The 8th International Purdue Symposium on Statistics
Session: Interactions Between Omics and Statistics: Analyzing High Dimensional Data
Sunday, June 24, 11:00am - 11:30am

Opportunities and Challenges of Statistical Genetics in Genome-wide Association Studies

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Outline

- GWAS: Opportunities and challenges
- Rare allele (CR-GWAS)
  - Arabidopsis
- Genomic distribution of trait-associated SNPs
  - Maize
- Examples of functional haplotypes
  - Sorghum

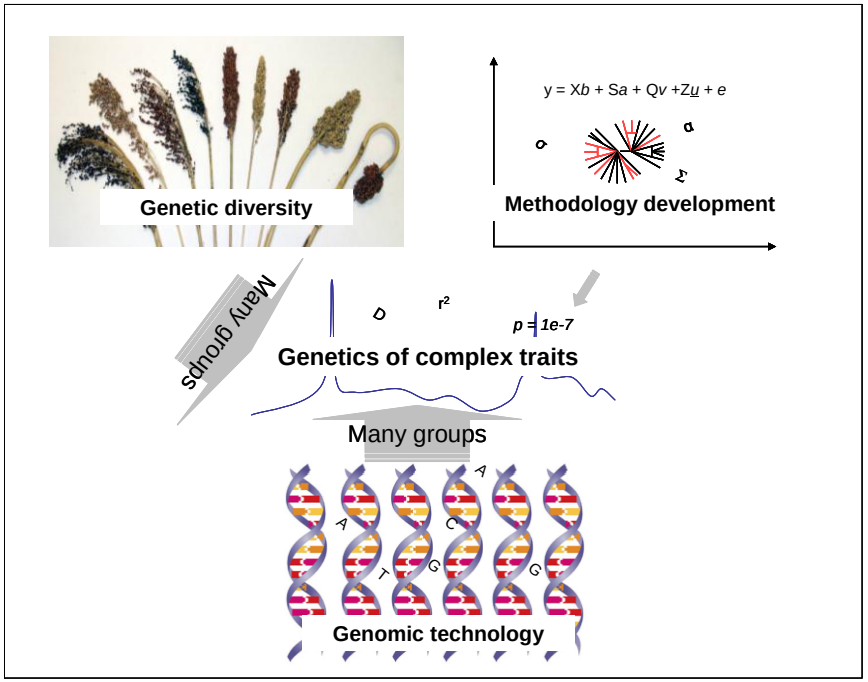
Geno→Pheno & Geno↔Pheno

- Genome-wide association study (GWAS)
  - Gene identification
  - Finding association "**signals**" with a large set of SNPs across diverse germplasm
  - Array-based genotyping & resequencing

- Genome-wide selection (GS)
  - "**Prediction**", breeding, genetic gain
  - Selection of individuals based on predicted phenotypic values using all markers, rather than only significant markers linked to QTL
  - Integration of genomic technology with plant breeding
    - Bernardo and Yu 2007, Crop Science 47:1082-1090

Opportunities of GWAS

- An additional strategy/tool in gene identification
  - Initiation/validation QTL cloning
  - Hypothesis of new functions of known genes
  - New genes/pathways
- The ability of rapidly nailing down genes for human diseases with relatively simple inheritance is impressive!
- Appreciation of the complexity and beauty of the natural variation
- Has the potential to offer a global view of **genetic architecture of complex traits**



Challenges of GWAS

- Population structure and relative kinship
  - Human genetics, plant and animal genetics
    - Structure; Mixed model QK; Dimension determination/model testing; Accuracy of variance-covariance matrix, R^2_{LR} for mixed model

Yu et al, 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38, 203-208

$$y = X\beta + S\alpha + Qv + Zu + e$$

y: Trait values

X: Environments, etc.

S: Candidate SNP effects

Q: Subpopulation effects

Z: Background Genetic effects, Var(*u*) = 2KV_A

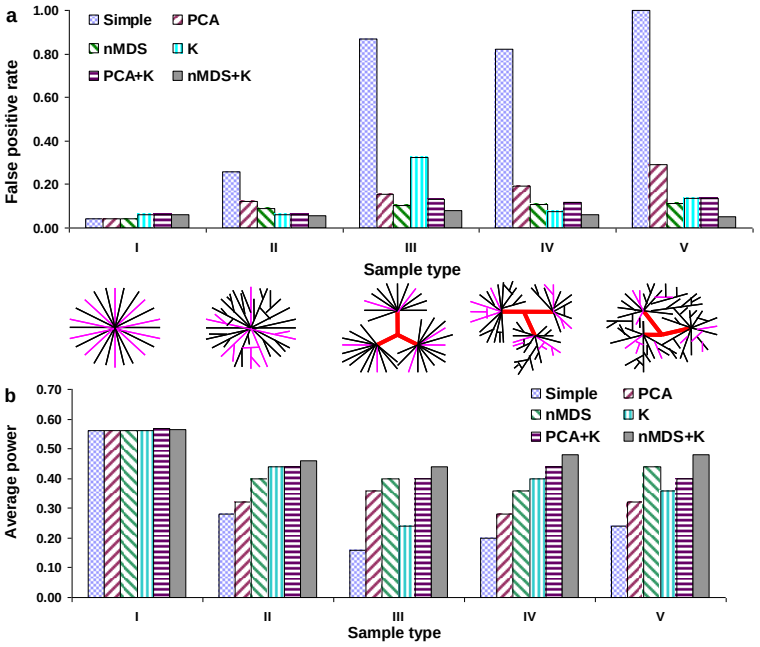
e

Q model

K model

QK model

Simple model



Zhu and Yu 2009, Genetics 182:875-888.

Challenges of GWAS

- Computational demand for mixed model
  - P3D, Compression, EMMA, EMMAX, and Fast-LMM
  - GEMMA, MLMM (Online first Nature Genetics)
- Multiple testing issue and significance threshold
- Missing heritability
- **Rare allele** and epistasis
- Genome, genetics, gene, coding region, allele, hapoltype, SNP, structural variation, etc.

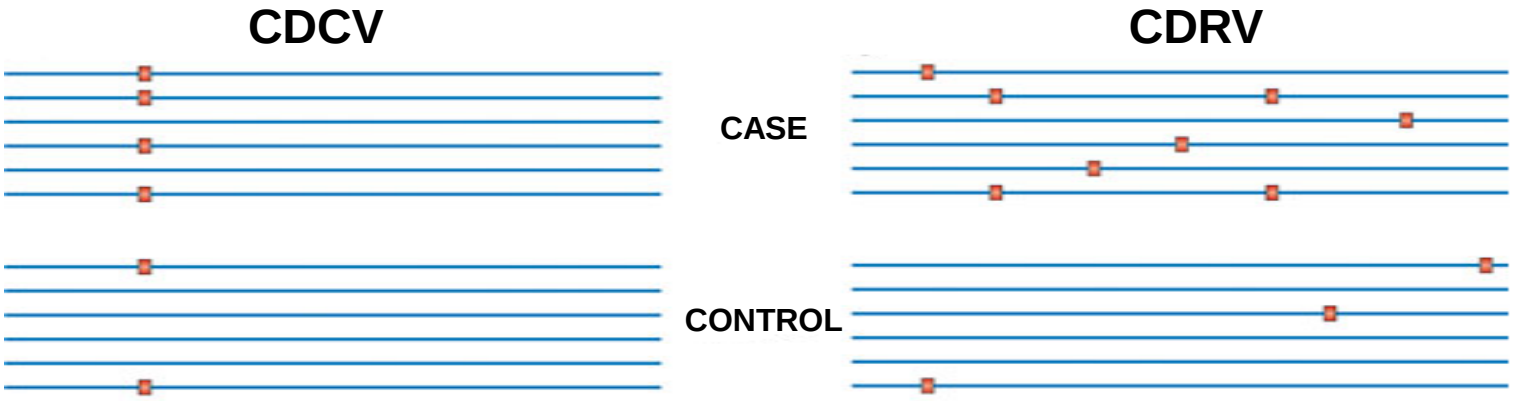
Blame "complex biology and genetics"

I. Composite Resequencing-based GWAS (CR-GWAS)

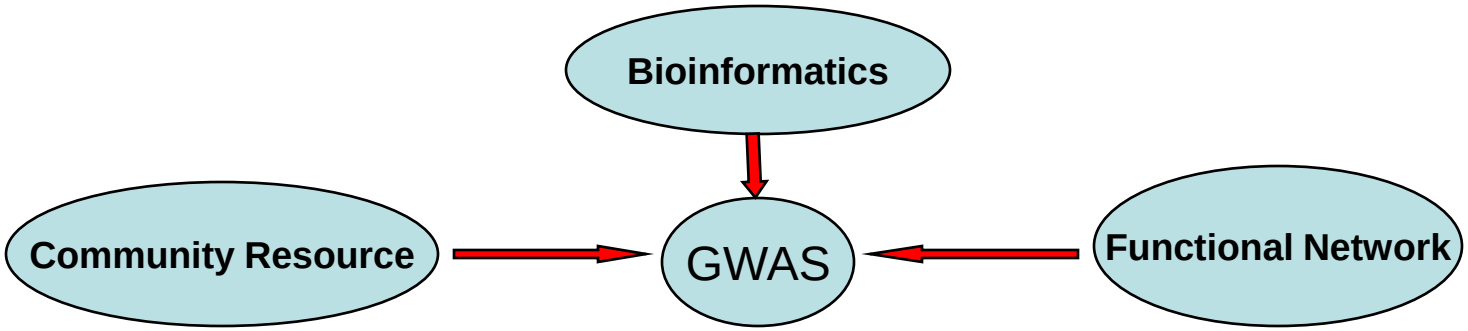
Hypothesis of GWAS: CDCV or CDRV

Rare allele: 4% from Genotyping versus 50% from Resequencing

Single-locus test after controlling for population stratification

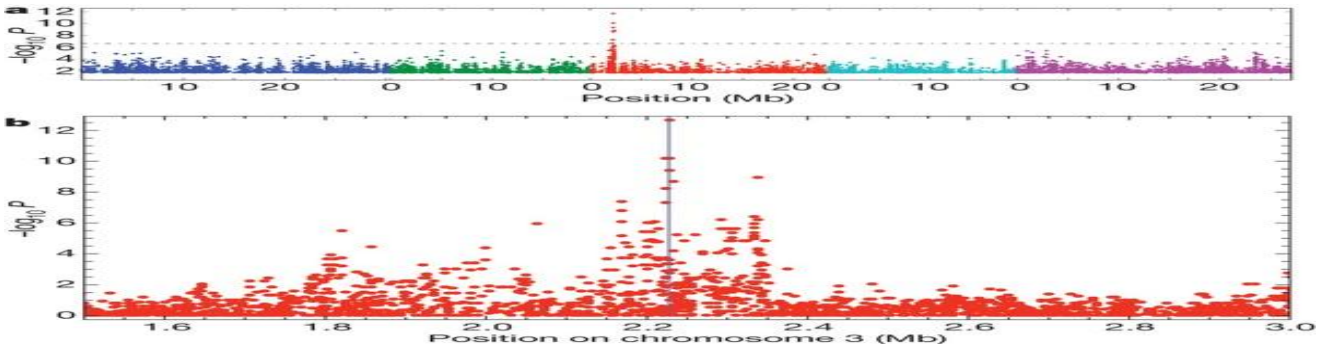


Nat Rev Genet **11, 773-785**

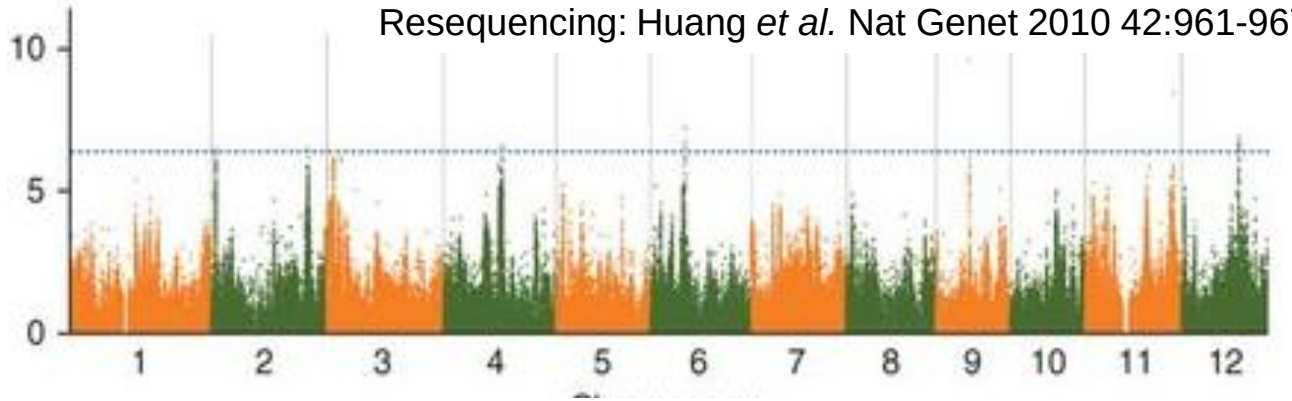


GWAS in Plants

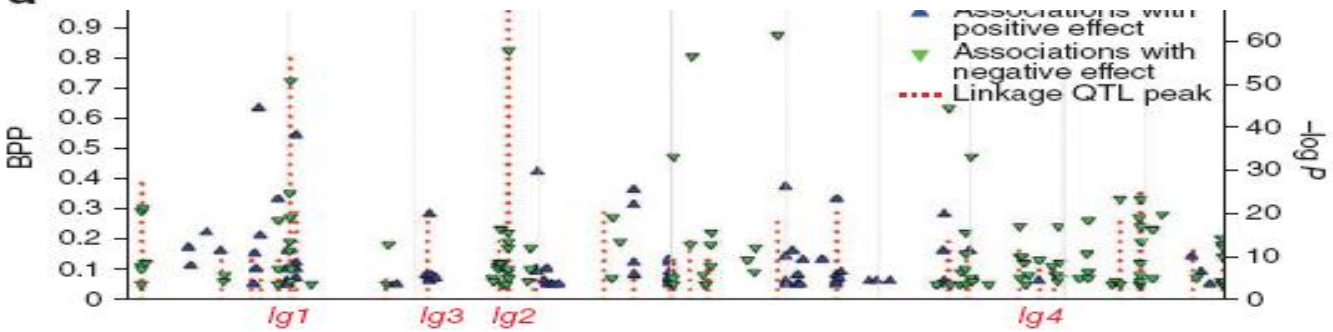
Genotyping: Atwell *et al.* Nature 2010 465:627-631
Resequencing: Zhao *et al.* PLoS Genet 2007 3:e3



Resequencing: Huang *et al.* Nat Genet 2010 42:961-967



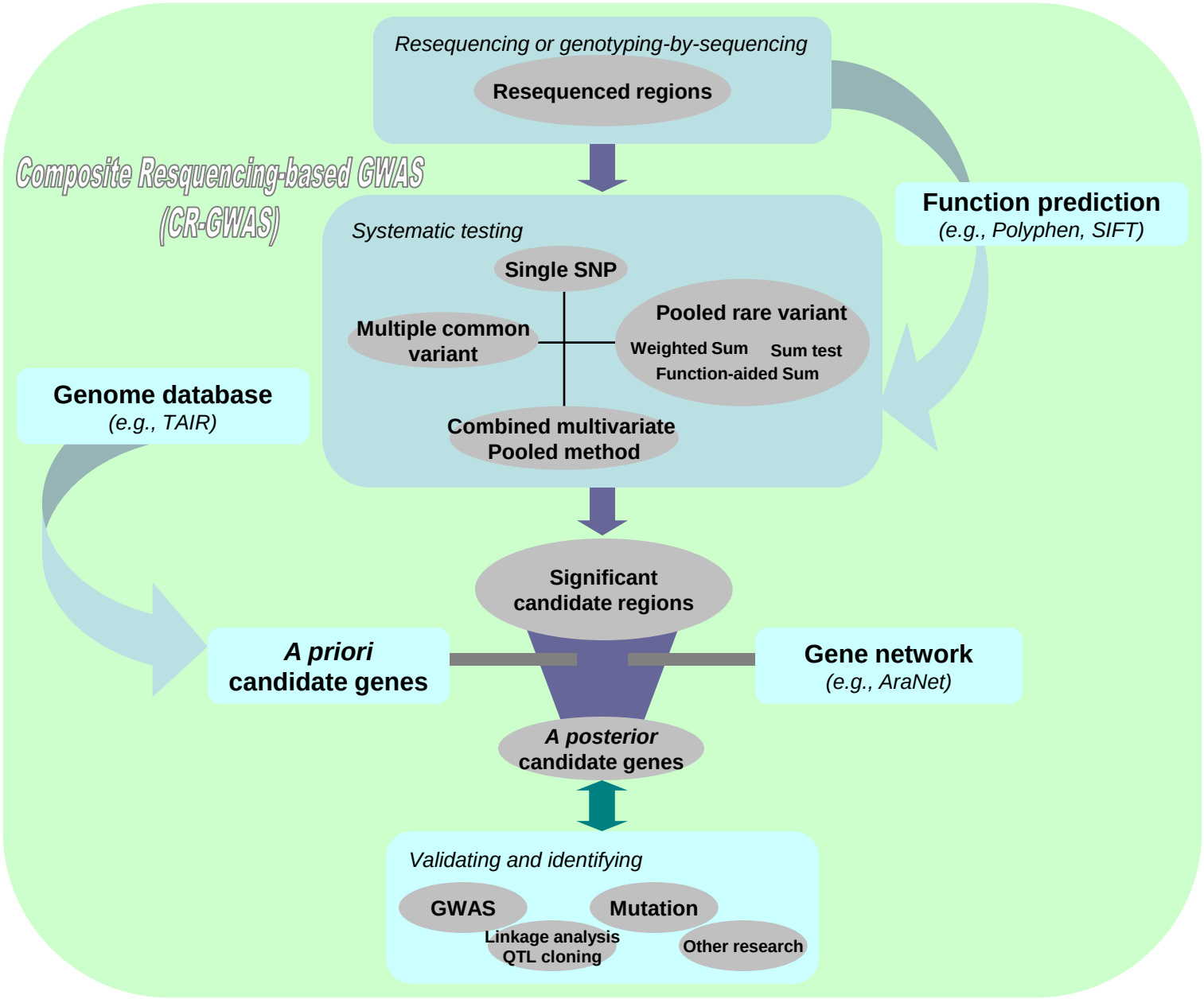
Genetic design + Resequencing: Tian *et al.* Nat Genet 2011 43:159-162
Genetic design + Resequencing: Kump *et al.* Nat Genet 2011 43:163-168

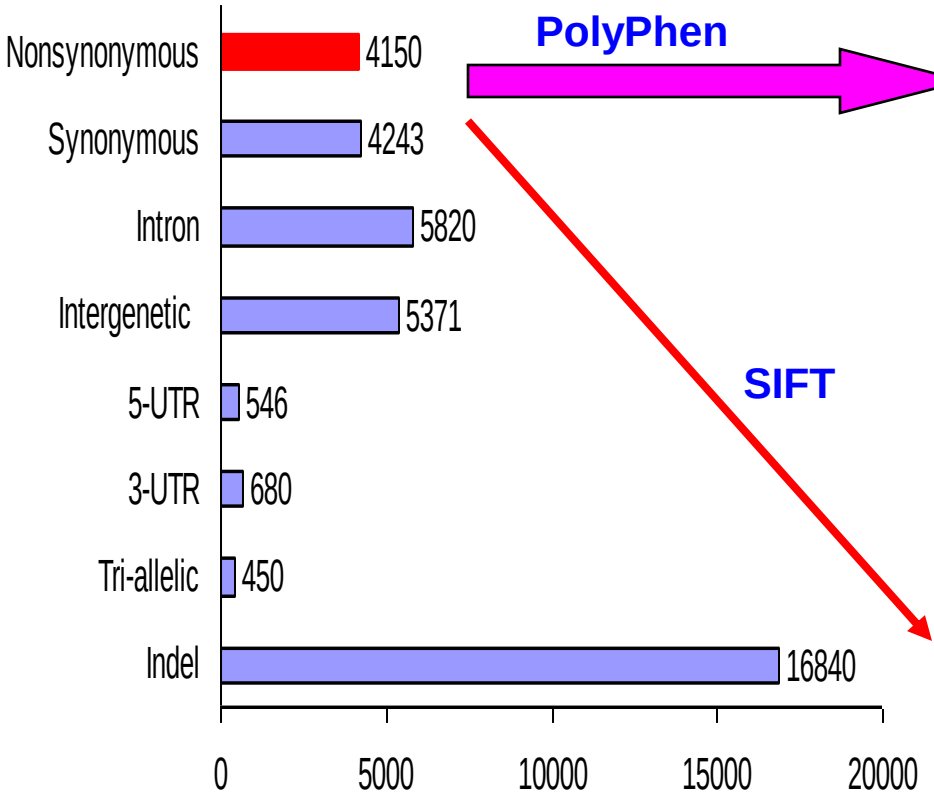


Critical Advances

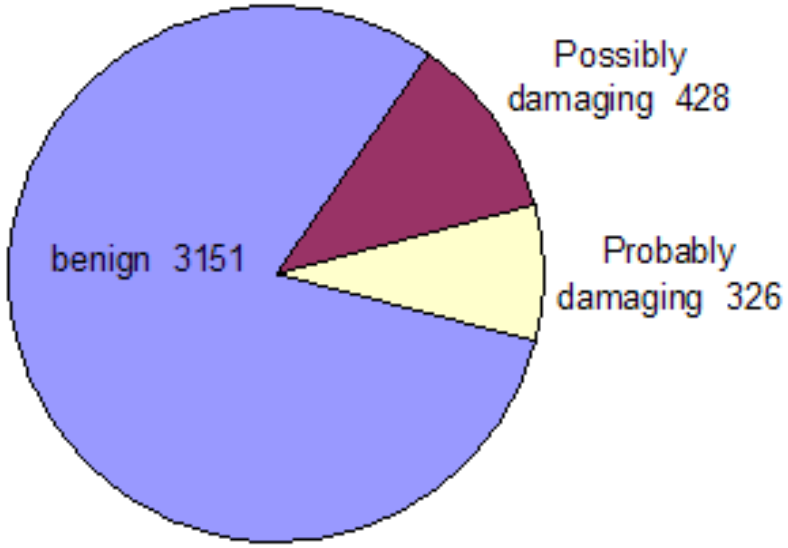
- With next generation sequencing technologies, **exome sequencing or whole genome resequencing** is now possible (*Shendure and Ji, 2008; Ansorge, 2009; Ng et al., 2010*).
- **Biological functions** of nucleotide polymorphisms can be **predicted** with the context sequence of genes (*Ramensky et al., 2002; Kumar et al., 2009*).
- Attention has been given to the **rare allele** issue (*Cohen et al., 2004; Bodmer and Bonilla, 2008; Nejentsev et al., 2009*) and some specific **statistics** have been developed to assess the significance of rare variants (*Morgenthaler and Thilly, 2007; Li and Leal, 2008; Madsen and Browning, 2009; Morris and Zeggini, 2010*).
- **Genome databases** and **gene networks** have been developed to aid the search and confirmation processes of gene-trait associations (*Lee et al., 2008; Lee et al., 2010; Lee et al., 2010*).

Composite Resequecing-based GWAS (CR-GWAS) integrates function prediction, genome database and gene network information, and common and rare variant testing

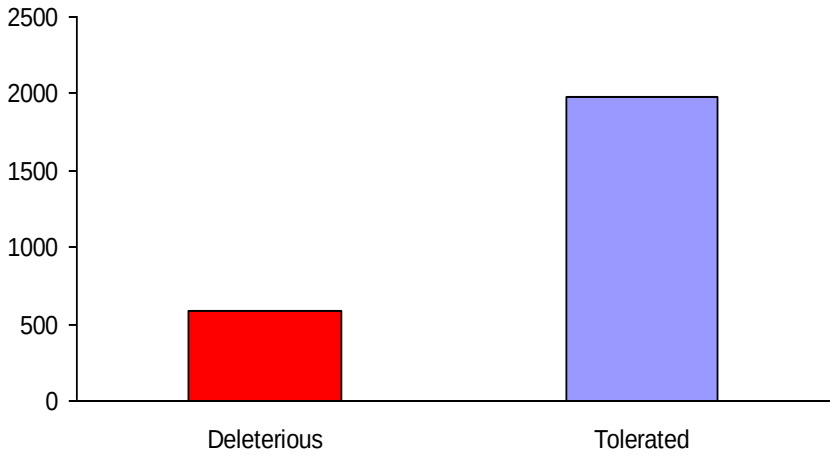




PolyPhen

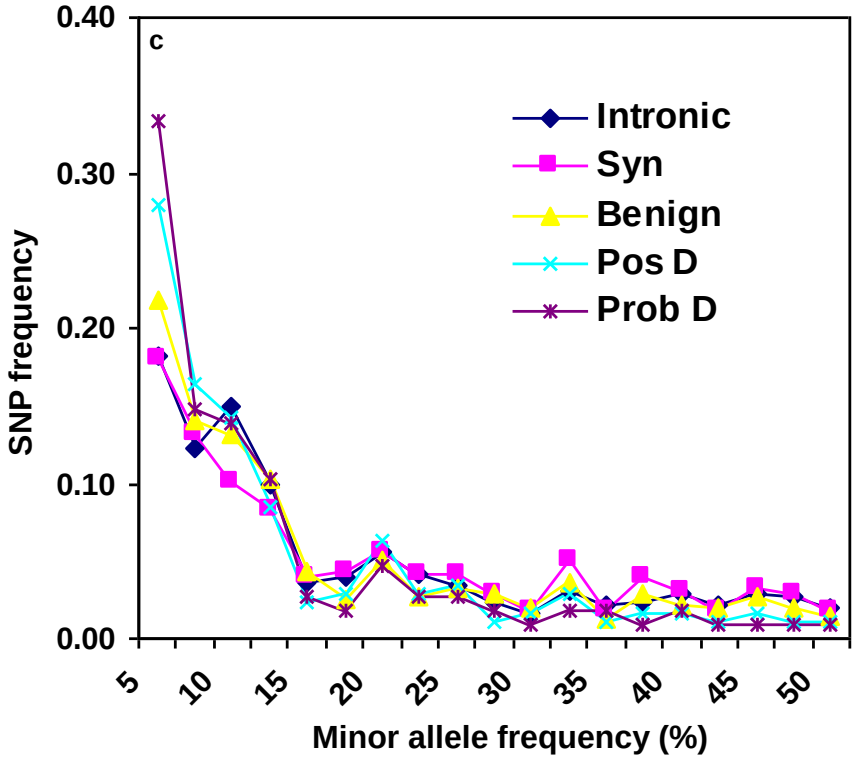


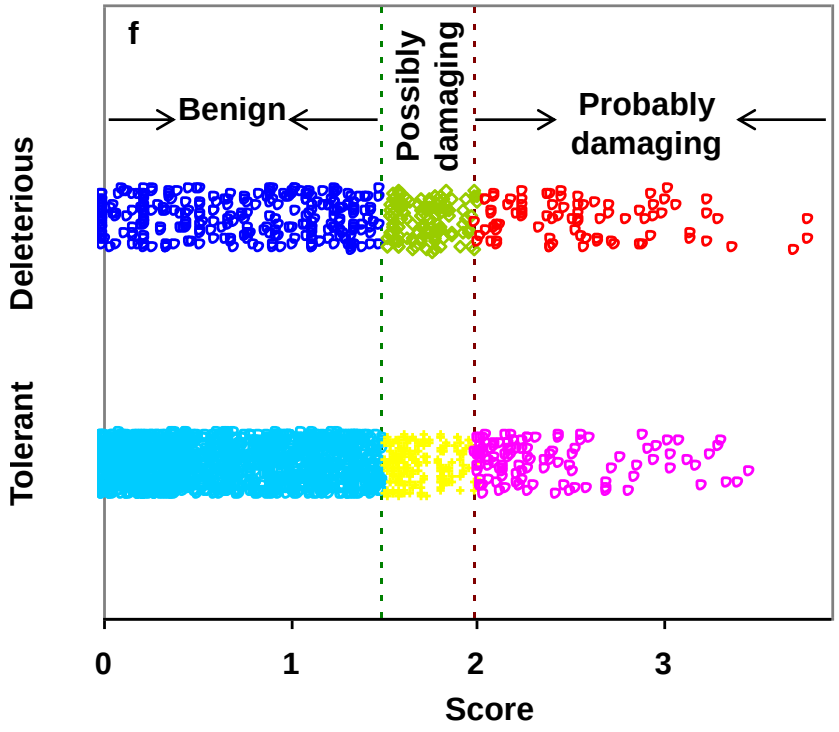
SIFT



Distribution of SNPs with different function prediction across different minor allele frequency

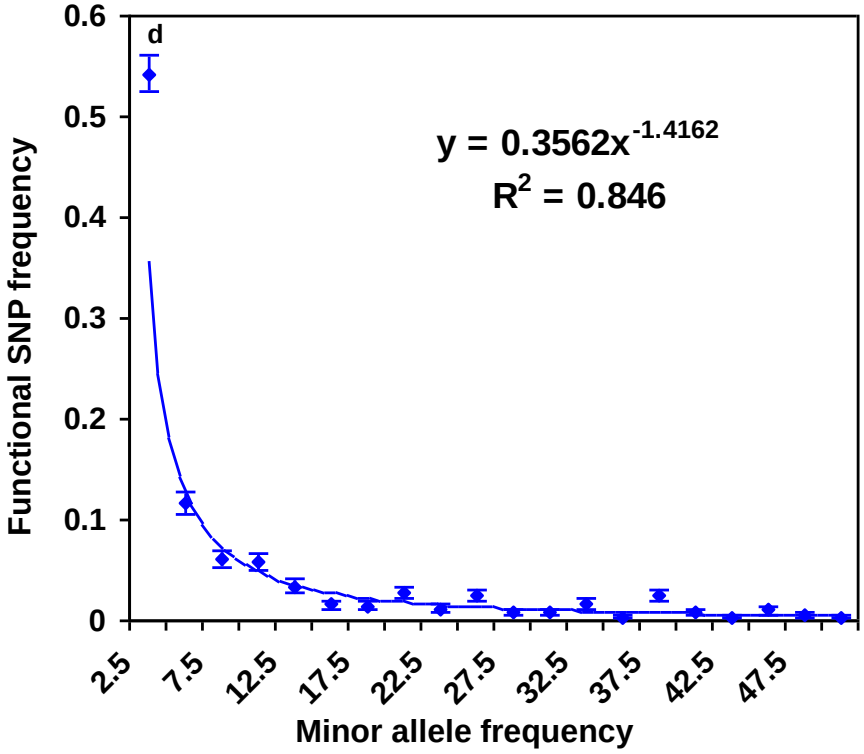
SIFT-predicted function class, deleterious or tolerant, and Polyphen-predicted score value.



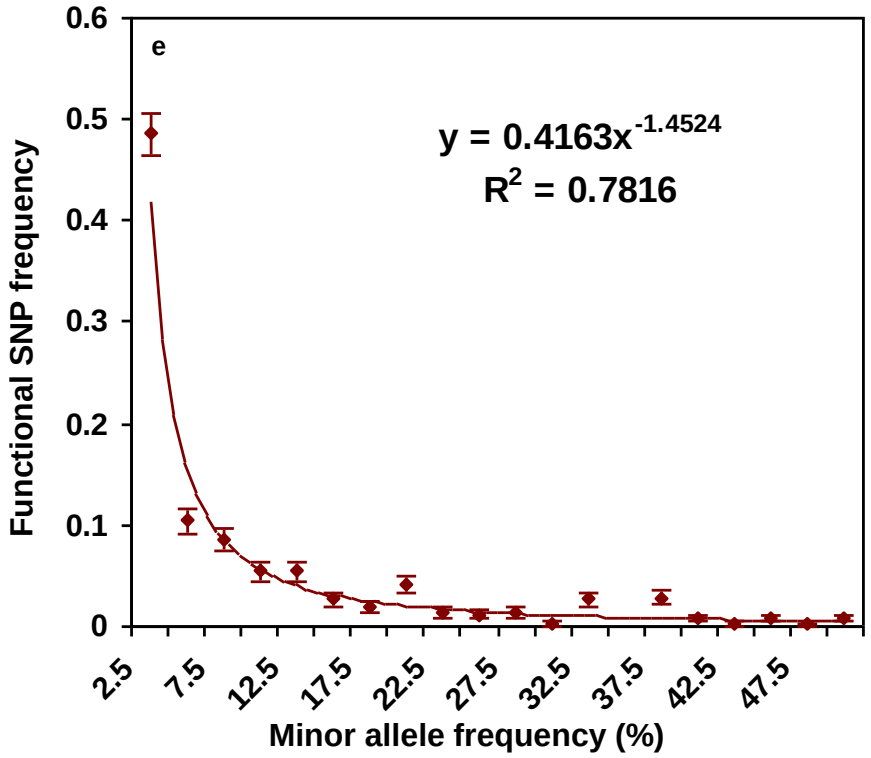


Functional SNP frequency & minor allele frequency

PolyPhen-predicted functional SNP frequency



SIFT-predicted functional SNP frequency



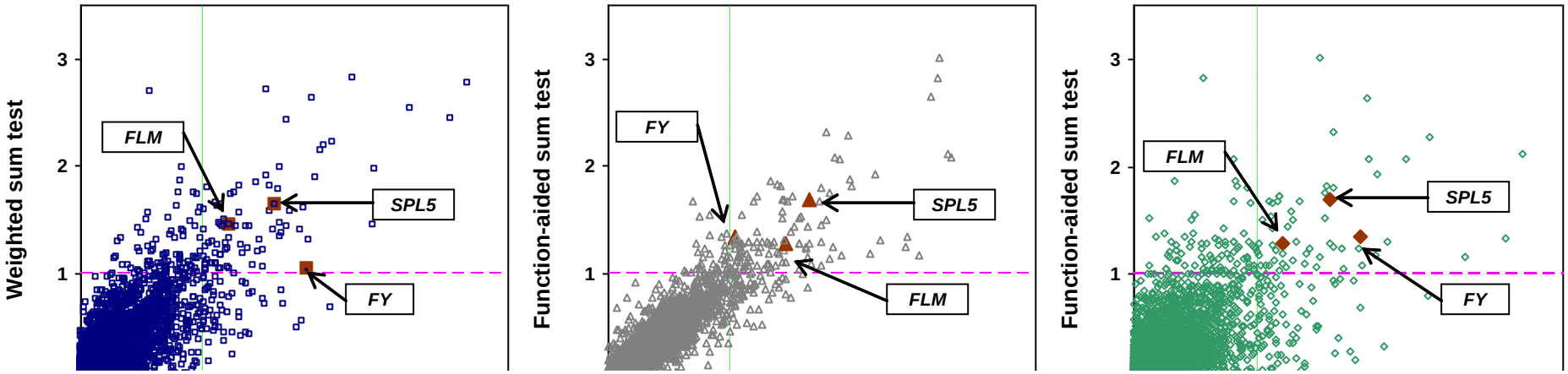
Rare allele statistics

$$y_i = \beta_0 + \beta_1 z_i + e_i$$

$$z_i = \sum_{j=1}^{m} \frac{x_{ij}}{m} \qquad z_i = \sum_{j=1}^{m} \frac{x_{ij}}{\sqrt{np_i(1-p_i)}} \qquad z_i = \sum_{j=1}^{m} S_j p_j^F x_{ij}$$

S = average probabilistic score of each class

$$p^F = 0.3562(p)^{-1.4162}$$



Candidate genes are over-represented among statistically significant associations.

Results for three genes (*FLM*, *SPL5*, and *FY*) with rare variants were consistent.

Genes with rare variants showing associations to flowering time

Gene & Gene ID	Sum test	Weighted sum	Function-aided sum	A priori candidate gene	Connected in AraNet	Supporting evidence
FLM AT1G77080	LD (3.32) JIC2W (4.78) JIC4W (3.23)	JIC2W (1.45)	JIC2W (3.12)	yes	yes	Scortecci *et al.* 2001 Werner *et al.* 2005
BAS1 AT2G26710	LD (4.19) SD (2.91) JIC2W (3.52)	LD (4.55) SD (3.47) JIC2W (3.69)	LD (4.33) SD (3.12) JIC2W (2.23)	yes	yes	Turk *et al.* 2005
SPL5 AT3G15270	JIC/USC (3.22)	JIC/USC (3.47)	JIC/USC (3.57)	yes	yes	Wu *et al.* 2009 Wu and Poethig 2006
FY AT5G13480	JIC2W (3.55) JIC8W (2.24)	JIC2W (4.05) JIC8W (2.31)	JIC2W (3.67)	yes	yes	Simpson *et al.* 2003 [Brachi *et al.* 2010]

Genes with common variants showing associations to flowering time

Gene & Gene ID	Single SNP test	Multiple common	Combined multivariate Pooled	A priori candidate Gene	Connected in AraNet	Supporting evidence [GWAS]
AP1 AT1G69120	JIC0W (2.91) FLC (2.85)	JIC0W (3.17) FLC (3.35) JIC4W (3.37) JIC8W (3.09)	JIC0W (3.28) FLC (3.72) JIC4W (3.48) VERN (3.77)	yes	yes	Gustafson-Brown *et al.* 1994 [Brachi *et al.* 2010] Mouradov *et al.* 2002
CR88 AT2G04030	JIC/USC (1.75)	JIC/USC (2.59)	JIC/USC (2.72)	yes	no	Cao *et al.* 2000
TIC AT3G22380	JIC4W (5.31)	JIC4W (1.87)	JIC4W (3.27)	yes	no	Ding *et al.* 2007
DCL2 AT3G03300	SDV (3.08)	SDV (3.55)	SDV (3.94)	no	yes	Henderson *et al.* 2006
FCA AT4G16280	±V(SD) (4.19)	±V(SD) (3.34)	±V(SD) (3.62)	yes	yes	Macknight *et al.* 1997 [Atwell *et al.* 2010] [Brachi *et al.* 2010] [Zhao *et al.* 2007]
FRI AT4G00650	FRI (14.78) FLC (4.13)	FRI (12.34) FLC (4.77)	FRI (9.43) FLC (3.68) JIC4W (4.23)	yes	yes	Johanson *et al.* 2000 Shindo *et al.* 2005 [Atwell *et al.* 2010] [Zhao *et al.* 2007]
FLC AT5G10140	SD/LD(V) (3.81)	SD/LD(V) (3.14) SDV (3.59)	SD/LD(V) (4.34) SDV (4.81)	yes	yes	Ratcliffe *et al.* 2001 [Atwell *et al.* 2010] [Zhao *et al.* 2007]

Summary

- We presented a CR-GWAS strategy to systematically exploit the collective biological information and analytical tools association analysis
- With the proposed strategy, we confirmed several well-known true positives and identified several new promising associations
- We identified AT3G03300 (*DCL2*) as a new target candidate in regulating flowering time in *Arabidopsis*
- We demonstrated that both common and rare variants contributed to the variation of flowering-time related traits

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Integrating Rare-Variant Testing, Function Prediction, and Gene Network in Composite Resequencing-Based Genome-Wide Association Studies (CR-GWAS)

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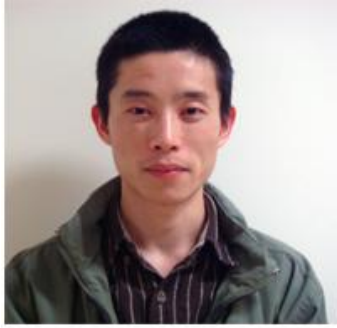
ABSTRACT High-density array-based genome-wide association studies (GWAS) are complemented by exome sequencing and whole-genome resequencing-based association studies. Here we present a composite resequencing-based genome-wide association study (CR-GWAS) strategy that systematically exploits collective biological information and analytical tools for a robust analysis. We showcased the utility

KEYWORDS
complex trait
dissection
association

II. Genomic Distribution of Trait-Associated SNPs (TAS)

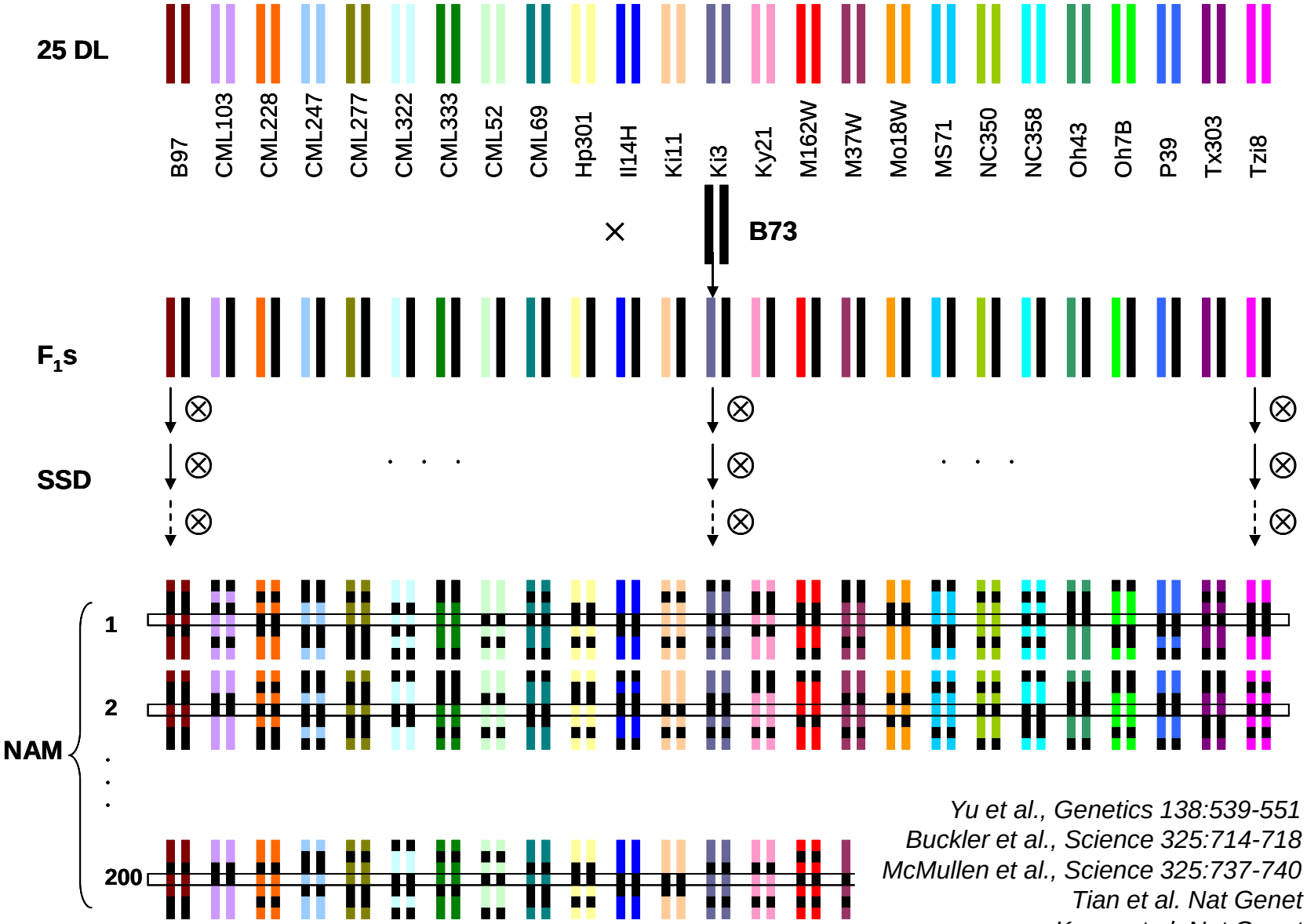


- Genetic Architecture of Complex Traits
  - **How many loci**?
  - What is the frequency of different alleles?
  - **Genomic location of these loci**?
  - **Genetic effects** (*e.g.,* homozygous, heterozygous, epistatic and pleiotropic effects) of these loci?
  - Gene x gene, gene x background, gene x environment interaction?

- Tabulation of previous QTL mapping results, information from QTL cloning experiments

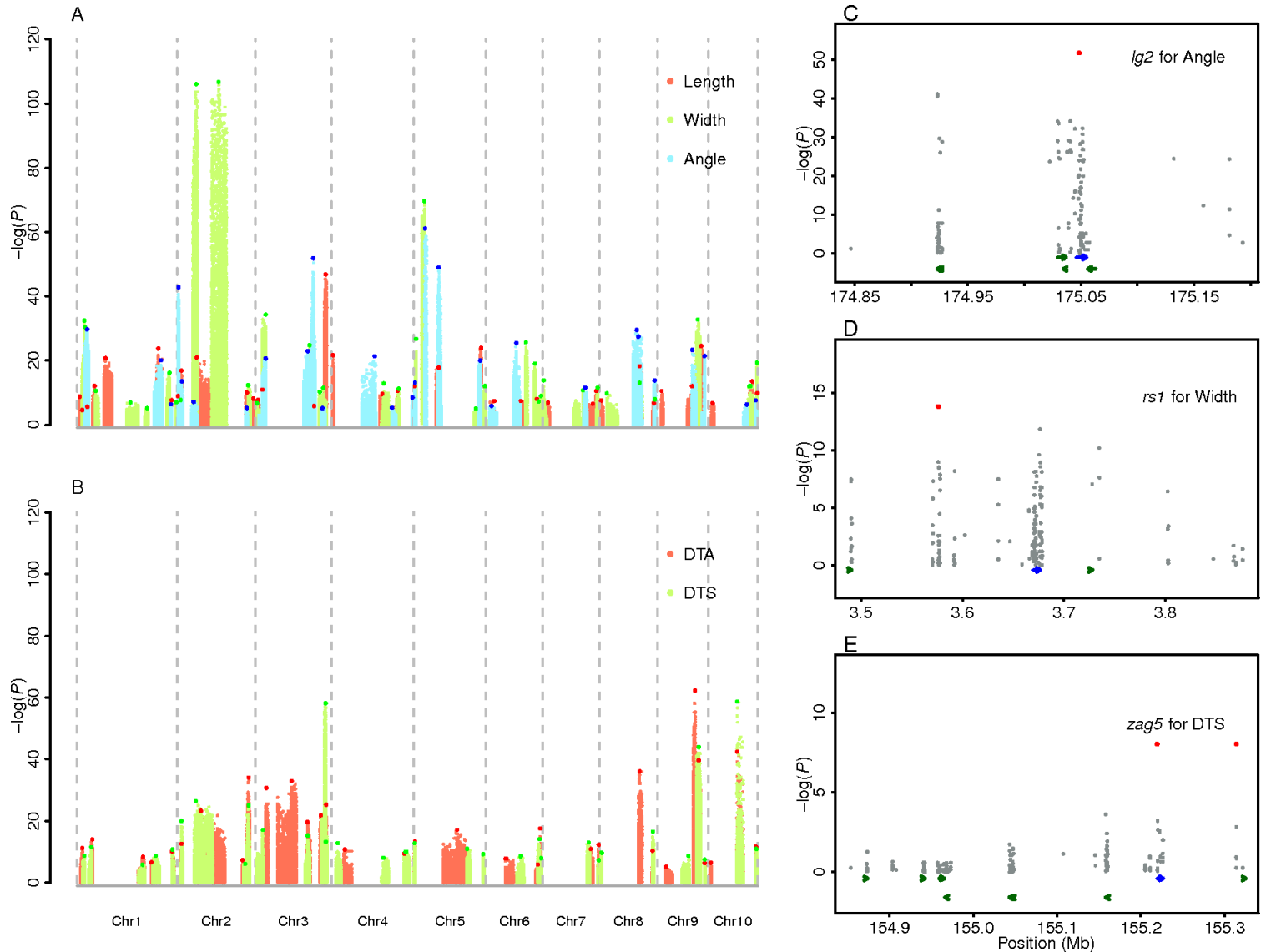
- Tabulation of Trait-Associated SNPs (TASs) from NAM-GWAS
  - Which part of the genome should be prioritized?

An integrated mapping strategy, NAM

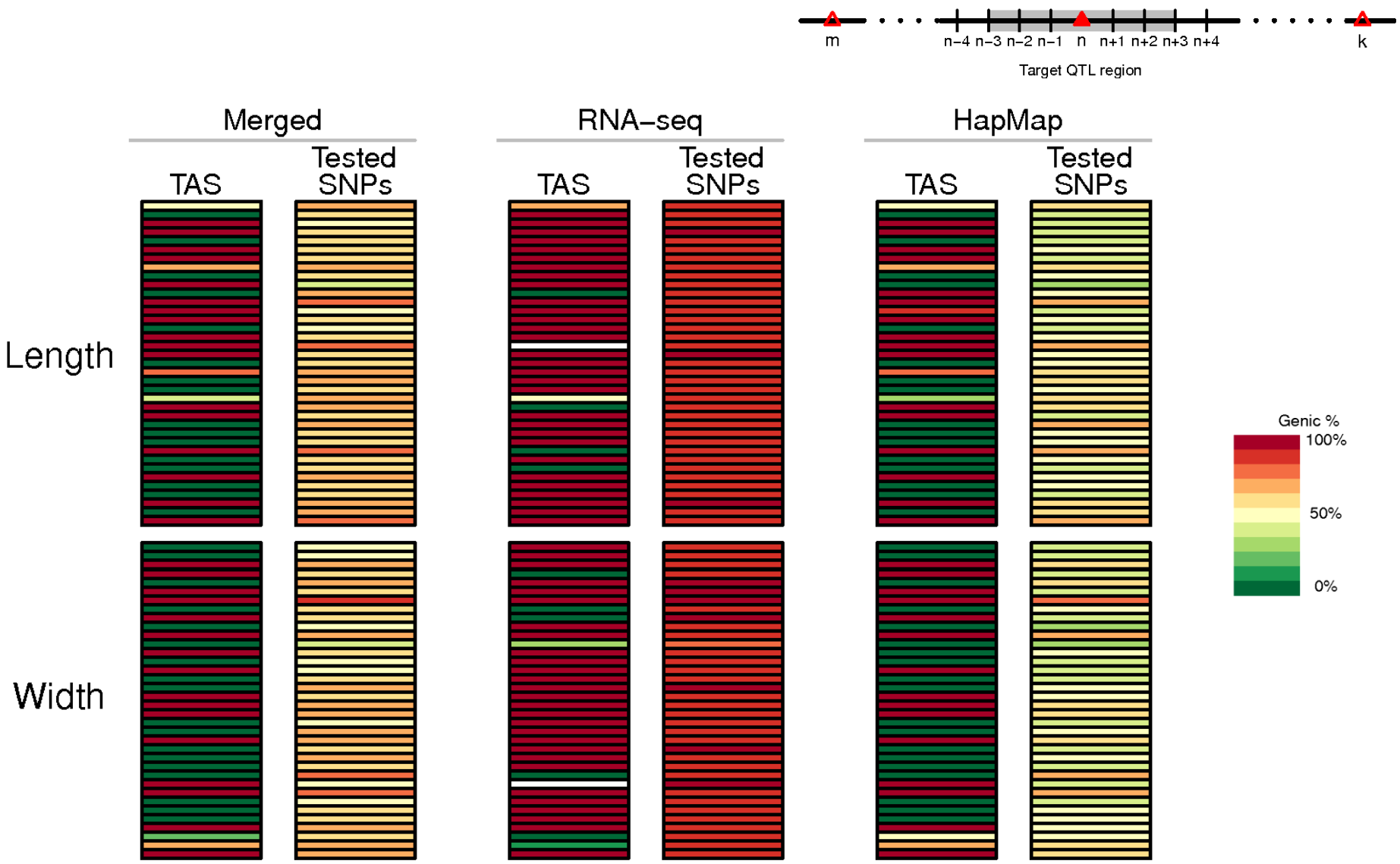


Yu et al., Genetics 138:539-551 (2008)
Buckler et al., Science 325:714-718 (2009)
McMullen et al., Science 325:737-740 (2009)
Tian et al. Nat Genet (2011)
Kump et al. Nat Genet (2011)

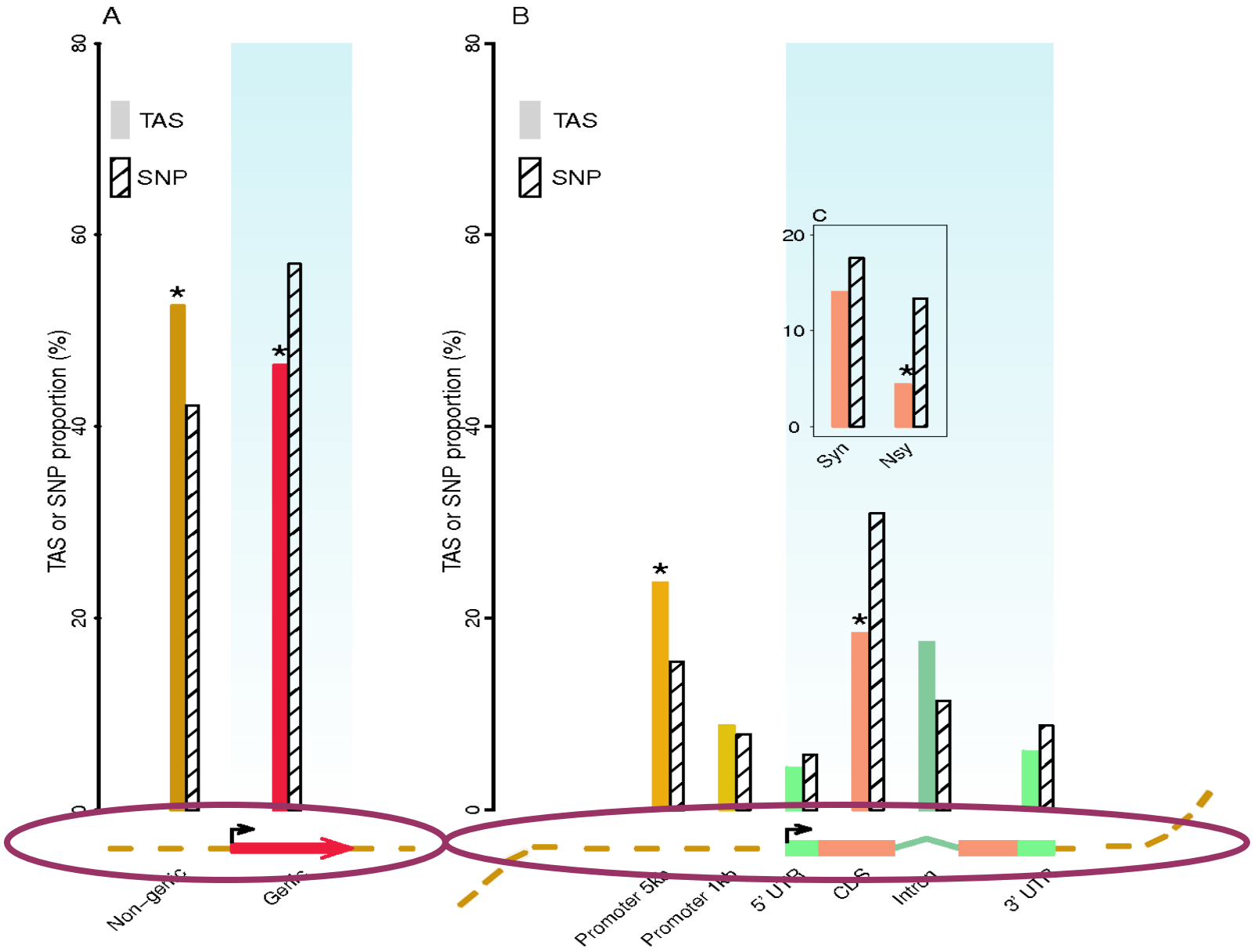
High Resolution of NAM-GWAS



Two-stage, targeted dissection genome scan



Genic + Promoter 5kb = 13% of maize genome, account for 71% of TASs



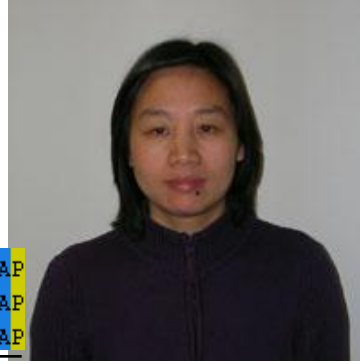
Summary

- Genic and non-genic TASs contribute approximately equally to phenotypic variation for maize quantitative traits

- Genic+promoter region, while comprises only 13% of maize genome, account for 71% of identified TASs and explain 79% of PVE of all TASs
  - *Evolutionary alterations in protein sequence appear to be quantitatively less important than changes in gene regulation in shaping the wide natural variation observed in maize.*

- Genotyping methods designed to discover SNPs in genes and their upstream regions would be the most economical approach for detecting genome-wide association signals
  - *The combination of RNA-seq and exome capture experiments using a long read (e.g., 454) and paired end (Illumina and 454) technologies*

III. Multiple functional haplotypes at a single locus



Tannin1 Gene Cloning, Wu *et al., 2012 PNAS (online first)*

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Tan1     MDLPKPPSTAASSSGAETPNPHAFTCELPHSIYALAFSPGAPVLASGSFLEDLHNRVSLLSFDPVRPSAASFRALPALSFDHPYPPTKLQFNPRAAAP
tan1-a   MDLPKPPSTAASSSGAETPNPHAFTCELPHSIYALAFSPGAPVLASGSFLEDLHNRVSLLSFDPVRPSAASFRALPALSFDHPYPPTKLQFNPRAAAP
tan1-b   MDLPKPPSTAASSSGAETPNPHAFTCELPHSIYALAFSPGAPVLASGSFLEDLHNRVSLLSFDPVRPSAASFRALPALSFDHPYPPTKLQFNPRAAAP

Tan1     SLLASSADTLRIWHAPLDDLSATASAPELRSVLDNRKAASEFCAPLTSFDWNEVEPRRIGTASIDTTCTVWDIDLGVVETQLIAHDKAVHDIAWGEA
tan1-a   SLLASSADTLRIWHAPLDDLSATASAPELRSVLDNRKAASEFCAPLTSFDWNEVEPRRIGTASIDTTCTVWDIDLGVVETQLIAHDKAVHDIAWGRP
tan1-b   SLLASSADTLRIWHAPLDDLSATASAPELRSVLDNRKAASEFCAPLTSFDWNEVEPRRIGTASIDTTCTVWDIDLGVVETQLIAHDKAVHDIAWGEA

Tan1     VFASVSADGSVRVFDLRDKEHSTIVYESHRPDTPLLRLAWNRSDLRYMAALLMDSSAVVVLDIRAPGVPVAELHRHRACANAVAWAPQATRHLCSAGD
tan1-a   SSPPCRPTAPSASSTSGTRNTPPSSTRAPAPTRRSSGWRGTALTSAIWPRCSWTAAPSSCSTYVRPGCRLPSCTGTGRAPTQSRGRRRPLGTSARLGT
tan1-b   VFASVSADGSVRVFDLRDKEHSTIVYESHRPDTPLLRLAWNRSDLRYMAALLMDSSAVVVLDIRAPGVPVAELHRHRACANAVAWAPQATRHLCSAGD

Tan1     DGQALIWELPETAAAVPAEGIDPVLVYDAGAEINQLQWAAAHPDWMAIAFENKVQLLRV   353 aa
tan1-a   TGRH                                                          298 aa
tan1-b   DGQALIWELPETAAGSGGCARRGD                                      318 aa
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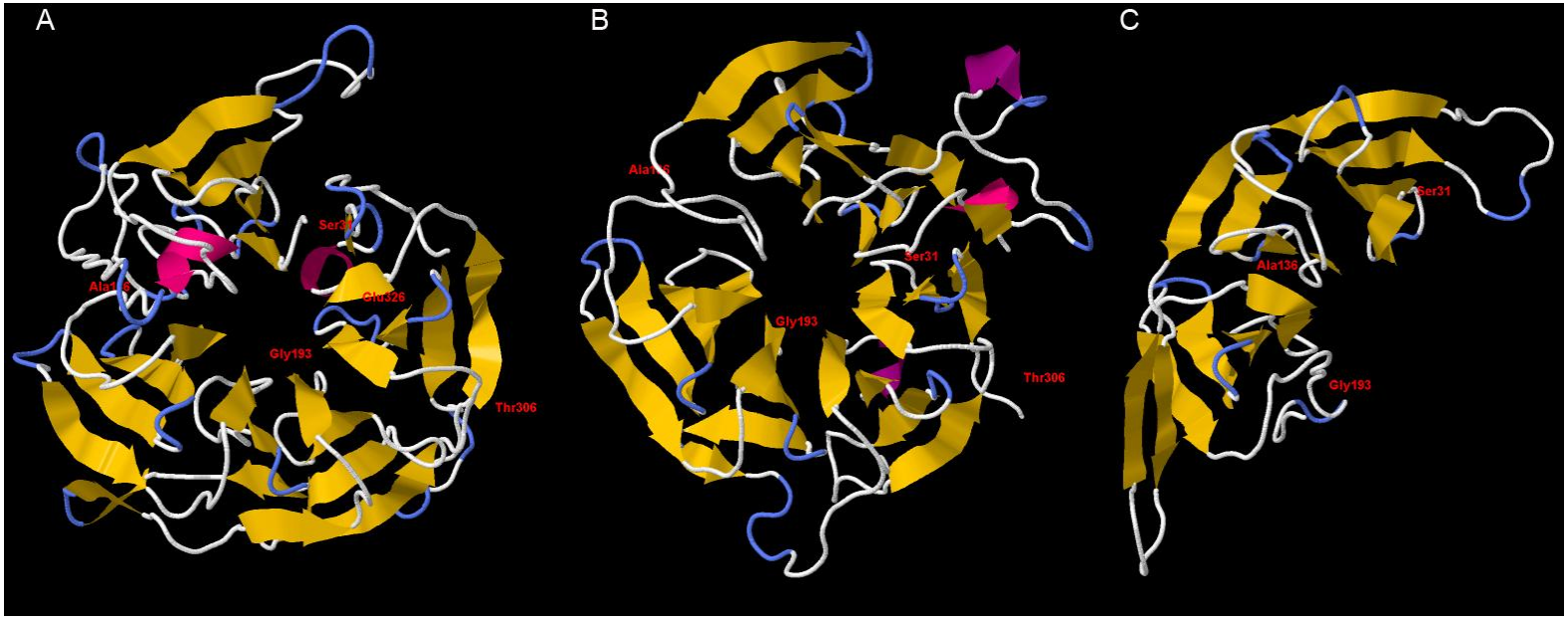
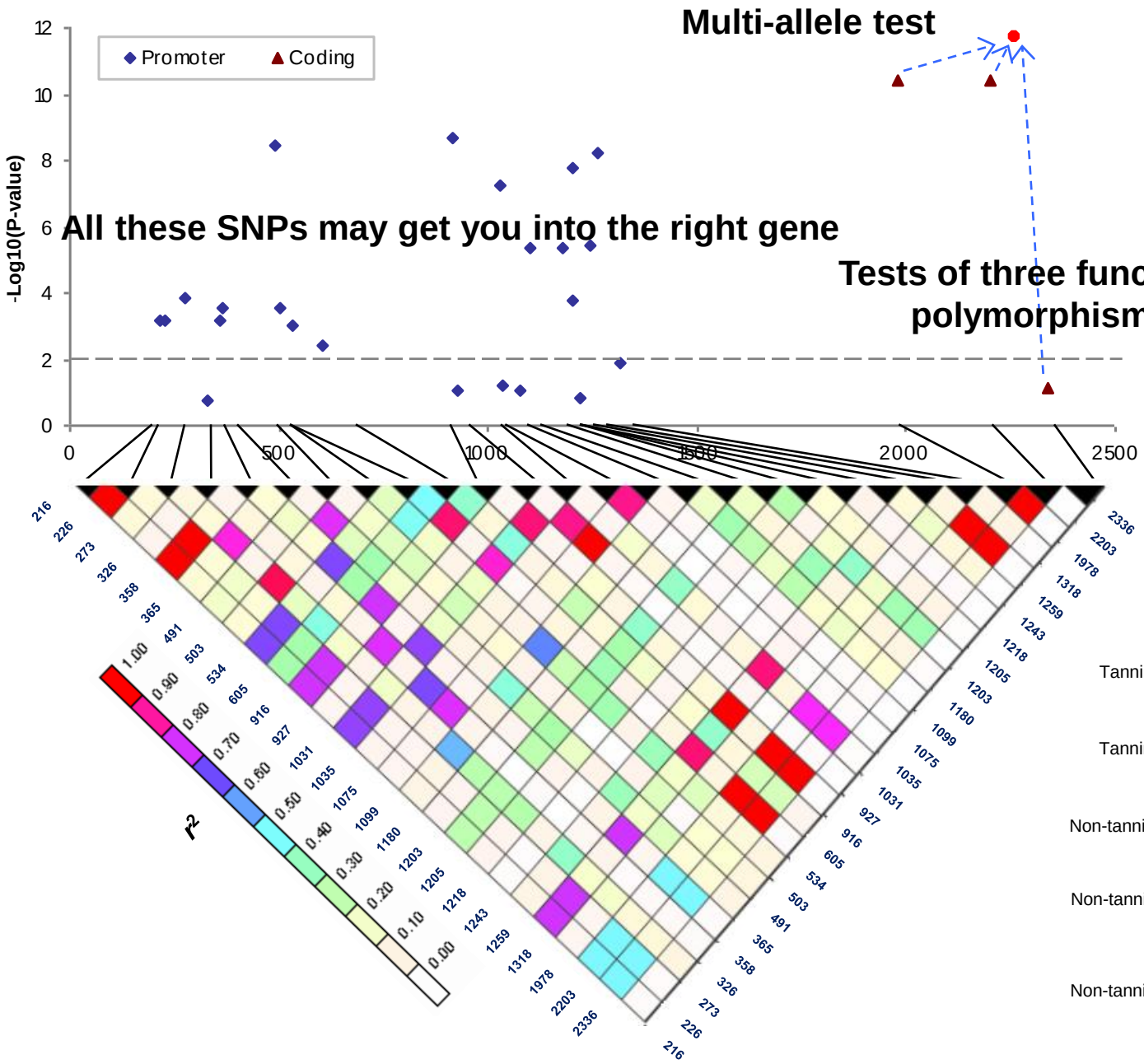
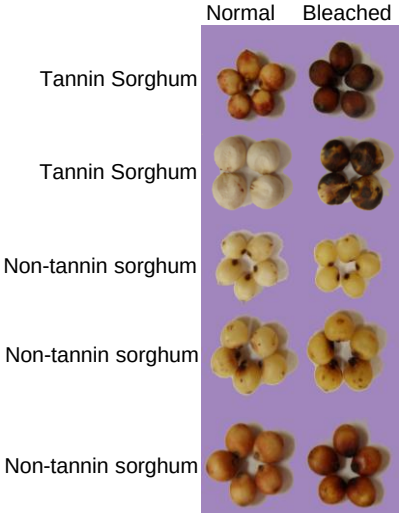


Tannin1 Gene Cloning, Wu *et al.*, 2012 *PNAS (online first)*





Shattering1 Gene Cloning, Lin *et al.*, 2012 *Nature Genetics 44:720–724*

a

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SV      1 MSAQQIAPVPEHVCYVHCNFCNTILAVSVPSHSMLNIVTVRCGHCTSLLSVNLRGLLQSL 60
Tx430   1 MSAQQIAPVPEHVCYVHCNFCNTILAVSVPSHSMLNIVTVRCGHCTSLLSVNLRGLLQSL 60
SC265   1 MSAQQIAPVPEHVCYVHCNFCNTILAVSVPSHSMLNIVTVRCGHCTSLLSVNLRGLLQSL 60
Tx623   1 MSAQQIAPVPEHVCYVHCNFCNTILALQRRGNVFLQHITDLLRKRYEGLKQATQT----- 55
          ************************.    .  *   *

SV     61 PVQNHYSQENNFKVQNFSFTENYPEYAPSSSKYRMPTMLSAKGDLDHMLHVRAPEKRQRV 120
Tx430  61 PVQNHYSQENNFKVQNFSFTENYPEYAPSSSKYRMPTMLSAKGDLDHMLHVRAPEKRQRV 120
SC265  61 PVQNHYSQENNFKVQNFSFTENYPEYAPSSSKYRMPTMLSAKGDLDHMLHVRGKRYEGLK 120
Tx623  55 ------------------------------------------------------------ 55

Sv    121 PSAYNRFIKEEIRRIKASNPDISHREAFSTAAKNWAHFPNIHFGLGPYESSNKLDEAIGA 180
Tx430 121 PSAYNRFIKEEIRRIKASNPDISHREAFSTAAKNWAHFPNIHFGLGPYESSNKLDEAIGA 180
SC265 121 QATQT------------------------------------------------------- 125
Tx623  55 ------------------------------------------------------------ 55

Sv    181 TGHPQKVQDLY 191
Tx430 181 TGHPQKVQDLY 191
SC265 125 ----------- 125
Tx623  55 ----------- 55
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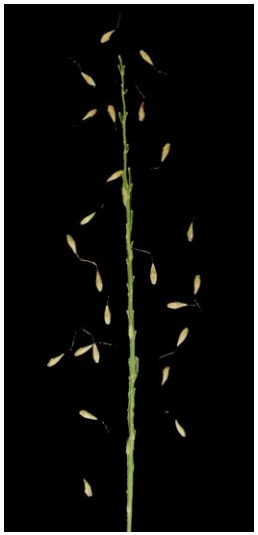

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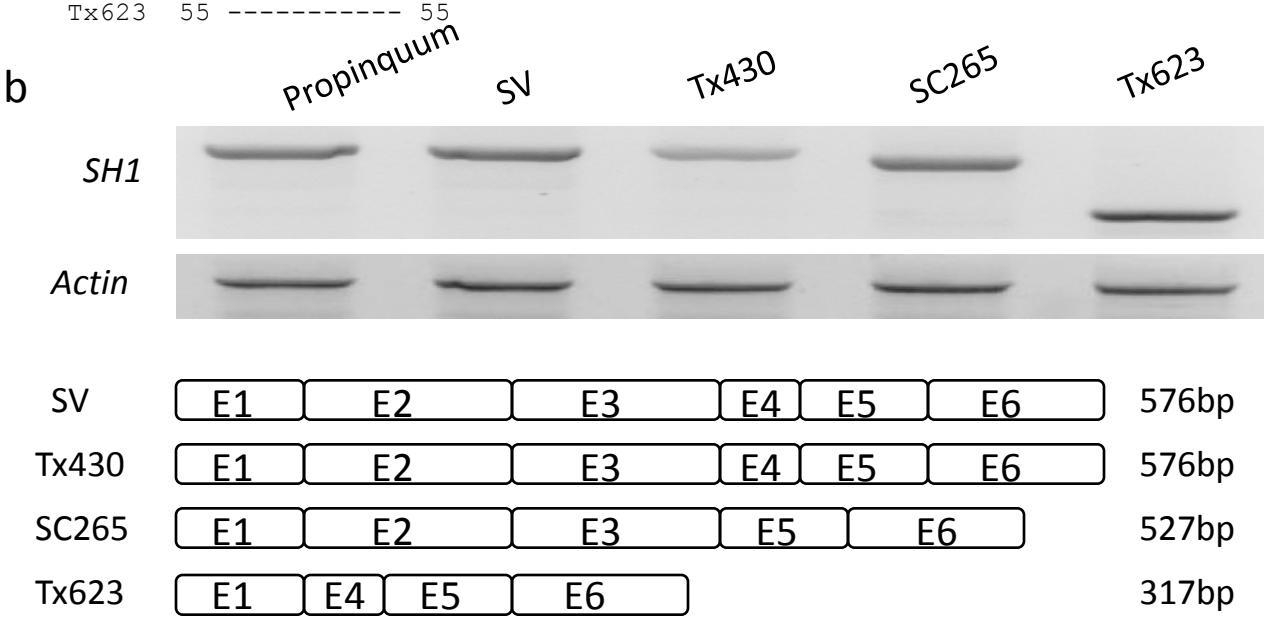
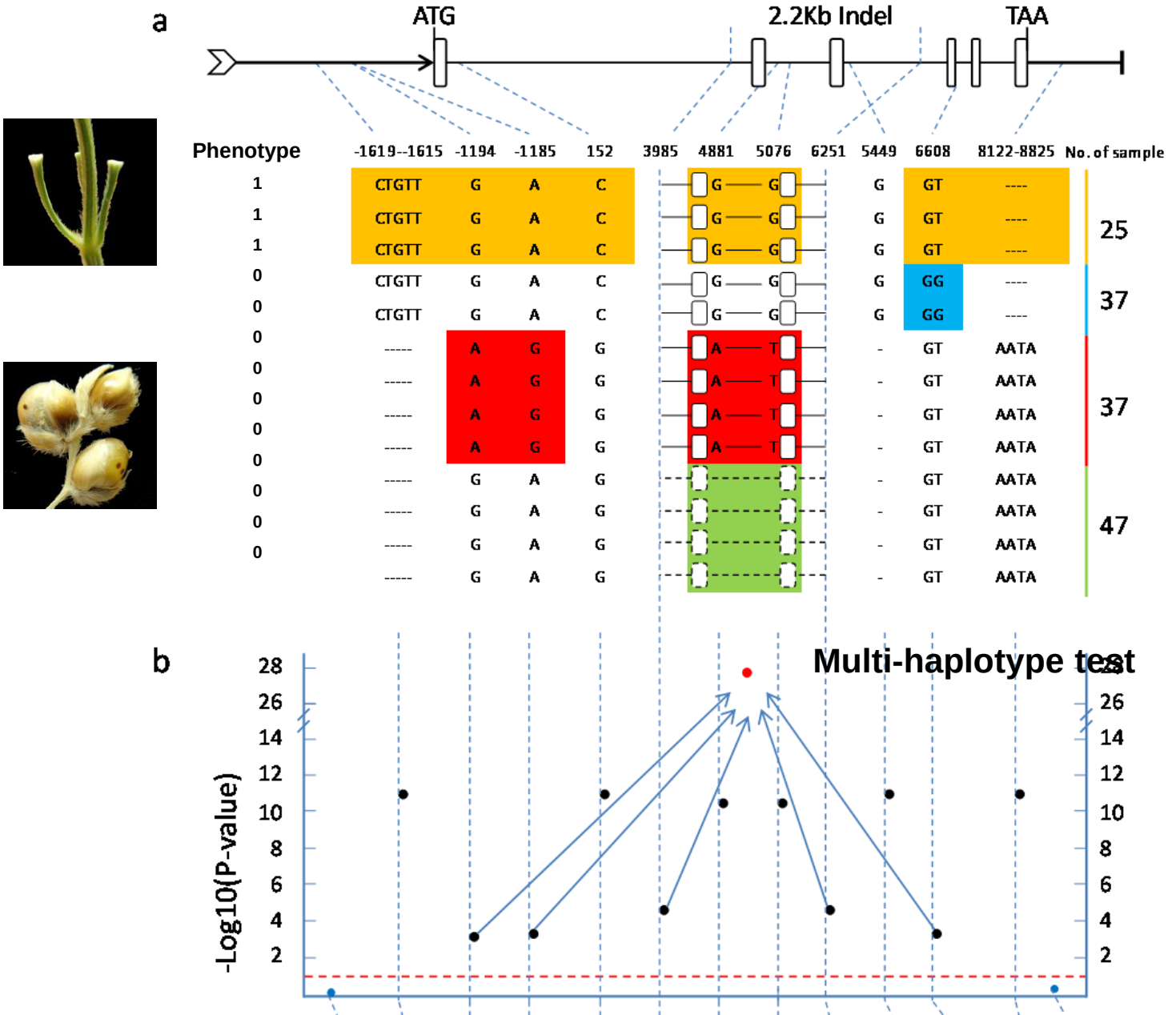


Shattering1 Gene Cloning, Lin *et al., 2012 Nature Genetics 44:720–724*



Overall Summary

- Traits are complex! Going genome-wide presents opportunities and challenges
- Many "**biological**" and "**statistical**" tools are available and it sure helps to use a combination of them
  - Geno→Pheno & Geno↔Pheno
- QTL → → TAS → → functional polymorphisms → → "**haplotypes**"
  - GWAS and GS
- Genic or non-genic region harbors functional polymorphisms?
  - Different functional polymorphisms/haplotypes for different allellic series, complex *versus* less complex traits
- How do we validate the findings of TAS/QTN through GWAS, with small-moderate effects, for complex traits?
  - ZFN, TALEs?

Communication

Statisticians	Biologists
These are the significant variables. All we can do so far.	Why my favorite genes are not tagged? No other choices?
These are the cut-off threshold that was used.	What if we changed that a bit? Will it make my genes significant?
These are the ones that are worth follow-up studies.	I only have one postdoc/student to chase after the strongest one.
We can look into other newly developed methods.	Well, I will have “HapMap X” data ready next week.
Statistical methods (generalized, less assumptions, higher power) Experimental design	Data collection (x, y, expression, protein, independent experiment, trangenic complementation, mutants) Biological questions

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