Date palm *(Phoenix dactylifera L.)* cultivar identification through Simple Sequence Repeats (SSR's): A practical molecular approach for next-generation commercial date palm micropropagation production and general Palmàprinting (Fingerprinting)

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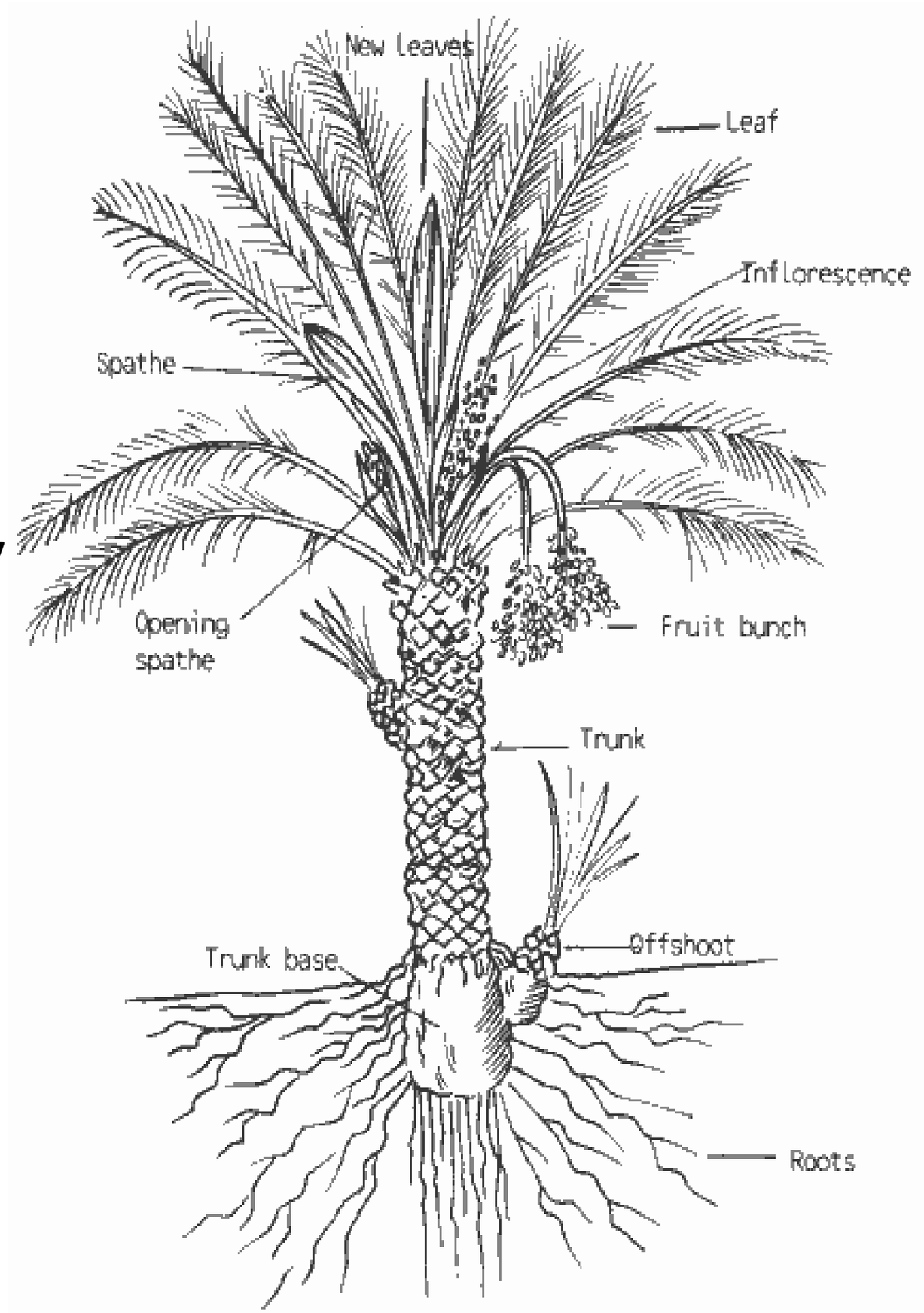
Applied Biotechnology of Date Palm



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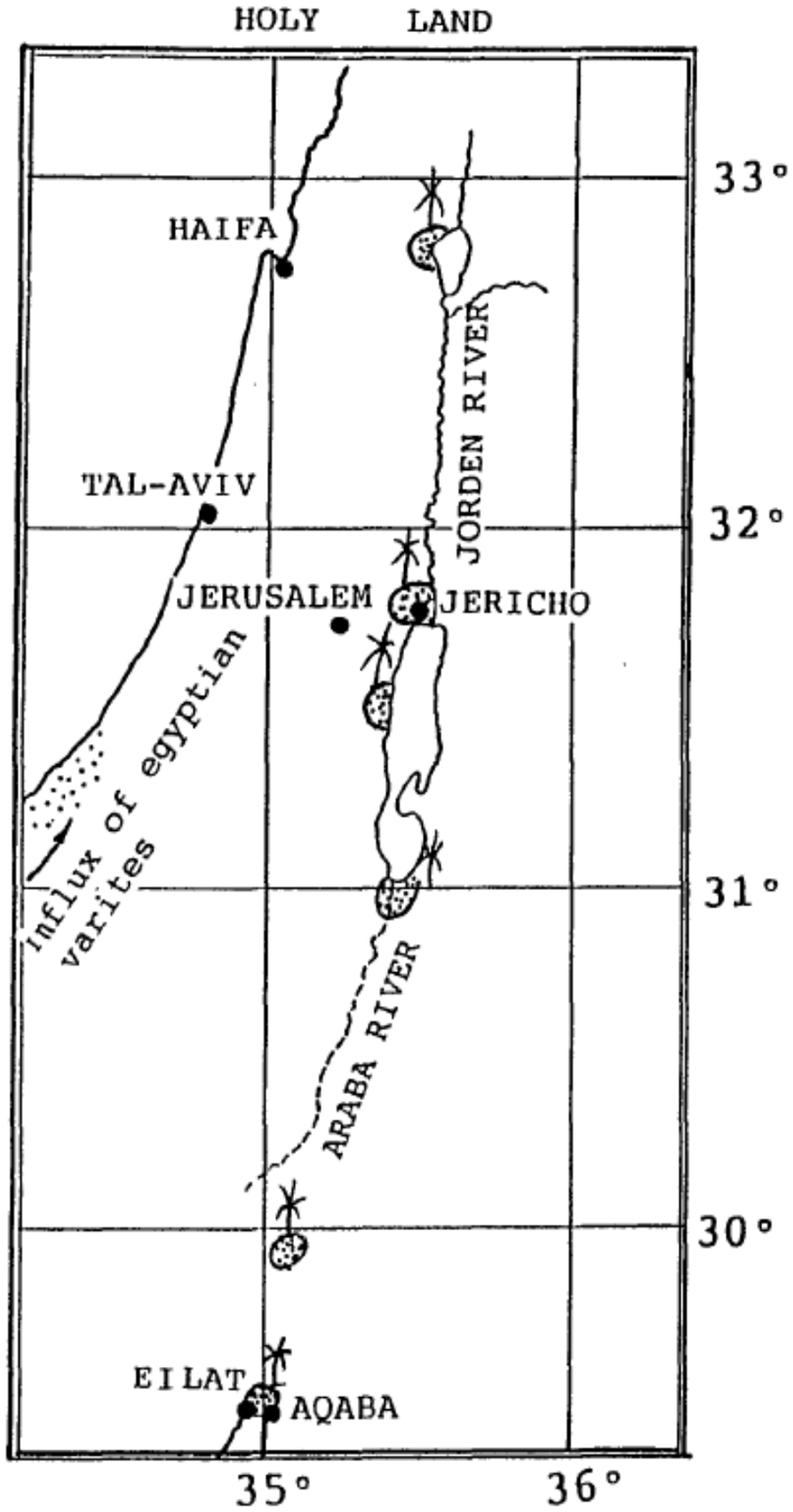
http://ressources.cieham.org/om/pdf/a28/96605880.pdf



Ancient Sites of Date Palm Oases

(Zohary & Spiegel-Roy 1975; Morton 1987).

Source:
https://www.dreamstime.com/royalty-free-stock-images-date-palm-phoenix-dactylifera

Date Palm

Phoenix dactylifera L.

Source: arcadianabe.blogspot.com/2013_01_01_archive.html

Date Palm

Phoenix dactylifera L.

Dioecious
Perennial monocot

Well known for its edible, sugar / nutrient-rich fruit

(Zohary & Spiegel-Roy 1975; Morton 1987).

Source: arcadianabe.blogspot.com/2013_01_01_archive.html

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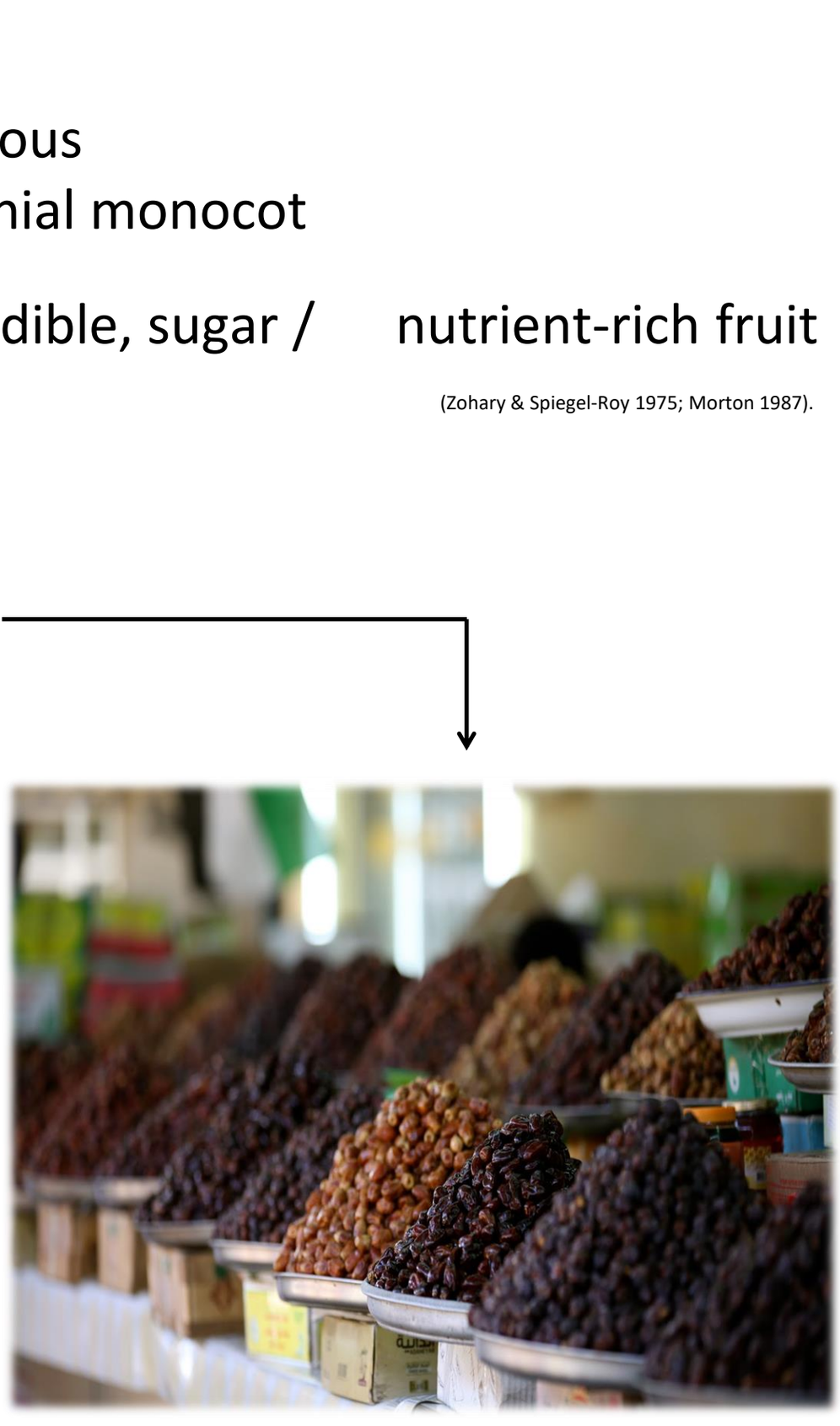
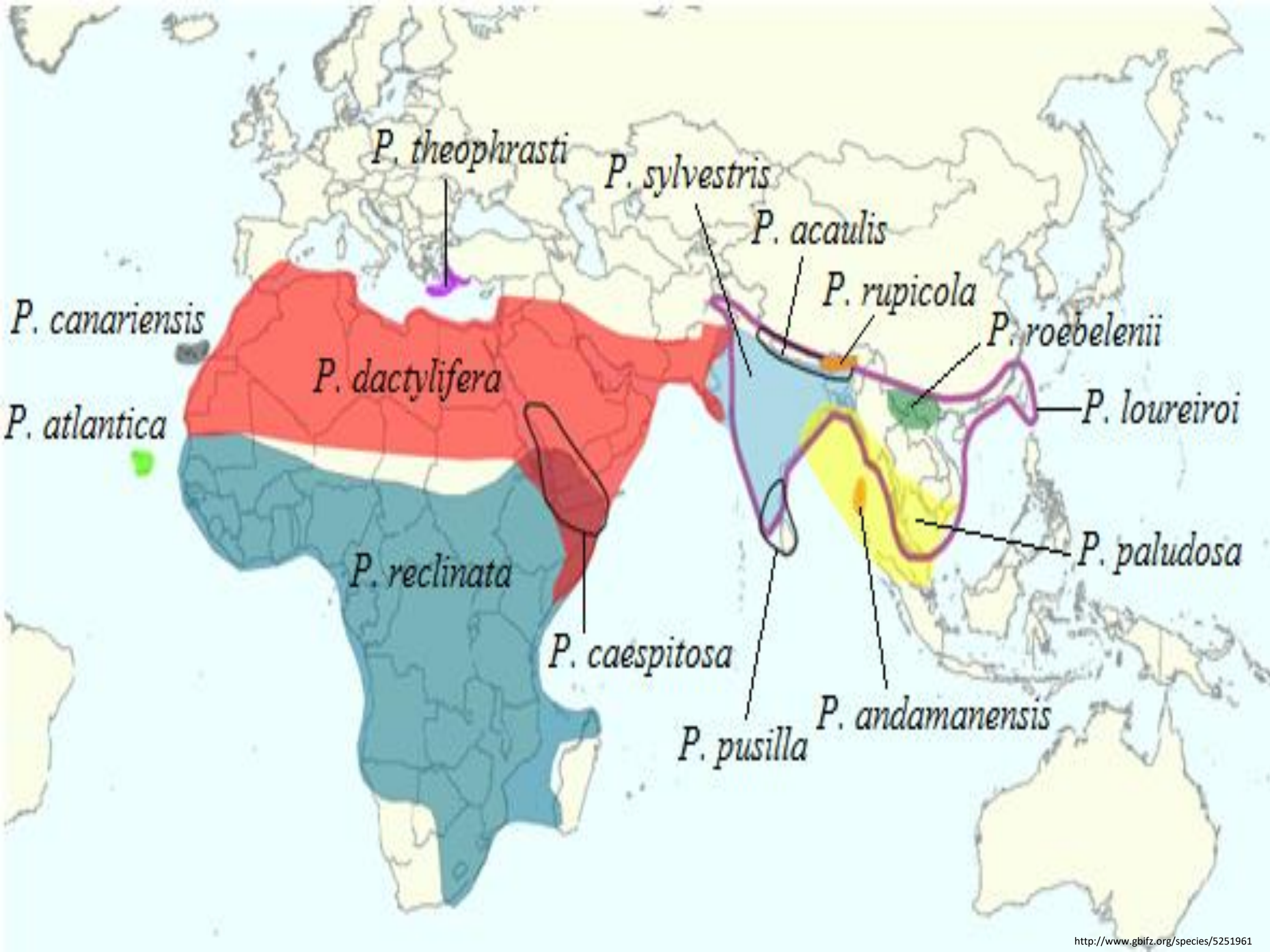
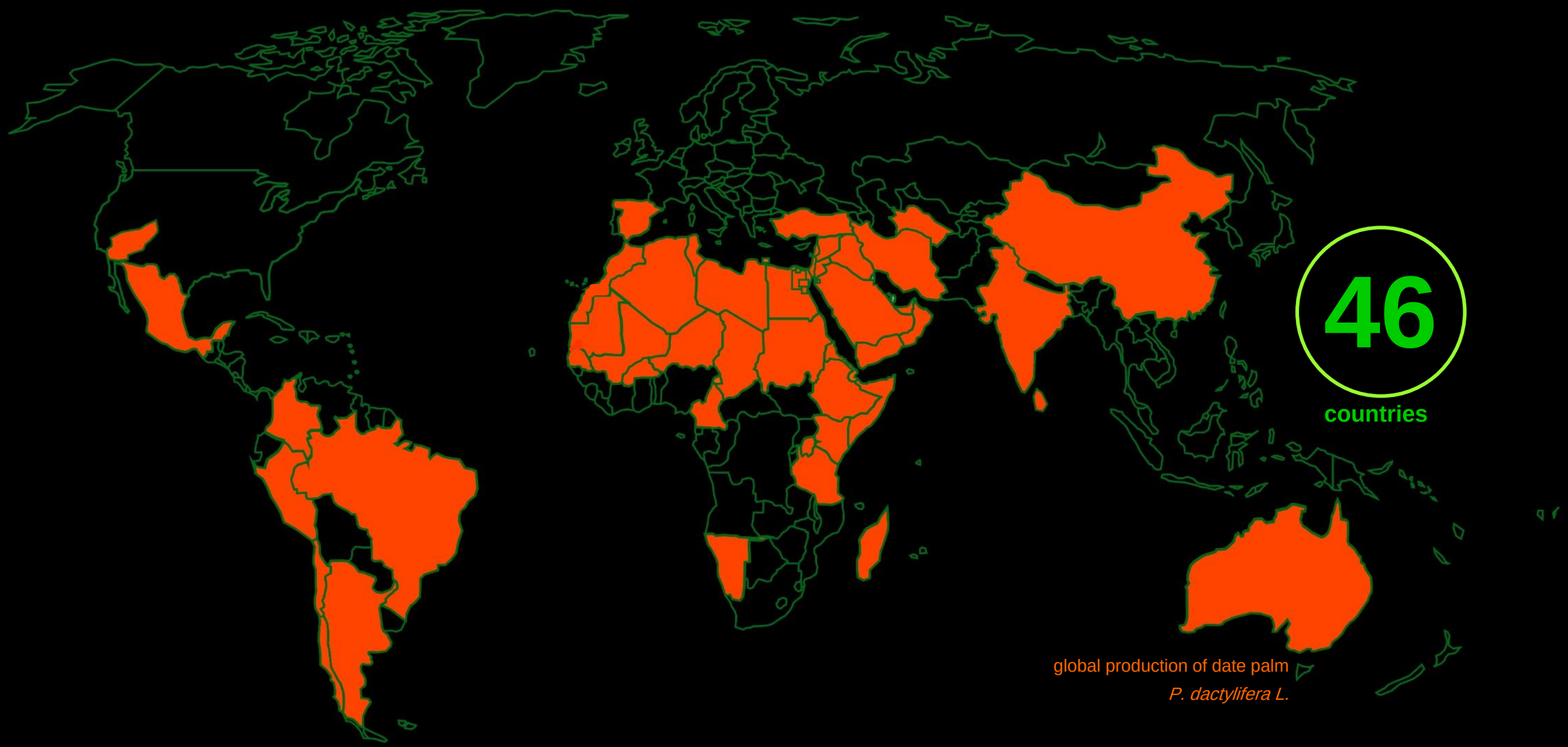


Source: arcadianabe.blogspot.com/2013_01_01_archive.html

Genome → Two 18 chromosome pairs

→ ≈ 670 Mb





global production of date palm

P. dactylifera L.

Industry -

Food

Construction

Products

Pharmaceutical

varieties & products

3,000

50

Source © Prof. A. Zaid Dr. & Dr. Mohammed Ben Salah Image

Rational

Date palm Bayoudh disease incidence

Fusarium oxysporum forma specialis albe dinis (Killian and Maire, 1930; Malencon, 1934 and 1936).

Rational

Date palm Bayoudh disease incidence

Fusarium oxysporum forma specialis albedini

S (Killian and Maire, 1930; Malencon, 1934 and 1936).

http://www.natureasia.com/en/nmiddleeast/article/10.1038/nmiddleeast.2010.220

http://www.gbifz.org/species/5251961

Rational

Date palm Bayoudh disease incidence

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http://www.fao.org/docrep/006/y4360e/y4360e0g.htm

Rational

Date palm Bayoudh disease incidence

Fusarium oxysporum forma specialis albedini

S (Killian and Maire, 1930; Malencon, 1934 and 1936).

Personal Photo, Location : Marrakech 2007

http://www.fao.org/docrep/006/y4360e/y4360e0g.htm

Rational

- Mixing of cultivars in large scale production through the Tissue Culture Organogenesis method

- Climate Change & need of large plant material production

http://be.convdocs.org/docs/index-106479.html

MIDDLE EAST

TURKEY
CYPRUS
SYRIAN ARAB REP.
LEBANON
ISRAEL
IRAQ
ISLAMIC REPUBLIC OF IRAN
AFGHANISTAN
PAKISTAN
TURKMENISTAN
KUWAIT
BAHRAIN
QATAR
UNITED ARAB EMIRATES
OMAN
SAUDI ARABIA
YEMEN
SOMALIA
EGYPT
LIBYAN ARAB JAMAHIRIYA
JORDAN

Mediterranean Sea
Caspian Sea
Persian Gulf
Gulf of Oman
Arabian Sea
Red Sea
Gulf of Aden
Sea of Hormuz

Tabrīz
Ashgabad
Mashhad
Tehrān
Herāt
Kandahār
Eṣfahān
Shīrāz
Halab
Al Mawṣil
Arbīl
Kirkūk
Baghdād
Tripoli
Beirut
Damascus
'Ammān
Alexandria
Port Said
Cairo
Suez
Al 'Aqabah
Al Başrah
Kuwait
Al Minyā
Asyūt
Aswān
Medina
Riyadh
Al Ḥufūf
Ad Dammām
Al Manāmah
Doha
Dubayy
Abū Dhabi
Muscat
Karachi
Jeddah
Mecca
Sanaa (Ṣan'ā')
Al Ḥudaydah
Al Mukallā
Aden
Djibouti
Suqutrá (Socotra)

55° 60° 65° 70°
35° 30° 25° 20° 15° 10°
45° 50° 55° 60° 65°

0 200 400 600 800 km
0 100 200 300 400 500 mi

Collected approximately 10 accessions for each of the 56 varieties from differing locations across the GCC region & North Africa

http://countries.bridgat.com/Middle_East.html

http://countries.bridgat.com/Middle_East.html

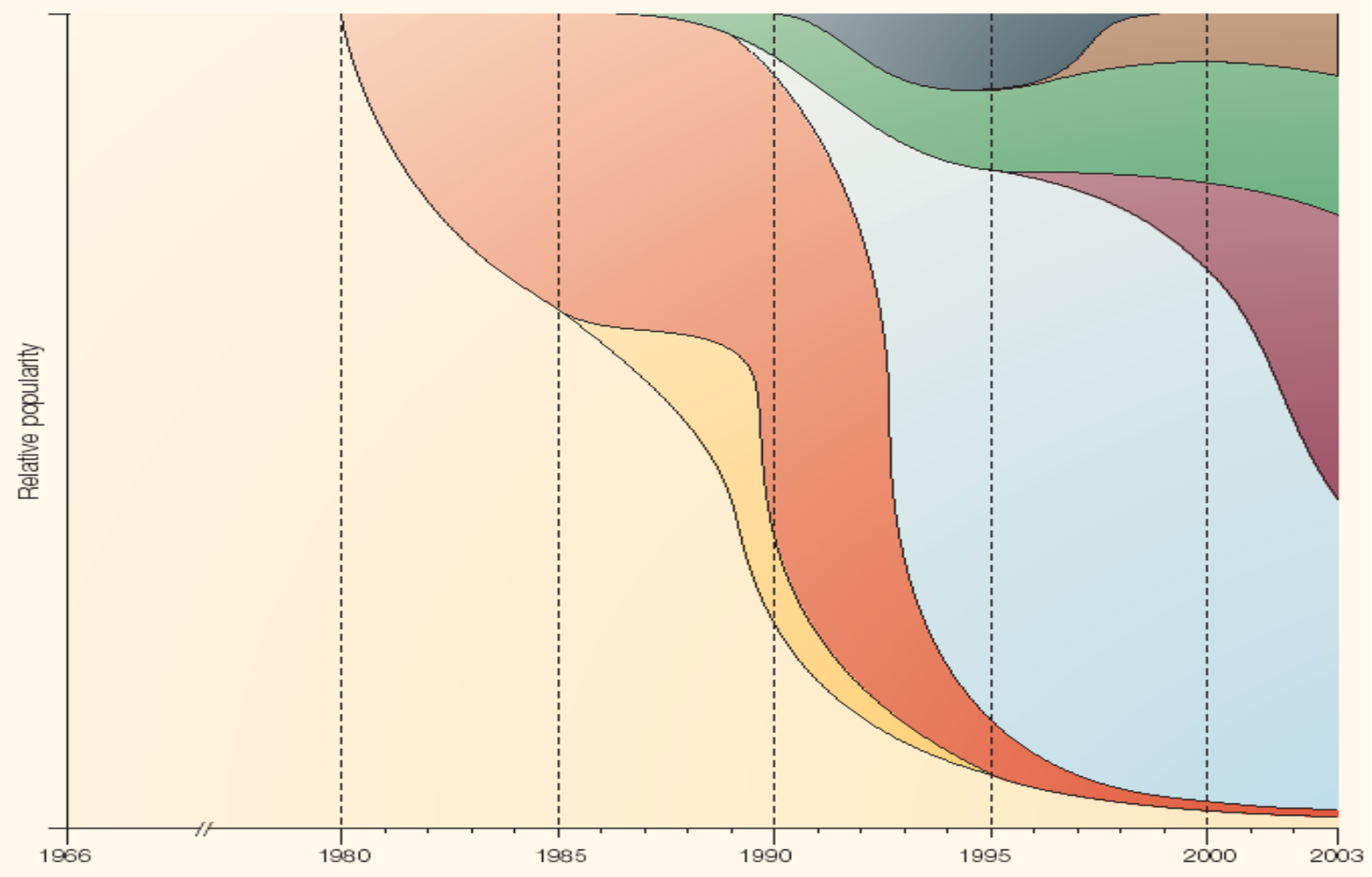
Methods

<u>Historically,</u>

- Indirect methods, such as Differences in Proteins or Phenotypes, were used to infer genetic differences among individuals, populations, and cultivars
- Direct methods
  - Sequencing a few genes or
  - section of genes

<u>Today,</u>

- Possible to detect variation among individuals or Cultivars at single nucleotide sites across entire genomes!



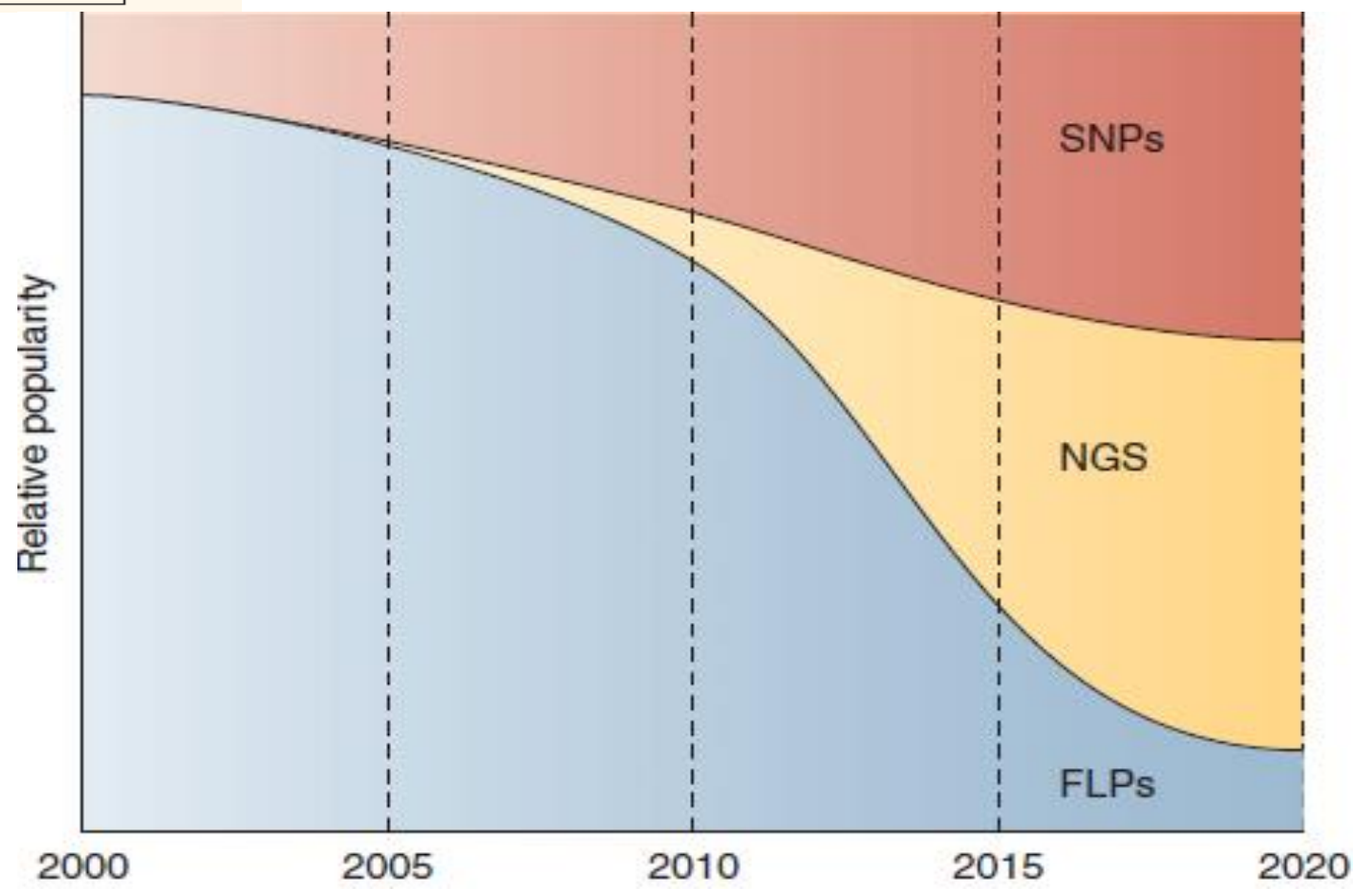
Changes in relative importance of genotyping strategies

1966 - 2020

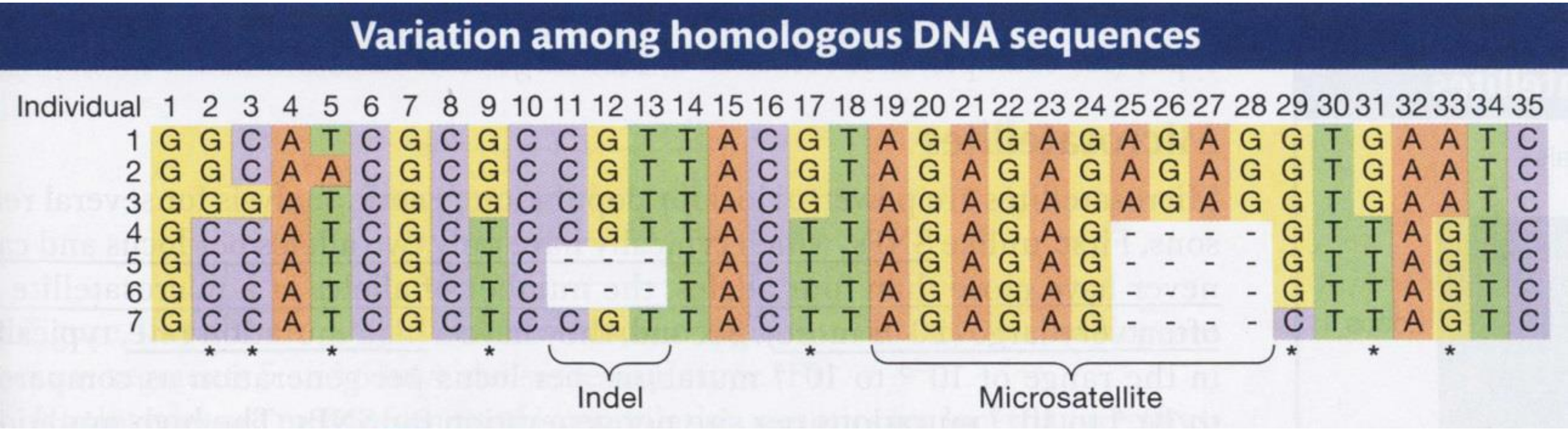
Schlotterer. 2004. Nature Rev. Gen.
Seeb et al. 2011. Mol.Ecol.Res.

Microsatellites =
Single Sequence (tandem) Repeats

SNP = Single Nucleotide Polymorphism
NGS = Next Generation Sequencing
FLP = Frangment length Polymorphism



Microsatellites



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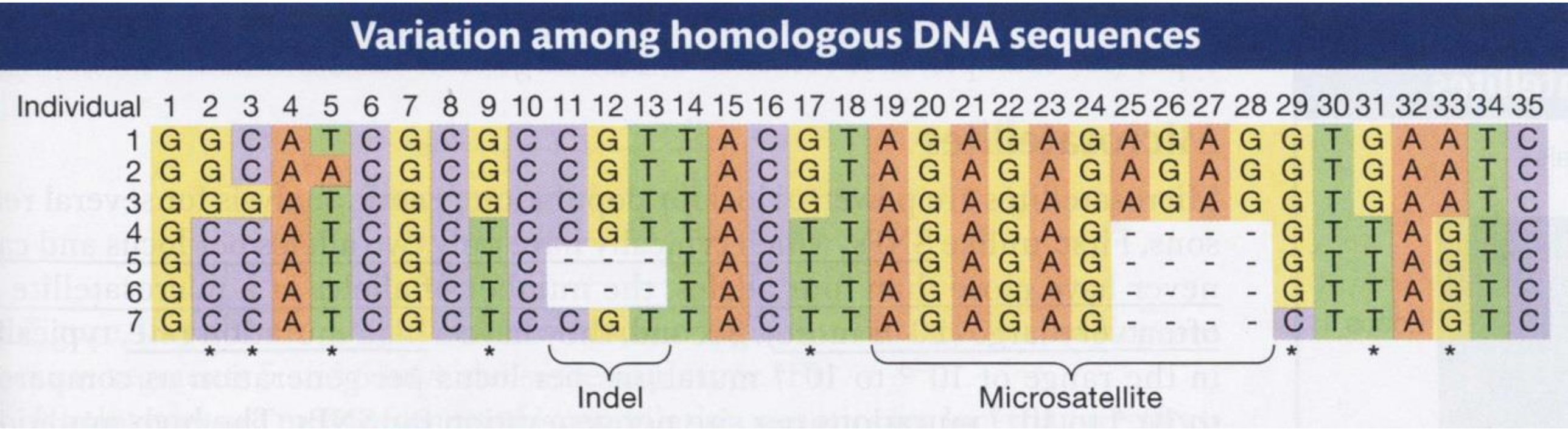

Can occur in Exons, Introns, Regulatory Regions but mainly in **Non-coding regions of DNA**

Present in the genomes of **all eukaryotes** and consists of several to over hundreds of repeats of a **1-6 n ucleotide motif**

Locus – Specific (in contrast to multi-locus markers - Minisat. & RAPDs)

Highly **polymorphic** (“hypervariable”) – Providing considerable Patterns

Useful at **range of scale** from **individual ID** to fine-scale Phylogenies

SSR Primers

Panel #	SSR Locus	Allelic Range (bp)	Optimal Ta (°C)	Primer Sequences (5' - 3')	Modification 5'
1	mPdCIR015	120–156	51.6	F: AGCTGGCTCCTCCCTTCTTA R: GCTCGGTTGGACTTGTTCT	6-FAM
	mPdCIR025	199–231	49.3	F: GCACGAGAAGGCTTATAGT R: CCCCTCATTAGGATTCTAC	6-FAM
	mPdCIR032	284–305	51.5	F: CAAATCTTTGCCGTGAG R: GGTGTGGAGTAATCATGTAGTAG	VIC
	mPdCIR085	152–183	50.4	F: GAGAGAGGGTGGTGTTATT R: TTCATCCAGAACCACAGTA	VIC
2	mPdCIR044	281–332	51.7	F:ATGCGGACTACACTATTCTAC R: GGTGATTGACTTTCTTTGAG	6-FAM
	mPdCIR048	156–192	51.4	F: CGAGACCTACCTTCAACAAA R: CCACCAACCAAATCAAACAC	6-FAM
	mPdCIR070	182–208	48.7	F: CAAGACCCAAGGCTAAC R: GGAGGTGGCTTTGTAGTAT	NED
	mPdCIR078	117–152	49.6	F: TGGATTTCCATTGTGAG R: CCCGAAGAGACGCTATT	NED
3	mPdCIR010	118–161	55.9	F: ACCCCGGACGTGAGGTG R: CGTCGATCTCCTCCTTTGTCTC	6-FAM
	mPdCIR035	175–221	53.9	F: ACAAACGGCGATGGGATTAC R: CCGCAGCTCACCTCTTCTAT	6-FAM
4	mPdCIR057	251–278	55.4	F: AAGCAGCAGCCCTTCCGTAG R: GTTCTCACTCGCCCAAAAATAC	6-FAM
	mPdCIR093	153–184	51.8	F: CCATTTATCATTCCCTCTCTTG R: CTTGGTAGCTGCGTTTCTTG	6-FAM
5	mPdCIR016	130–138	51.7	F: AGCGGGAAATGAAAAGGTAT R: ATGAAAACGTGCCAAATGTC	6-FAM
	mPdCIR090	142–175	48.6	F: GCAGTCAGTCCCTCATA R: TGCTTGTAGCCCTTCAG	6-FAM

Primers Selected

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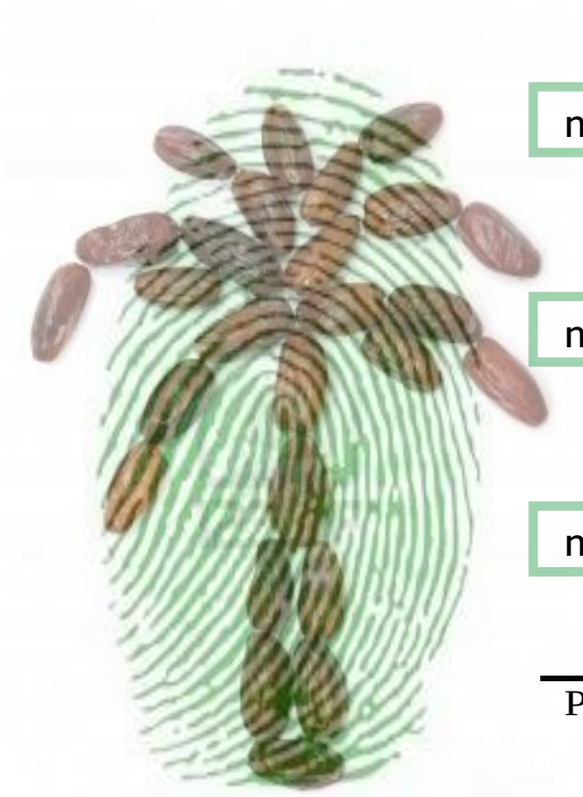
Currently, microsatellites still represent an easy, robust and cost-effective way of performing variety determination for such institutions.

Results



SSR locus	Primer sequence (5′ →'3′)	5′ Modification
mPdCIR015	F: AGC TGG CTC CTC CCT TCT TA	6-FAM
	R: GCT CGG TTG GAC TTG TTC T	
mPdCIR025	F: GCA CGA GAA GGC TTA TAG T	6-FAM
	R: CCC CTC ATT AGG ATT CTA C	
mPdCIR032	F: CAA ATC TTT GCC GTG AG	VIC
	R: GGT GTG GAG TAA TCA TGT AGT AG	
mPdCIR085	F: GAG AGA GGG TGG TGT TAT T	VIC
	R: TTC ATC CAG AAC CAC AGT A	

Provided courtesy of Billotte *et al*. (2004)

Results

Currently, microsatellites still represent an **easy**, robust and **cost-effective** way of performing **variety determination** for such institutions.

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	R: GCT CGG TTG GAC TTG TTC T	
mPdCIR025	F: GCA CGA GAA GGC TTA TAG T	6-FAM
	R: CCC CTC ATT AGG ATT CTA C	
mPdCIR032	F: CAA ATC TTT GCC GTG AG	VIC
	R: GGT GTG GAG TAA TCA TGT AGT AG	
mPdCIR085	F: GAG AGA GGG TGG TGT TAT T	VIC
	R: TTC ATC CAG AAC CAC AGT A	

Provided courtesy of Billotte *et al*. (2004)

Results / Conclusion

Currently, microsatellites still represent an easy, robust and cost-effective way of performing variety determination for such institutions.

Variety name	SSR locus – allele sizes (bp)							
	mPdCIR015		*mPdCIR025*		*mPdCIR032*		*mPdCIR085*	
Aboumaan	122	124	211	213	287	294	155	163
Ajwa	122	128	226	226	292	296	163	172
Ashal Hassa	128	128	211	213	287	298	155	174
Baqlat Bent Manii	122	128	211	230	287	298	153	172
Barhee	120	132	213	230	287	300	155	172
Chichi	132	136	211	230	287	294	153	172
Dibbas	122	122	211	213	287	298	172	176
Fard White	120	128	200	211	294	296	172	172
Ghannami*†	124	124	211	230	300	304	172	176

Ability to distinguish between **54** out of **56** samples (Varieties & Cultivars).

18 Unconfirmed Cultivars / Var.

Abu Badia
Abu Zabd
Ashal Khass
Ayassha
Diyala Sukkarri
Ganda
Hamdiya
Hilali Senee
Kuweitat
Maymona
Nabtat Dakhil
Nabtat Moneef
Nagal Hilali
Rotana Masfoot Salmia
Sheikha
Thinal
Umm Thaq

* Male date palm var.

18 Unconfirmed Cultivars / Var.

Genetically Distinct & Unique

Sympatric speciation

?

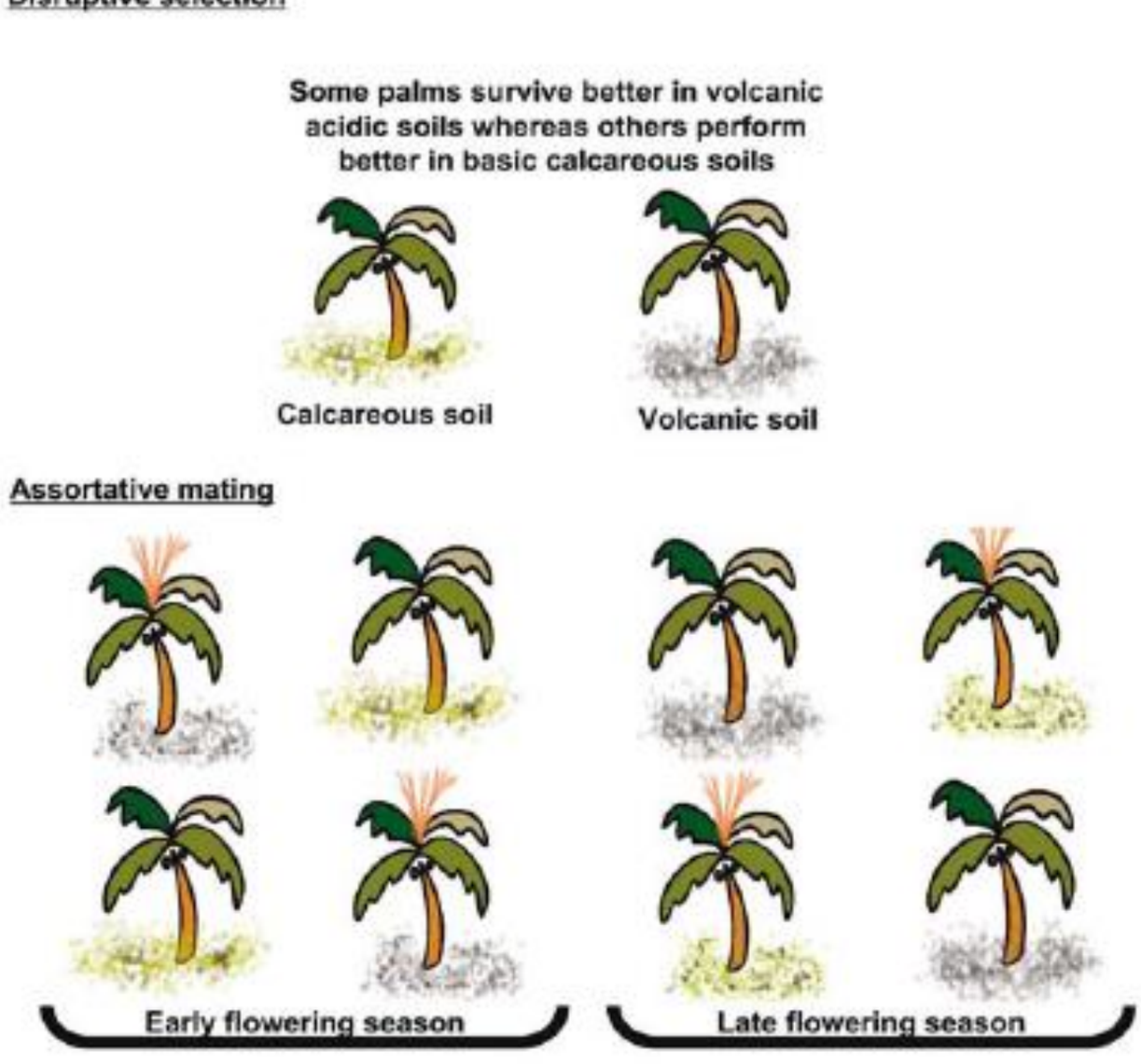
Abu Badia
Abu Zabd
Ashal Khass
Ayassha
Diyala Sukkarri
Ganda
Hamdiya
Hilali Senee
Kuweitat
Maymona
Nabtat Dakhil
Nabtat Moneef
Nagal Hilali
Rotana Masfoot Salmia
Sheikha
Thinal
Umm Thaq

* Male date palm var.

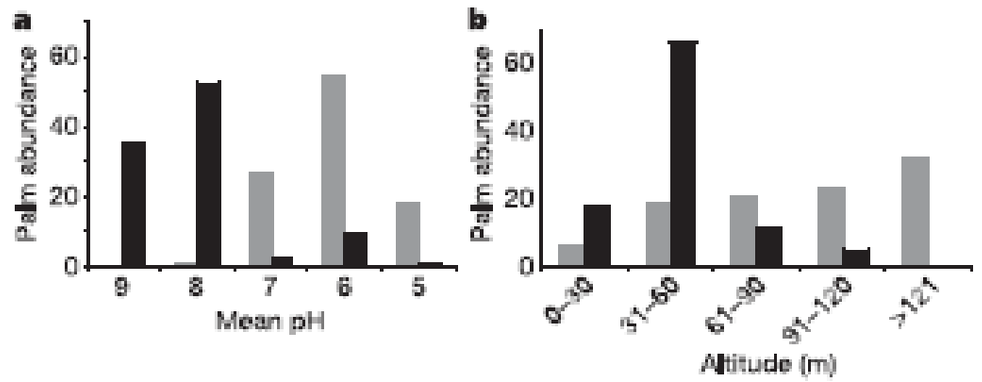
Sympatric speciation

Requires: Genetic correlation (one gene, linkage disequilibrium between genes) between trait subjected to disruptive selection and assortative mating

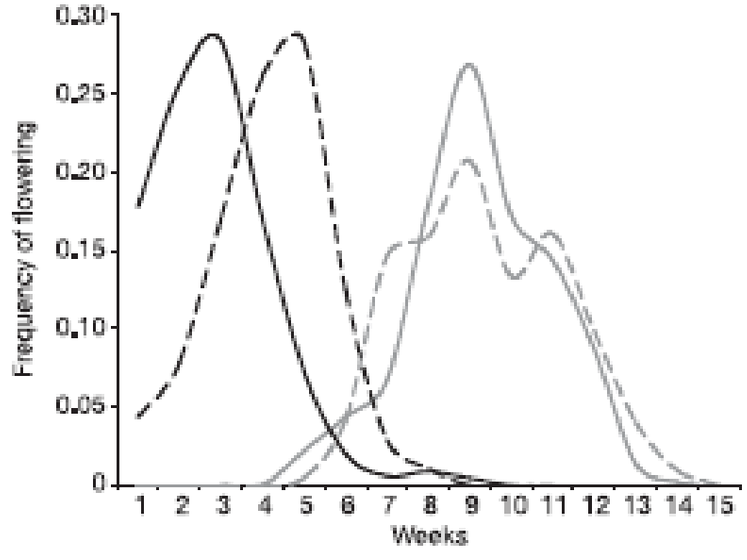
Example: Lord Howe palms (*Howea belmoreana* and *H. forsteriana*)



Ortiz-Barrientos D, Rieseberg LH 2006. Splitting when together. Divergence 97: 2-3.

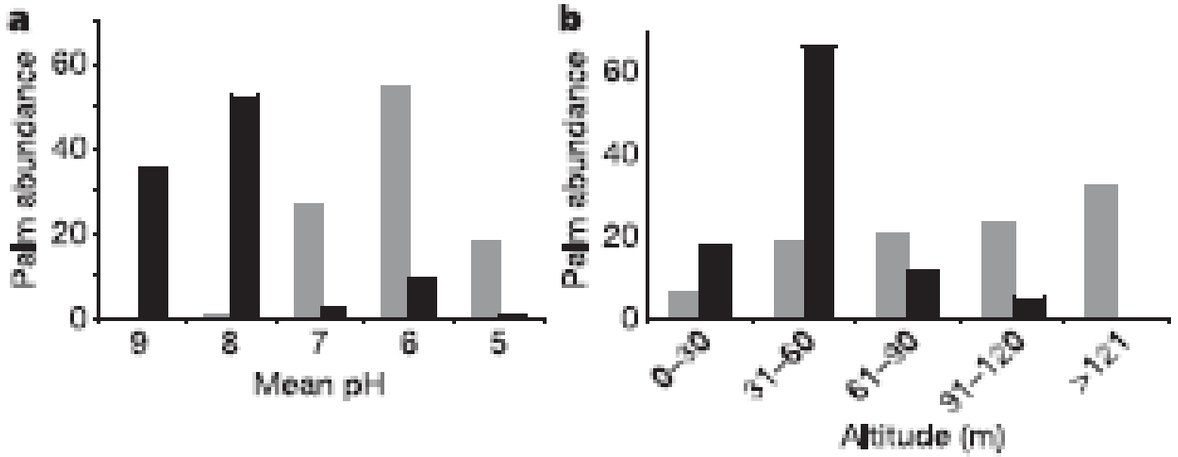


Distribution by pH and altitude: *H. belmoreana* (gray); *H. forsteriana* (black)



Flowering phenology:
***H. belmoreana* (gray) *H. forsteriana* (black)**
Male phase (solid) Female phase (dotted)

Savolainen V. et al. 2006. Sympatric speciation in palms. Nature 44: 210-213.



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Howea belmoreana (gray)
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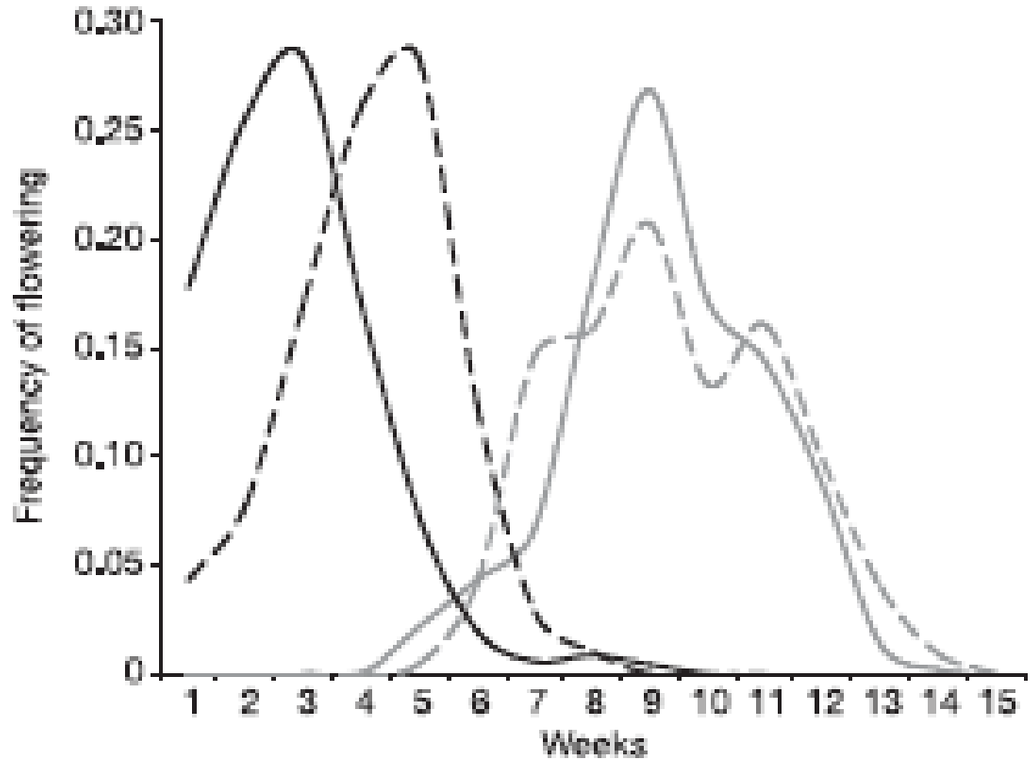

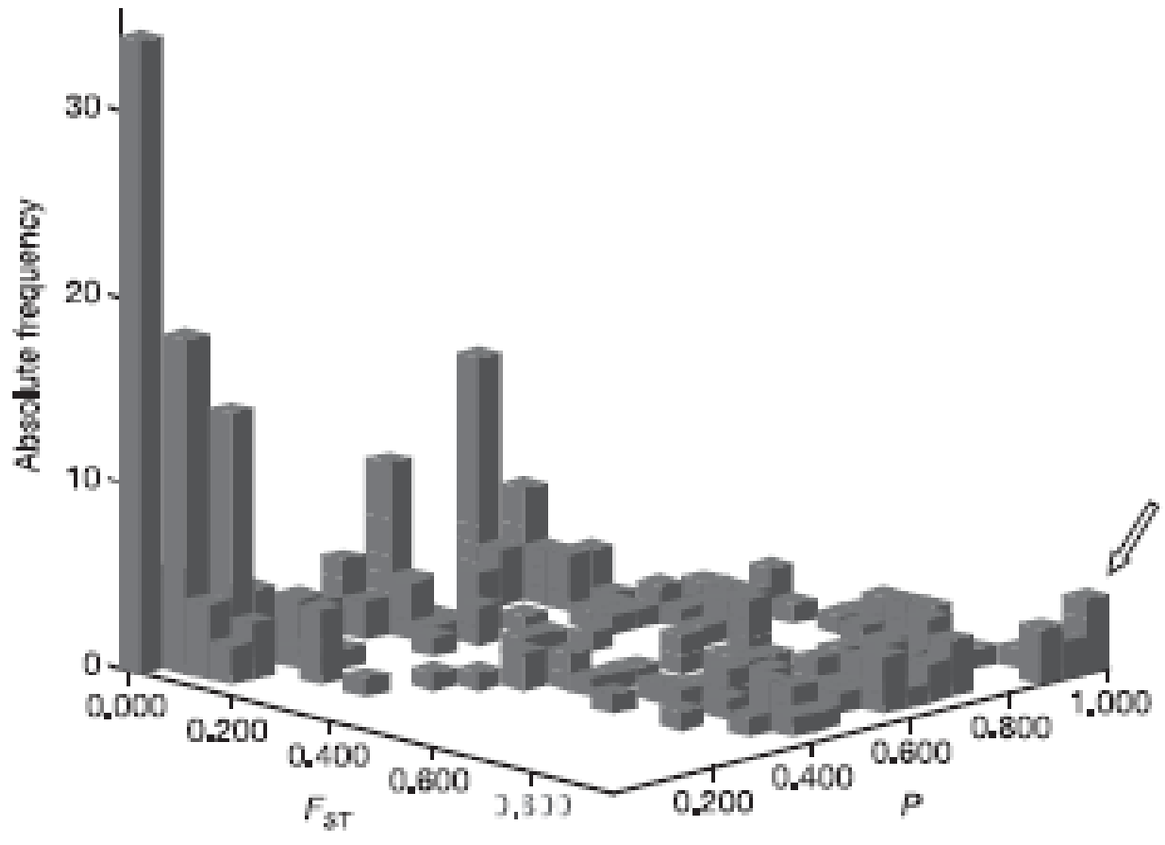

Interspecific differentiation:
AFLP genome scan (274 loci).

Flowering phenology:
Howea belmoreana (gray) *H. forsteriana* (black)
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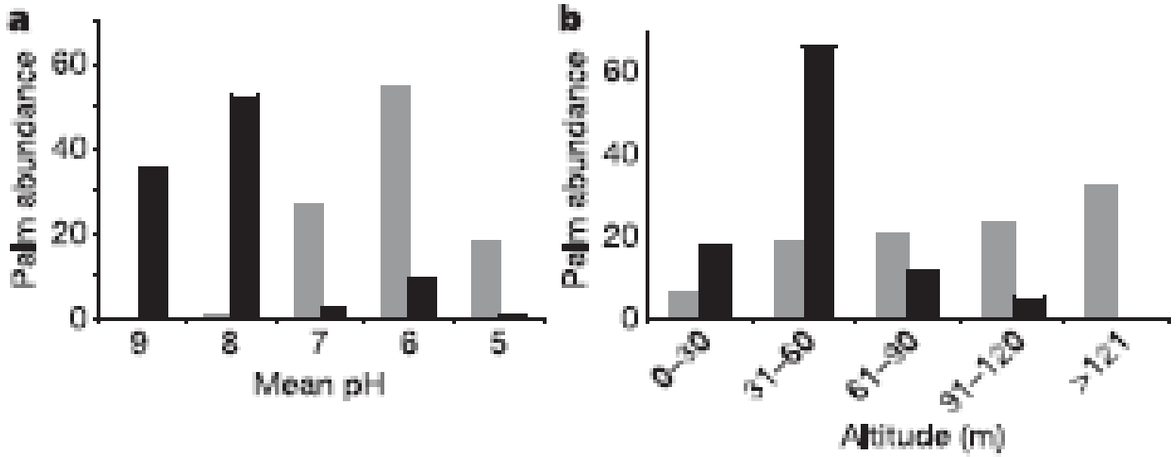

Distribution by pH and altitude:
Howea belmoreana (gray)
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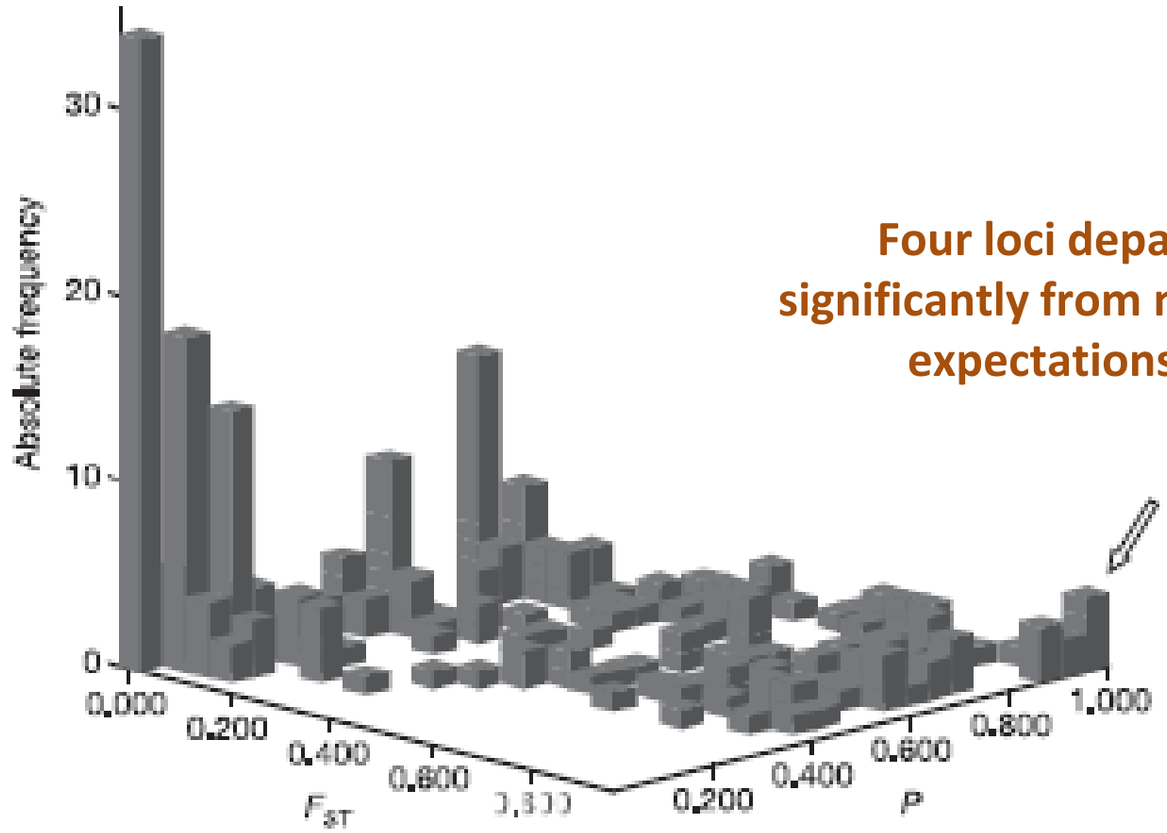
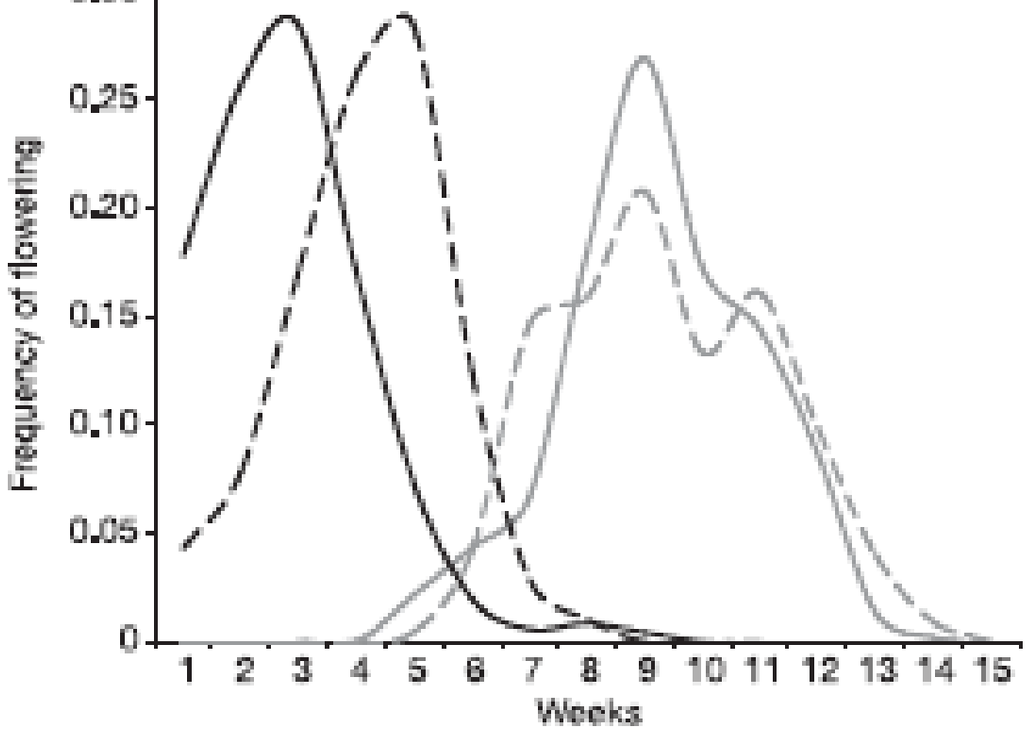


Four loci depart significantly from neutral expectations

(probability of departure from neutrality)

Interspecific differentiation:
AFLP genome scan (274 loci).

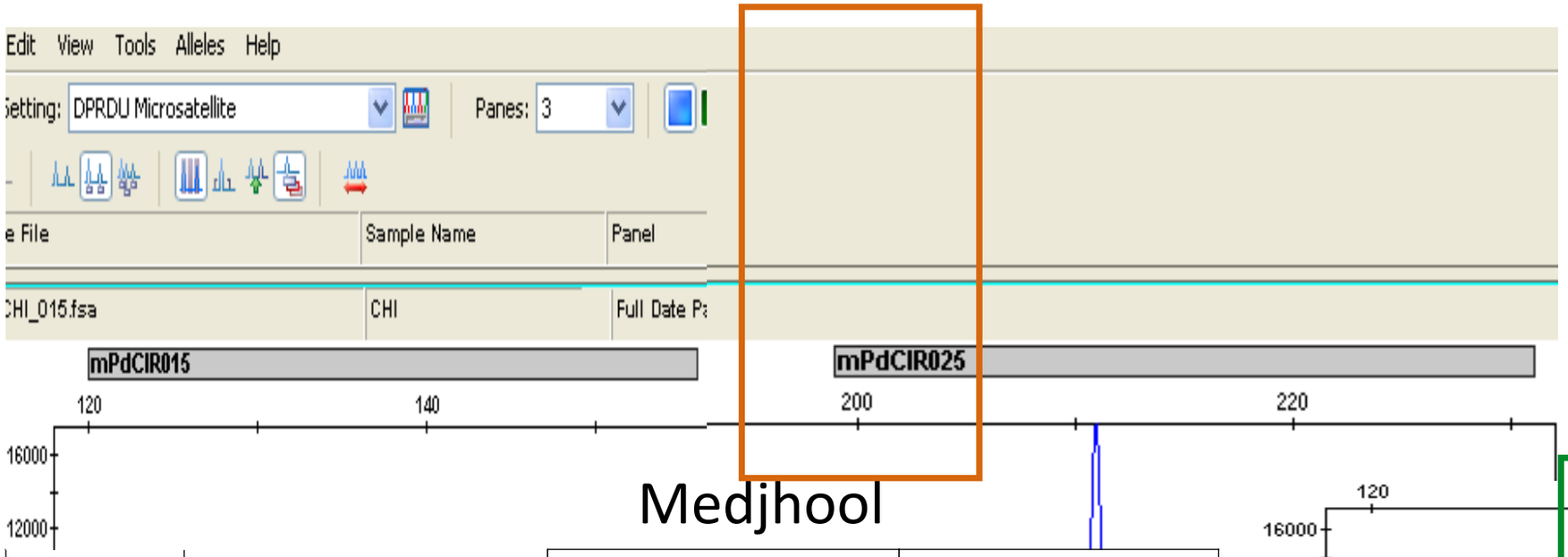


Flowering phenology:
Howea belmoreana (gray) *H. forsteriana* (black)
Male phase (solid) Female phase (dotted)

18 Unconfirmed Cultivars / Var.

- Miscalled Primers mpdCIR015 & 25

- Fragment being marginally outside the de fined allelic range.



Medjhool

PANEL	MICROSATELLITE	MJH		HEL	
1	mPdCIR015	136	136	120	122
	mPdCIR025	199	213	211	211
	mPdCIR032	294	295	292	300
	mPdCIR085	153	176	172	172
2	mPdCIR044	300	300	-	-
	mPdCIR048	155	155	182	190
	mPdCIR070	190	190	194	195
	mPdCIR078	117	123	132	136
3	mPdCIR010	120	160	134	134
	mPdCIR035	184	194	186	188
4	mPdCIR057	262	266	253	253
	mPdCIR093	167	171	171	171
5	mPdCIR016	129	129	135	135
	mPdCIR090	159	165	157	159

Abu Badia
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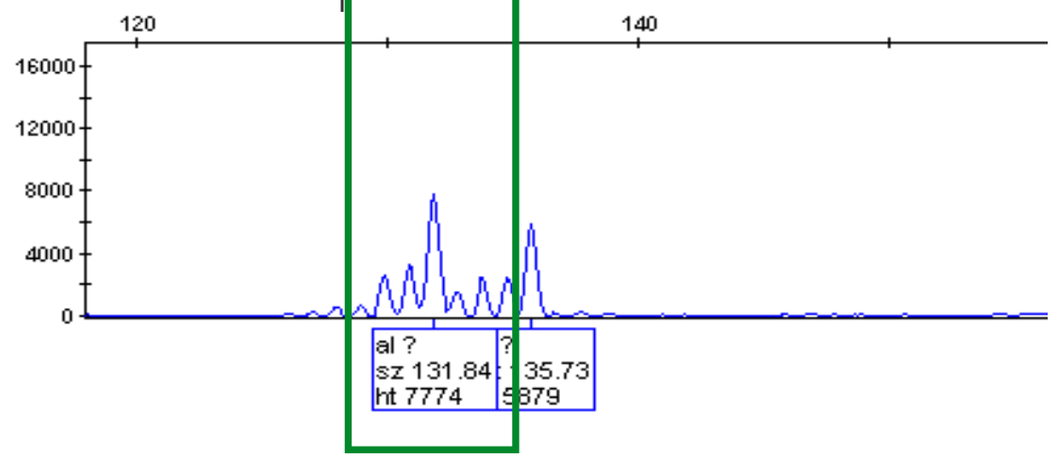

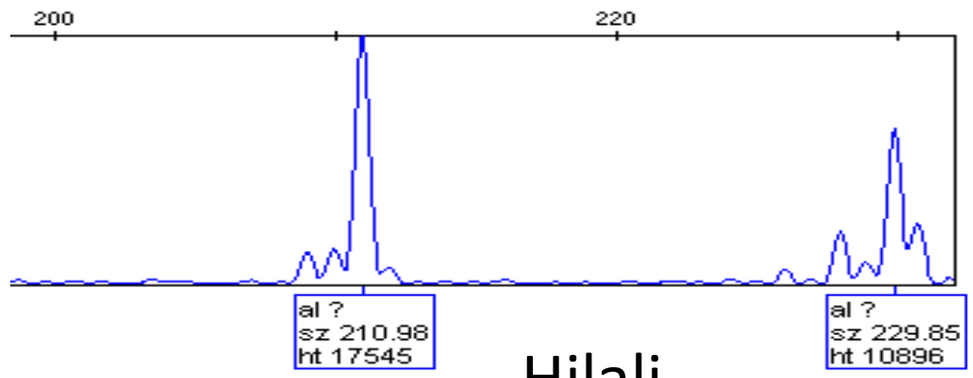

Hilali

38 Confirmable Cultivars / Var.

Aboumaan
Ajwa
Ashal Hassa
Baqlat Bent Manii
Barhee
Chichi
Dibbas
Fard White
Ghannami*
Ghareef*
Hilali
Hilali Red
Jabri
Jech Fatima
Jech Ramli
Kadri
Khadraoui
Khenezi
Khenezi White
Khisab
Khlass
Lulu
Madayan
Maktoumi
Mdasry*
Medjhool
MP (Al Ain Male)*
Mumtaz
Nabtat Breem
Nabtat Mazroui
Nabtat Seif
Nadira
Nawader
Sakaii
Sekka*
Sukkarri
Sultana
Zamli

* Male date palm var.

Revealed that each were genetically distinct and unique

6-FAM labeled primers for *mPdCIR010* and *mPdCIR035*

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Routine Var. Typing

Microsatellite panel - 4 SSR Loci

Microsatellite panel - 4 SSR Loci

Quality Control

Microsatellite panel - 4 SSR Loci

Facilitating management

Quality Control

Microsatellite panel - 4 SSR Loci

Applicable in any Date Palm tissue culture facility

Facilitating management

Quality Control

Honey Goat Cheese Da
tes with Walnuts

Source: table.io/honey-goat-cheese-dates/