I		14		12	13		13
27		NC	EBR	2	1	I		14		12	13		13
28	0	Dhio	-MF	213	1	I		14		12	12		13
29	0	Dhio	-MF	213	1	1		14		12	12		13

A matrix where the data for individuals are in rows, the loci are in column

- *n* consecutive rows have the data for each individual of *n*-ploid species
- **Integer** should be used for coding genotype
- Missing data should be indicated by a number which doesn't occur elsewhere in the data (e.g. -1)
- The data file should be a text file (.txt) not an excel file (.xls) for running STRUCTURE

Summary

- Three computer programs, MSA, GGT, and STRUCTURE were introduced for SNP data analysis by providing the following information:
 - What can the programs do?
 - Where can you download them?
 - How can you format input data for each program?



United States Department of Agriculture

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Downstream analysis with SNP markers Part II: The use of STUCTURE software for association mapping of bacterial spot resistance in tomato

Sung-Chur Sim The Ohio State University, OARDC SolCAP workshop

Bacterial spot in tomato

- A disease complex caused by species of *Xanthomonas* bacteria.
- ✤ Five physiological races: T1-T5
- Sources of resistance from close relatives of cultivated tomato (Solanum lycopersicum L.) or S. pimpinellifolium
 - Hawaii 7998 (T1)
 - Hawaii 7981 (T3)
 - PI128216 (T3)
 - PI114490 (T1, T2, T3, and T4)



Association analysis models incorporate a correction for population structure

Unified mixed model (Yu et al. 2006)

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Adding a matrix, Qw, of population structure can correct for pseudo-linkage and can add insight to which crosses, pedigrees, subpopulations have the highest breeding value

Format marker data

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13	6115S3	11	13	14	4 1	.3 1	13 1	1 14	11	14	1 1	2 -	1 12	2 13	3 11	11	11	13	14	11	13	12	: 12	2
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The marker data file used in this example is available on the workshop URL: <u>http://pbgworks.org/tomato-workshop</u> (file name: STRUCTURE_InputData.txt)



Burnin length: how long to run the simulation before collecting data to minimize the effect of the starting configuration (Recommendation: 10,000 ~100,000)

MCIC length: how long to run the simulation after the burnin to get accurate parameter estimates (Recommendation: 500,000~1,000,000)



Inference of best K (number of populations)

 \odot The log likelihood for each K, Ln P(D) = L(K)

Two approaches to determine the best K

1. Use of L(K): When K is approaching a true value, L(K) plateaus (or continues increasing slightly) and has high variance between runs (Rosenberg et al. 2001, Evanno et al. 2005).

⇒ nonparametric test (Wilcoxin test)

2. Use of an ad hoc quantity (ΔK): Calculated based on the second order rate of change of the likelihood (ΔK) (Evanno et al. 2005). The ΔK shows a clear peak at the true value of K.

 $\Rightarrow \Delta K = m([L"K])/s[L(K)]$



A. L(K)

Evanno et al. 2005. Molecular Ecology 14: 2611-2620

Structure

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🖻 ·· 🍌 500000		Parameter	Run Name	K	Ln P(D)	Var[LnP(D)]	01	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5	Fst_6	Fst_7	
Settings		500000	500000_run_9	4	-3278.6	206.2	0.2052	0.3857	0.4197	0.6008	0.2779	-	-	-	
🖻 🕌 Results		500000	500000_run_8	4	-3279.7	207.8	0.2056	0.2752	0.4202	0.3860	0.6039	-	-	-	Ξ
500000_run_100 (K=8)		500000	500000_run_7	4	-3277.9	204.8	0.2047	0.5993	0.3861	0.2764	0.4203	-	-	-	
500000_run_101(K=9)		500000	500000_run_6	4	-3279.0	206.8	0.2052	0.4188	0.6014	0.3874	0.2763	-	-	-	
500000_run_102 (K=9)		500000	500000_run_5	4	-3278.7	205.8	0.2046	0.2757	0.3860	0.4199	0.6002	-	-	-	
500000_run_103 (K=9)		500000	500000_run_4	4	-3279.1	207.0	0.2043	0.3842	0.5991	0.2756	0.4204	-	-	-	
500000_run_104 (K=9)		500000	500000_run_3	4	-3279.6	208.0	0.2060	0.6010	0.2762	0.3868	0.4202	-	-	-	
500000_run_105 (K=9)		500000	500000_run_2	4	-3279.0	06.9	0.2045	0.3843	0.6003	0.4195	0.2769	-	-	-	-
500000_run_106 (K=9)		500000	500000_run_20	-	-3280.8	10.4	0.2047	0.5984	0.2766	0.3868	0.4198	-	-	-	-
500000_run_107(K=9)		500000	500000_run_1		-3278.8	06.3	0.2051	0.4198	0.2768	0.6013	0.3864	-	-	-	-
500000_run_108 (K=9)		500000	500000_run_19		-3278.5	06.0	0.2041	0.5977	0.3862	0.4193	0.2777	-	-	-	-
500000_run_109 (K=9)		500000	500000_run_18	4	-3278.3	04.9	0.2047	0.4190	0.6000	0.2746	0.3856	-	-	-	-
500000_run_10 (K=4)		500000	500000_run_17	4	-3278.5	205.7	0.2039	0.4193	0.5983	0.3855	0.2763	-	-	-	-
500000_run_110 (K=9)		500000	500000_run_16	4	-3280.1	209.1	0.2051	0.5992	0.3861	0.4203	0.2767	-	-	-	-
500000_run_111(K=9)		500000	500000_run_15	4	-3279.4	207.5	0.2059	0.4196	0.2770	0.6027	0.3875	-	-	-	-
500000_run_112 (K=9)		500000	500000_run_14	4	-3279.4	207.4	0.2051	0.4187	0.3867	0.2751	0.6023	-	-	-	-
500000_run_113 (K=9)		500000	500000_run_13	4	-3279.2	206.9	0.2055	0.4193	0.3863	0.2768	0.6024	-	-	-	-
500000_run_114(K=9)		500000	500000_run_12	4	-3278.8	206.1	0.2048	0.5975	0.2769	0.4198	0.3849	-	-	-	-
500000_run_115 (K=9)		500000	500000_run_11	4	-3279.9	2	0.2044	0.4187	0.2748	0.3849	0.6022	-	-	-	
500000_run_116 (K=9)		500000	500000 run 10	4	3279.4	20.7.6	0.2060	0.2757	0.3890	0.4195	0.6033	-	-	-	•
500000 run 117 (K=9)	-						•		111					•	

Log likelihood values

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Inference of best K using the delta K method

	5	ب ا						-	-	The best K a	nalysis -	Microsoft	Excel			-	_	
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4	24	-3279	.7	5	-3221 0.14	13 87348	58 11	-34.6	34.6	2 493966		12 -	\ `			, 003		
5	34	-3277	.9	6	-3197.52	17.41567	23.51	-22.705	22.705	1.303711		10	/					
6	44	-327	79	7	-3196.71	44.95482	0.805	31	31	0.689581		10						
7	54	-3278	.7	8	-3164.91	5.927498	31.805	-77.76	77.76	13.11852		8						
8	64	-3279	.1	9	-3210.86	61.8554	-45.955	-143.045	143.045	2.312571		6		+			Series1	
9	74	-3279	.6	10	-3399.86	52.43314	-189	104.2	104.2	1.987293		4						
10	84	-327	79	11	-3484.66	56.18421	-84.8	49.195	49.195	0.875602								
11	94	-3280	.8	12	-3520.27	74.40753	-35.605	27.065	27.065	0.36374		2	\sim			~		
12	10 4	-3278	.8	13	-3528.81	88.5478	-8.54	5.855	5.855	0.066122		o —			1 1			
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Structure

File Project Parameter Set Plotting View Help



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SAS codes

```
%macro Mol(mark);
proc mixed data = three;
class & mark gen rep;
model T1 = pop1 pop2 pop3 pop4 pop5 pop6 pop7 pop8 &mark /
solution;
random gen rep;
                                      JW
%mend:
                                                               Markera
         %Mol(M1);
         %Mol(M2);
         %Mol(M3);
         %Mol(M4);
run;
                        The SAS code used in this example is available on the
                        workshop URL: <a href="http://pbgworks.org/tomato-workshop">http://pbgworks.org/tomato-workshop</a>
                        (file name: SAScode.txt)
```

Summary

- STRUCTURE is a useful tool to detect population subdivision
- The use of the Q-matrix can correct for subpopulations during association analysis in breeding populations; avoids detection of false-positives
- The SNP resources from SolCAP are a powerful survey tool; we should be thinking beyond bi-parental populations toward analysis of complex breeding populations