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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

PUTTING THE EDV CONCEPT INTO PRACTICE FOR MAIZE:
SSRS TODAY AND SNPS TOMORROW? (REVISED)

Document prepared by an expert from the United States of America

Abbreviations used in this document:

ASTA:	American Seed Trade Association
CVIS:	Cultivar Variety Identification Sub-Committee
EDV:	Essentially Derived Variety
Off-PVP:	Varieties with Expired Plant Variety Protection
PIC:	Polymorphic Information Content
PVP:	Plant Variety Protection
SEPROMA:	*Chambre Syndicale des Entreprises Françaises de Semences de Maïs*
SNP:	Single Nucleotide Polymorphism
SSR:	Simple Sequence Repeat
US:	United States of America

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Putting the EDV concept into practice for Maize: SSRs today and SNPs tomorrow?

Slide 2

Status of ASTA CVIS Project: Selection of SSR Set for US EDV

ASTA = American Seed Trade Association: 550 members
CVIS = Corn Variety Identification Sub-Committee

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Inbreds Analyzed

- 100 inbreds
- Publically available germplasm
  - 86 yellow dent, 9 Sweet corn, 7 white dent
- Includes Mikel and Dudley[1] 'widely used inbreds' in US germplasm
  - Off-PVP (expired PVP): 13 Pioneer, 8 Holden, 5 Dekalb, 2 Syngenta, 1 Asgrow, 1 Northrup King

[1]Crop Sci. 2006 46: 1193-1205

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SSR data: Biogenetics

- Gel-based SSR system (3% high-resolution agarose)
- 98 inbreds with data
  - 2 inbreds failed to germinate
- 285 SSRs
  - selected for analysis by ASTA based on being high quality and highly polymorphic in ASTA member labs
- Biogenetics selected a sub-set of 150 SSRs based on quality (ability to score), polymorphism and genome distribution
- 60 SSRs common to SEPROMA 163 SSR set and ASTA 285 SSR set

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Missing Data and Heterozygotes for 285 SSRs

- Average missing data 0.23% (range 0-6.7%)
- Average heterozygotes 2.5% (range 0-31%)
  - Sample with high hets is Minn13; population
  - Range 0-13% without Minn13

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Comparison of Number of Alleles in Different Studies

Marker Set	Reported by member companies on own inbreds – sequencing systems?	ASTA/Biogenetics study on ASTA inbreds - agarose	SEPROMA study on SEPROMA inbreds – sequencing system
285 ASTA SSRs	11.4 (2-40)	3.4 (1-8)	
150 ASTA SSRs	13.2 (3-40)	3.7 (2-8)	
60 common to SEPROMA set and 285 ASTA SSRs	13.4 (4-26)	4.1 (2-8)	5.9 (3-11)

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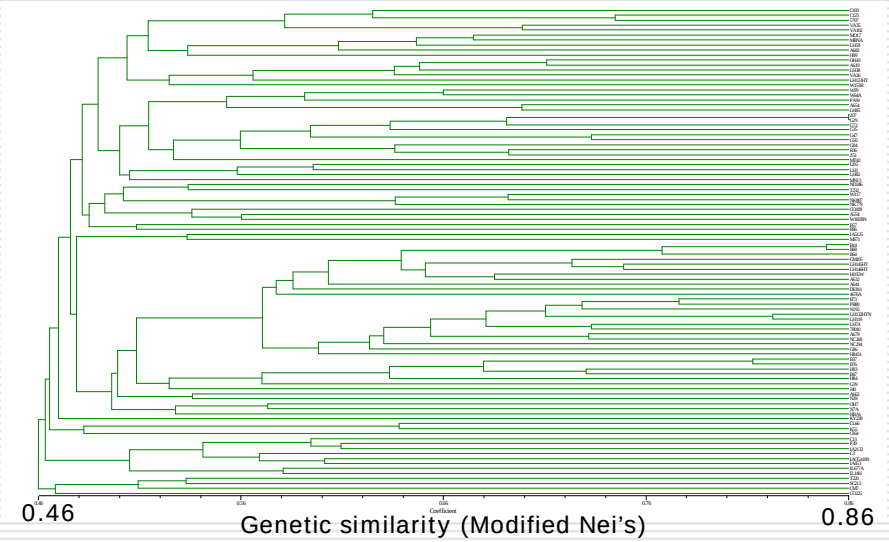
Genome coverage (IBM2 2005)

- 285 SSR marker set
  - Genome coverage 90.5%
  - 94% bins have 1 or more markers
  - Average Distribution 25.0 cM
    - Range 0-101.7 cM, 3 markers > 100 cM
- 150 SSR Marker Set
  - Genome coverage 88.8%
  - 90% bins have 1 or more markers
  - Average Distribution 47.2 cM
    - Range 0-195.9 cM, 8 > 100 cM

Slide 8

Dendrogram 98 ASTA inbreds



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Status of ASTA CVIS Project: Consideration of 'Next Generation' Technology: SNPs

Slide 10

Why Consider Moving to SNPs?

- SSRs – resolution of different systems results in different numbers of alleles in different systems and **potentially different determinations of EDV**
- Results from one SSR system can not be easily compared to another
- Most companies use SNPs in maize for greater throughput and to reduce costs

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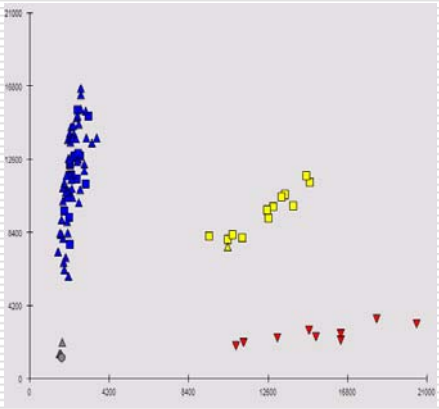
Why SNPs?



- Same number of alleles with different systems - consistent determination of EDV across chemistries and platforms
- Results from across laboratories can be compared
- Results can be standardized world-wide
- Lower error rates, lower cost, compared with SSRs
- In, or next to, transcribed genes

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Using SNPs to Determine EDVs

- Loss of polymorphism for SNPs on a single-locus basis: SSRs multi-allelic: SNPs bi-allelic
- How many SNPs?
- How do SNP based distances compare with SSR distances?
- How do SNP based distances compare with pedigree relatedness?
- What are equivalent thresholds for determining EDV using SNPs?

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SNP Profiling on ASTA Inbreds

- Availability: Over 6,000 SNPs currently available on www.panzea.org
- 465 public SNP markers selected based on being previously validated, polymorphic among the ASTA inbreds and having map locations
  - Average 9.9 % missing data (range 0 – 39.6%)
  - Average 3.9% heterozygous loci (range 4.2-25.1%)
- Higher missing data than for SSRs – reflects difficulty in scoring non-fixed inbreds using cluster analysis?
  - This should not be an issue for EDV assessment of uniform varieties, therefore we did not remove markers based on missing data (markers have proven to be good quality in previous projects)
- Similar level of heterozygous loci compared with SSRs
- A sub-set of 320 SNPs were selected: removed markers at same location, selecting highest PIC markers

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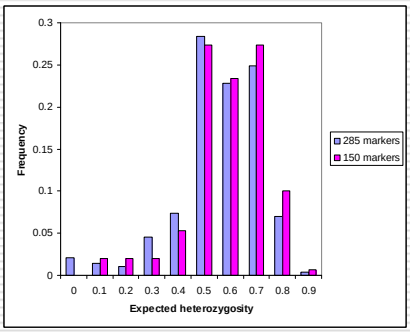
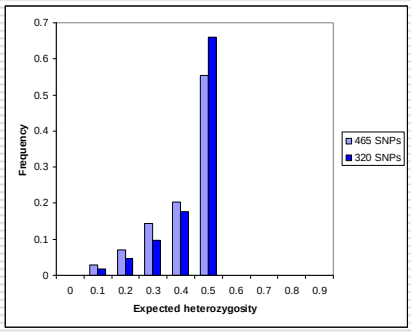
Expected Heterozygosity

SSRs



SNPs



- 285 marker average = 0.51
- 150 marker average = 0.54
- 465 marker average = 0.38
- 320 marker average = 0.41

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Genome distribution SNP markers 465 and 320 marker sets

- Genome coverage 87.8%
- No bin information for SNP markers
- Average Distribution 21.4 cM
  - Range 0.2-112.8 cM, 3 markers > 100 cM
- Equivalent coverage to SSR set

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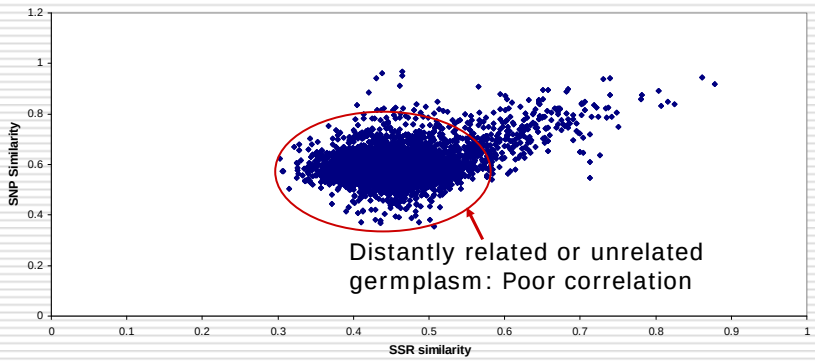
Correlation between SSRs and SNPs for ASTA Genotypes



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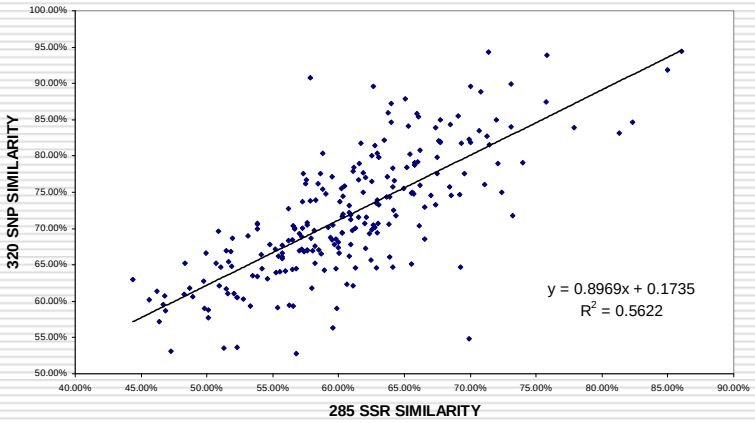
Correlation between SSRs and SNPs for *RELATED* ASTA Genotypes (Malecot's > 0.2)



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Equivalent Analysis of Pioneer Germplasm with SSRs and SNPs

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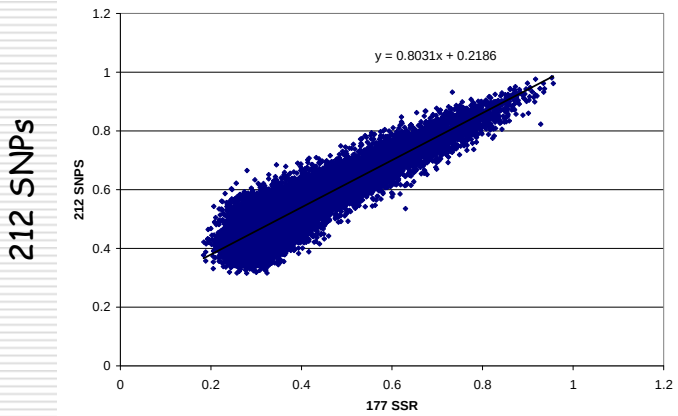
Correlation Between Genetic Similarities with SSRs and SNPs in Pioneer US PVPd Inbreds



212 SNPs

$R^2 = 0.92$
Slope = 0.80

Similar correlation for EU PVPd set with SAME markers

177 SSRs

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Equivalent Thresholds for SNPs Using Equation for Line of Best Fit and Pioneer PVPd Inbreds

SSR threshold % similarity	SNP threshold % similarity
82	87-90
90	93-94

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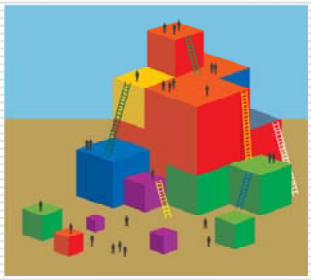
SSRs in EDV: Status

- Industry in Europe has established a set of SSRs to help in determination of EDV
- Industry in US is on track to reach that goal



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SNPs Tomorrow?

- Technology is here and rapidly replacing SSRs
- Research on EDVs comparing with SSRs with SNPs is the first step in helping industry look forward to this new technology

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