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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

ADDENDUM

THE SPANISH EXPERIENCE (GESLIVE-IRTA) ON THE ENFORCEMENT OF PLANT VARIETY RIGHTS: DNA-FINGERPRINTING

Document prepared by experts from Spain

The Spanish Experience (GESLIVE-IRTA) on the Enforcement of Plan Variety Rights: DNA Fingerprinting. Part 2

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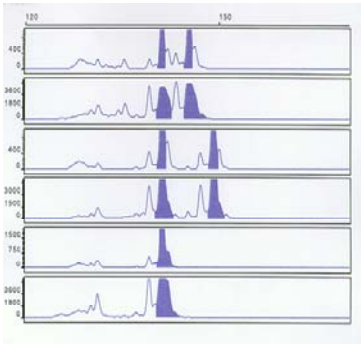
HISTORICAL

1982-96	**Previous IRTA experience on variability analysis with markers**
1996	**Selección Plantas Sevilla S.L. (COLLABORATION WITH INDUSTRY STARTS)**
1996-97	**Identification with AFLPs**
1998	**SSRs substitute AFLPs**
1999	**IRTA's GENETIC ANALYSIS SERVICE CREATED (IRTAGen)**
2003	**GENETIC DATABASE AGREEMENT WITH GESLIVE (*Prunus*)**
2003-present	**Contract application and extension to other species**

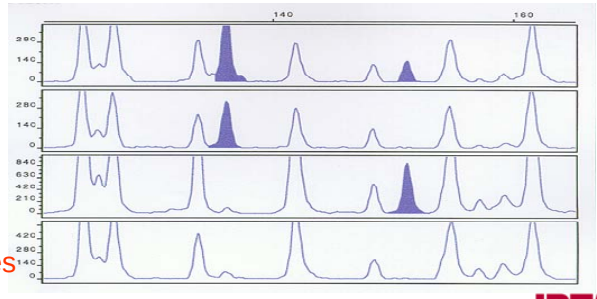


Microsatellites (SSRs)

Highly polymorphic
Abundant
Codominant (pedigree inferences)
Highly reproducible
Simple, robust method
Do not need high quality DNA
Same in different tissues
Transferable
Many SSRs freely available

AFLPs

Highly polymorphic
Abundant
Dominant
Reproducible
Robust method
Need high quality DNA
Not reliable in different tissues
Poorly transferable
Proprietory



IRTA

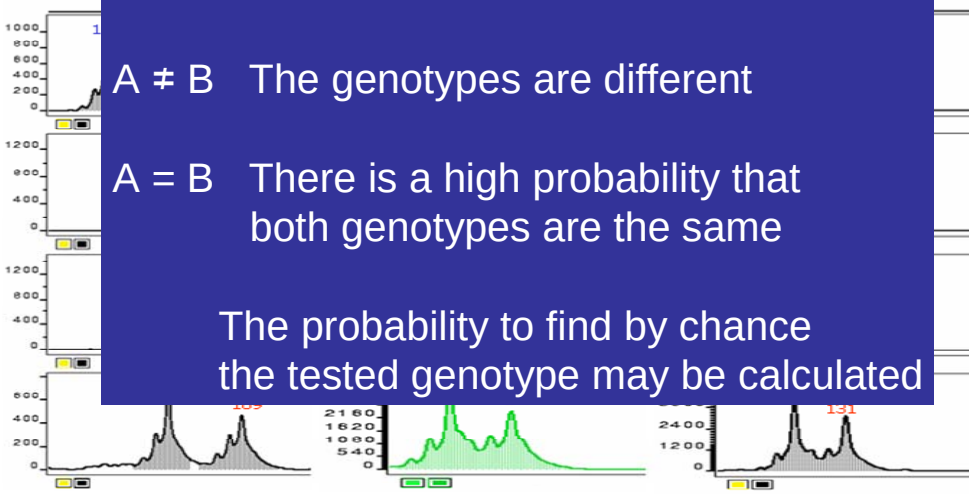
A LARGE COLLECTION OF MARKERS
>1100 SSRs: 6-700 mapped

TEST of IDENTITY

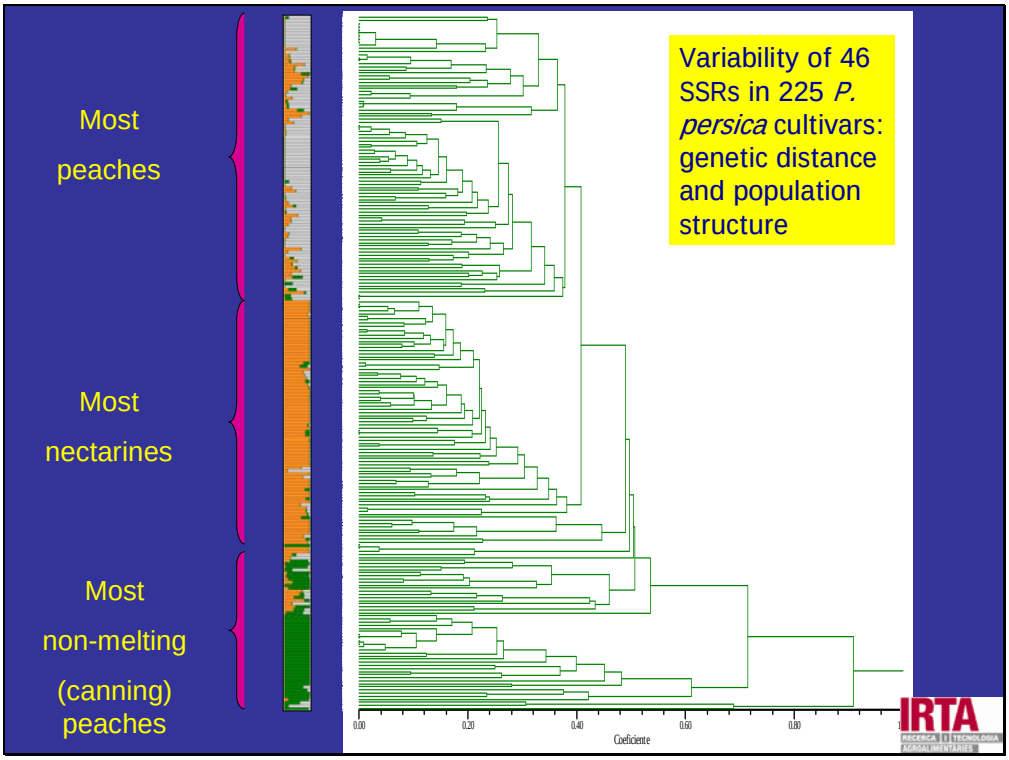
A ≠ B The genotypes are different

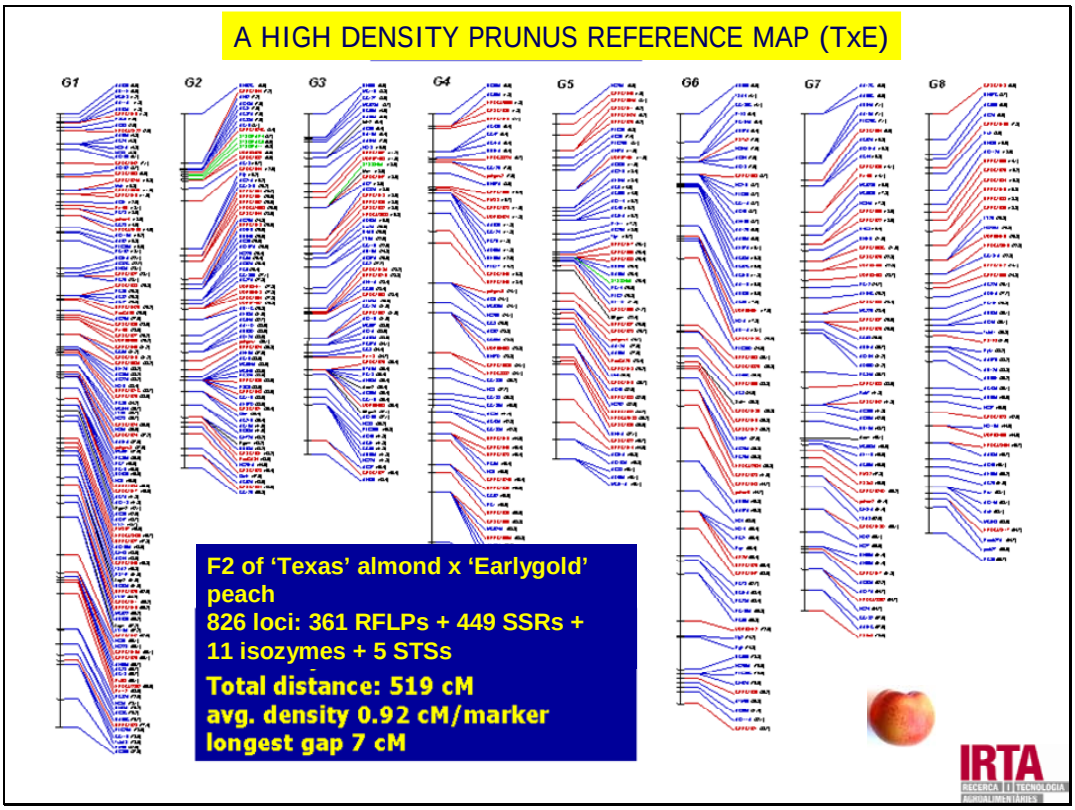
A = B There is a high probability that both genotypes are the same

The probability to find by chance the tested genotype may be calculated



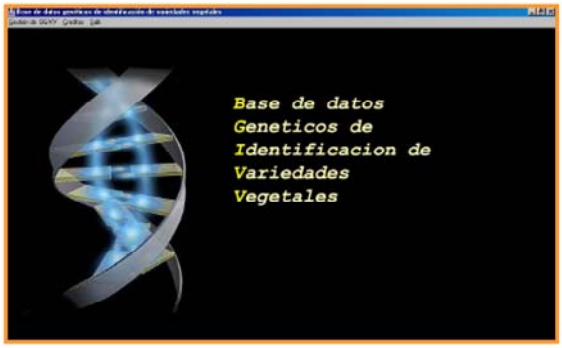
IRTA


Most peaches
Most nectarines
Most non-melting (canning) peaches
Variability of 46 SSRs in 225 P. persica cultivars: genetic distance and population structure
0.00
0.20
0.40
0.60
0.80
Coeficiente
IRTA


A HIGH DENSITY PRUNUS REFERENCE MAP (TxE)
G1
G2
G3
G4
G5
G6
G7
G8
F2 of 'Texas' almond x 'Earlygold' peach
826 loci: 361 RFLPs + 449 SSRs + 11 isozymes + 5 STSs
Total distance: 519 cM
avg. density 0.92 cM/marker
longest gap 7 cM
IRTA

GENETIC DATABASE FOR PLANT VARIETY IDENTIFICATION

Signed between IRTA and GESLIVE in 2003



GESLIVE

IRTA

GENETIC DATABASE FOR PLANT VARIETY IDENTIFICATION

OWNED BY GESLIVE

DEVELOPED, CURATED AND USED BY IRTA

SAMPLES FOR DNA EXTRACTION
HARVESTED AT OFFICIAL REPOSITORIES

CULTIVAR FINGERPRINTS OF GESLIVE'S MEMBERS

ONLY FOR GESLIVE'S MEMBERS

GESLIVE

IRTA

What is now in GESLIVE's database?

SPECIES	# cvs.
Peach/nectarine	151
Carnation	110
Wheat	28
Apricot	27
Grape	3
Strawberry	2
Plum	1

Collections of SSRs and genotyped cultivars at IRTA

Species	own SSRs	EST SSRs	Published SSRs	General collection of genotypes	Company collection of genotypes
Fruit trees					
Peach/nectarine	***	***	***	***	***
Almond	**	**	*	**	**
Japanese plum	**			**	**
Apricot		*	**	*	**
Cherry			*		*
Hazelnut			*	*	**
Citrus			**	**	***
Olive			*	*	**
Other vegetatively propagated crops					
Strawberry	***			*	***
Rubus			*		**
Blueberry			*		**
Carnation	**			*	**
Rose	*			*	
Vegetables					
Pepper		**	*		**
Eggplant	*	**	*		**
Tomato		***	**		**
Cucumber		**	**		**
Melon	***	**	*	***	**
Eggplant		*	**		**
Squash		*	**		**

CURRENT CHALLENGES

- **High mutation rates of SSRs (10^{-3}-10^{-5})**

  Small differences may not imply detection of different cultivars

- **Allele consistency across time or between different labs for SSRs**

  Control genotypes
  Use of trinucleotide microsatellites

- **Subpopulation structures (all markers)**

  Values of probability of uniqueness of the genotype may be underestimated

- **Linkage disequilibrium (all markers)**

  In species with high conservation of LD, markers have to be selected knowing their map position

IRTA

FUTURE

SSRs or other sequence-based markers (**SNPs**)

Towards markers based on **genic sequence**
- genic SSRs (EST-SSRs and others)
- gene-based SNPs

Gene-based **chips** with 300-1500 SNPs for many species probable next step

Standard marker sets or procedures are desirable, but **the area will keep moving fast**

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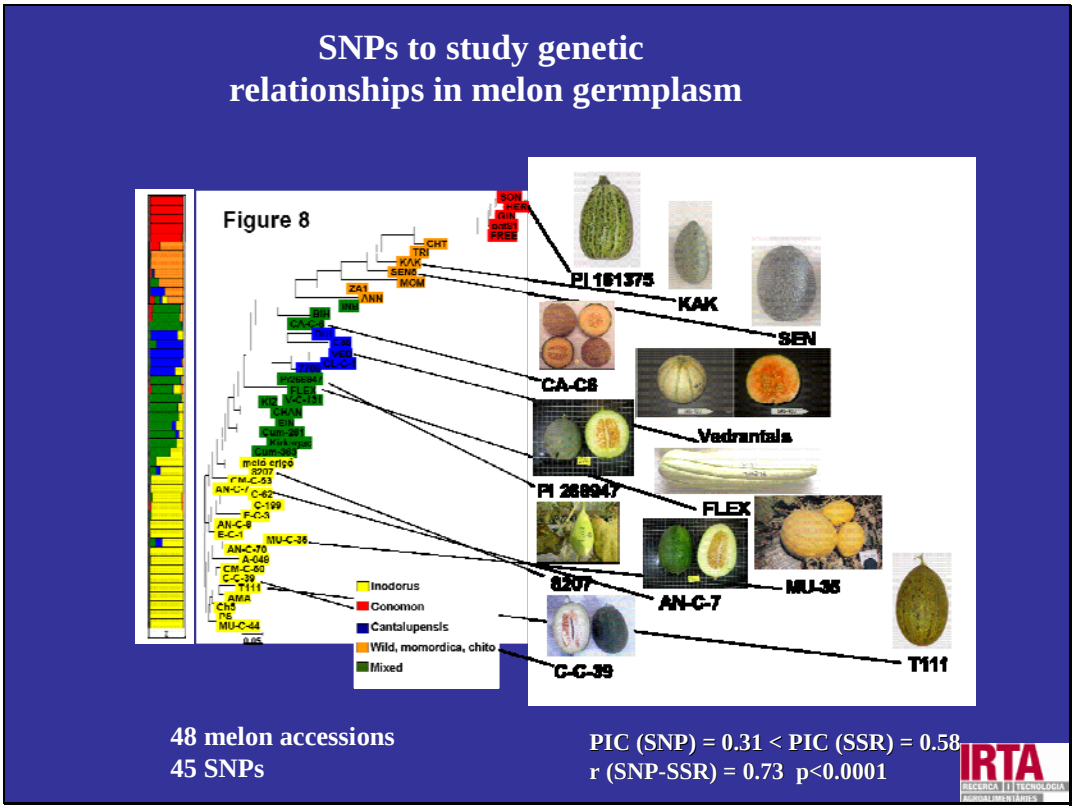

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