"Life cycle variation and host specialisation in Myzus persicae*"*

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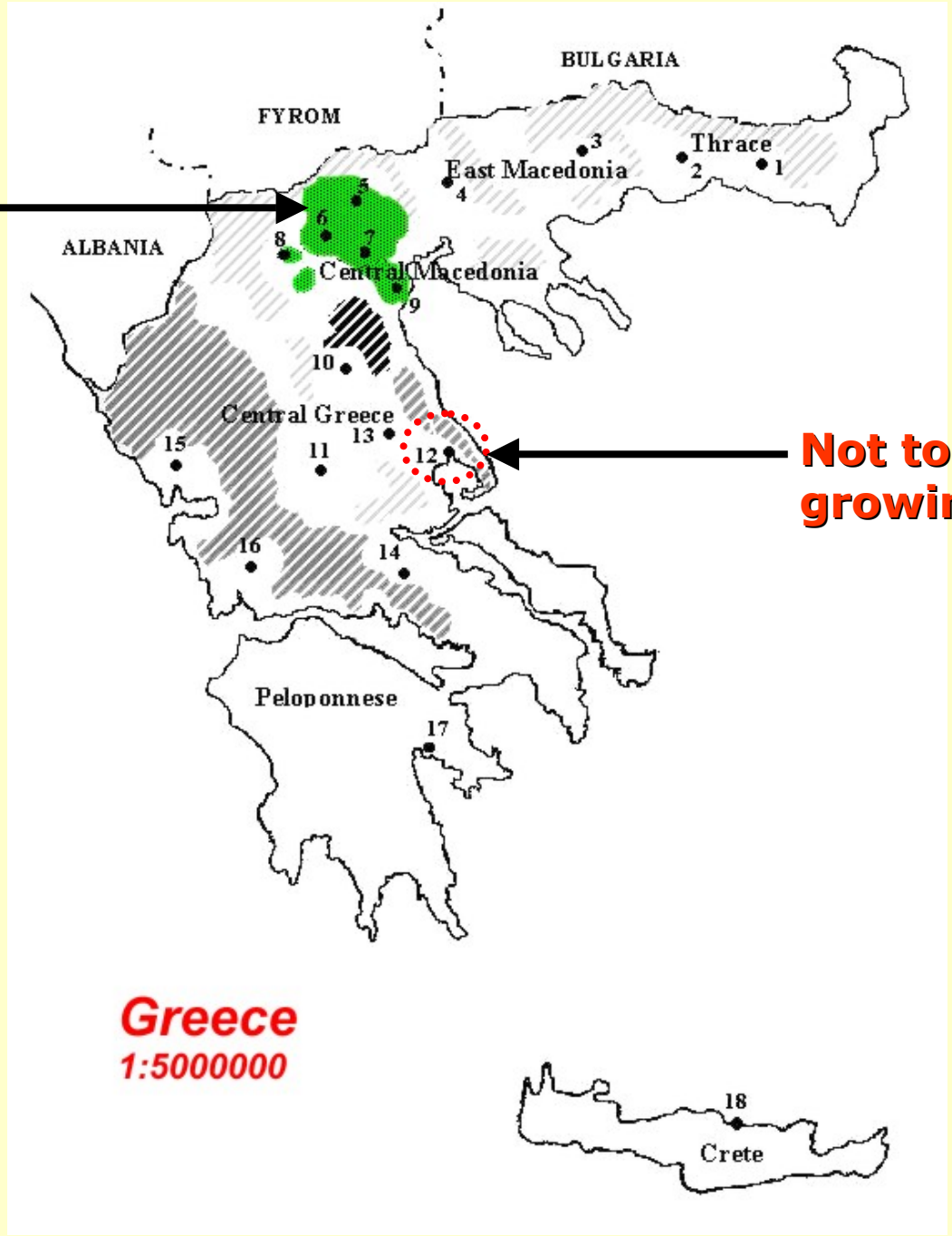
Myzus persicae

- Cosmopolitan species, many secondary hosts.
- In 1987 tobacco-feeding populations were described as a new species *Myzus nicotianae* Blackman (**distinct morphology**).
- Blackman & Spence (1992) found polymorphism in **glutamate oxoacetate transaminase (GOT).**
- Field *et al*. (1994) reported **identical** fragments of the **E4 and FE4 genes.**

- What is the status of populations on tobacco?
- Does the distinct morphology reported in 1987 and 1992 hold in recent specimens?
- If so, does this reflect genetic divergence?
- Does host specialisation exist in *Myzus persicae*?
- What is the relevance of the overwintering strategies of the species?

Main peach growing regions



Not tobacco growing region

Cultivated area (Ha) (1994-98)

•Peach	52,500
•Tobacco	64,680
•Pepper	3,350
•Potato	42,451

Aim of the study

I. *RAPD-PCR*
II. *Morphometrics*

Clones from tobacco and other hosts

Life cycles

Greece-Europe, near and away peach growing regions

Performance and choice

On tobacco and pepper plants

Genetic variation between clones collected from different hosts

"Is *Myzus nicotianae* a distinct species?"

RAPD-PCR

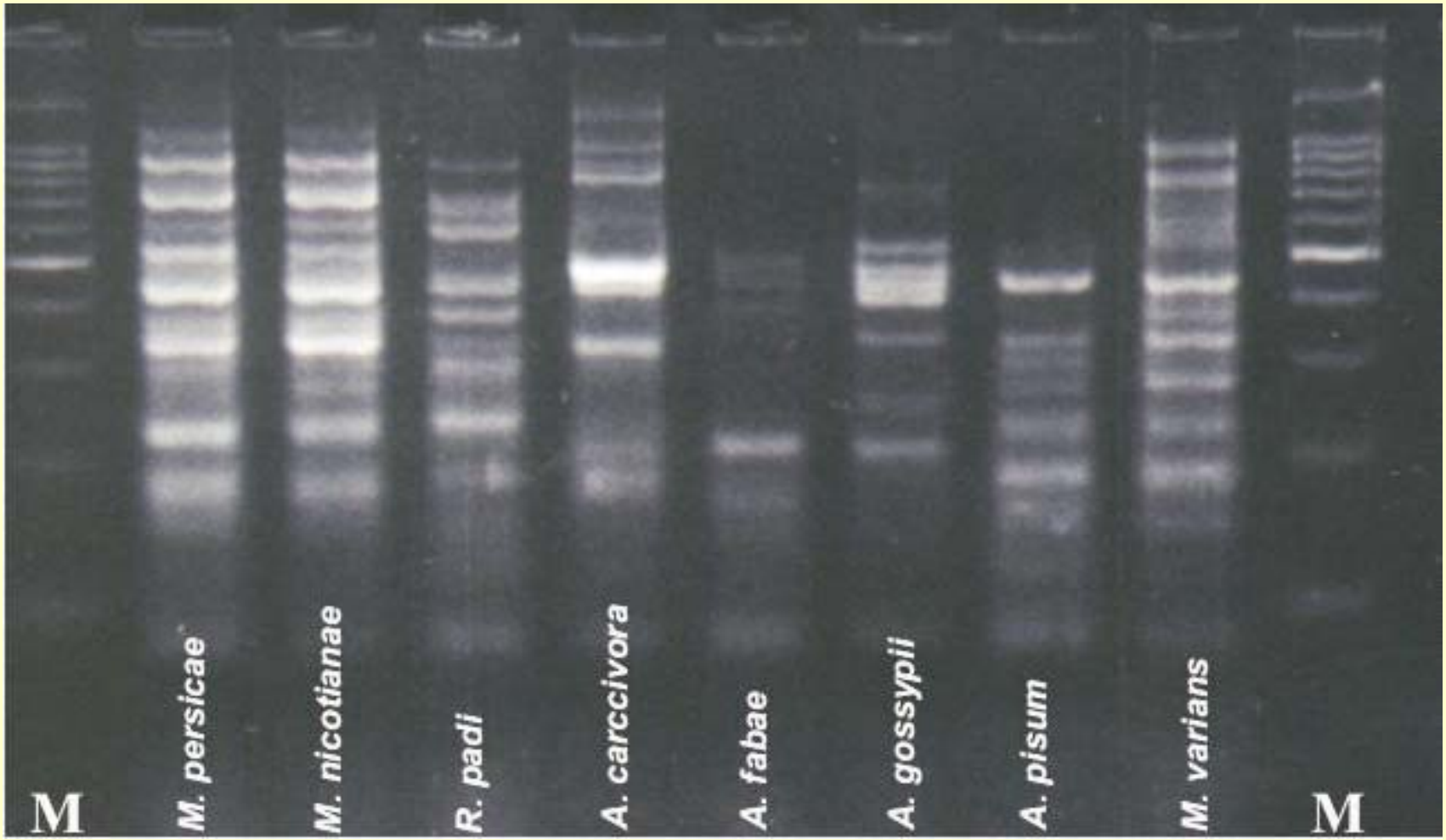
- *Aphis fabae*
- *Acyrthosiphon pisum*
- *Aphis gossypii*
- *Myzus varians*
- *Aphis craccivora*
- *Rhopalosiphum padi*

64 decamer random primers

- *M. persicae group*
  - tobacco 22
  - peach* 17
  - pepper* 7
  - cabbage* 1

*Away from tobacco growing regions

Banding pattern in different aphid species using primer OPA-18



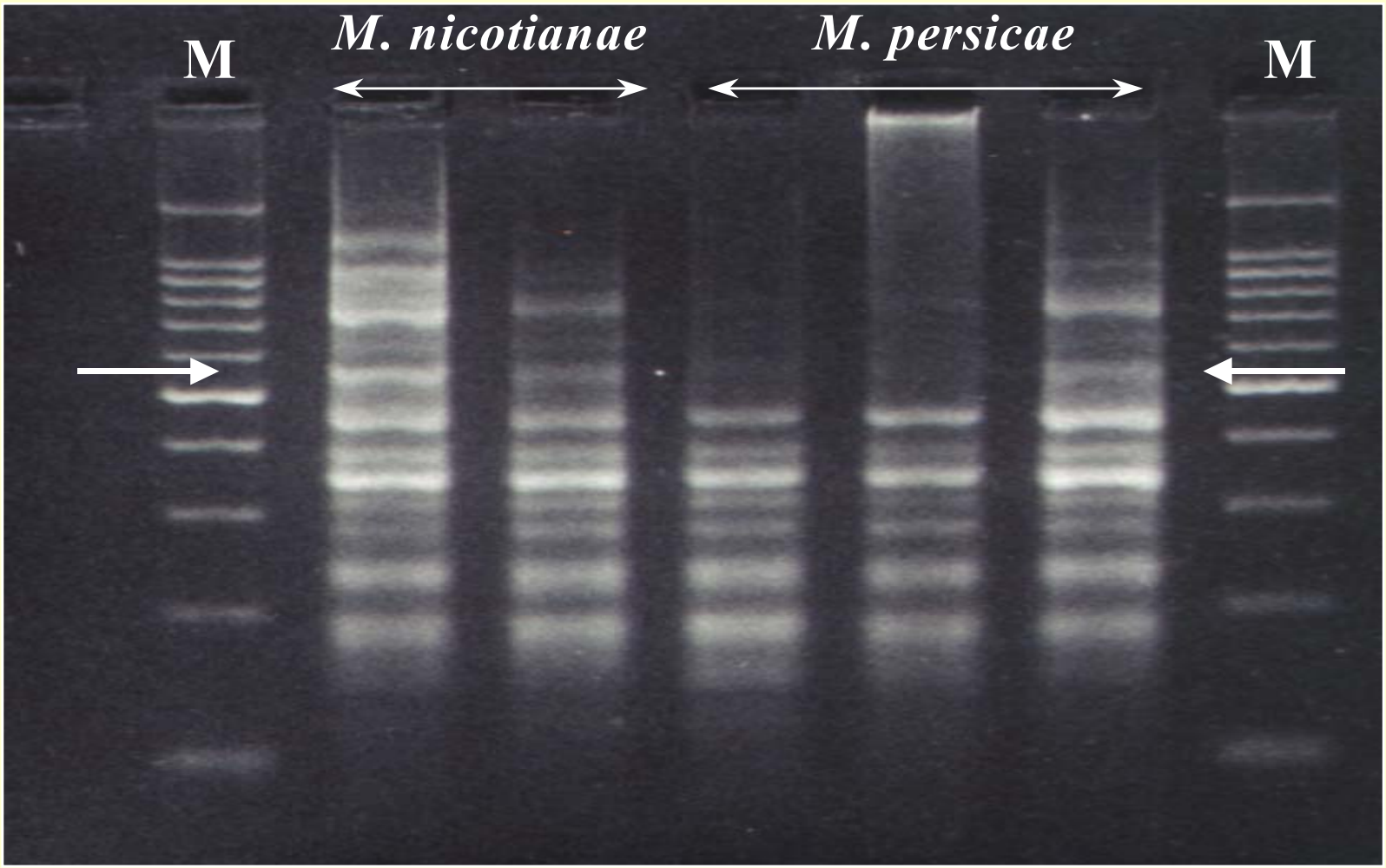
- 61 primers $\rightarrow$ **Little variation** (not species specific) **in banding patterns** between clones collected on tobacco and those on other hosts (away tobacco-growing regions).

- 3 primers failed to produce any band.

- Almost all differences unable to discriminate the two taxa.

- **Single band difference** associated with host-plant origin.

Banding pattern in clones of M. persicae *using primer OPA-18*



Presence or absence of 550 b.p. band in clones of M. persicae *using primer OPA-18*

Species	Host	Clones with band	Clones without band	Total
M. nicotianae	Tobacco	**22**	**0**	22
M. persicae	Peach	5	12	17
M. persicae	Pepper	1	6	7
M. persicae	Cabbage	0	1	1
Total		28	19	47

(χ^2=28.8, df=3, P<0.001)

Restriction enzymes

- Alu I, Hae III, Taq I & Mbo I
  - Four cutter base restriction enzymes
- 21 co-migrating bands (>800 b.p.)
- **Identical pattern** after digestion with the restriction enzymes

✓The almost identical banding pattern between tobacco-feeding clones and those on other secondary hosts suggests that the two groups are **co-specific**.

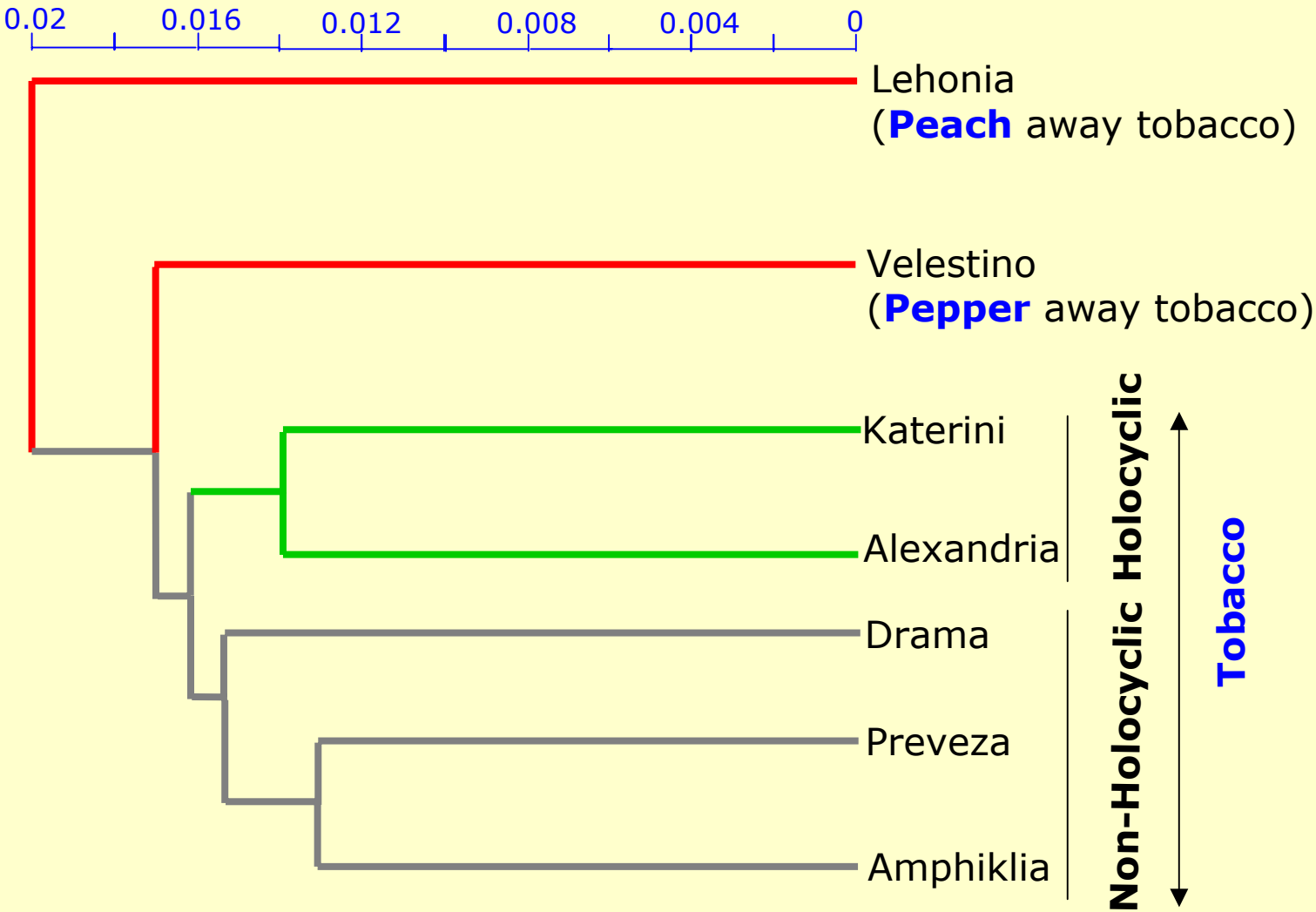
✓However, the single band difference denotes the existence of a separate taxon on tobacco.

RAPD-PCR

- 96 clones
- 7 populations
  - 5 from tobacco (South-Central to North Greece)
  - 2 from peach and pepper, away from tobacco growing regions (Central-Eastern Greece)
- 10 decamer random primers

- "Allele" frequency (Lynch & Milligan, 1994)
- Nei's genetic distance
- Estimated heterozygosity

UPGMA dendrogram based on Nei's genetic distances between samples of *M. persicae* collected from different host-plants and regions of Greece



- The results showed a genetic divergence between the tobacco-feeding form and those on other hosts.

- Life cycle and host origin are important factors affecting the genetic structure of *M. persicae* populations

What multivariate morphometrics tell us about host specialisation

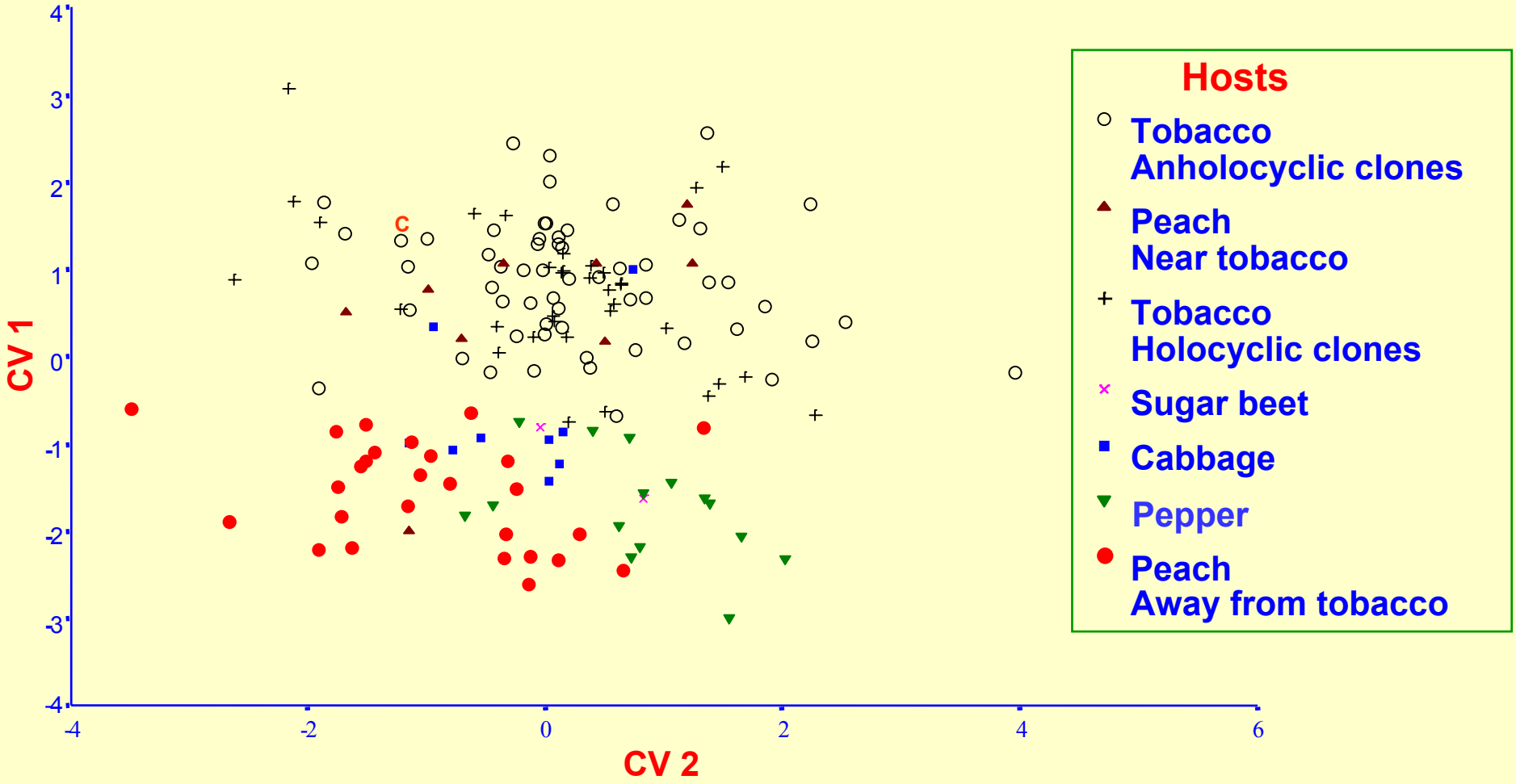
"Environmental effects

or

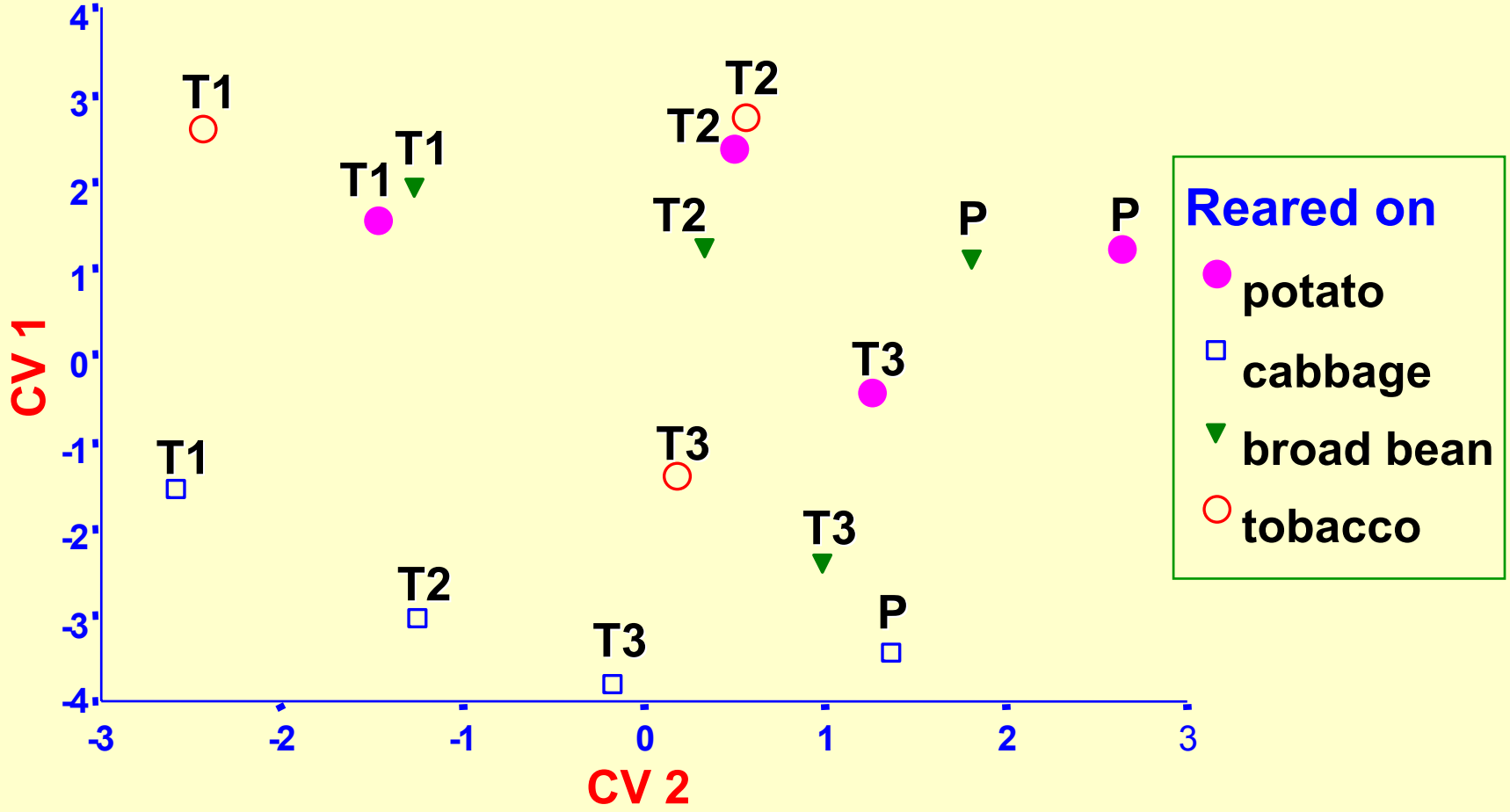
genetic differences?"

Morphometrics

- **Total: 157 clones from Greece**
  - tobacco 94
  - peach 37
  - pepper 15
  - cabbage 9
  - sugar beet 2
- Tobacco and non-tobacco growing regions
- 9 morphological characters (Blackman, 1987)
- 10-12 adult apterous per clone
- 1-3 parthenogenetic generations on potato. **COMMON** environment
- Five clones after 1-3 years rearing on
  - cabbage
  - tobacco
  - broad bean
- **Total: 1723 apterae**



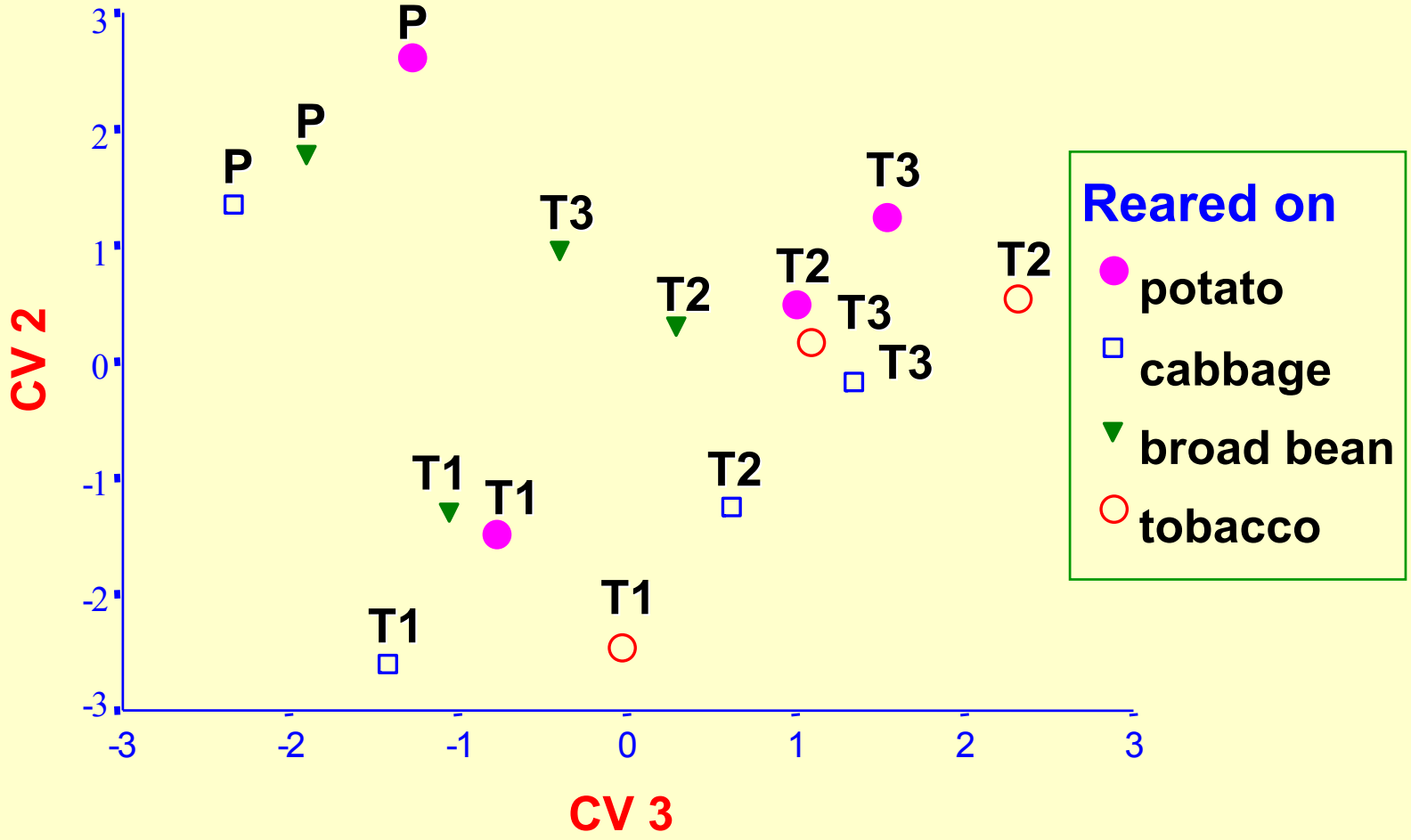
Plots of the scores of the first canonical variates for 157 clones of *M. persicae* originated from different hosts and regions of Greece.



Plots of the scores of second and third CVs of four clones of *M. persicae* reared on different plants.

T1-T3: collected from tobacco

P: collected from peach away from tobacco growing regions



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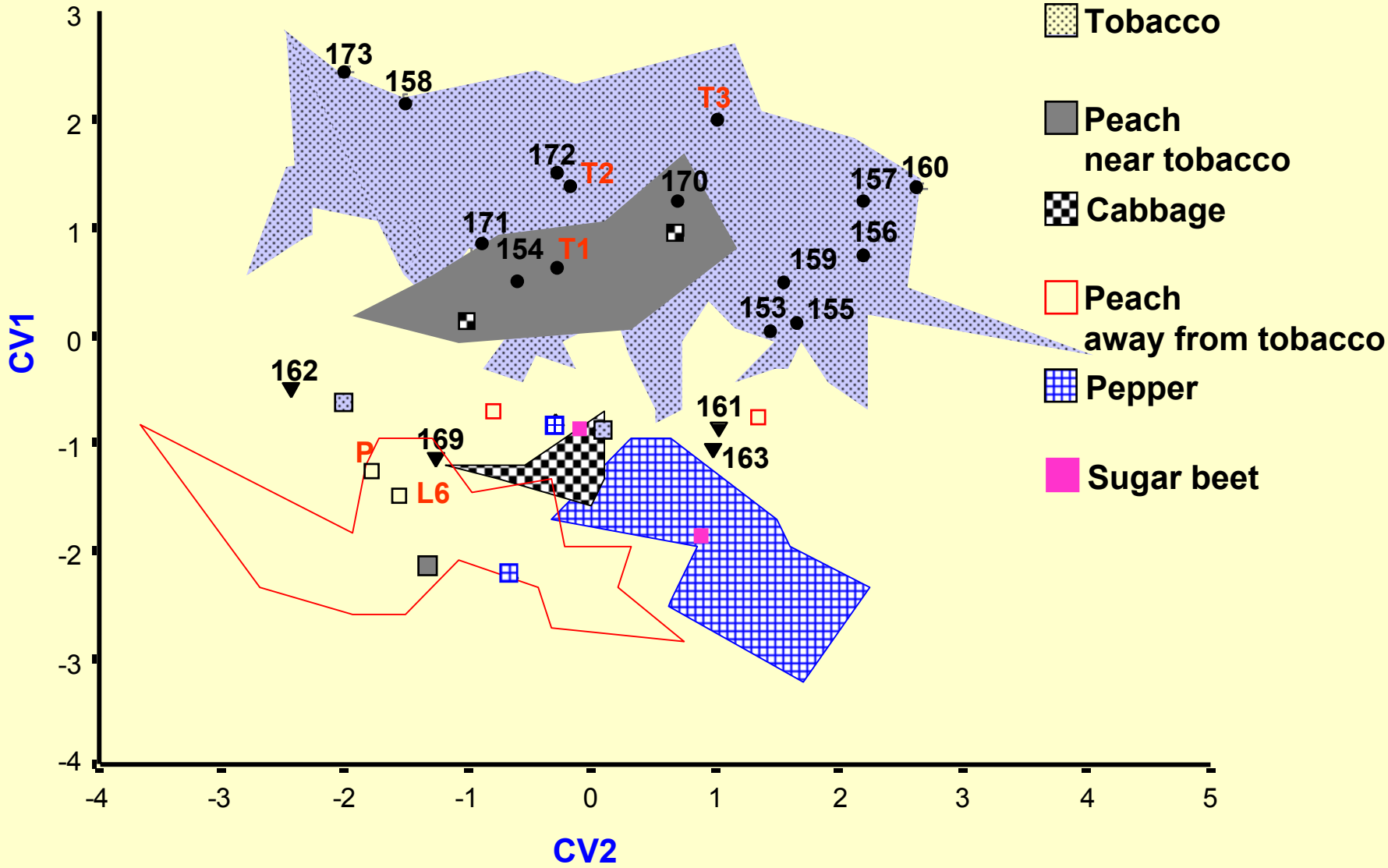
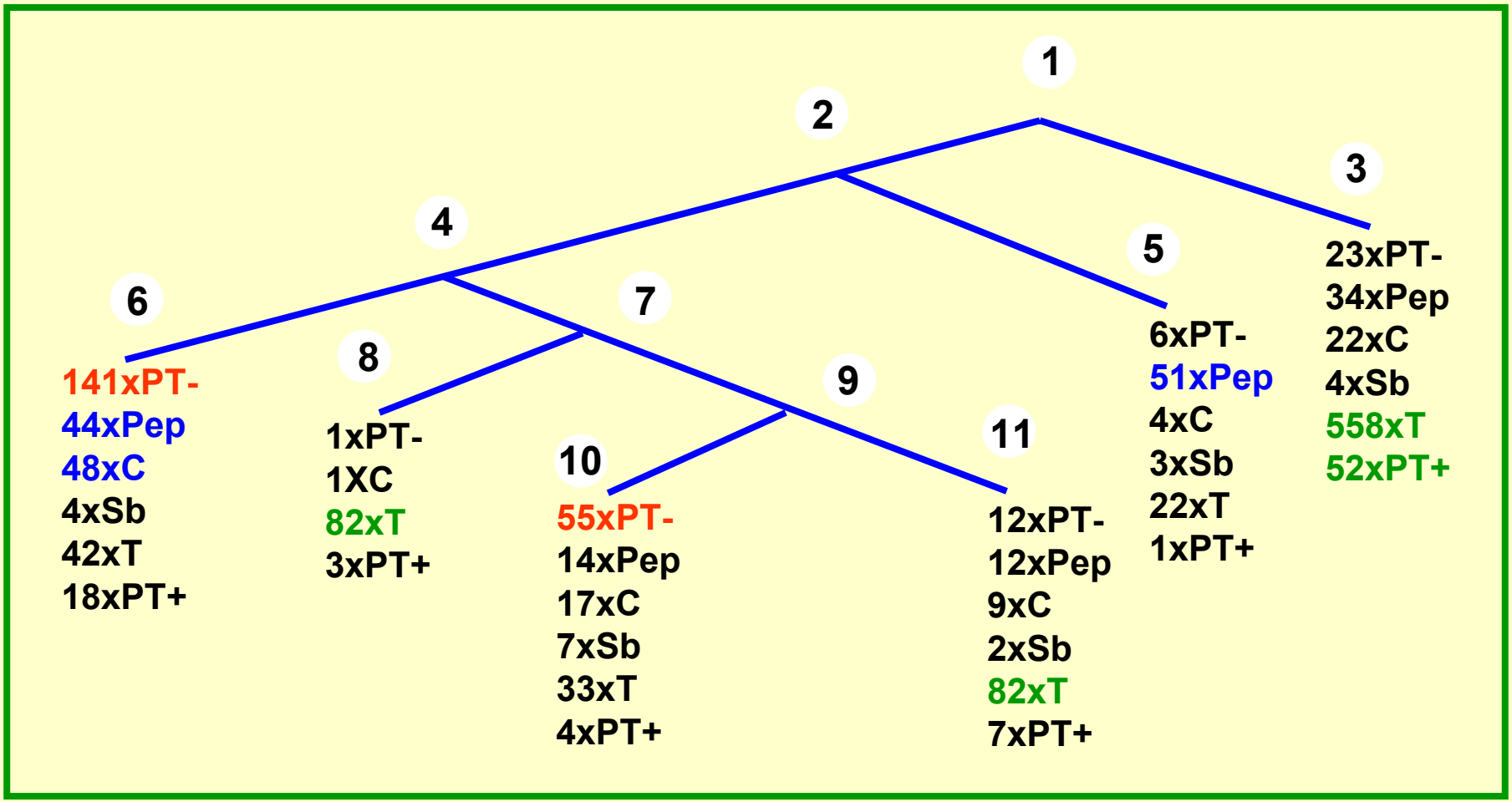


Plot of the scores of the first two CVs for 157 *M. persicae* clones and samples of them after various treatments

Non-parametric classification tree for the specimens of 157 clones of *M. persicae* originated from different hosts



PT-: peach away tobacco, **Pep:** pepper, **C:** cabbage, **Sb:** Sugar beet **T:** tobacco, and **PT+:** Peach near tobacco

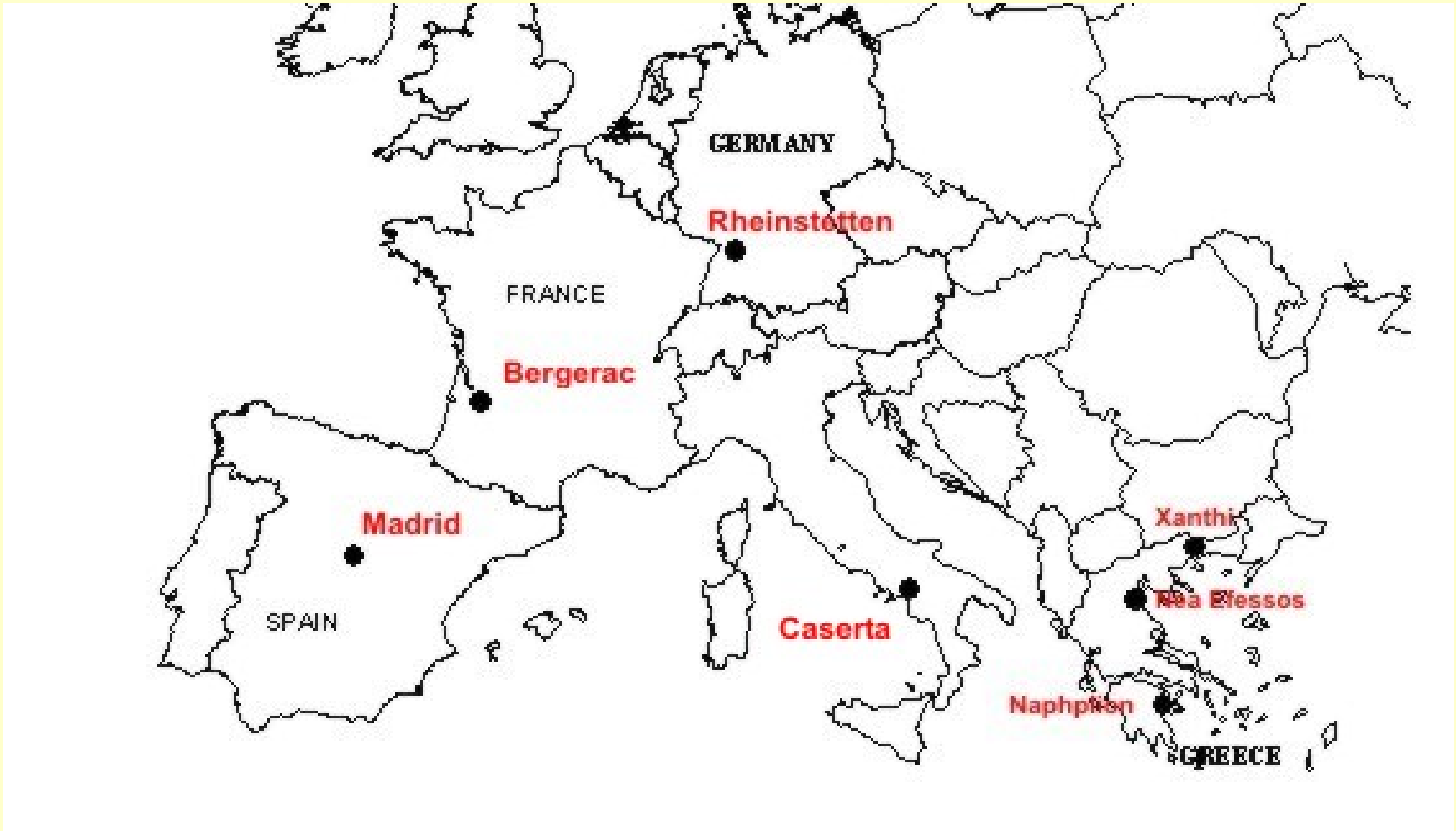
Does the separation hold in samples from other European countries?

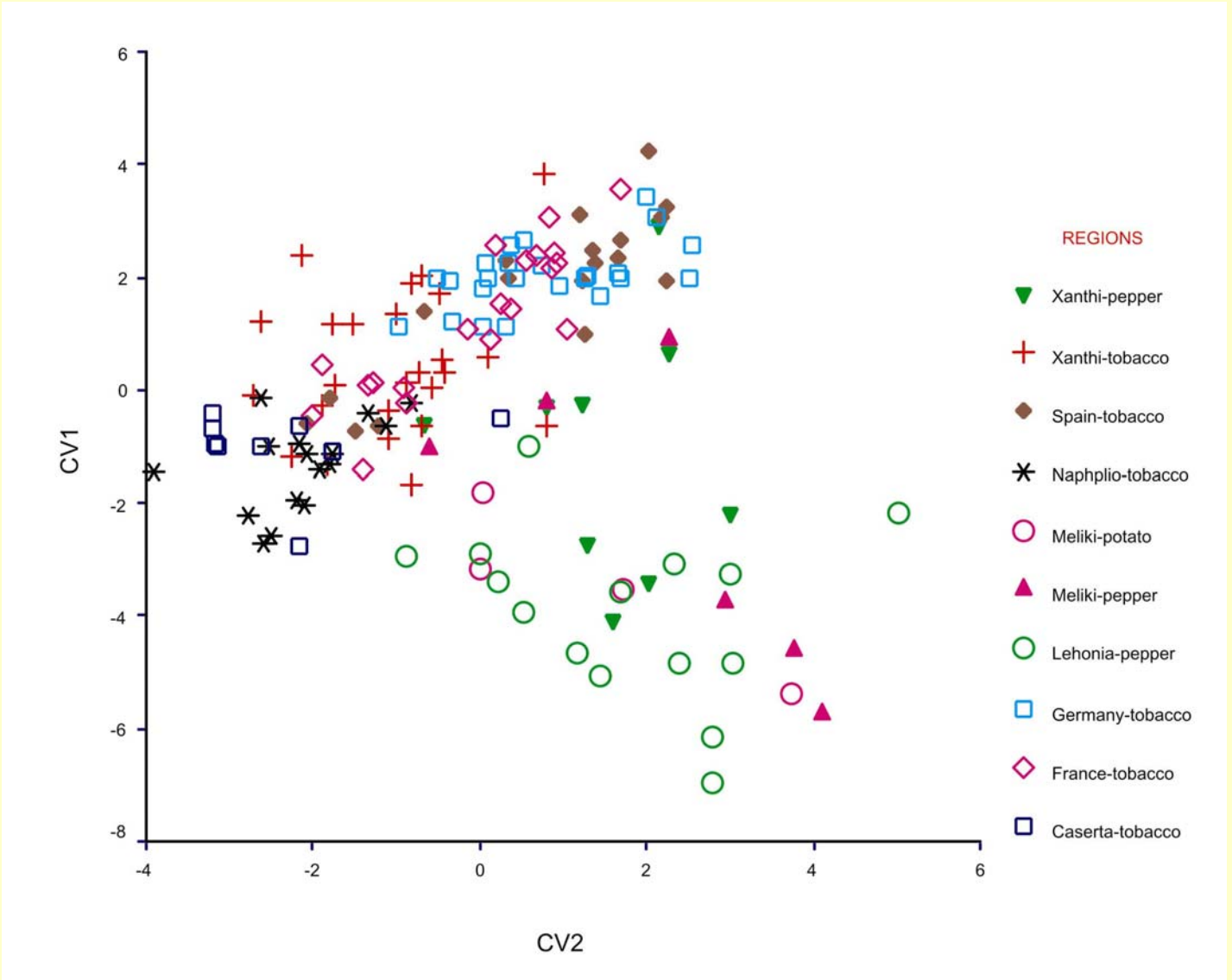
Aphid samples

- **Tobacco clones**
  - –Greece and Europe
- **Potato-pepper clones**
  - –Greece

Total: 147 clones

Clones collected from different regions of Europe

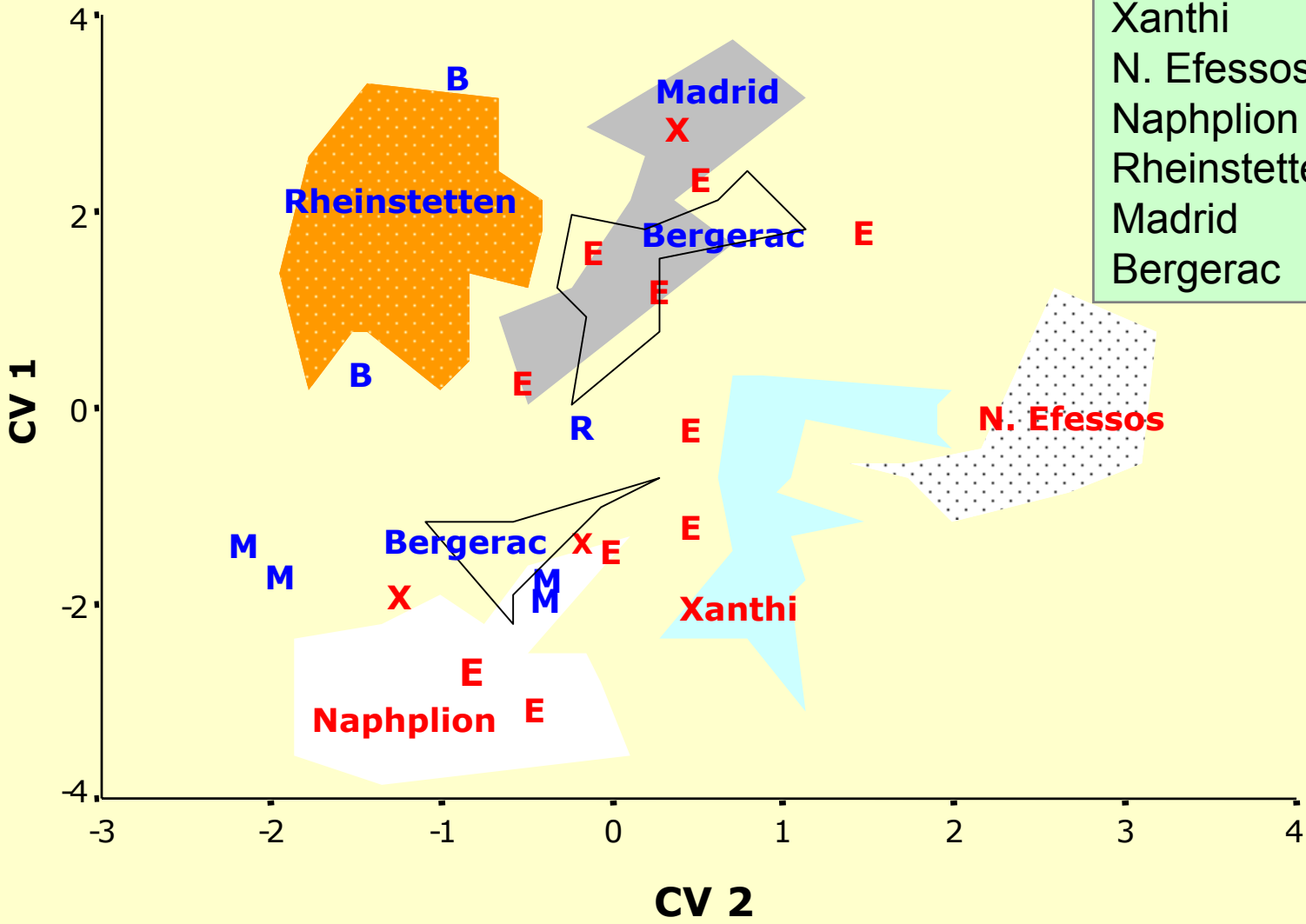




Plot of the scores of the first two CVs for 147 *M. persicae* clones originated from tobacco and other hosts in Greece and other regions in Europe

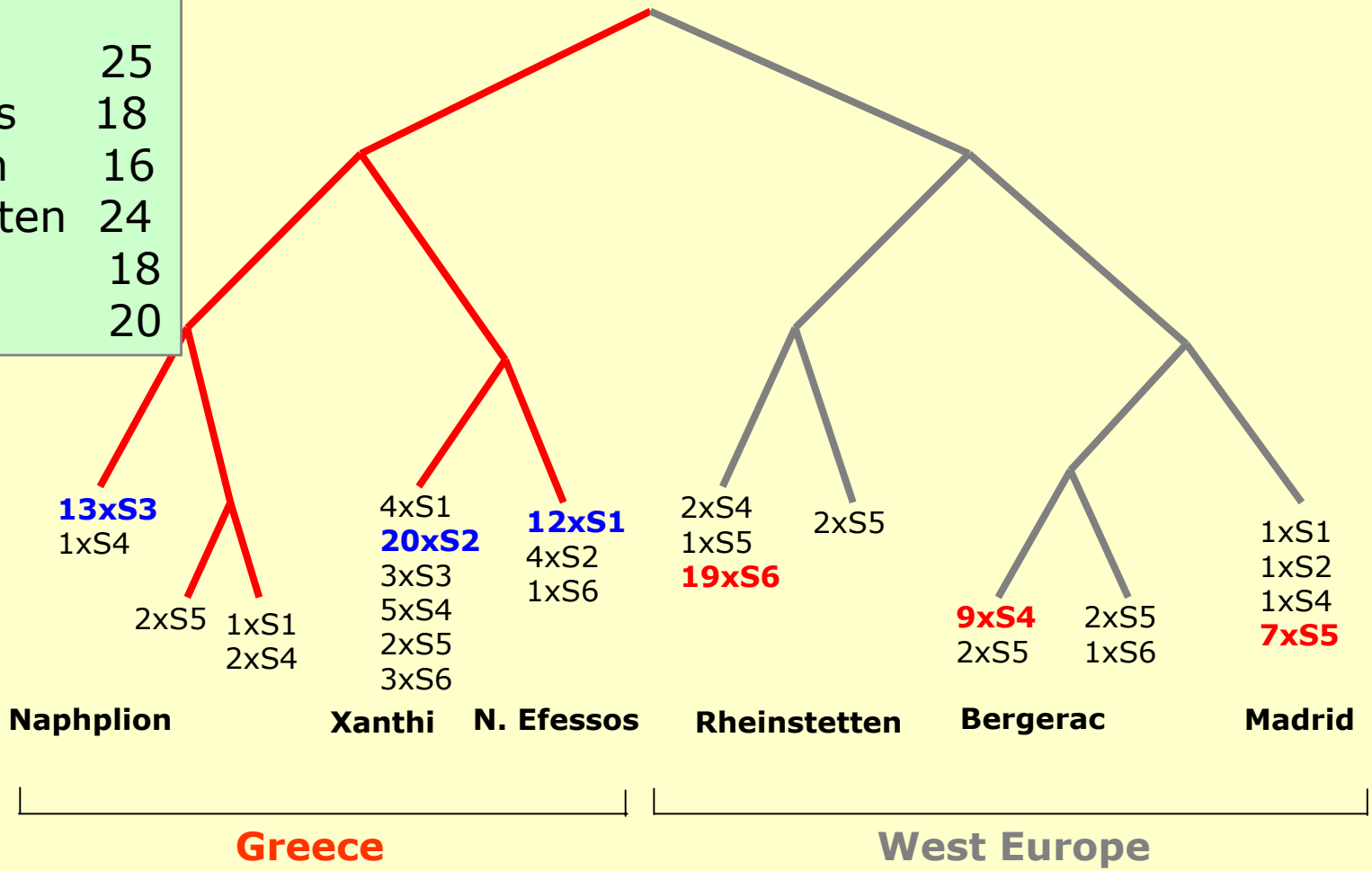
- ✓ The clones collected on **tobacco** or on **peach in tobacco-growing regions separated** from those originating on **other crops**.
- ✓ The morphological differences are constant and **reflect genetic differentiation**.
  - All clones were reared in a **COMMON environment**.
- ✓ The tobacco-feeding form was not found in areas where tobacco is not cultivated.
- ✓ Long term parthenogenetic rearing and the host on which clones were reared affected aphid morphology. However, both groups retained their distinct morphology.
- ✓ Both taxa retain their host-related differences even in regions where the species have a bisexual generation on peach.

Are populations on tobacco from different parts of Europe genetically divergent?



Plot of the scores of the first two CVs for 121 clones of *M. persicae* collected from tobacco from different regions of Europe

Clones	
Xanthi	25
N. Efessos	18
Naphplion	16
Rheinstetten	24
Madrid	18
Bergerac	20



Non parametric classification tree obtained from individuals of 121 clones of *Myzus persicae* collected from tobacco from different regions of Europe

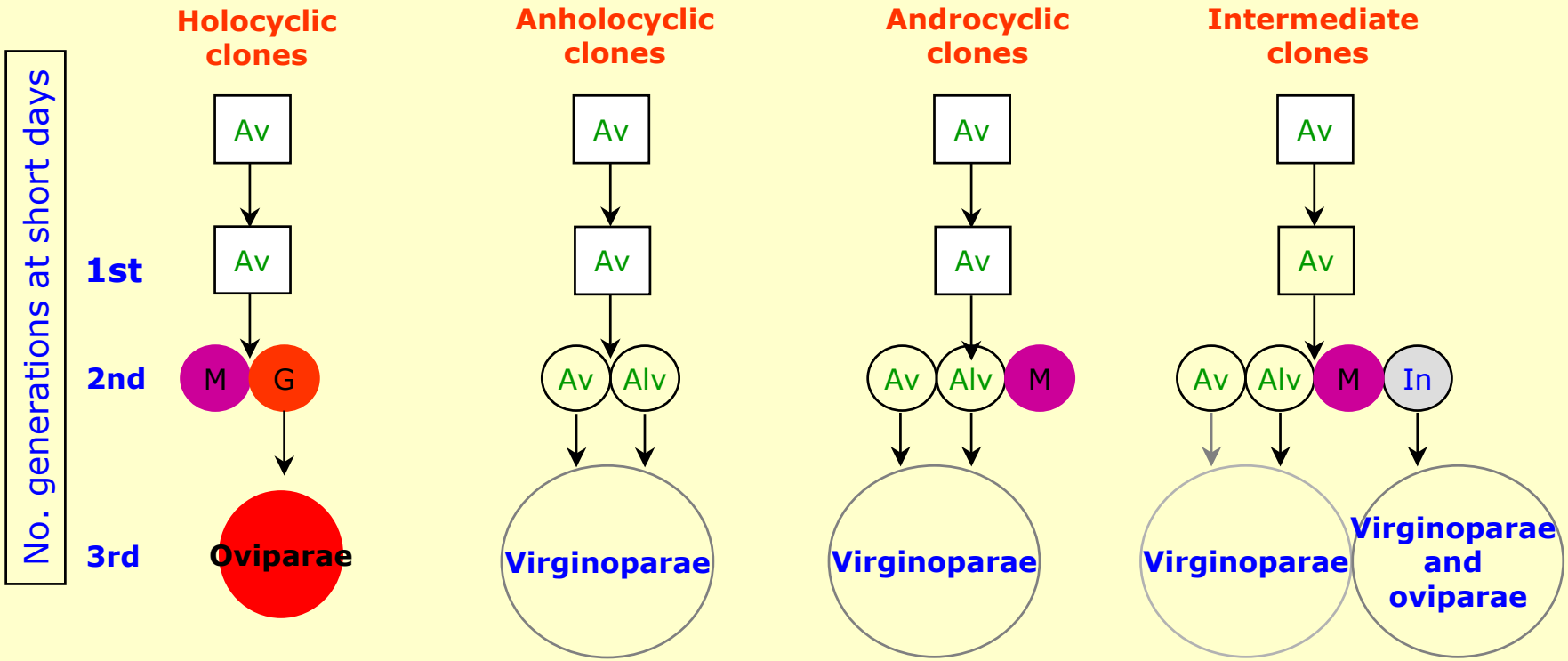
Life cycle variation of M. persicae

"factors affecting overwintering strategy"

Aphid samples

- **Greece**
  - peach 556
  - tobacco 1982 (field and seedbeds)
  - Other hosts 259
- **Spain (Madrid)**
  - tobacco 18
- **Italy (Caserta)**
  - peach 22
  - tobacco 51
- **France (Bergerac)**
  - tobacco 20
- **Germany (Rheinstetten)**
  - tobacco 24

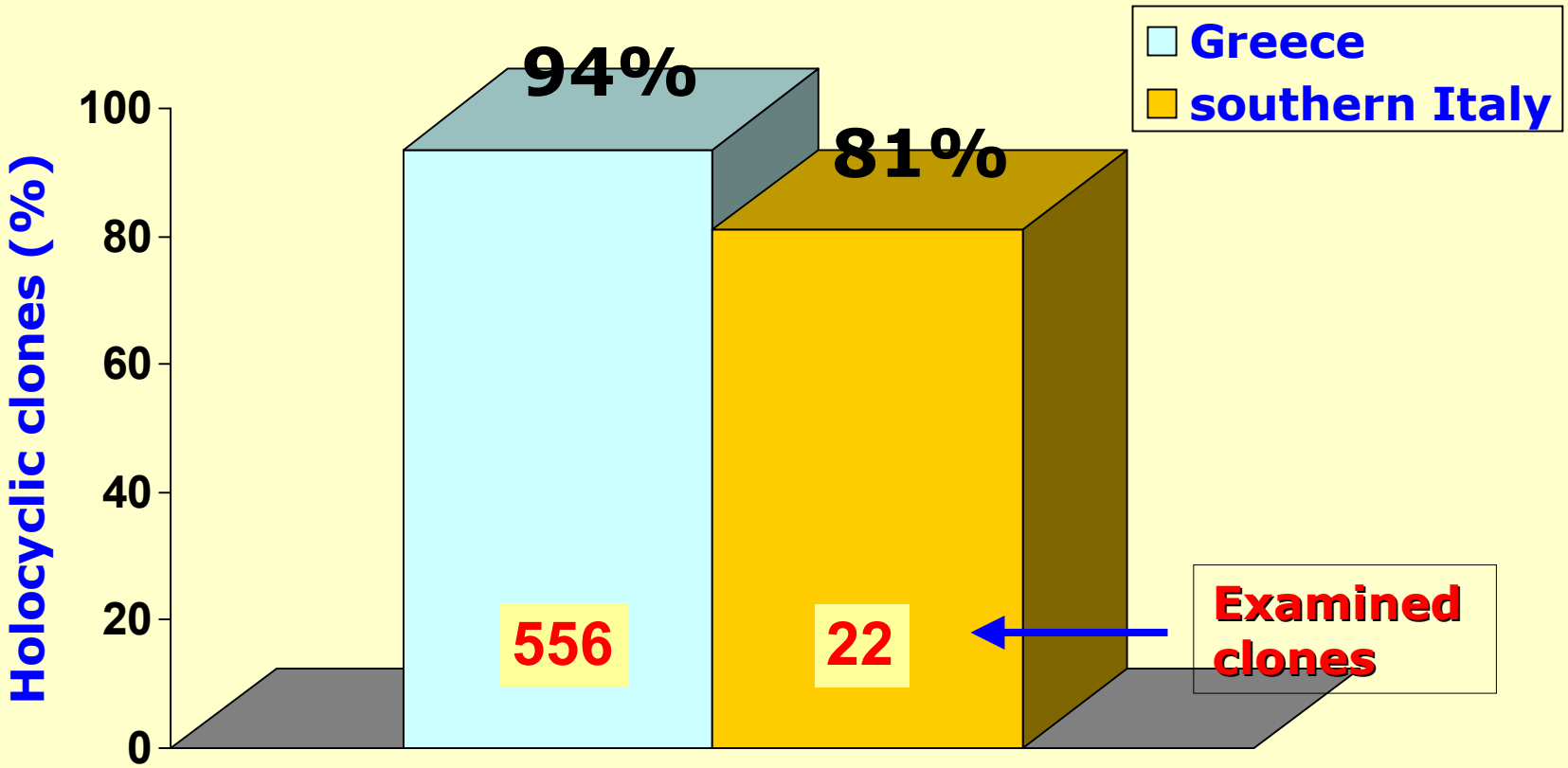
- ***Total: 2,931 clones***
- ***Sampling years: 1995-1999***



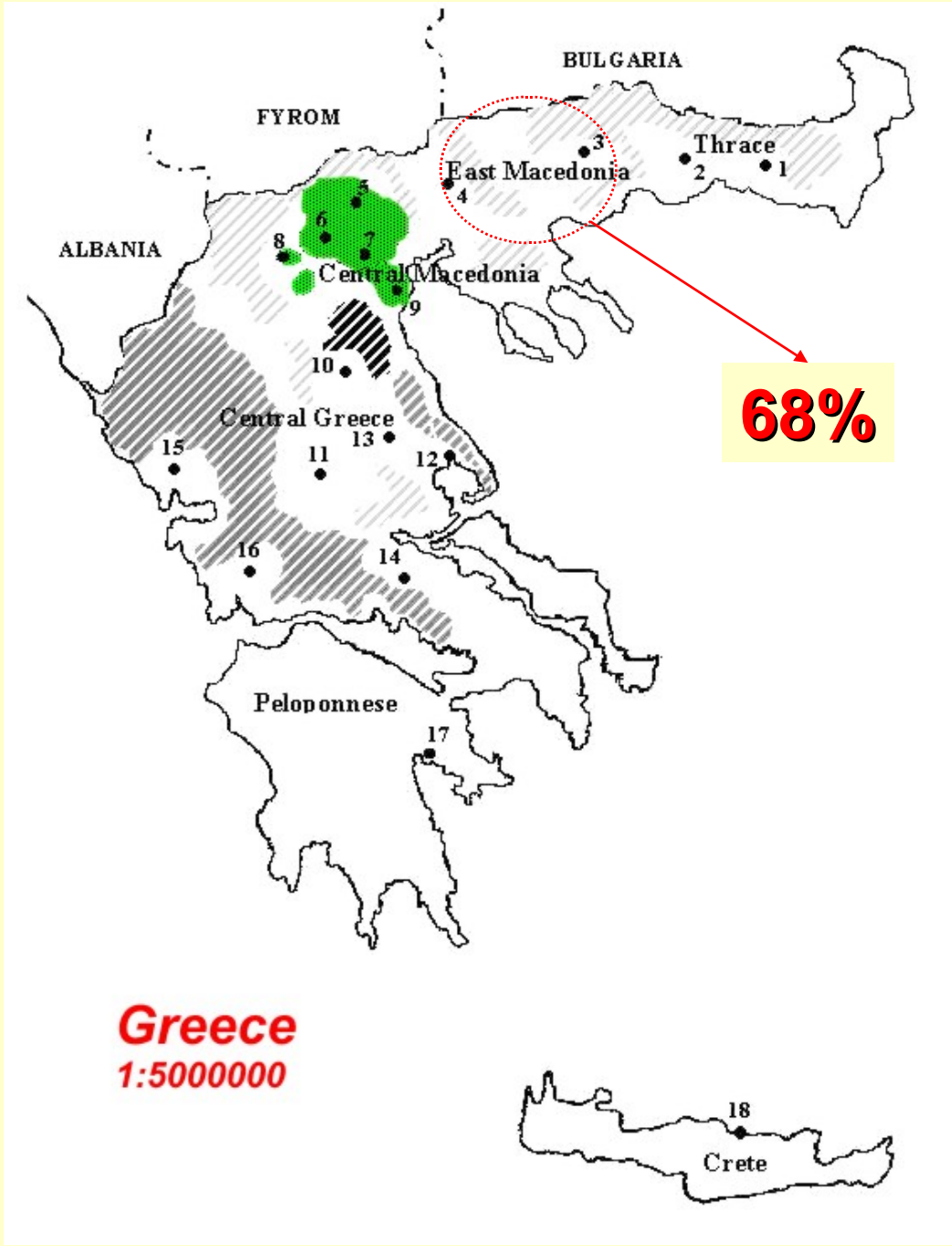
Life cycle categories of *M. persicae*

Av: apterous virginoparous females M: males, G: gynoparae, Alv: alate virginoparous females, In: intermediate alate females.

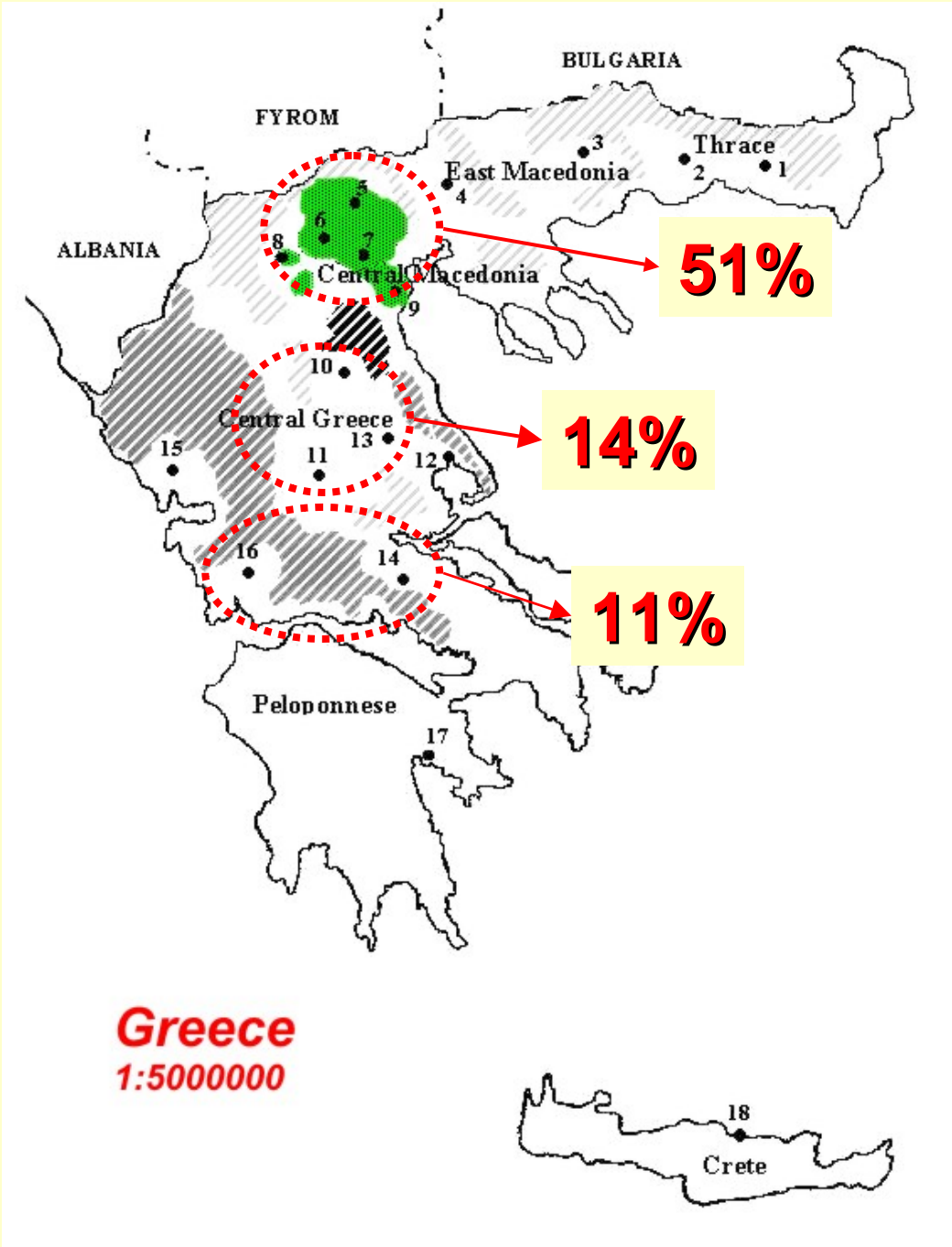
Holocyclic clones (%) of *M. persicae* collected from peach in Greece and southern Italy



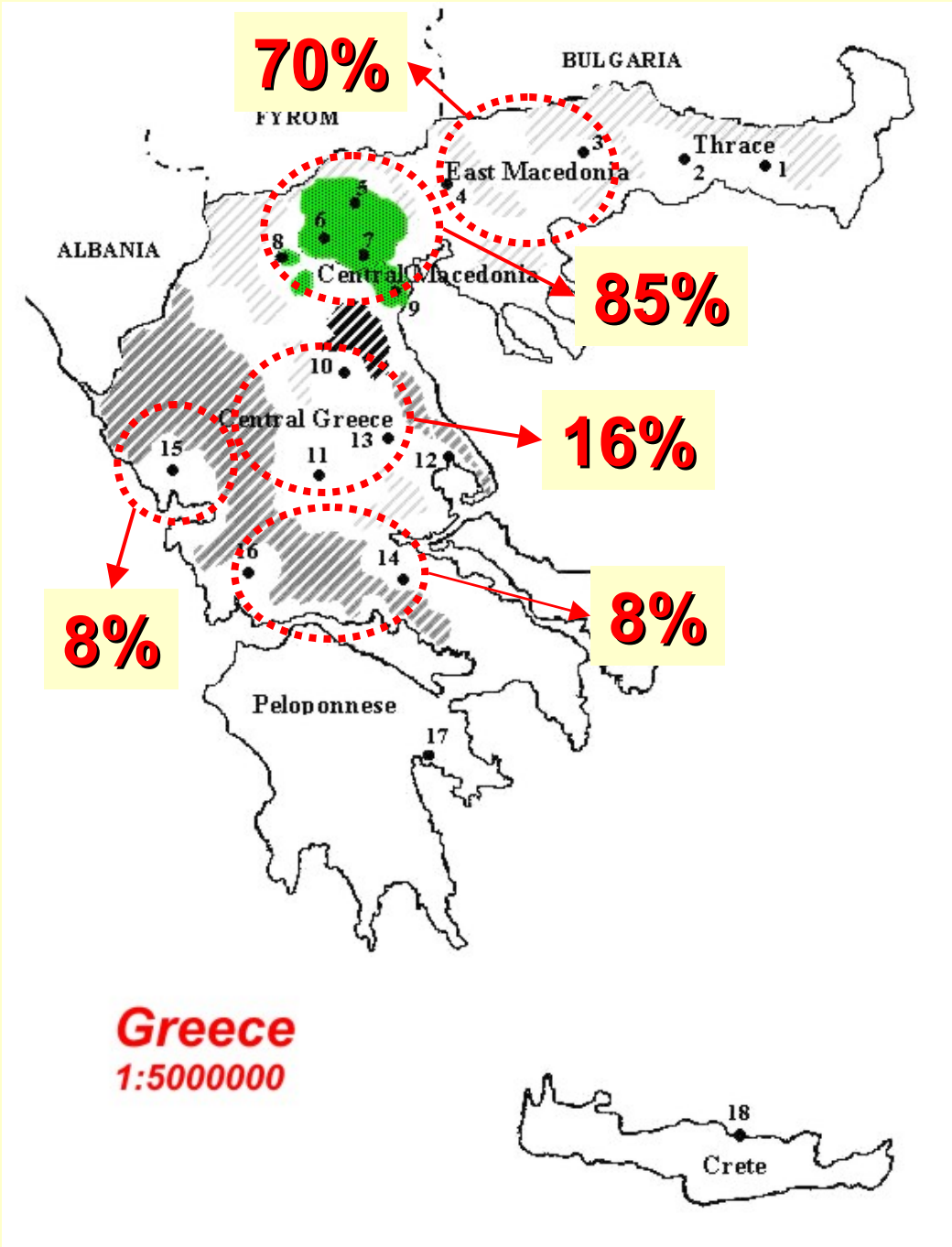
Holocyclic clones on tobacco



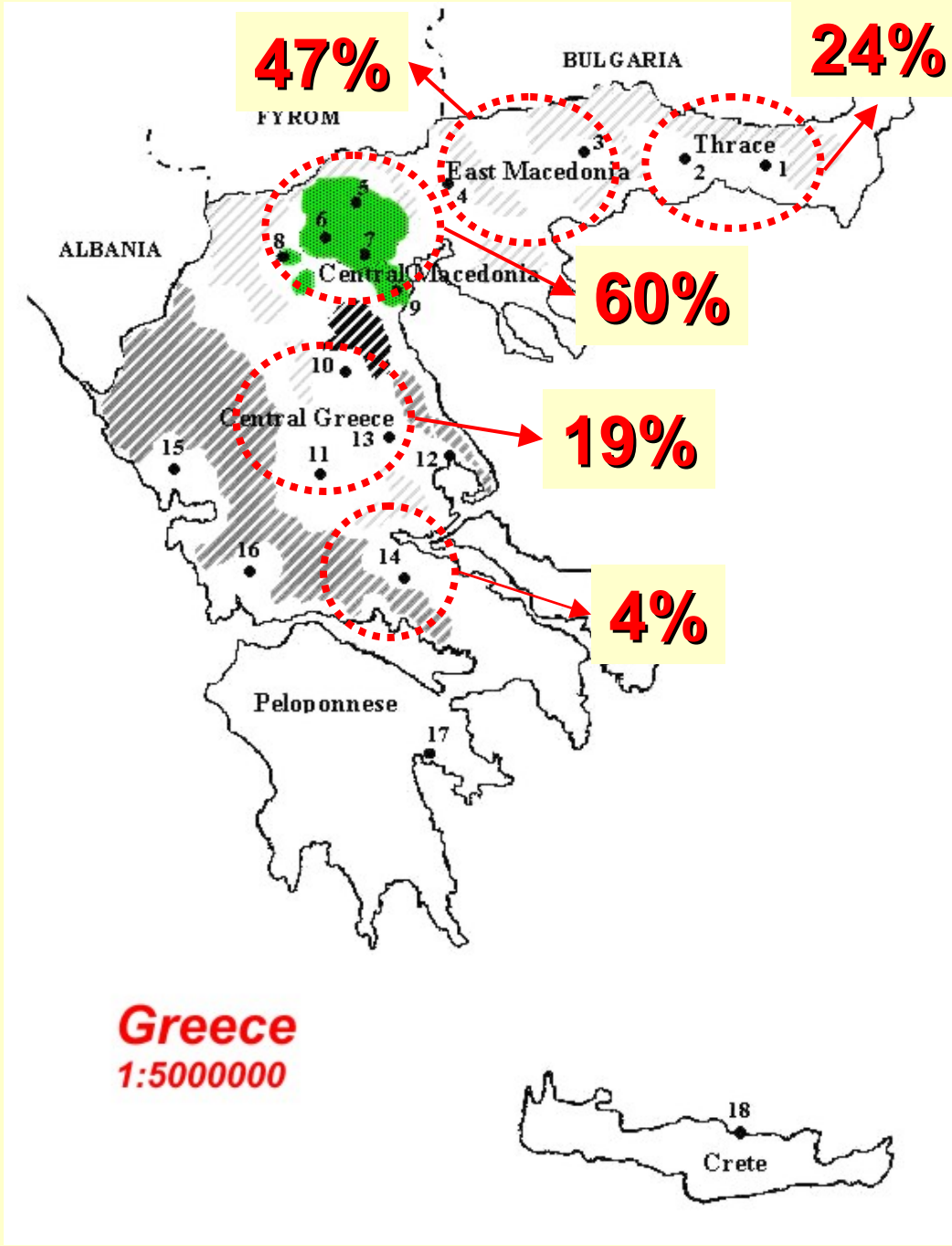
Holocyclic clones (%) on tobacco in 1995



Holocyclic clones (%) on tobacco in 1996

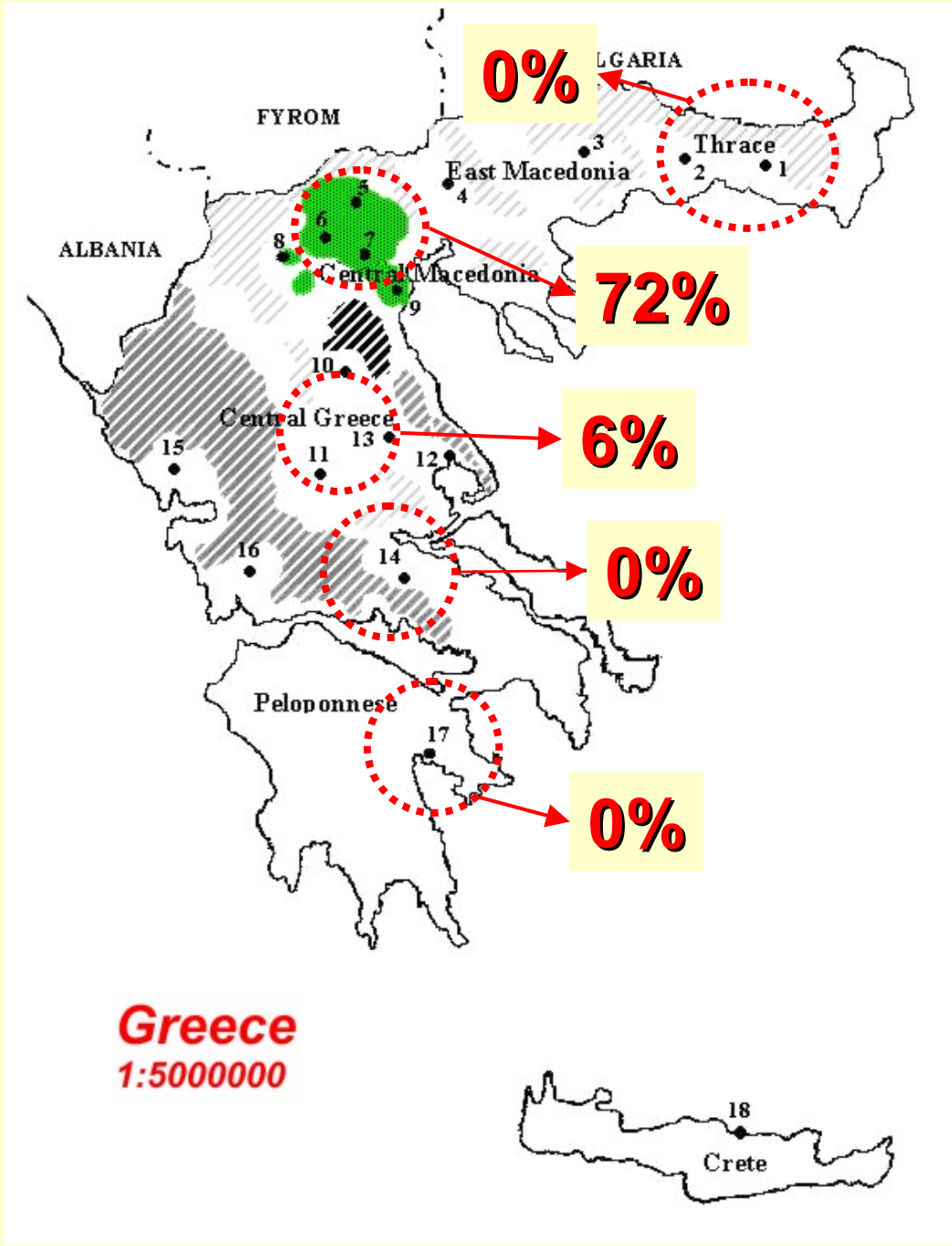


Holocyclic clones (%) on tobacco in 1997

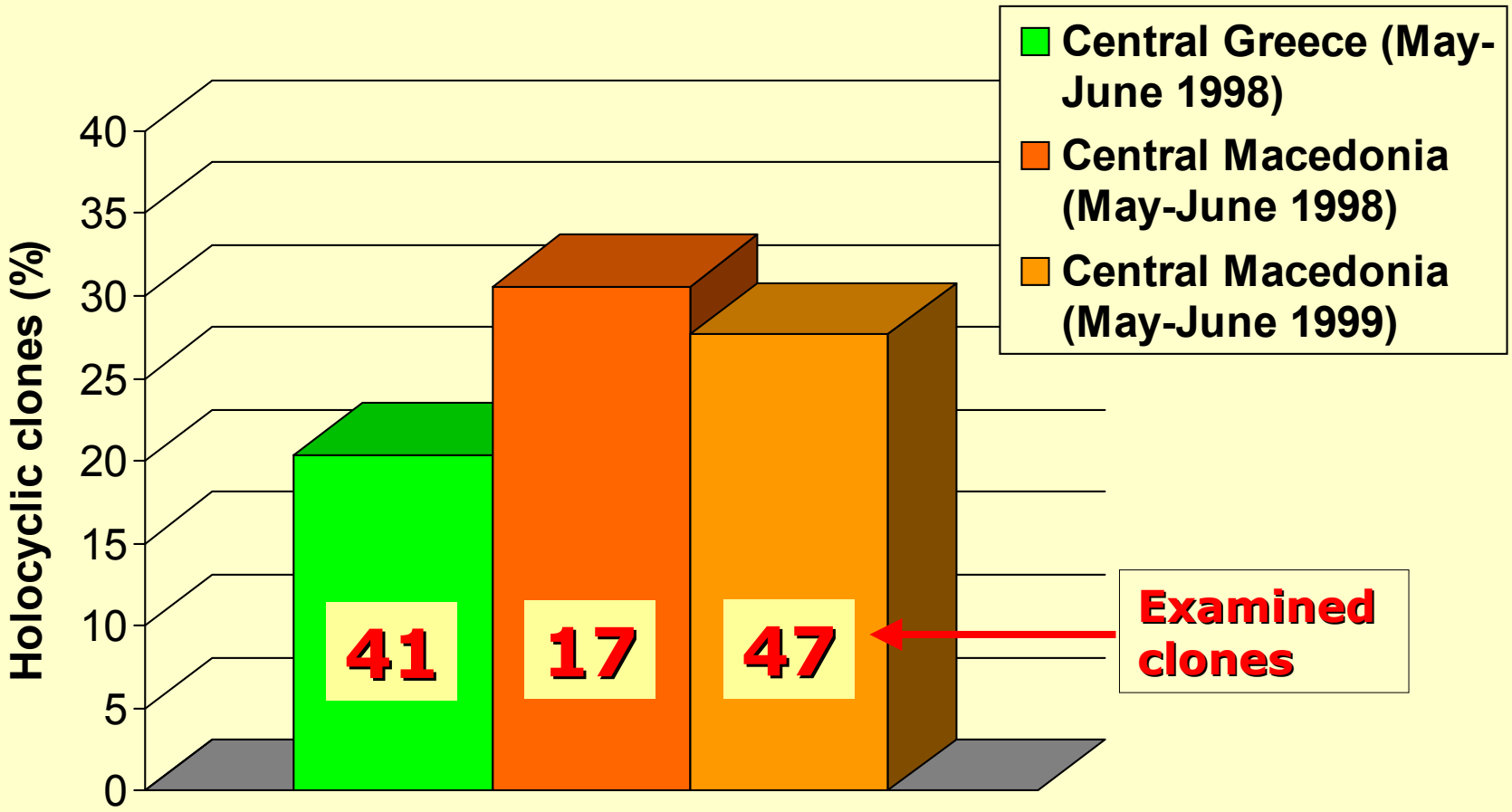


Holocyclic clones (%) on tobacco in 1998

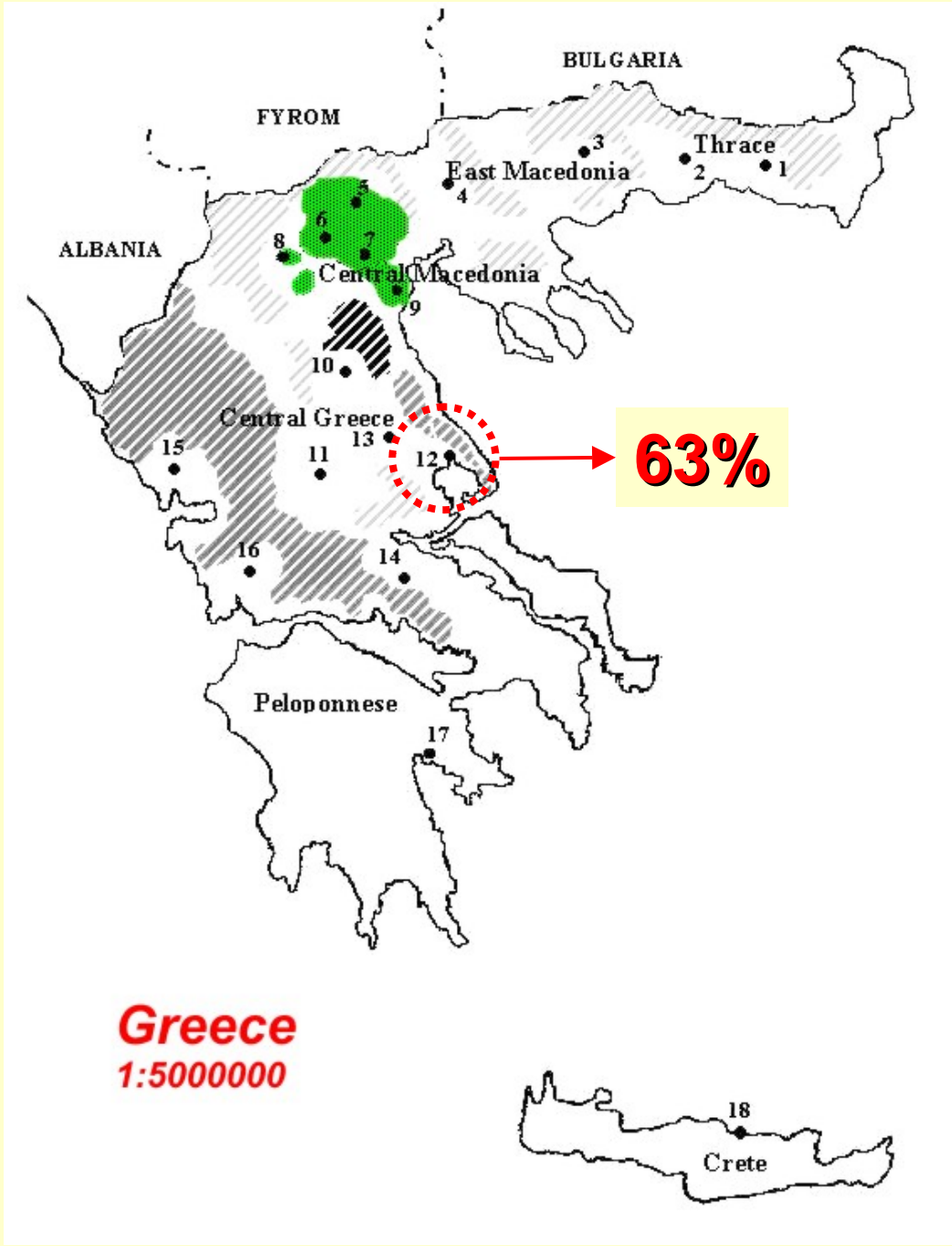
Holocyclic clones (%) on tobacco in 1999



Holocyclic clones (%) collected on tobacco seedbeds

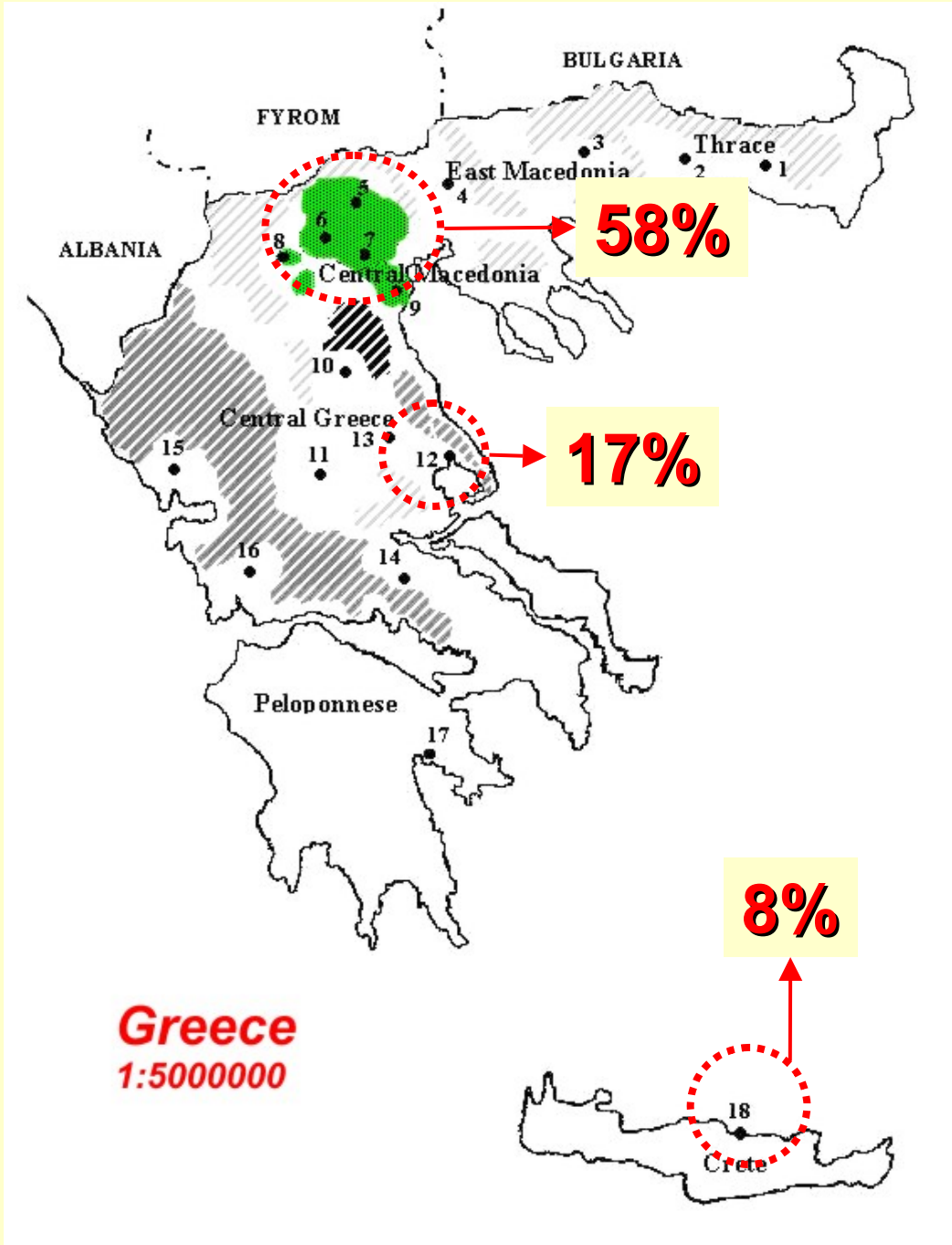


Holocyclic clones on other crops

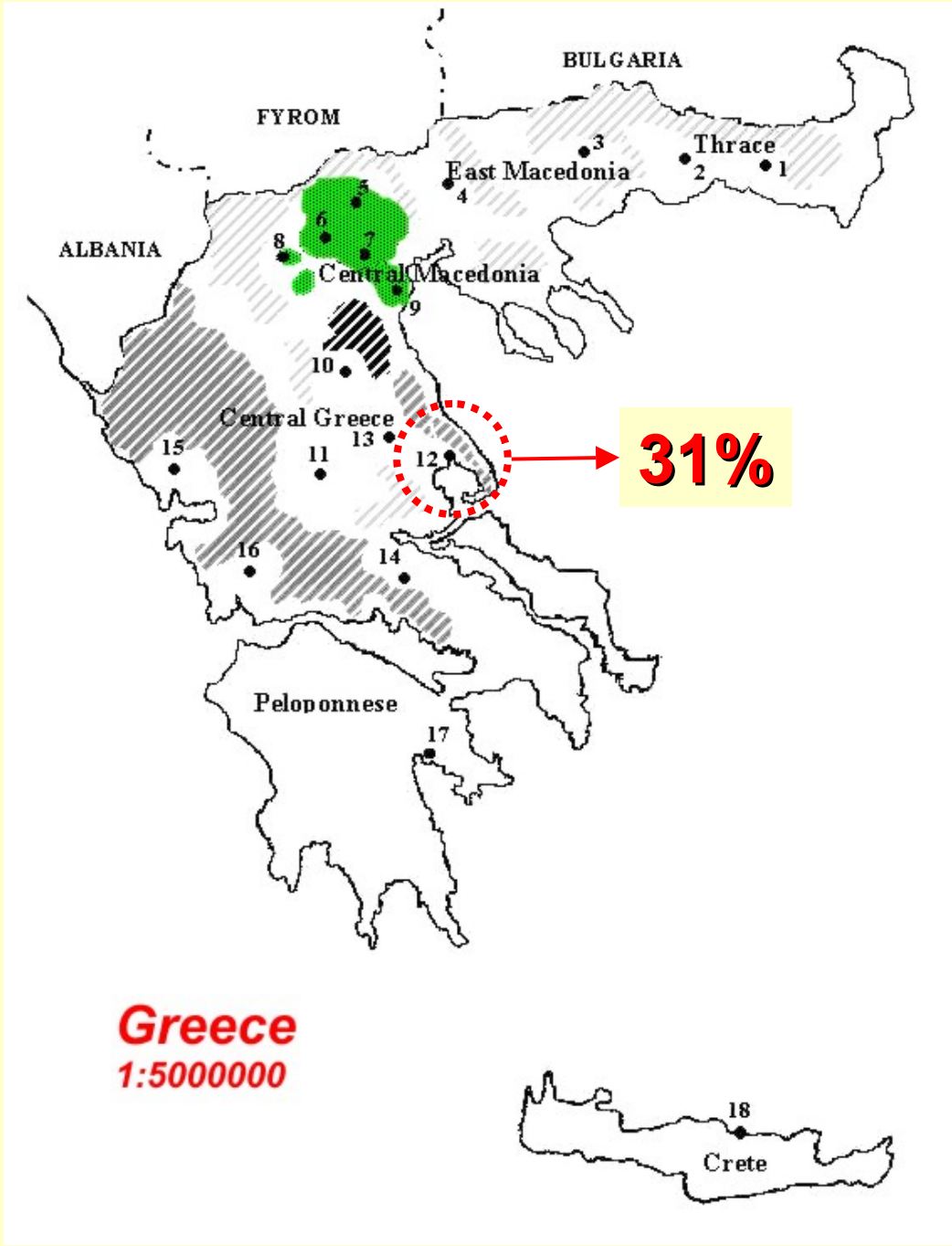


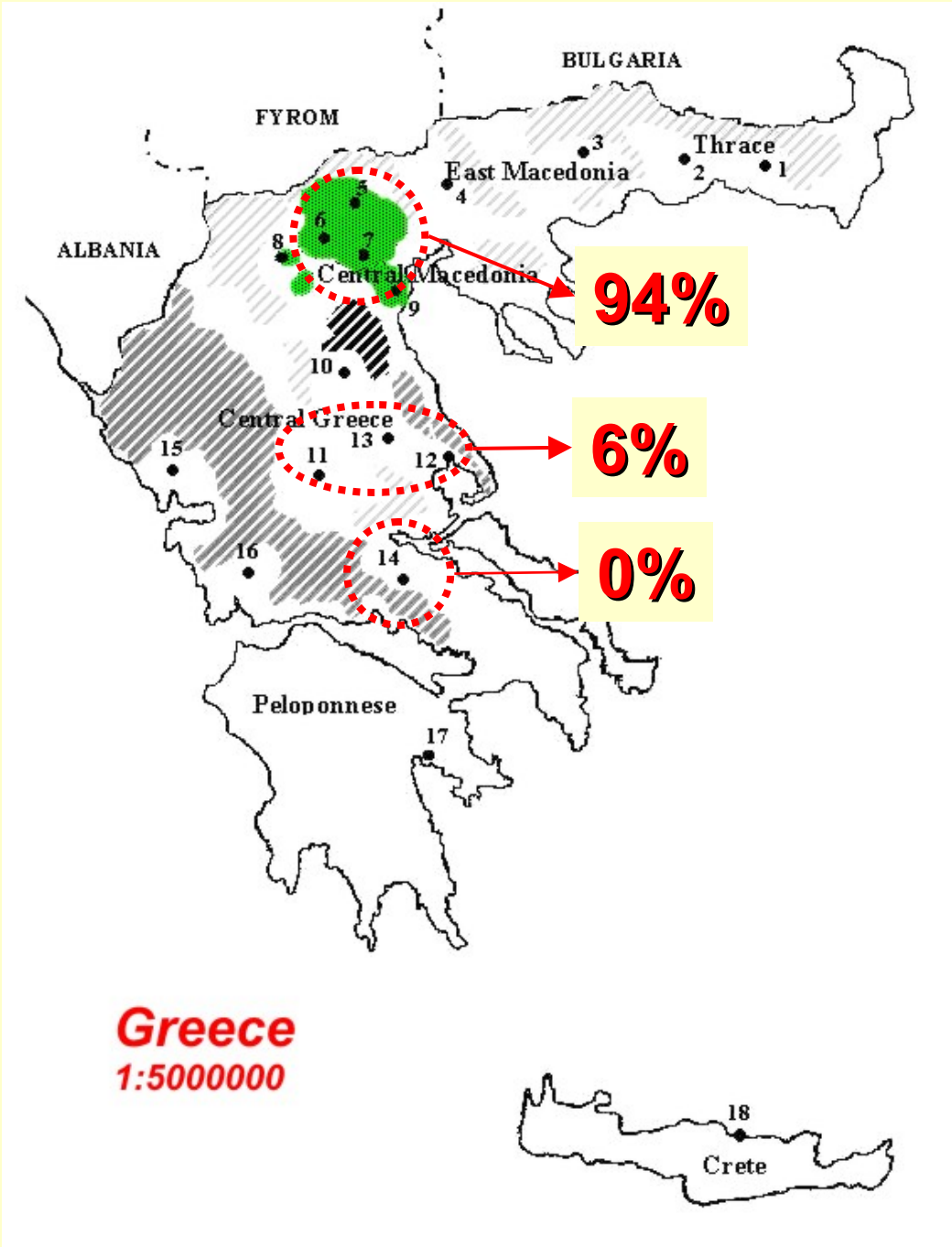
Holocyclic clones (%) on pepper in 1995

Holocyclic clones (%) on other crops in 1996

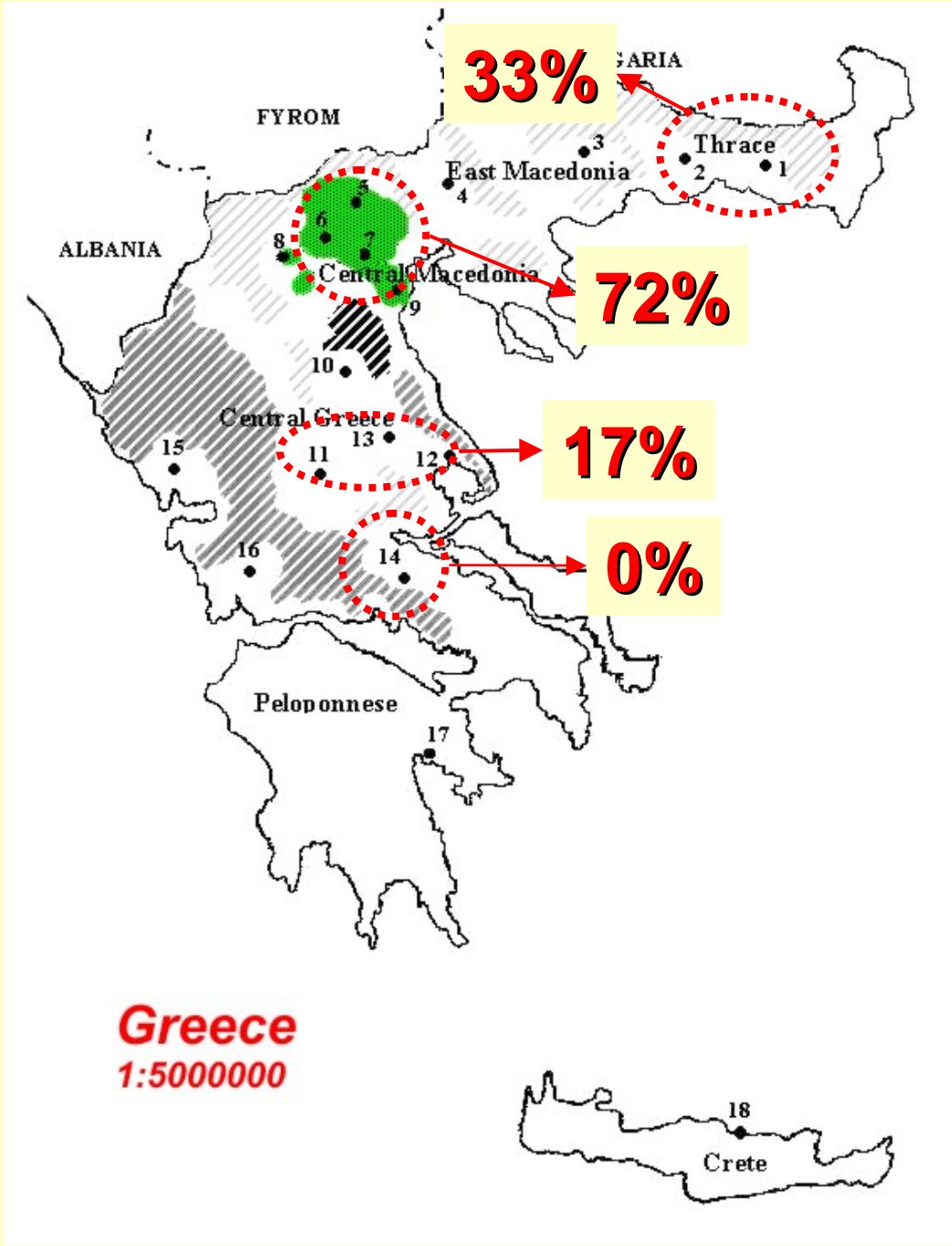


Holocyclic clones (%) on other crops in 1997





Holocyclic clones (%) on other crops in 1998



Holocyclic clones (%) on other crops in 1999

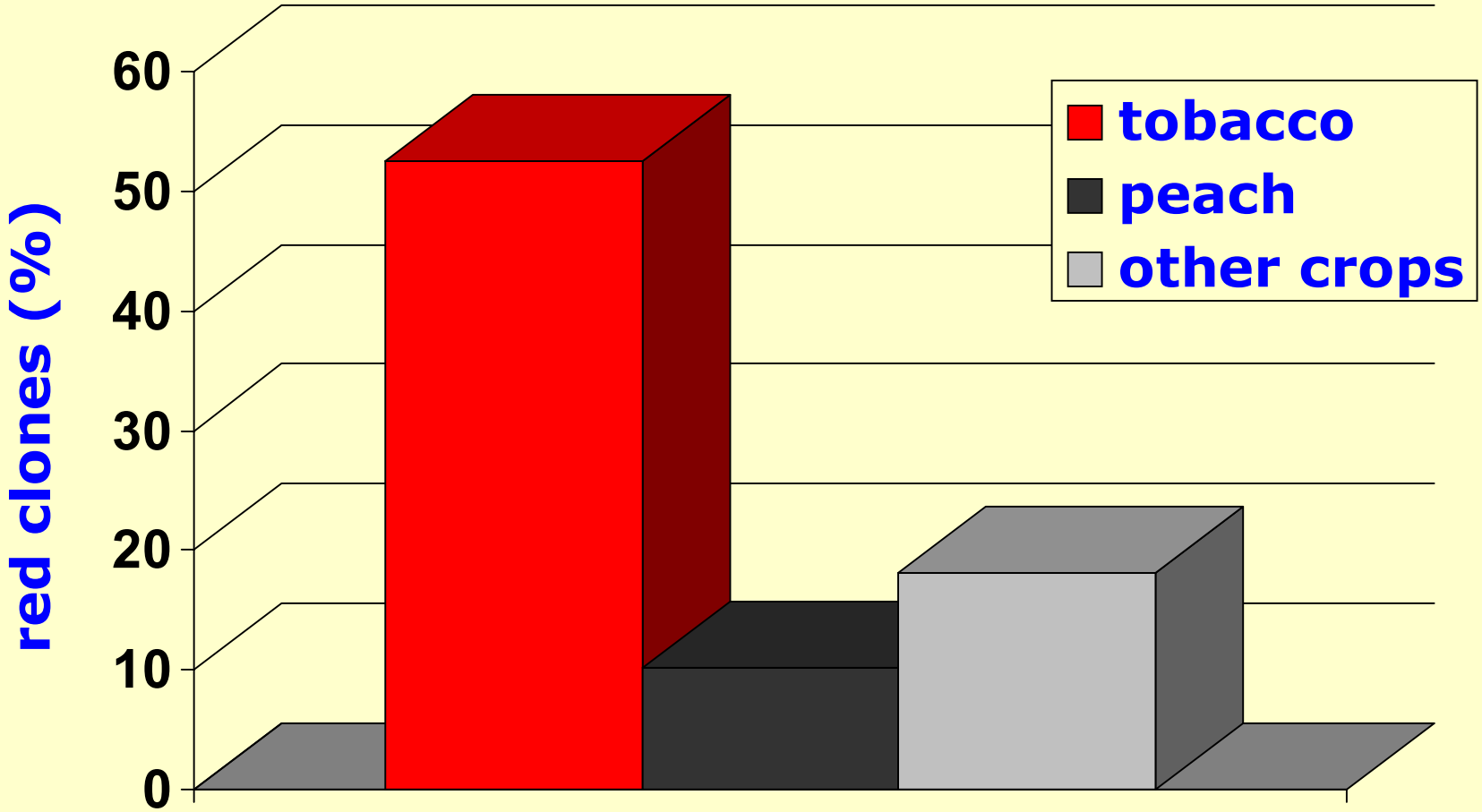
Androcyclic and intermediate clones of *M. persicae* found in Greece during 1995-99

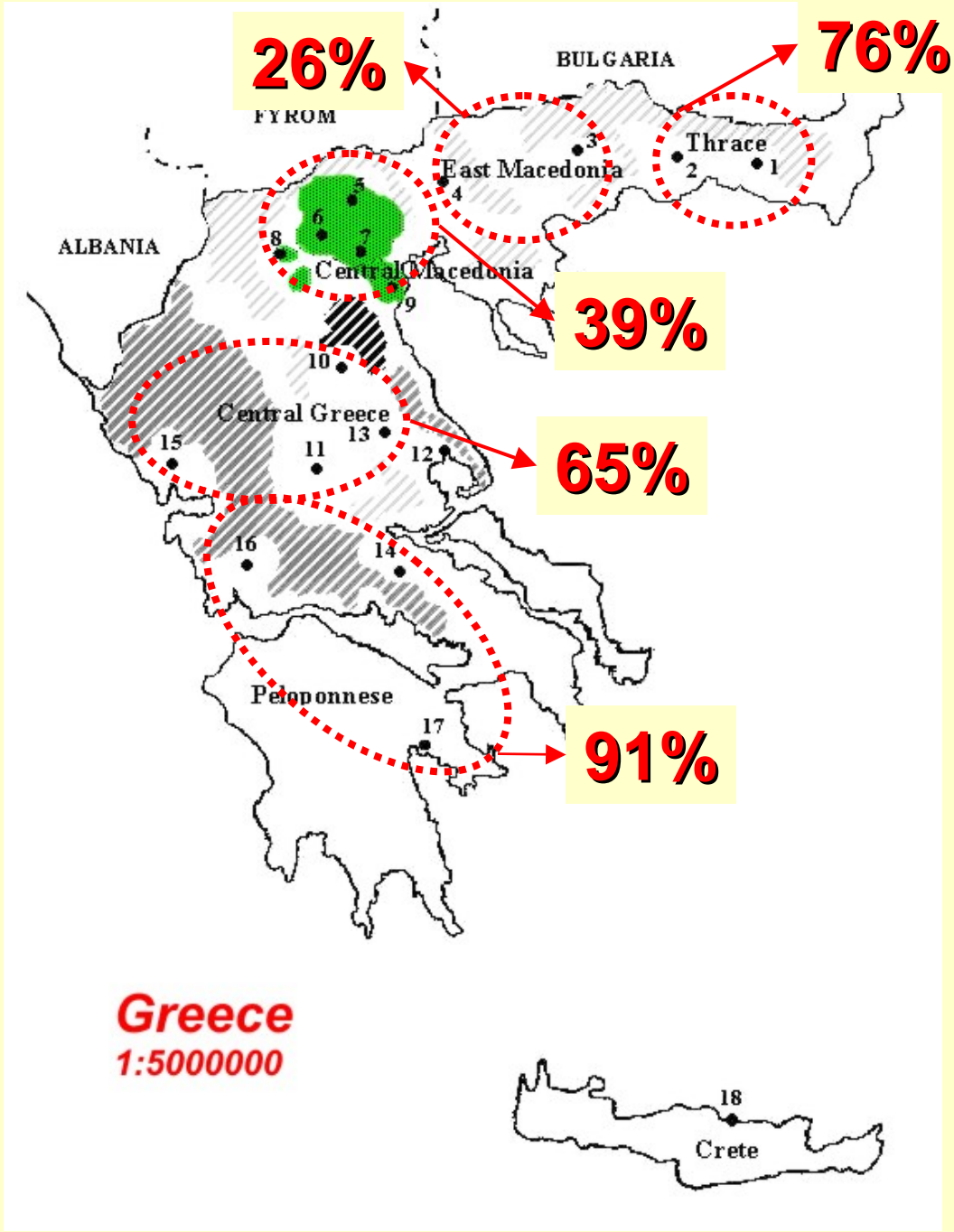
Non-holocyclic clones examined	Intermediate clones (%)
1457	**6.9%**
Non-holocyclic clones examined	Androcyclic clones
149	**57.0%**

Colour morphs

"Red vs. Green"

Red clones found on different hosts in Greece



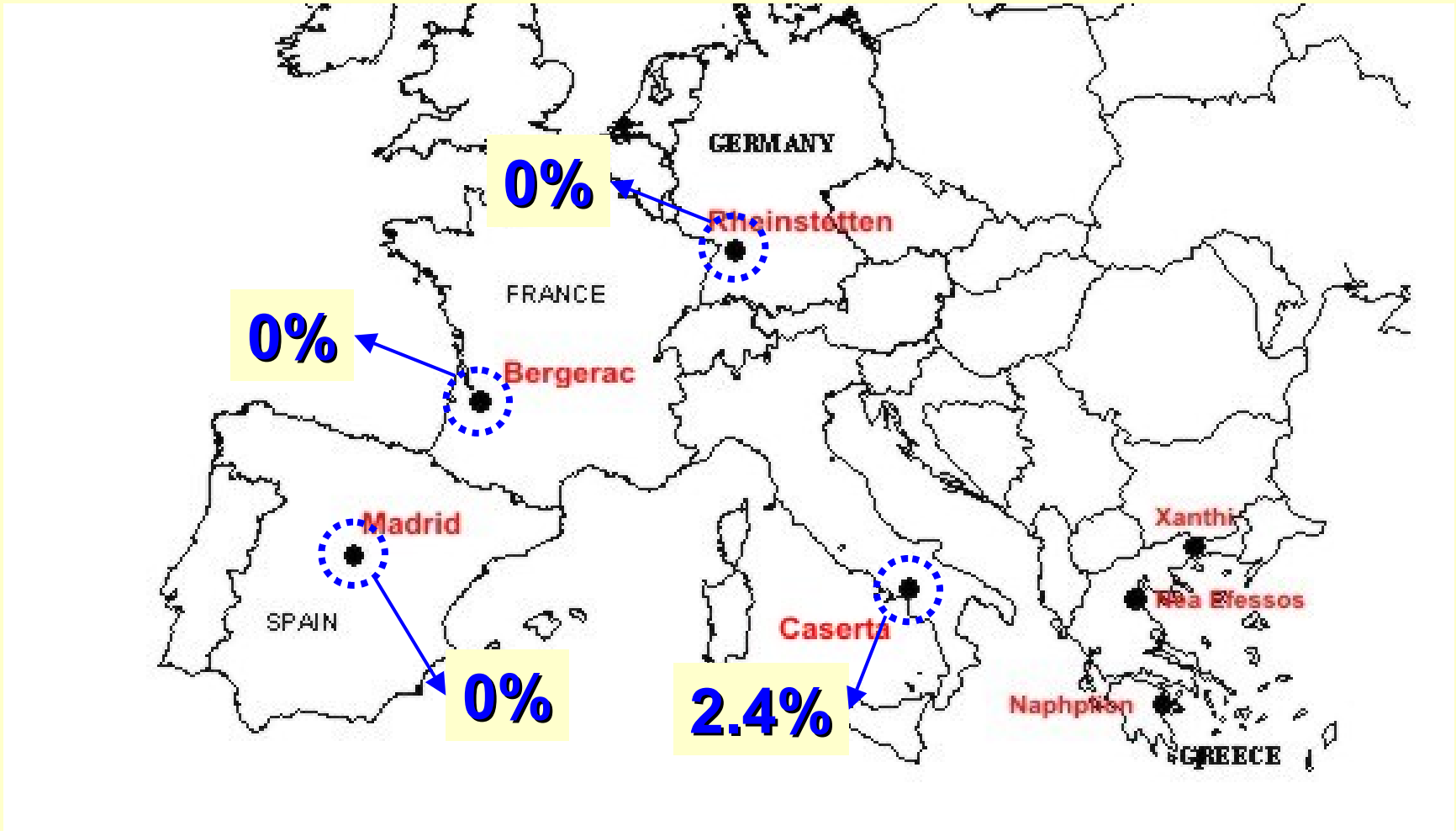


Red clones (%) on tobacco during 1995-99

Samples from different parts of Europe

"Anholocycle in colder regions?"

Holocyclic clones (%) collected on tobacco from different regions in Europe



- ✓ In Greece, four life cycles categories
  - Relative advantage in terms of adaptation, survival and production of novel genotypes.

- ✓ Correlation between **abundance of peach trees** and frequency of **holocyclic genotypes** on secondary hosts.

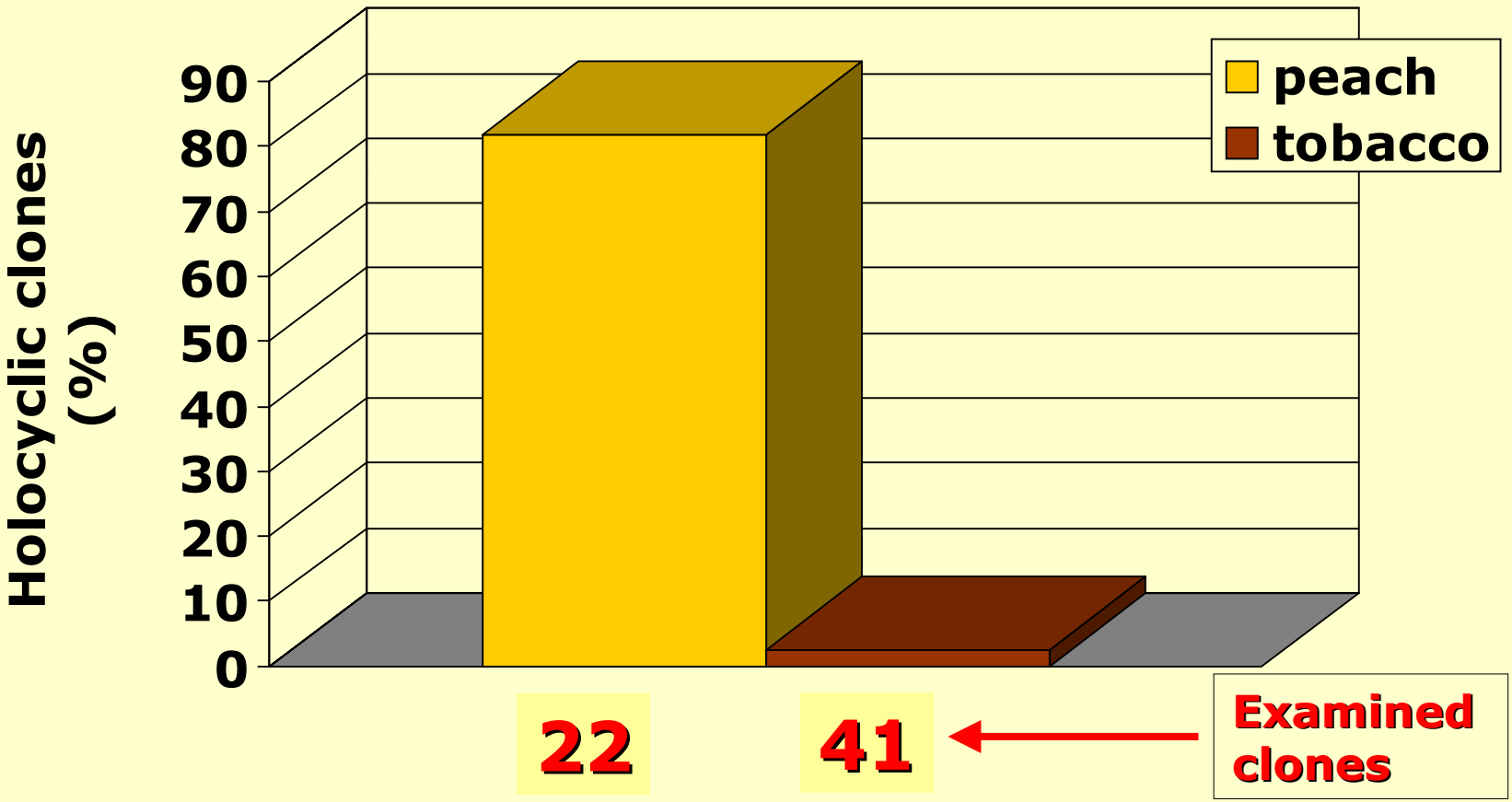
- ✓ High frequency of androcyclic genotypes.
  - Denotes **link** between populations investing in different reproductive strategies.

- ✓ Geographical variation in colour morphs parallel to that of life cycle.
- ✓ High risk of virus infections in seedbeds due to the colonization by aphids from weeds.
- ✓ Anholocycle overwintering strategy in colder regions.

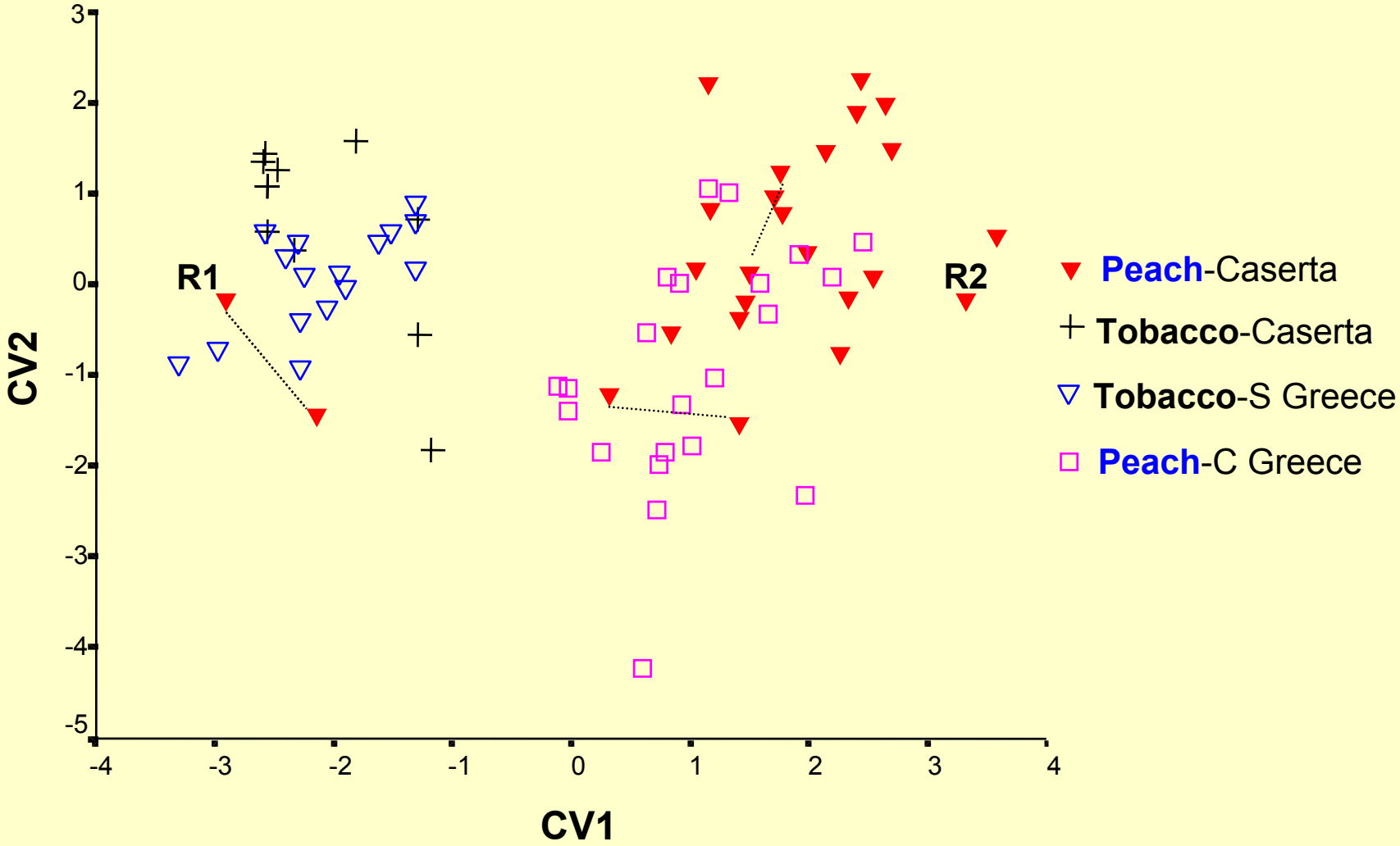
Populations in Southern Italy

"A different story"

Holocyclic clones (%) collected on tobacco and peach in Caserta, southern Italy



Plot of the scores of the first two CVs for clones of *M. persicae* collected from tobacco and peach in southern Italy and Greece



- ✓ ***Coexistence*** of the two groups in the same area
  - Aphids collected on peach have different morphology from those on tobacco.
  - Tobacco aphids have not the ability of sexual reproduction.

- ✓ **Other factors** than the presence of peach trees have also an influence on the relative frequency of holocyclic genotypes.
  - **Strong founder effect**?

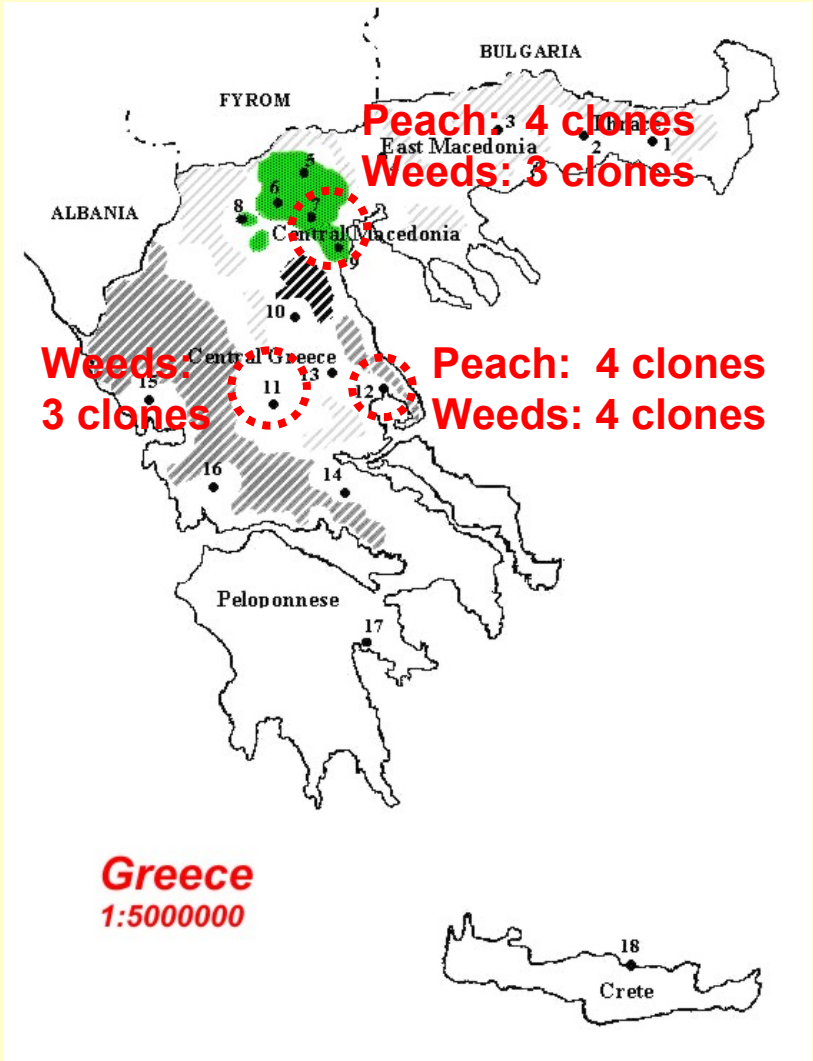
- ✓ Could the present situation change?

- ✓ Difference in ecology and population structure of *M. persicae* in neighbouring counties of the Mediterranean area with similar climate and cultivated crops.

Host specialisation

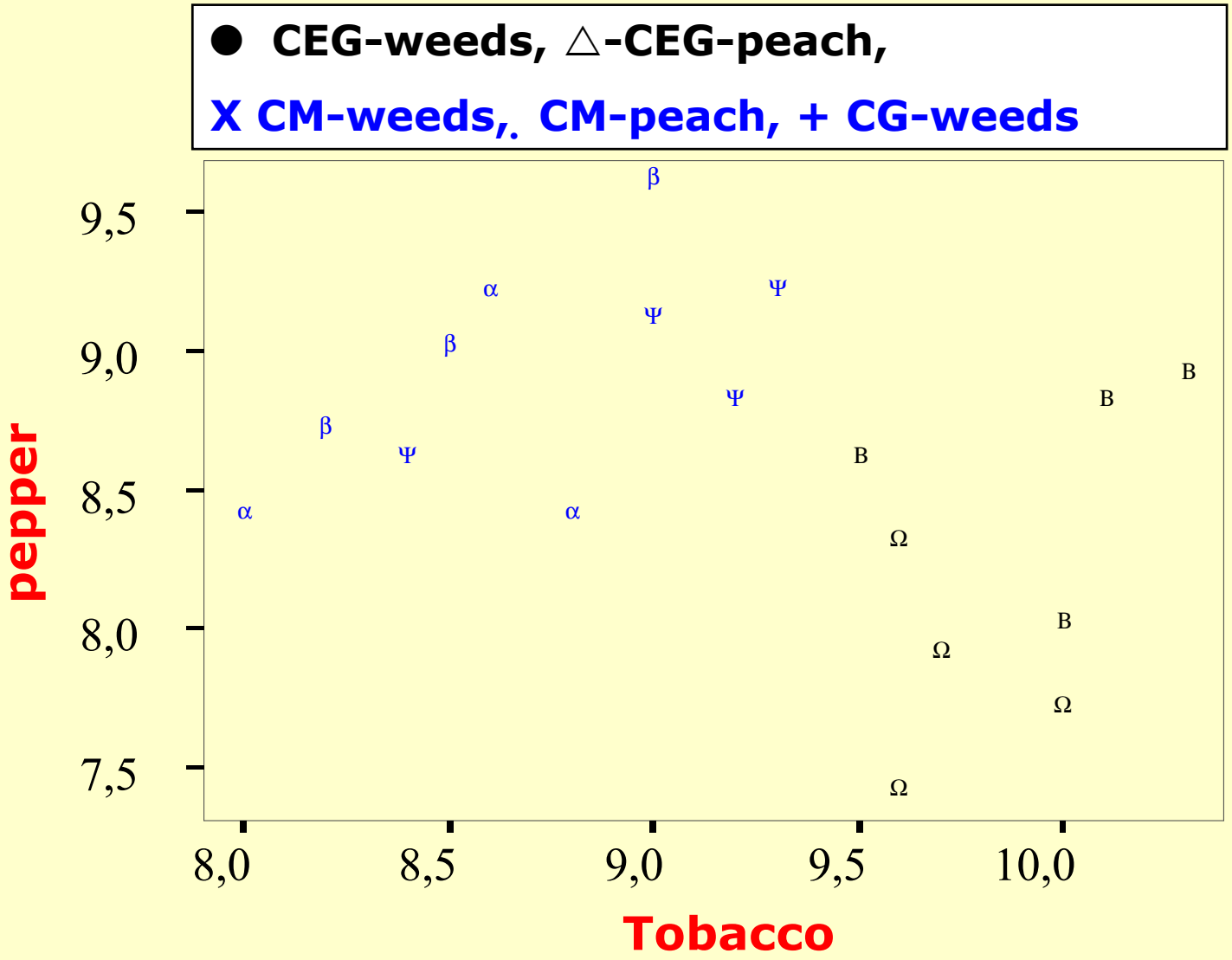
"Performance or preference?"

Performance and choice trials



- ***Performance***
  - **18 clones**
  - **12 replications per clone**
  - **Tobacco, pepper**
- ***Choice test***
  - **18 clones**
  - **200 individuals per clone**
  - **Tobacco, pepper**

Developmental time (days) of 18 *M. persicae* clones reared for two generations on tobacco and pepper

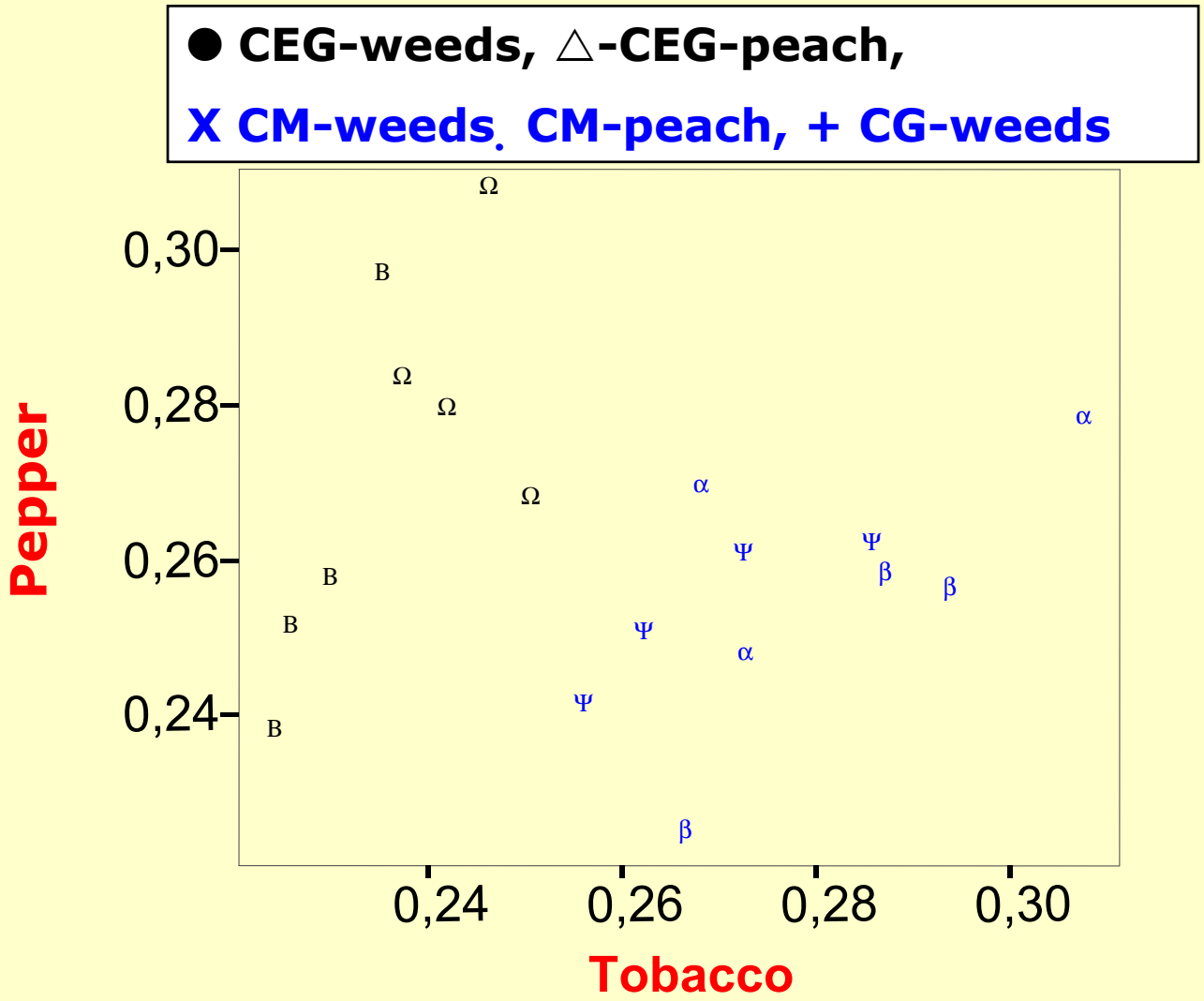


Developmental time (days) of 18 *M. persicae* clones (pooled data) reared for two generations on tobacco and pepper

Region/host origin	N	Developmental time	
		tobacco	pepper
CEG/weeds	4	9,7A	7,8A
CEG/peach	4	10,0B	8,6B
CM/weeds	3	8,6C	9,1C
CM/peach	4	9,0D	8,9C
CG/weeds	3	8,5C	8,7BD

N=number of clones

Intrinsic rate of increase of 18 *M. persicae* clones reared for two generations on tobacco and pepper

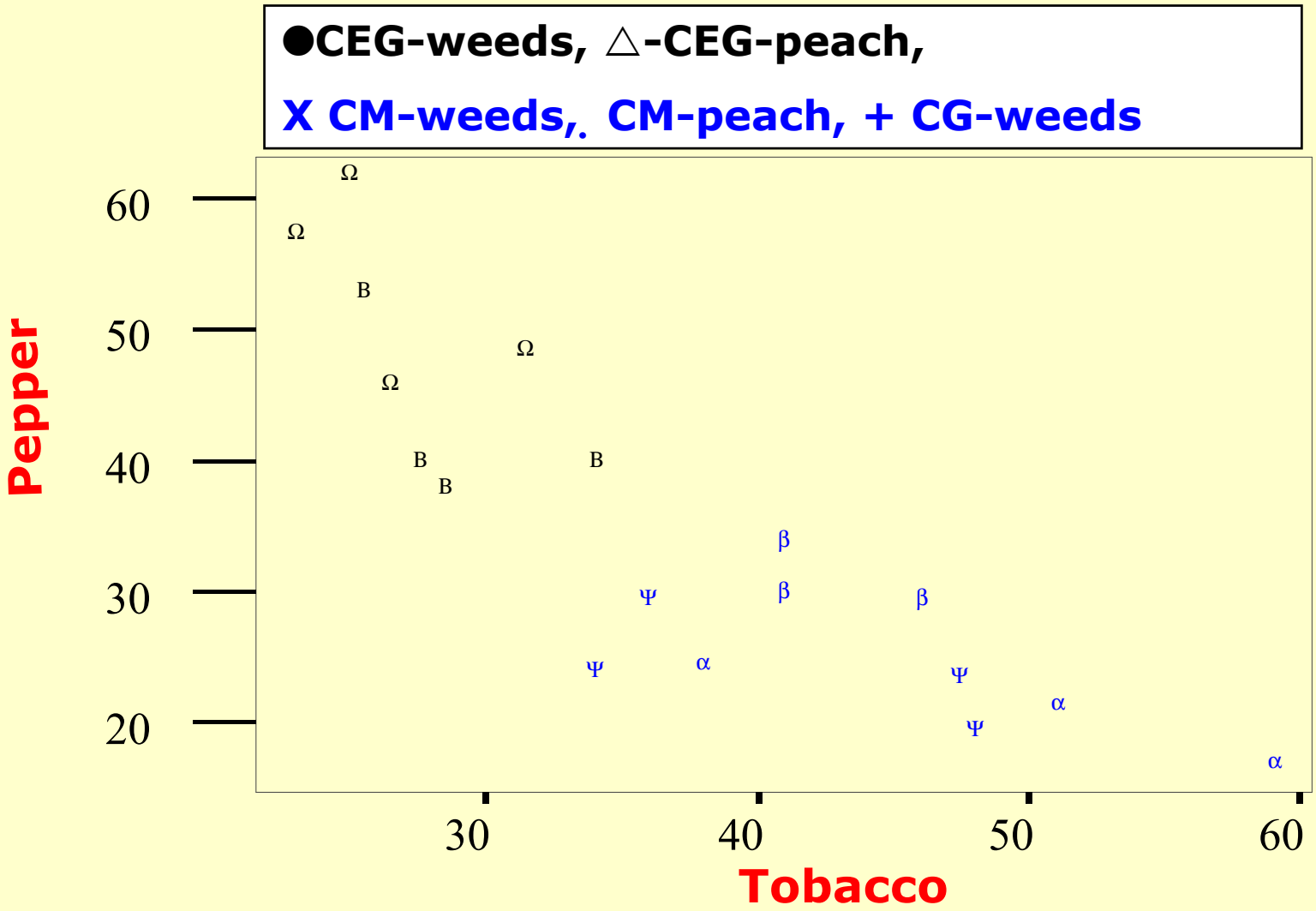


Intrinsic rate of increase of 18 *M. persicae* clones (pooled data) reared for two generations on tobacco and pepper

Region/host origin	N	Intrinsic rate of increase	
		tobacco	pepper
CEG/weeds	4	0,2439A	0,2838A
CEG/peach	4	0,2286B	0,2600BC
CM/weeds	3	0,2828C	0,2463D
CM/peach	4	0,2689D	0,2528CD
CG/weeds	3	0,2834C	0,2644B

N=number of clones

Host preference (%) of 18 *M. persicae* clones between tobacco and pepper



Host preference (%) of 18 *M. persicae* clones between tobacco and pepper (pooled data)

Region/host origin	Percentage of aphids preferred	
	tobacco	pepper
CEG/weeds	26,5A	53,0A
CEG/peach	29,0A	42,5B
CM/weeds	42,7B	30,5C
CM/peach	41,5B	23,5CD
CG/weeds	49,5B	20,5D

- Aphids from **tobacco-growing regions** (CM and CG) **performed better on tobacco** than those collected in an area where tobacco is not cultivated (CEG). The opposite was observed on pepper.

- However, differences were not too strong.

- Probably, host specialisation results from **differences in host-plant preference**.
  - A higher proportion of apterae from CM and CG choose tobacco from pepper leaf-rings.

- The host-plant selection behaviour by alatae is under examination.

Conclusions

- **Host specialisation** is an important feature of the ecology of *M. persicae.*
- Both multivariate morphometrics and RAPD analyses revealed the differentiation of tobacco-feeding populations from those on other secondary hosts.
- Host specialisation may result from differences in host-plant preference.
- Considerable life cycle variation allows the species to produce new genotypes and to counteract different environmental conditions.

Conclusions

- In Greece, the presence of peach orchards is a important factor affecting the distribution and the genetic structure of the species populations.

- However, other factors (e.g. founder effect) may have evolved in southern Italy.

- Overwintering strategy and virus epidemiology.

Acknowledgments

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