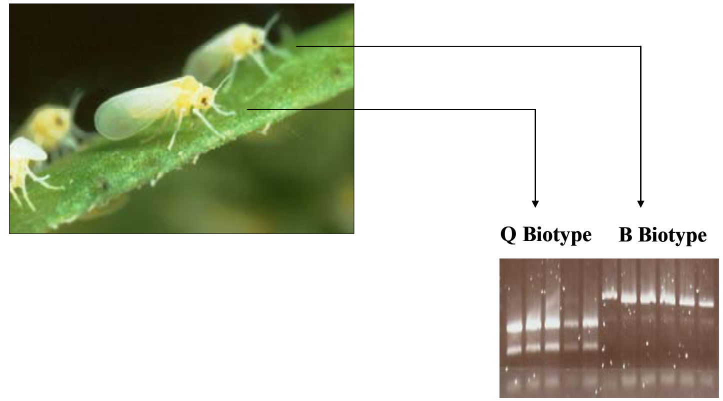
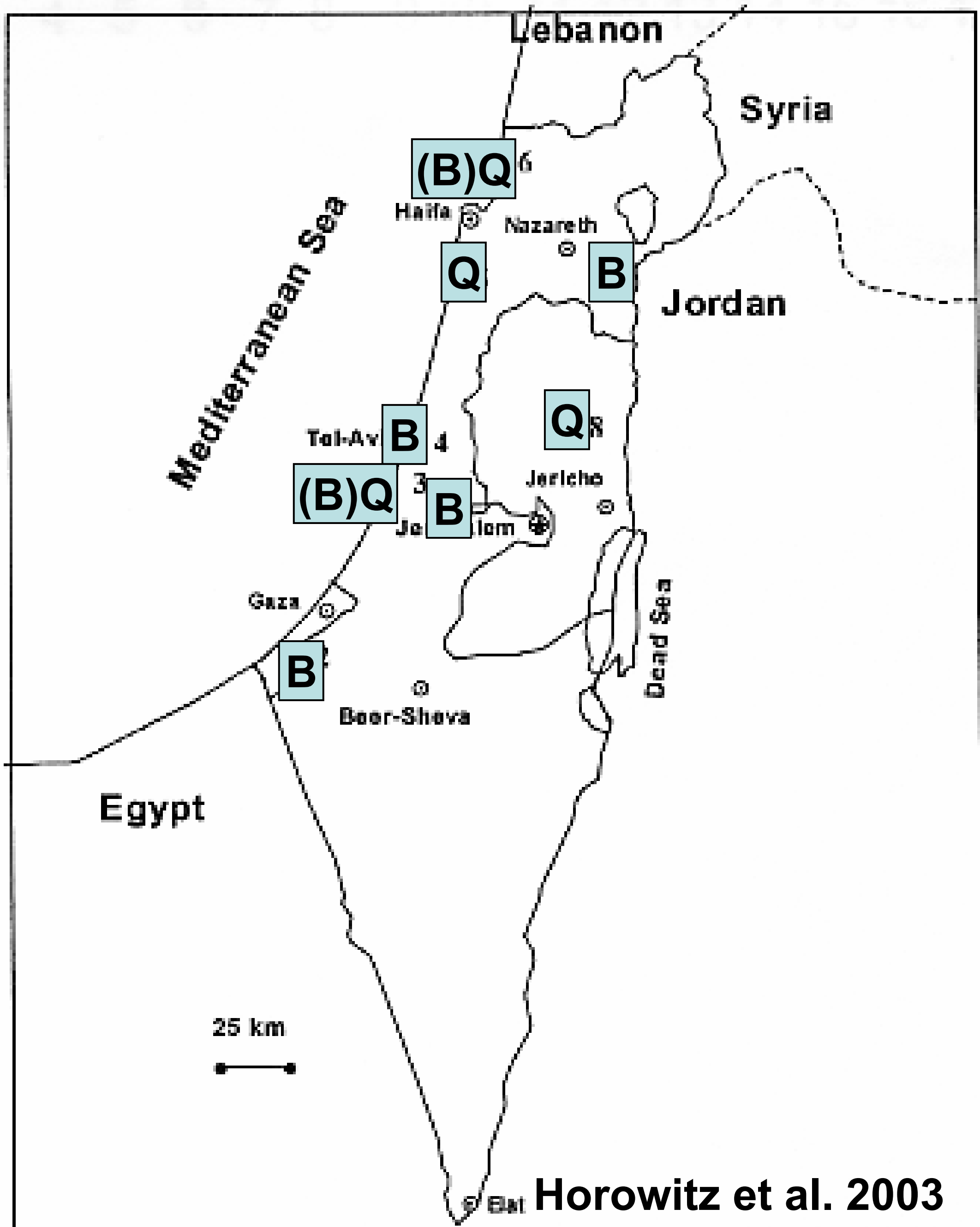
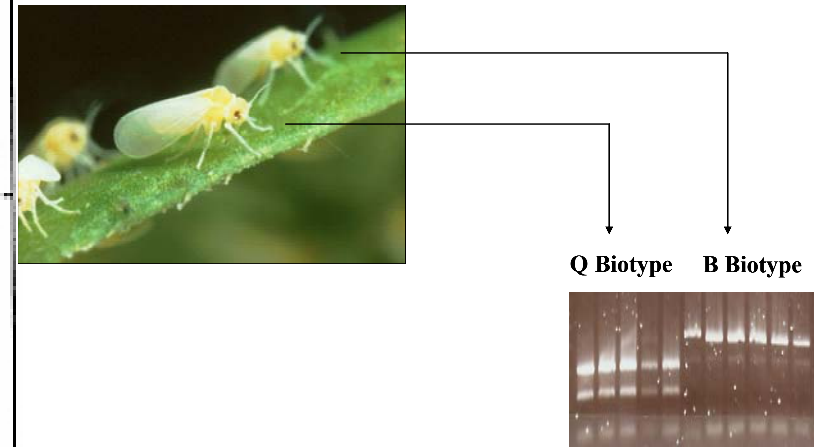


Evolution of insecticide resistance in the B and Q sympatric biotypes of *Bemisia tabaci* - single or multuple origins?

Shai Morin, Department of Entomology
Faculty of Agriculture, The Hebrew University of Jerusalem





Horowitz et al. 2003

Are the B and Q biotypes of *Bemisia tabaci* genetically isolated?

Strains used in the study

Strain	Biotype	Year	Country	Host
B ref	B	1987	Israel	Cotton
Ashalim	B	2003	Israel	Melons
PyriS	Q	1991	Israel	Roses
Ayalon	Q	2002	Israel	Cotton

Strains used in the study

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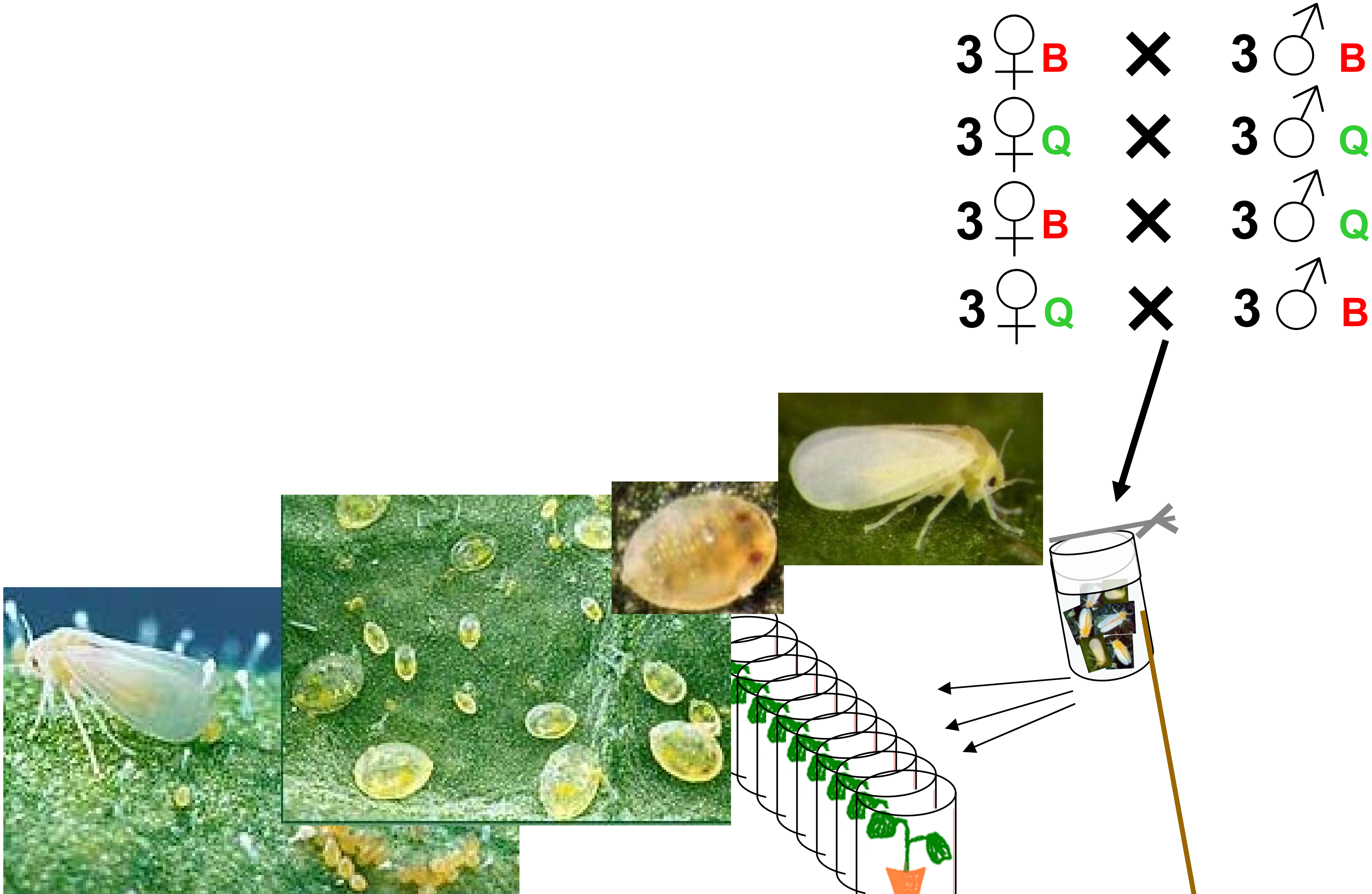
Ayalon	Q	2002	Israel	Cotton

Cross design for inter and intra biotype mating

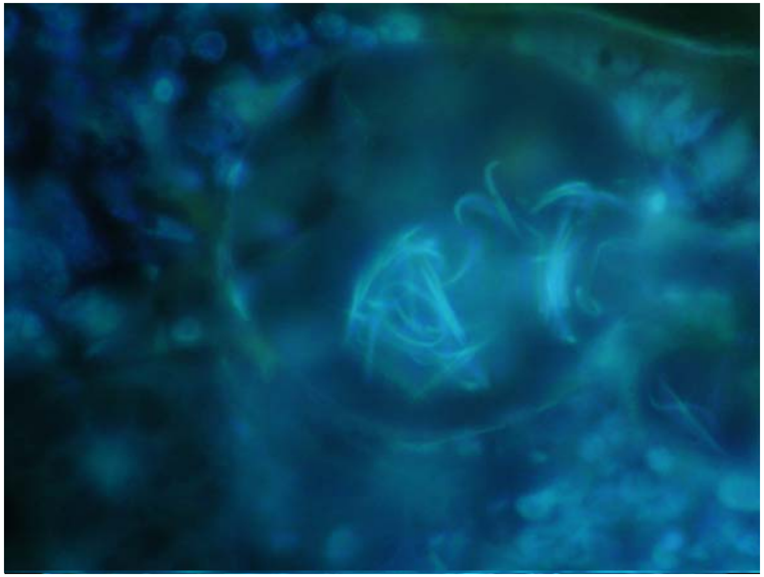


1. While males and females progeny are produced within each biotype, no female progeny are produced by crosses between biotypes

B Ashalim X Q Ayalon

	♂B	♂Q
♀Q	Total ♀=0 Total ♂= 78	Total ♀=46 Total ♂=90
♀B	Total ♀=26 Total ♂= 35	Total ♀=0 Total ♂= 78

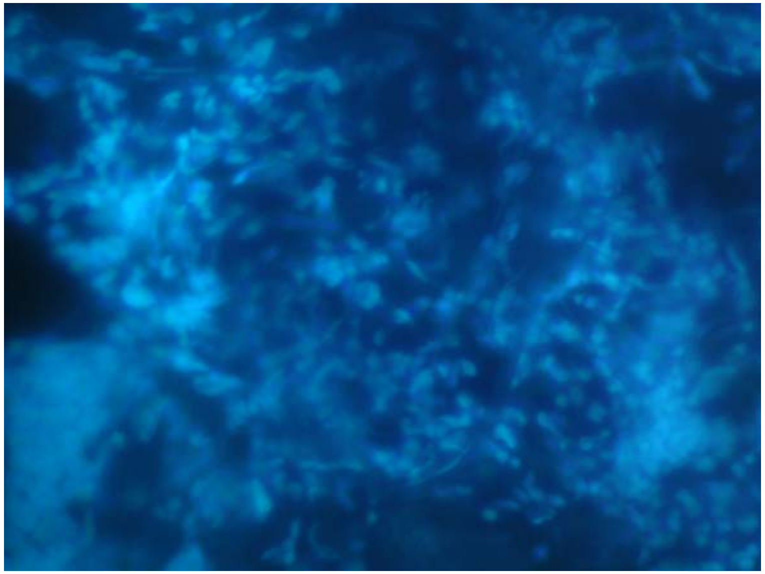
2. No sperm is transferred between biotypes – Pre-zygotic barrier



BXB

QXQ

BXQ or Virgin Females



3. Disturbed courtship and mating behavior between the B and Q biotypes

5. Wing movement

6. Copulatory organ contact.

1. Female contact and male orientation.

4. Body pushing

3. Abdominal movement

2. Antennal drumming.

4. The possible involvement of symbionts is unlikely

Strain/symbionts	*Wolbachia* sp.	*Rickettsia* sp.	*Hamiltonella defensa*
B Ashalim	---	-- / +	+++
B ref	---	+++	+++
Q Ayalon	---	-- / +	---
Q PyriS	---	+++	---

5. Unfertile female progeny are produced by crosses between biotypes collected ~20 years ago

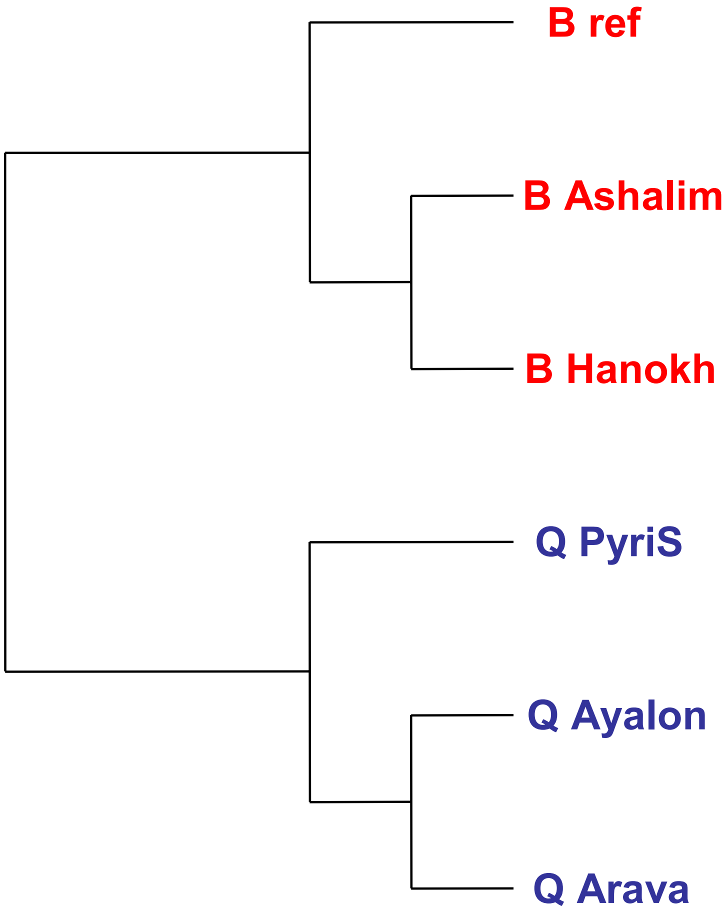
B ref X Q PyriS

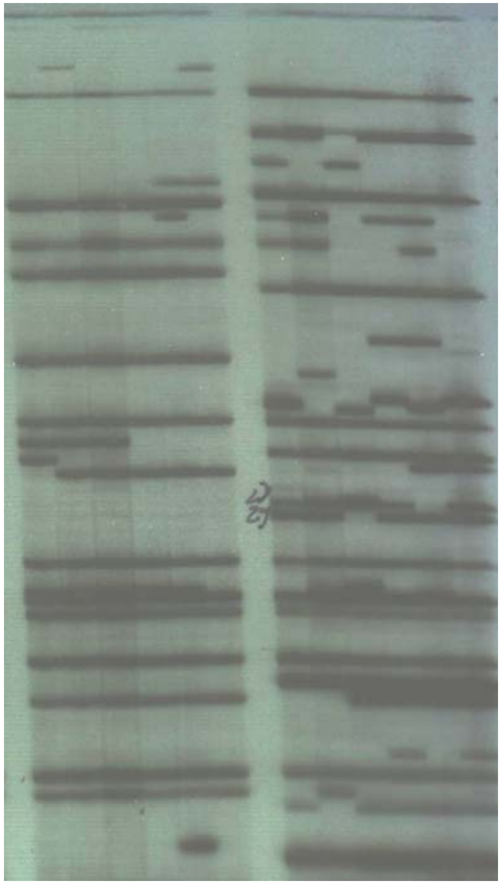
	♂B	♂Q
♀Q	Total ♀=0 Total ♂= 136	Total ♀=29 Total ♂=18
♀B	Total ♀=77 Total ♂=102	Total ♀=**2BQ** Total ♂= 72

6. AFLP analysis of molecular variance between populations of the B and Q biotypes shows significant reduction in gene flow

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Between biotypes	1	535.333	33.87111 Va	67.84
Among populations within biotypes	4	109.067	2.80333 Vb	5.62
Within populations	24	318.000	13.25000 Vc	26.54
Total	29	962.400	49.92444	

	1	2	3	4	5	6
1 BAshalim	-	0.07653	0.13265	0.48980	0.53571	0.52551
2 BHanokh	15	-	0.09694	0.48469	0.52041	0.51020
3 Bref	26	19	-	0.46939	0.48469	0.48469
4 QPyriS	96	95	92	-	0.13776	0.15816
5 QAyalon	105	102	95	27	-	0.16327
6 QArava	103	100	95	31	32	-





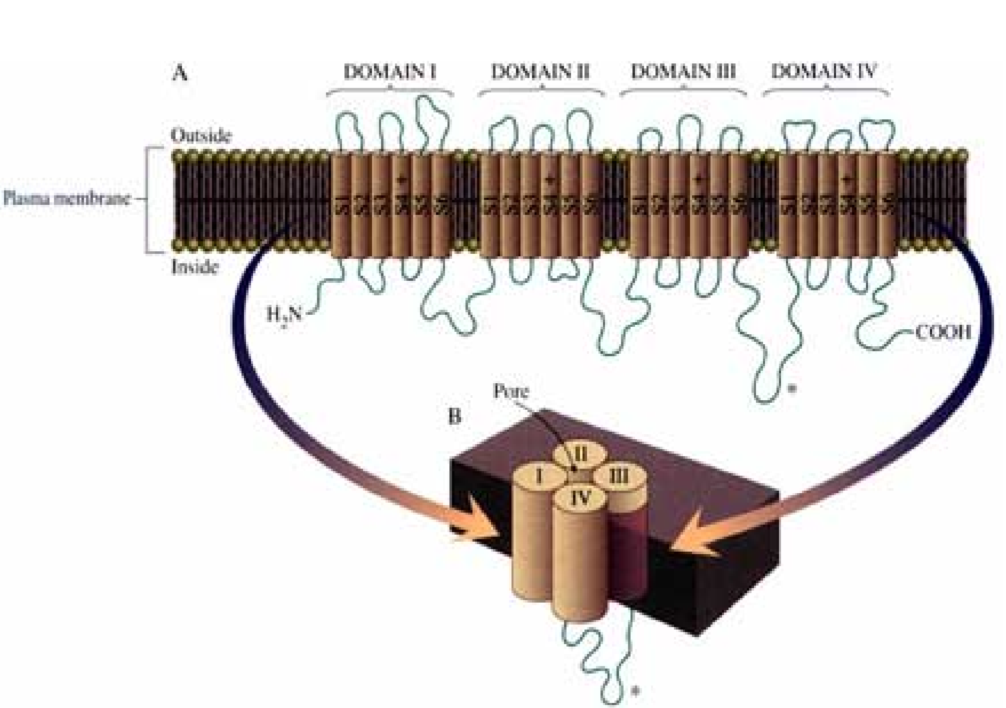
Scientific Questions

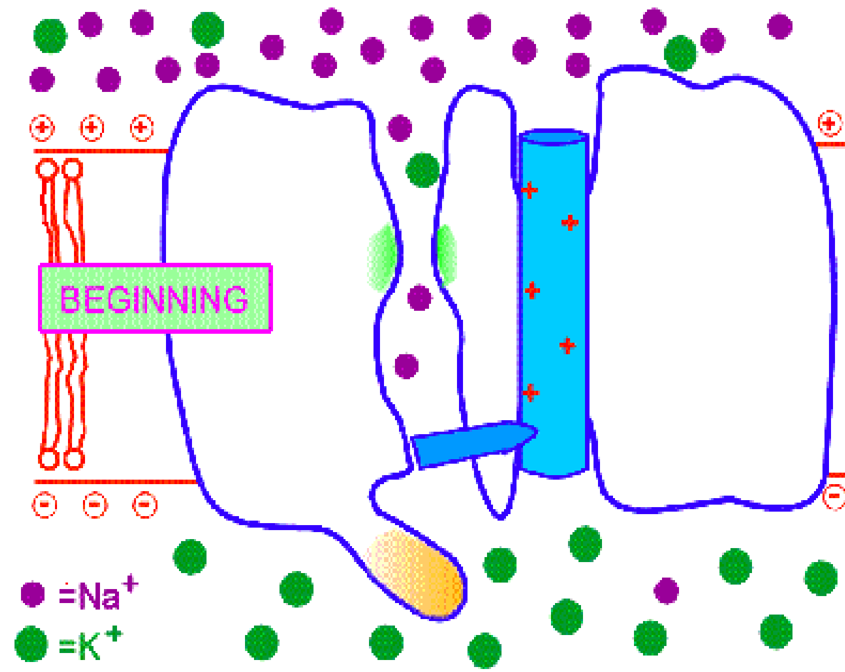
- Did similar insecticide selection in the B and Q biotypes result in similar genetic changes

- Did the genetic changes arise once in the common ancestor
- Arise once in one biotype and was subsequently transferred to the other biotype
- Arise independently in each biotype (parallel evolution)

Multiple Origins of Pyrethroid Resistance in Sympatric Biotypes of *Bemisia tabaci*

The effect of pyrethroids on the *para*- type voltage gated sodium channel





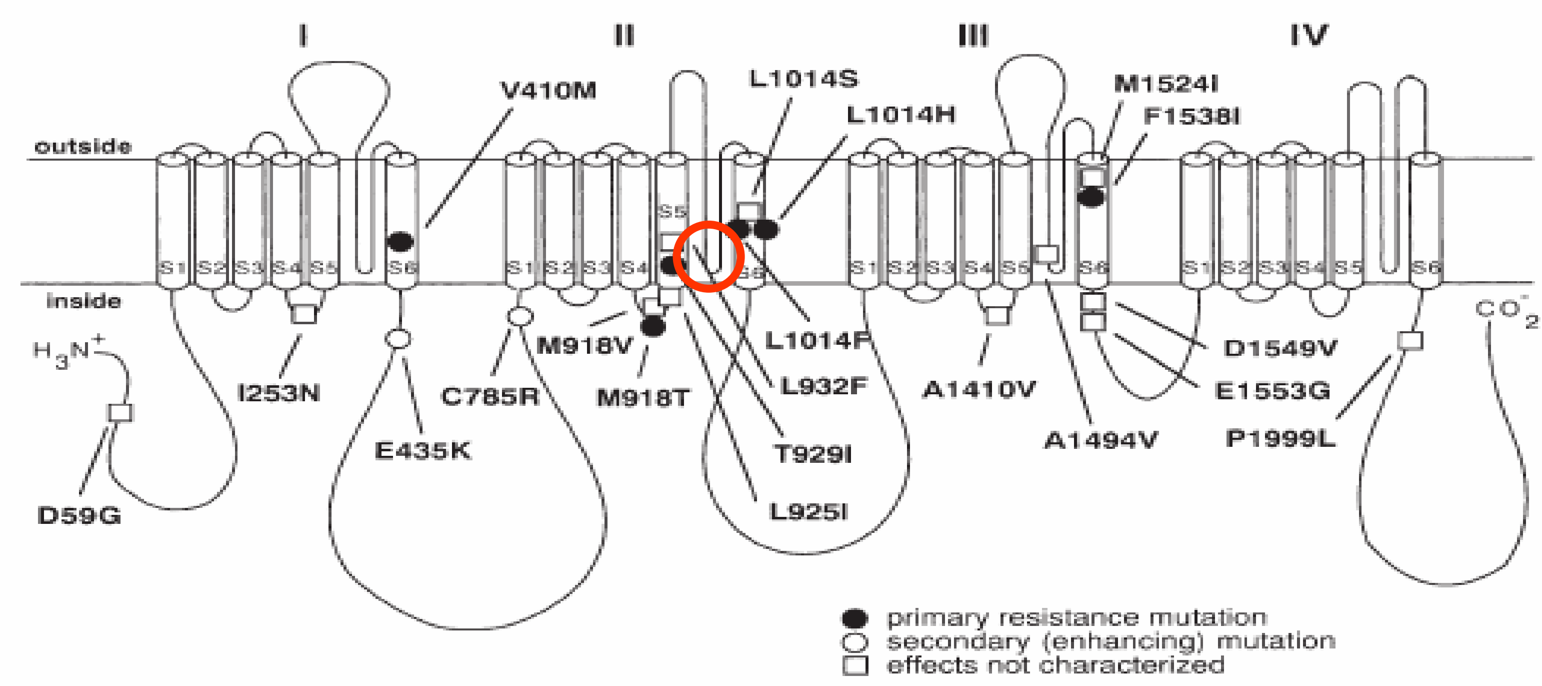
Control

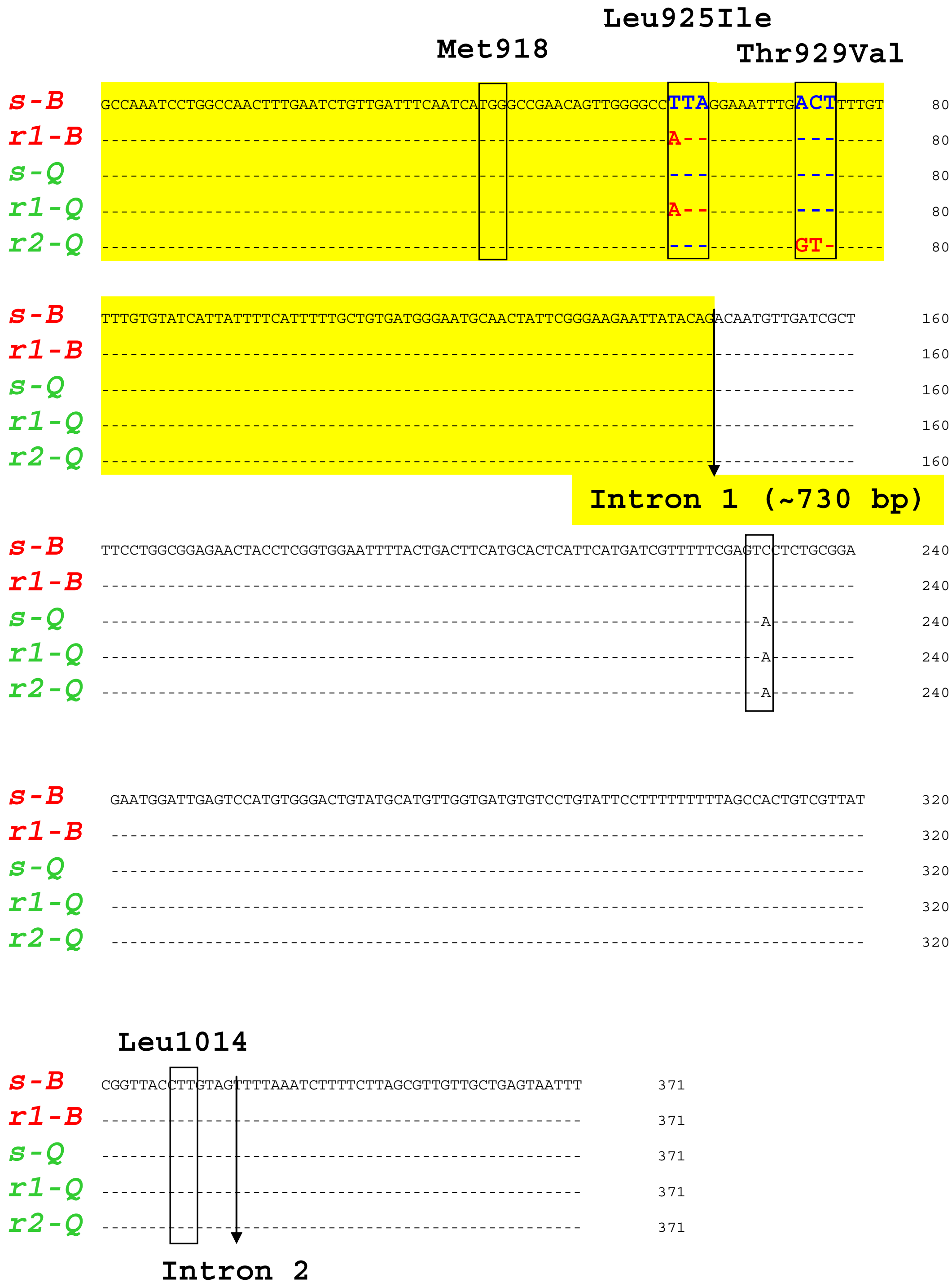
After pyrethroid treatment

Resistance to pyrethroids caused by mutations in the *para*- type voltage gated sodium channel

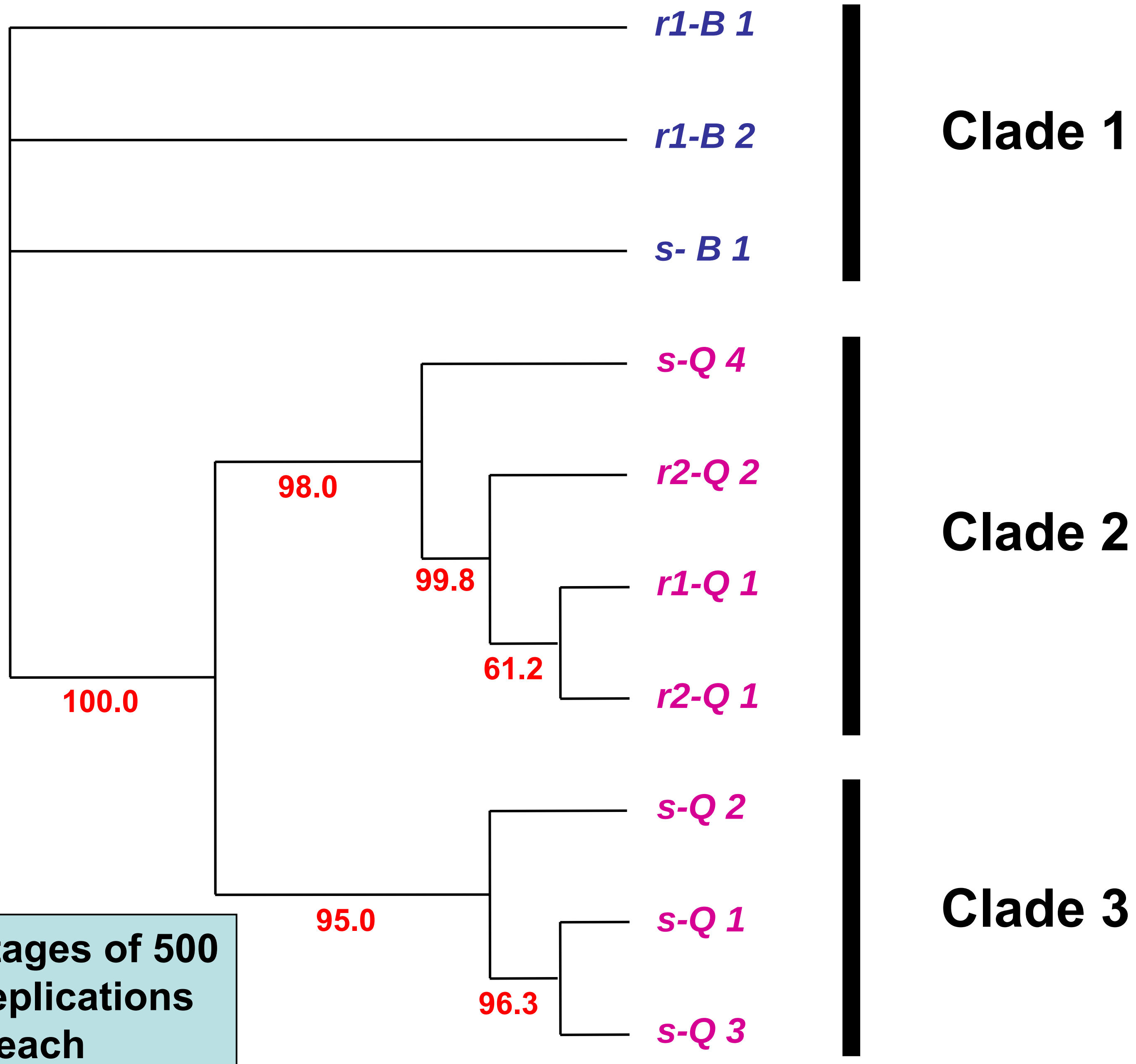
Resistance mechanism: lower sensitivity of the target site =

Knockdown Resistance - kdr





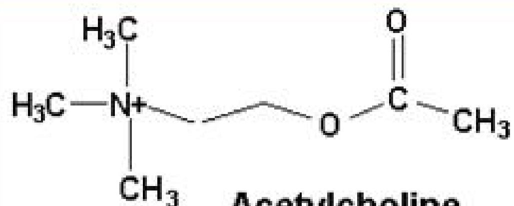
Leu925Ile
Met918
Thr929Val
s-B
r1-B
s-Q
r1-Q
r2-Q
GCCAAATCCTGGCCAACTTTGAATCTGTTGATTTCAATCATGGGCCGAACAGTTGGGGCCTTAGGAAATTTGACTTTTGT 80
A--
GT-
TTTGTGTATCATTATTTTCATTTTTGCTGTGATGGGAATGCAACTATTCGGGAAGAATTATACAGACAATGTTGATCGCT 160
Intron 1 (~730 bp)
TTCCTGGCGGAGAACTACCTCGGTGGAATTTTACTGACTTCATGCACTCATTCATGATCGTTTTTCGAGTCCTCTGCGGA 240
A
GAATGGATTGAGTCCATGTGGGACTGTATGCATGTTGGTGATGTGTCCTGTATTCCTTTTTTTTTAGCCACTGTCGTTAT 320
Leu1014
CGGTTACCTTGTAGTTTTAAATCTTTTCTTAGCGTTGTTGCTGAGTAATTT 371
Intron 2



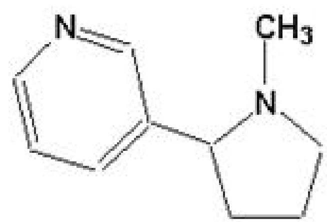
The percentages of 500 bootstrap replications supporting each branch are shown

A Possibility for a Single Origin of Cytochrome P450 Dependent Neonicotinoids Resistance in the B and Q biotypes of *Bemisia tabaci*

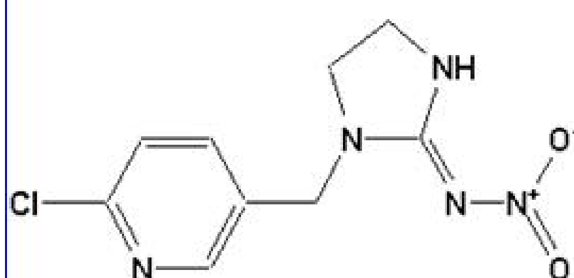
Neonicotinoids - Nicotinic Acetylcholine receptor agonists



Acetylcholine

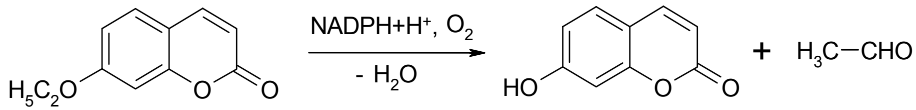


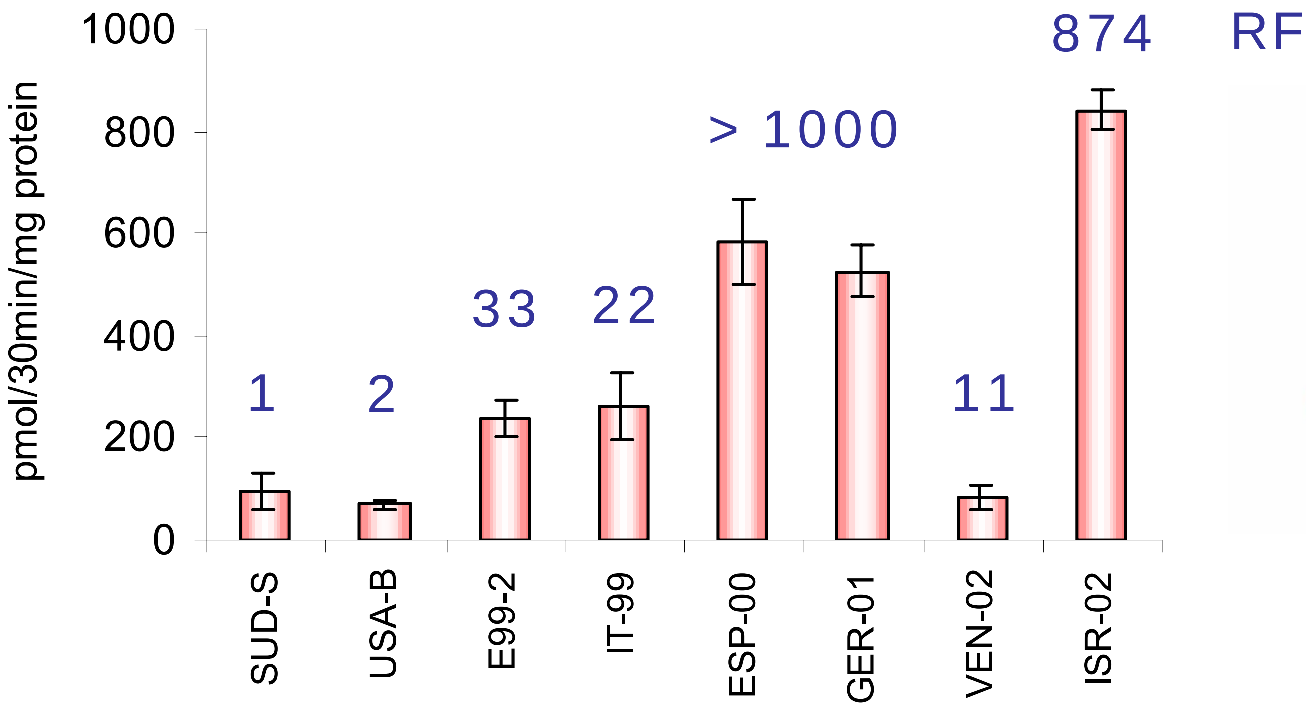
Nicotine (55 mg/kg)

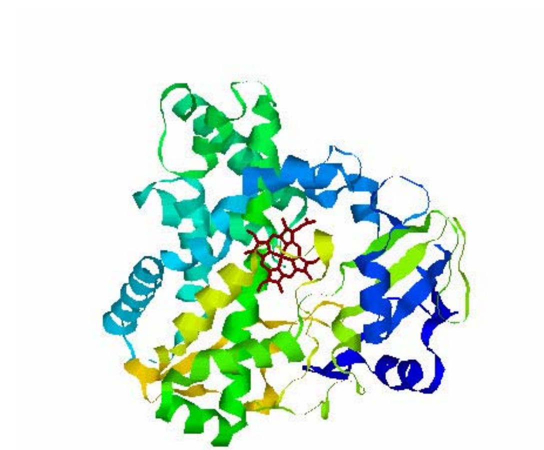


Imidacloprid (424-475 mg/kg)

P450s Activity Confer Neonicotinoid Resistance in *Bemisia tabaci*



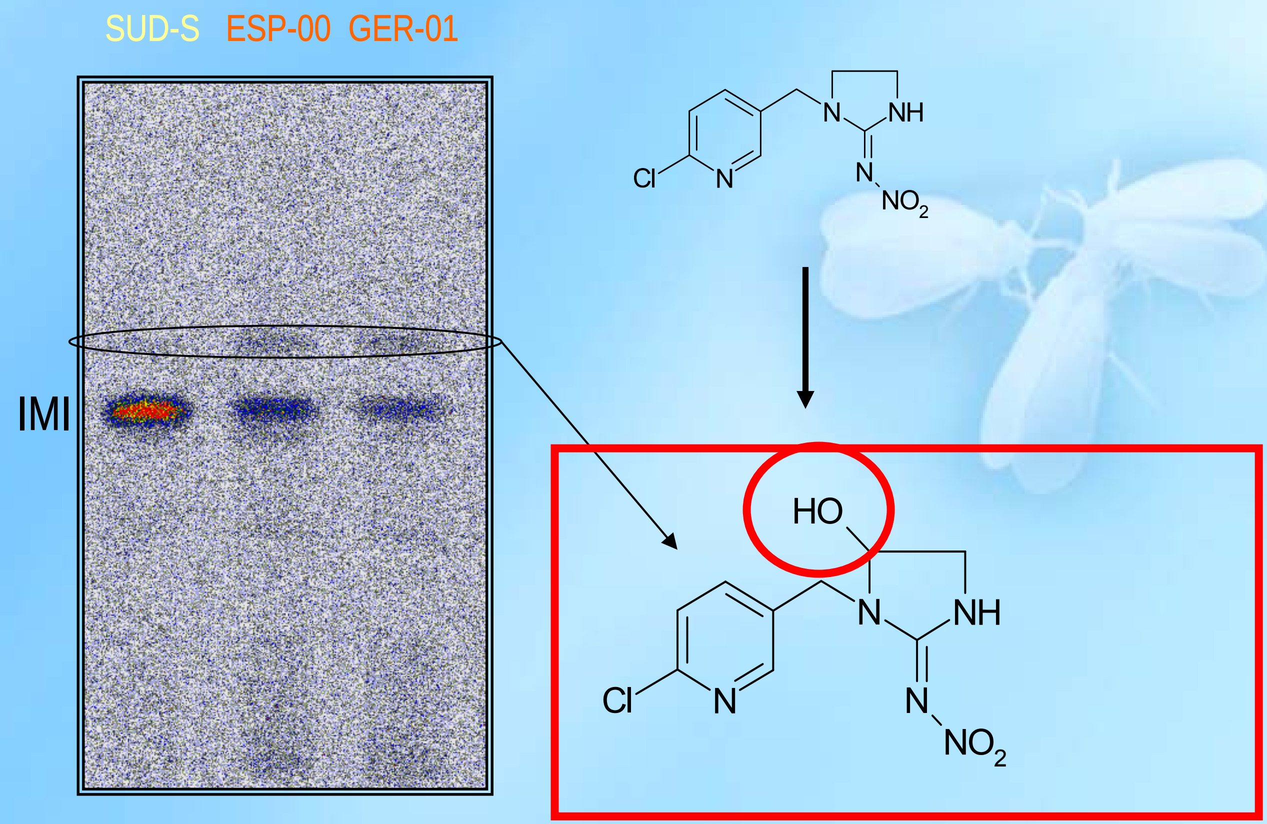




Rauch & Nauen (2003) Arch. Insect Biochem. Physiol. 54

7-Ethoxycoumarin-O-deethylase activity is a biochemical marker linked to neonicotinoid resistance in ***Bemisia tabaci***

P450s Can Metabolize Imidacloprid to Five-Hydroxy-Imidacloprid



Rauch & Nauen (2003) Arch Insect Biochem Physiol. 54

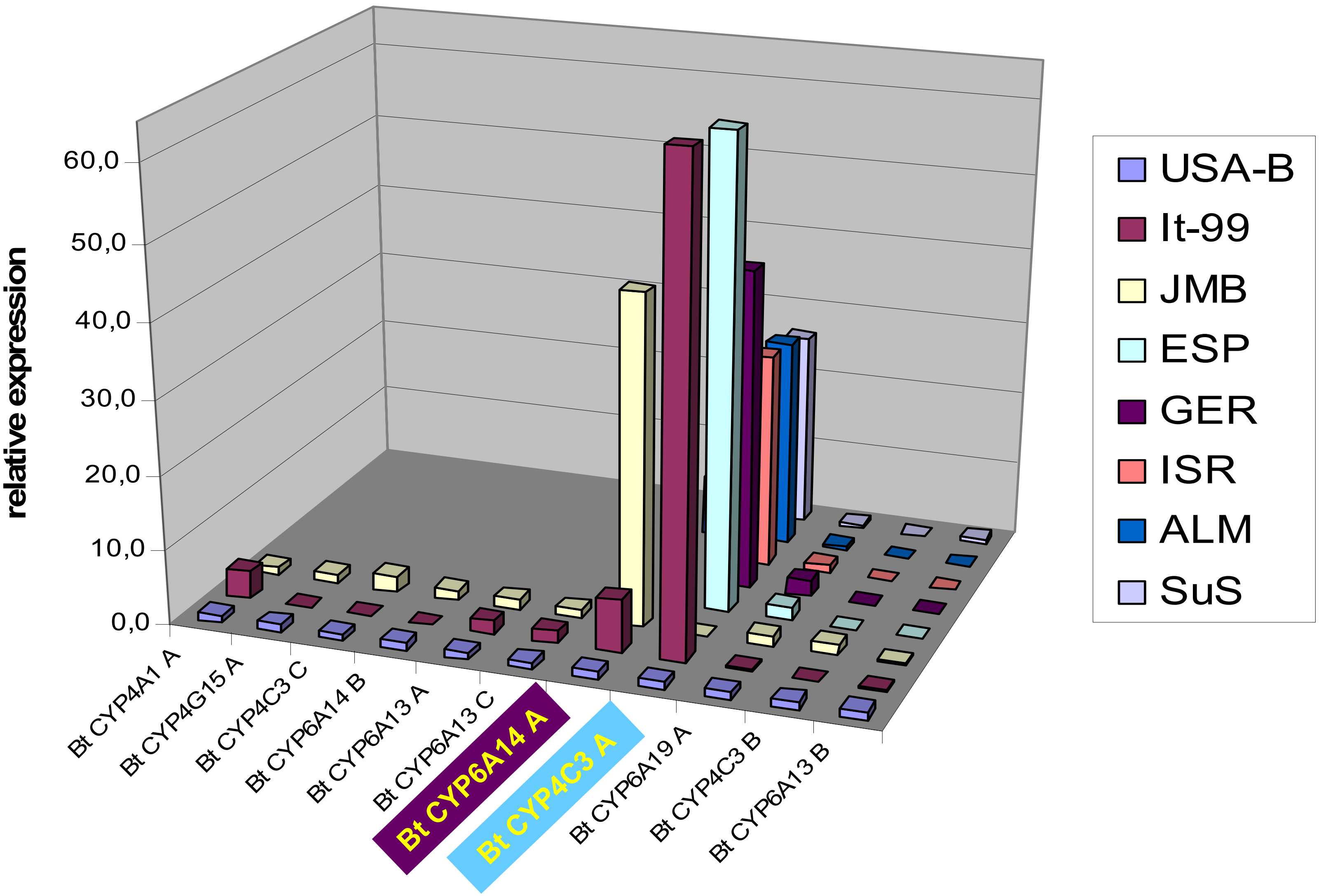
Neonicotinoids Resistance is Found in Both the B and Q Biotypes

Strain	Year of collection	Origin	Biotype	Neonicotinoid-resistance	Resistance factor *
SUD-S	1978	Sudan	-	No	Reference
E99-2	1999	Spain	Q	Moderately	33
IT-99	1999	Italy	Q	Moderately	22
ESP-00	2000	Spain	Q	High	>1000
GER1-01	2001	Germany	Q	High	>1000
USA-B	1994	California	B	No	2
VEN-02	2002	Venezuela	B	Moderately	11
JMB	2003	Brazil	B	High	874**

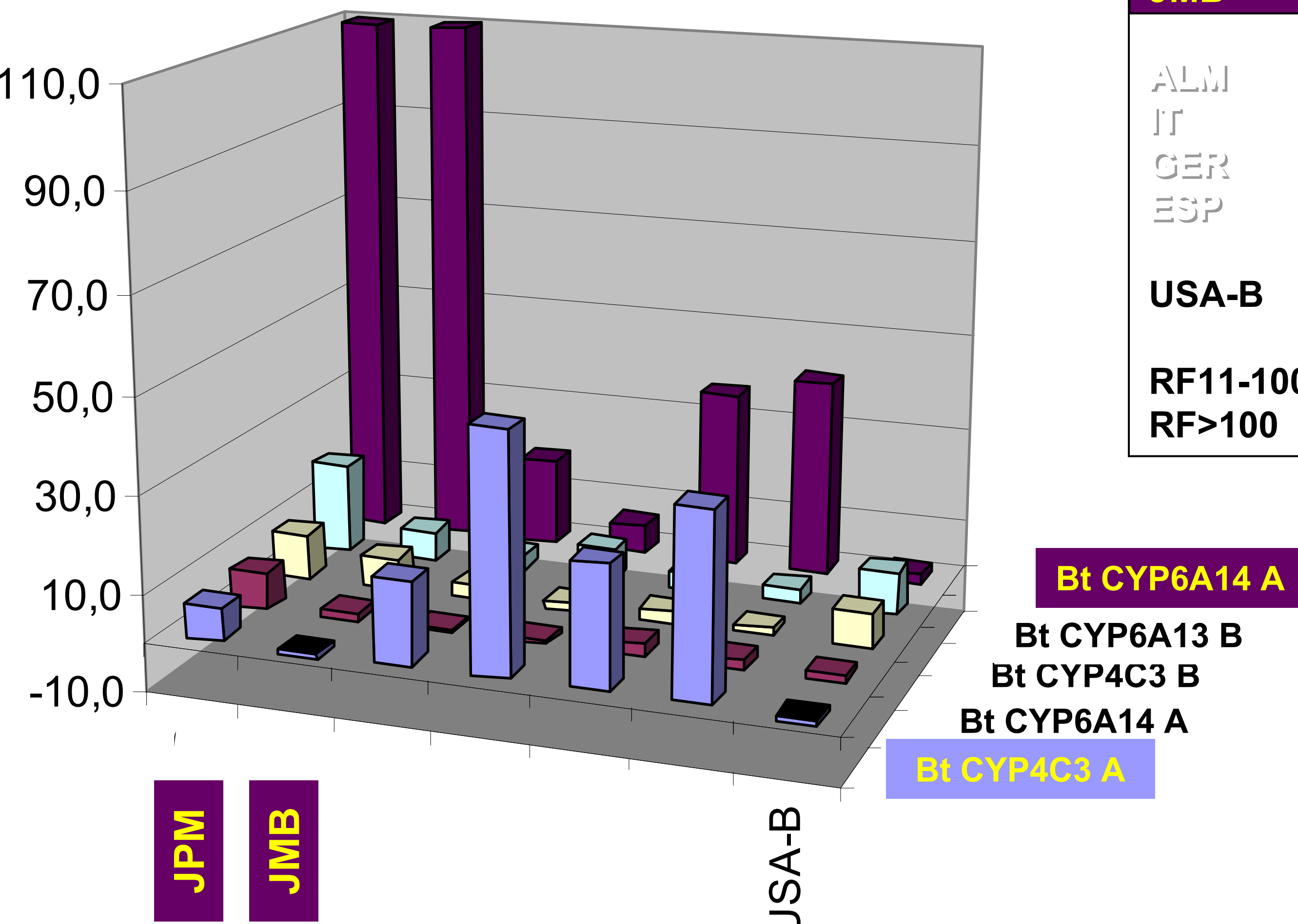
* Leaf-dip bioassay with imidacloprid (72h)

Rauch & Nauen (2003) Arch Insect Biochem Physiol. 54

Real-time PCR-analysis of the 11 p450 genes showed that two of them are over-expressed in resistant strains

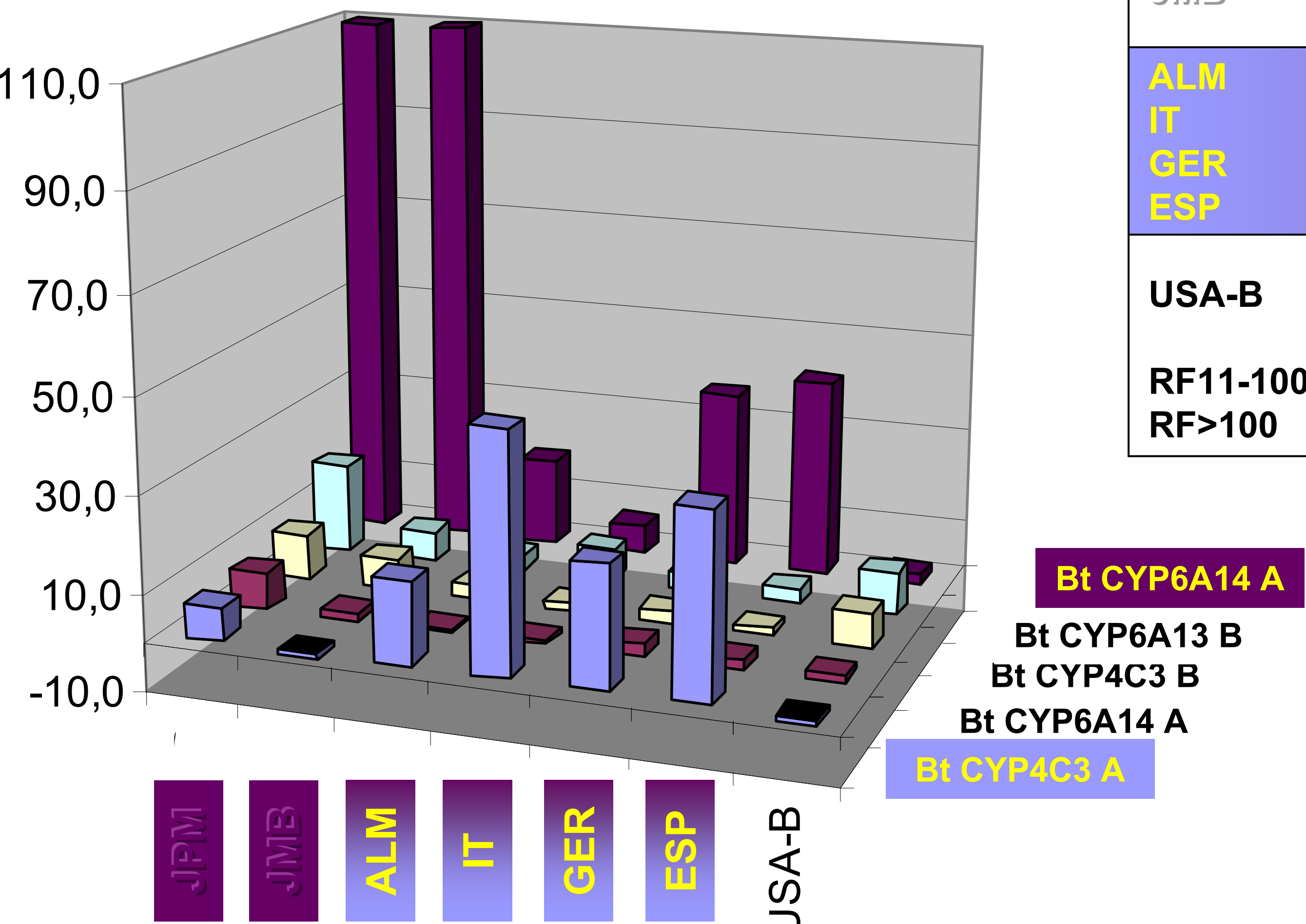


Only one gene is correlated with resistance in both the B and Q biotypes



Strain	RF*	Comments
JPB	+++	sys
JMB	+++	sys
ALM	++	spray
IT	++	spray
GER	+++	leaf dip (2001)
ESP	+++	spray
USA-B	No	leaf dip
RF11-100	++	
RF>100	+++	

Only one gene is correlated with resistance in both the B and Q biotypes



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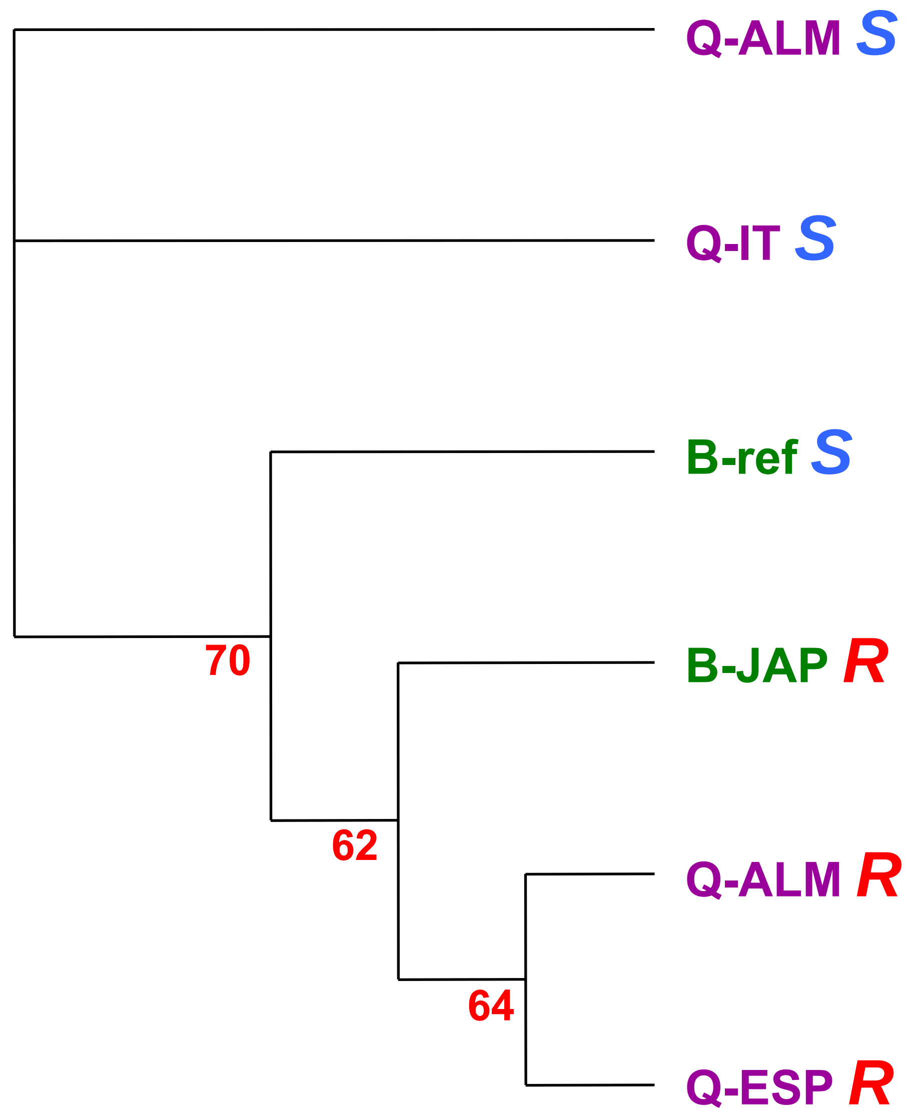
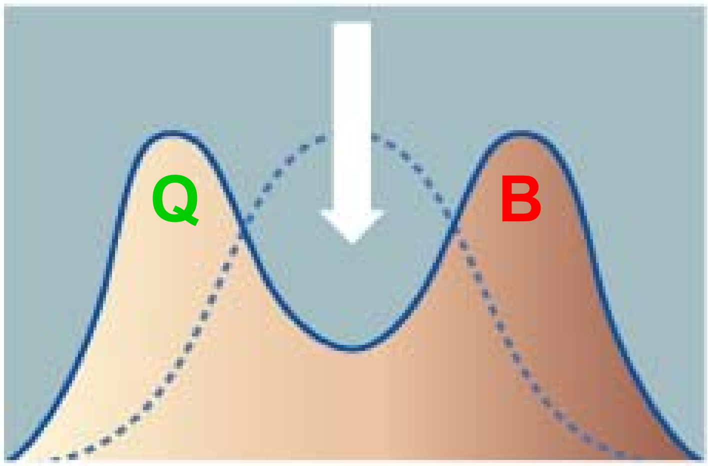
GER	+++	leaf dip (2001)
ESP	+++	spray
USA-B	No	leaf dip
RF11-100	++	
RF>100	+++	

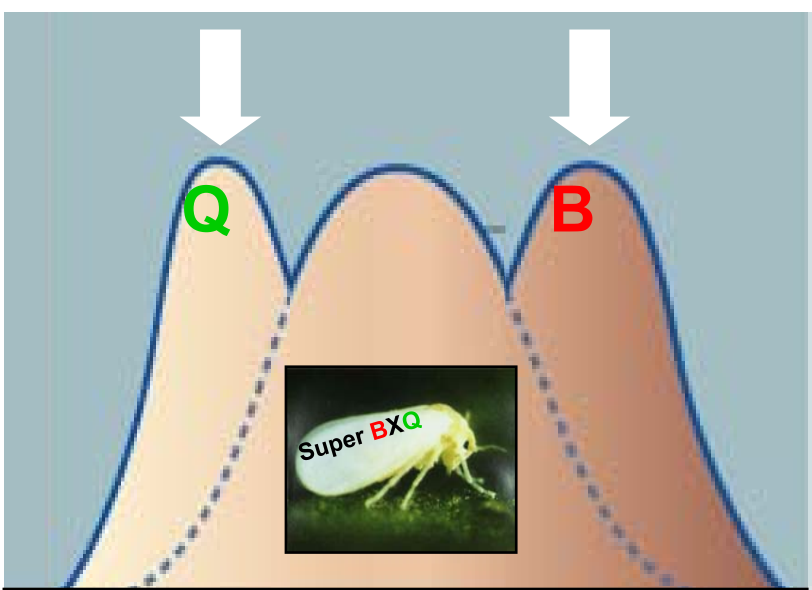
Unrooted maximum likelihood tree of *Bemisia tabaci*, produced from the first 0.3 kb intron sequence of the Bt CYP6A14 A gene

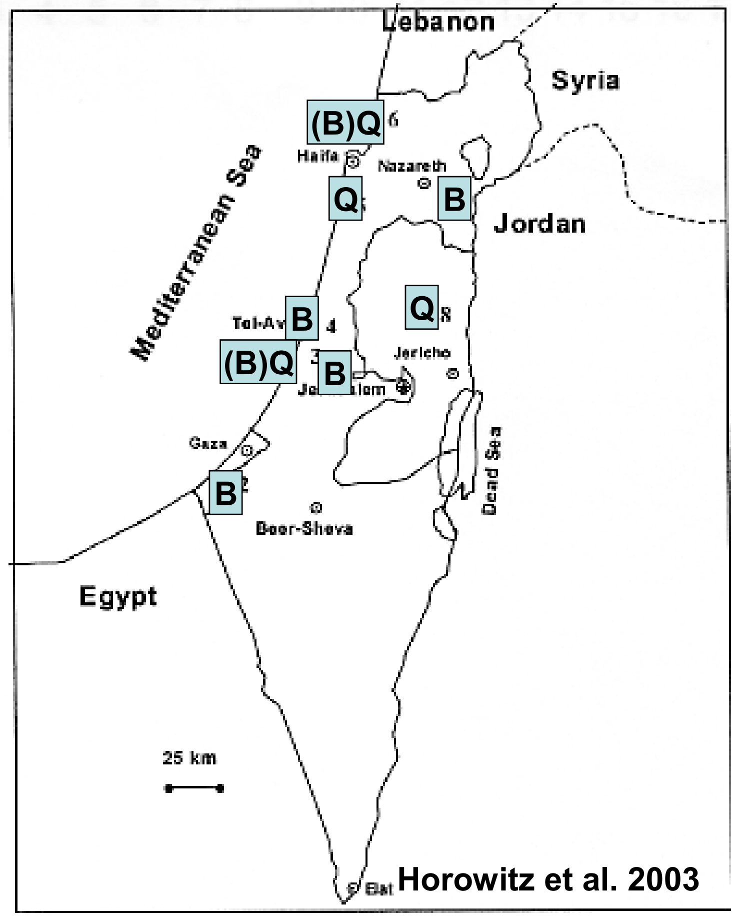


The percentages of 500 bootstrap replications supporting each branch are shown



Allopatric speciation with secondary contact





Collaborators:

Juergen Benting	Bayer CropScience
Ralf Nauen	Bayer CropScience
Rami Horowitz	The Israeli Agricultural Research Organization

Students in the lab:

Iris Karunker	P450s and neonicotinoid resistance
Navit Lahav	Reproductive isolation between biotypes
Michal Alon	Evolution of resistance to pyrethroids
Moshe Elbaz	Mating behaviour
Dina Berlovitch	Mating behaviour