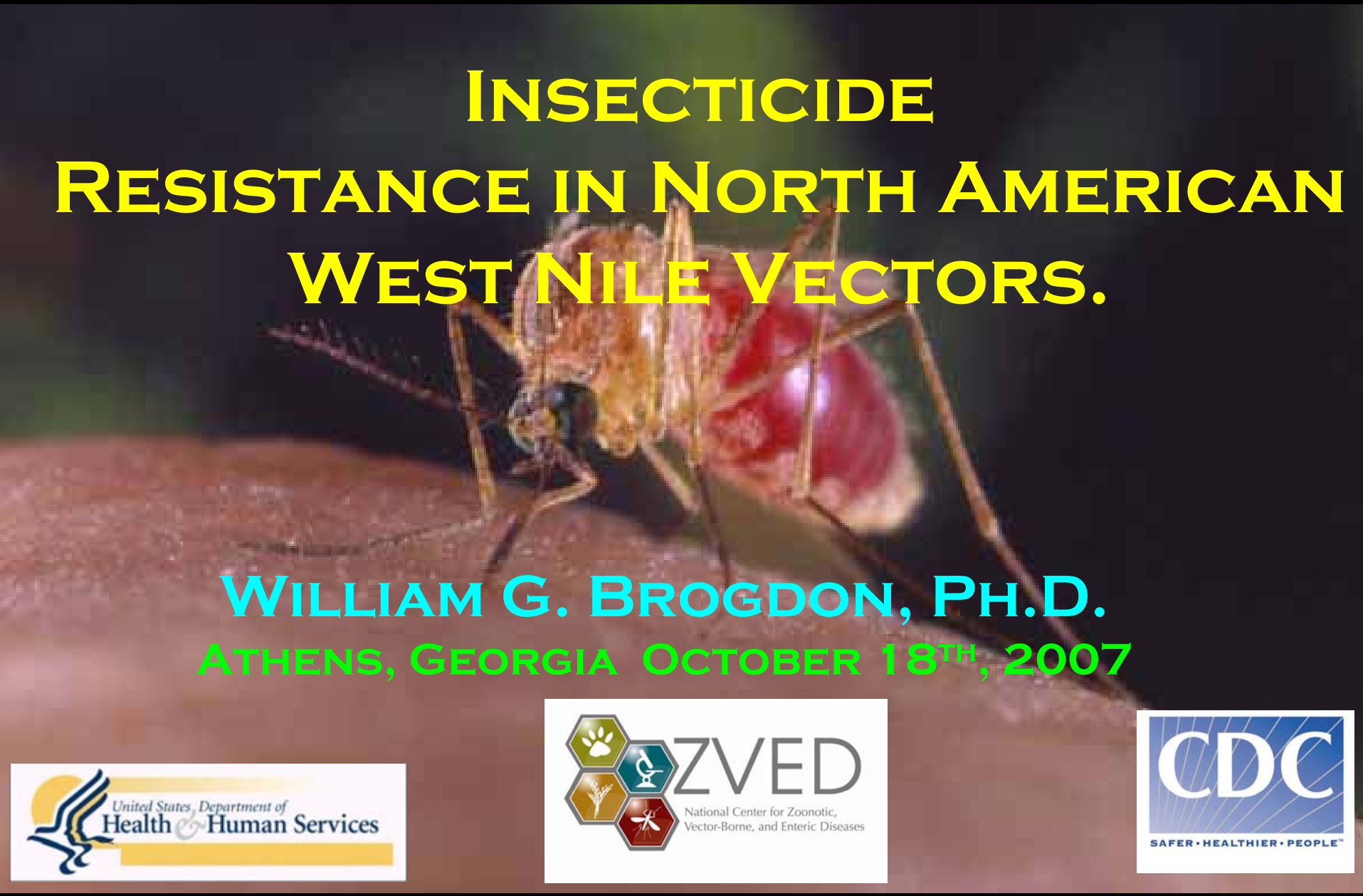


Insecticide Resistance in North American West Nile Vectors.

William G. Brogdon, Ph.D.

Athens, Georgia October 18th, 2007

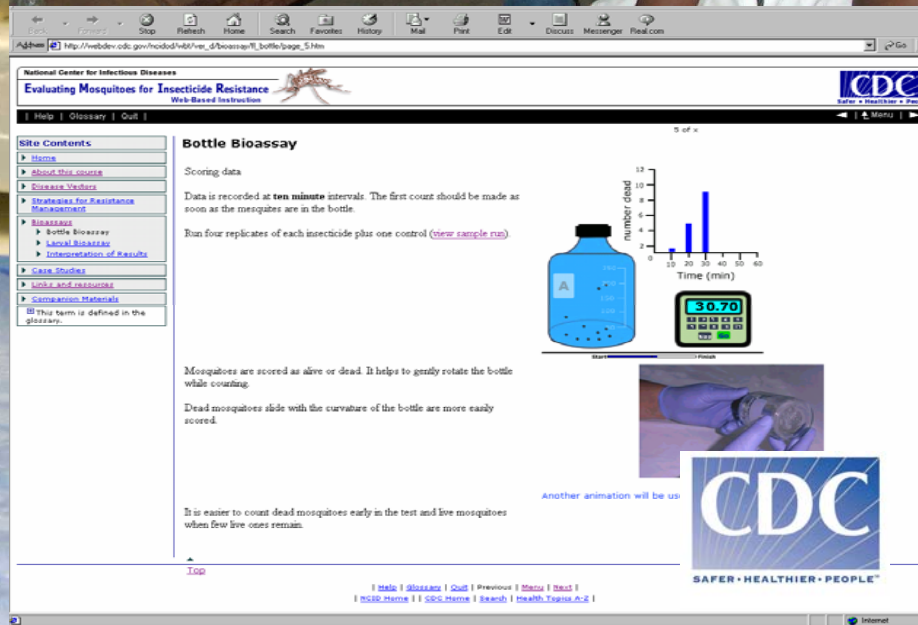
United States Department of Health & Human Services

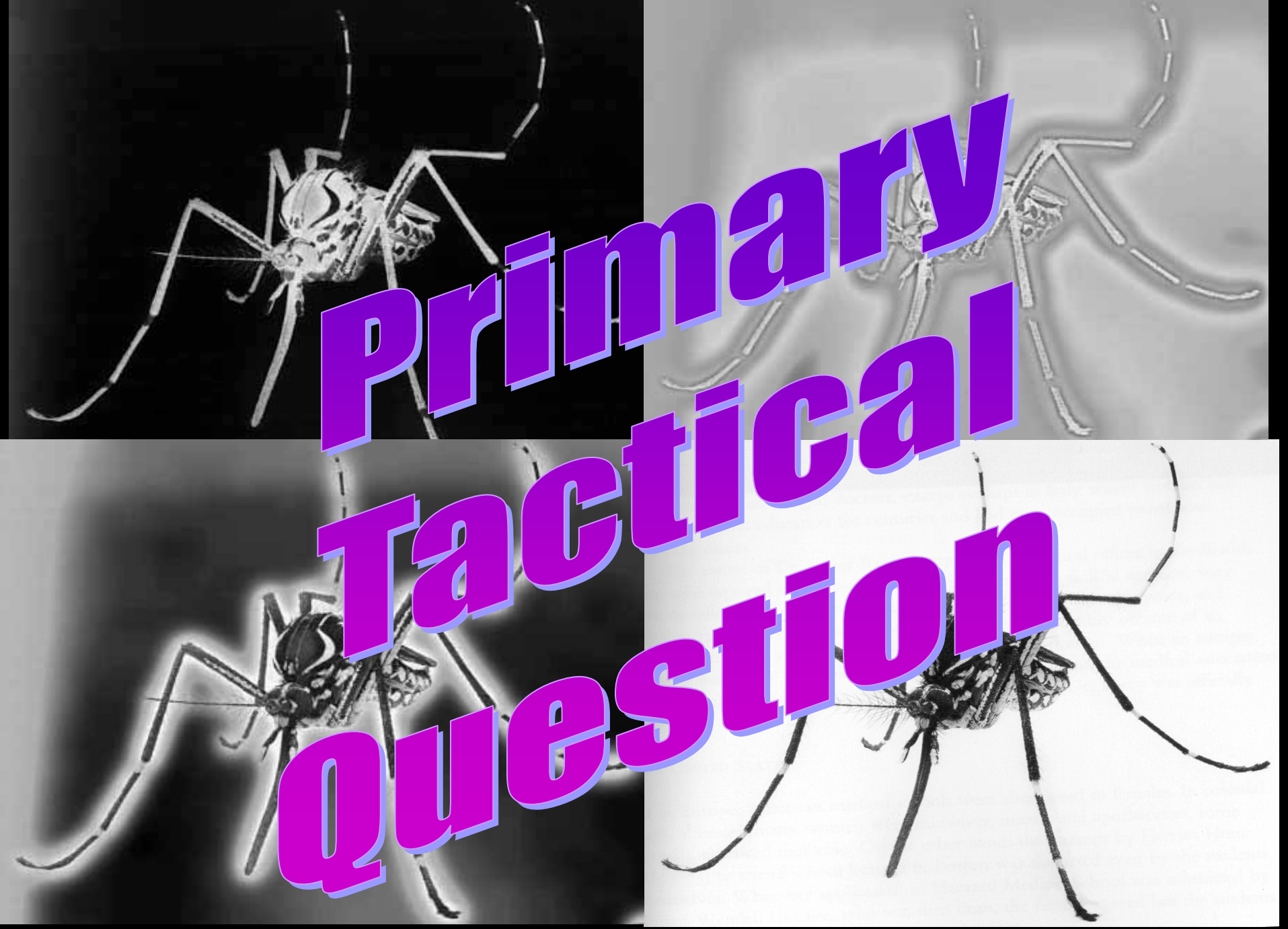
ZVED
National Center for Zoonotic, Vector-Borne, and Enteric Diseases

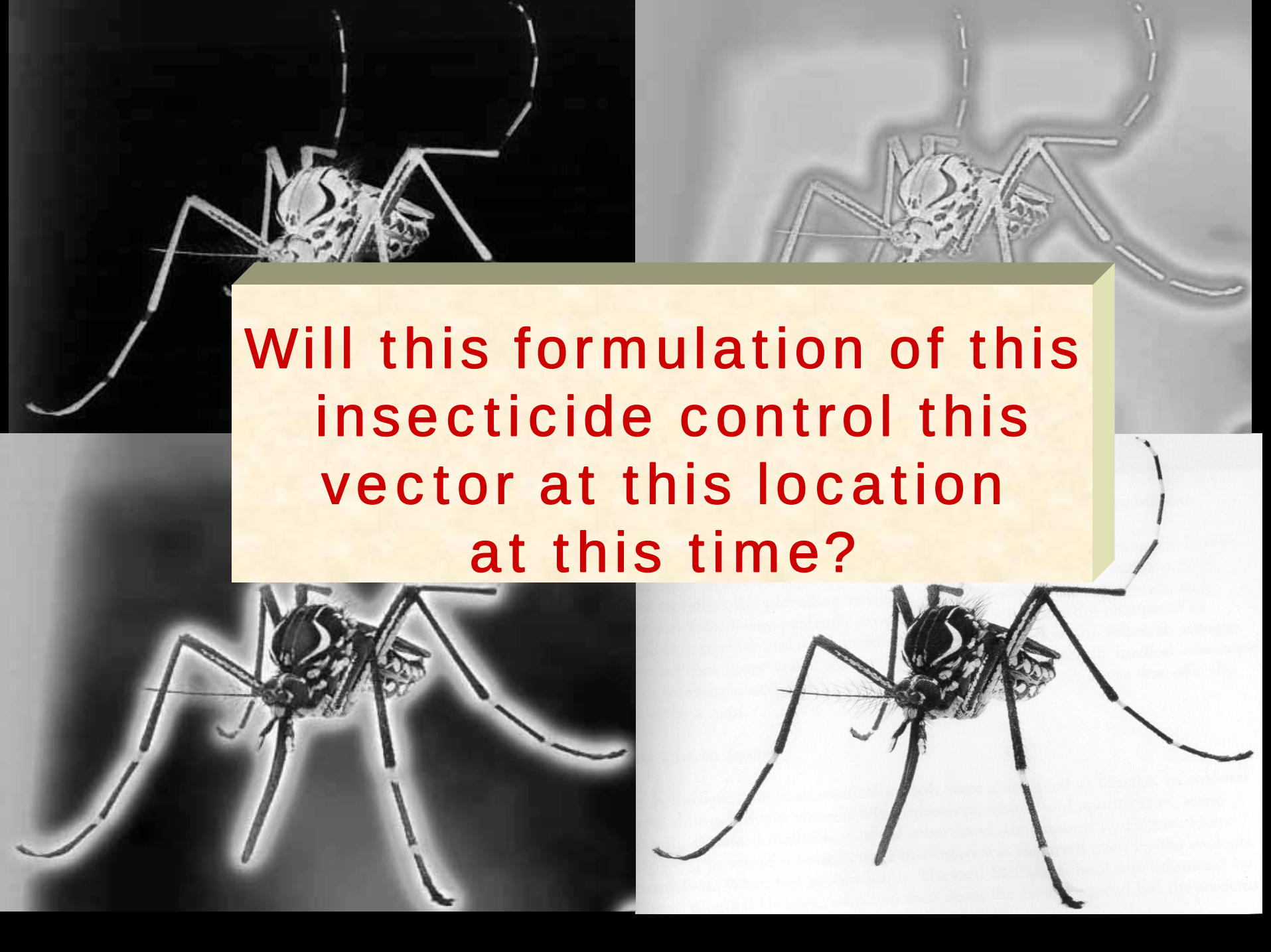
CDC
SAFER·HEALTHIER·PEOPLE™

The most practical method for proving the presence of resistance is a good bioassay.

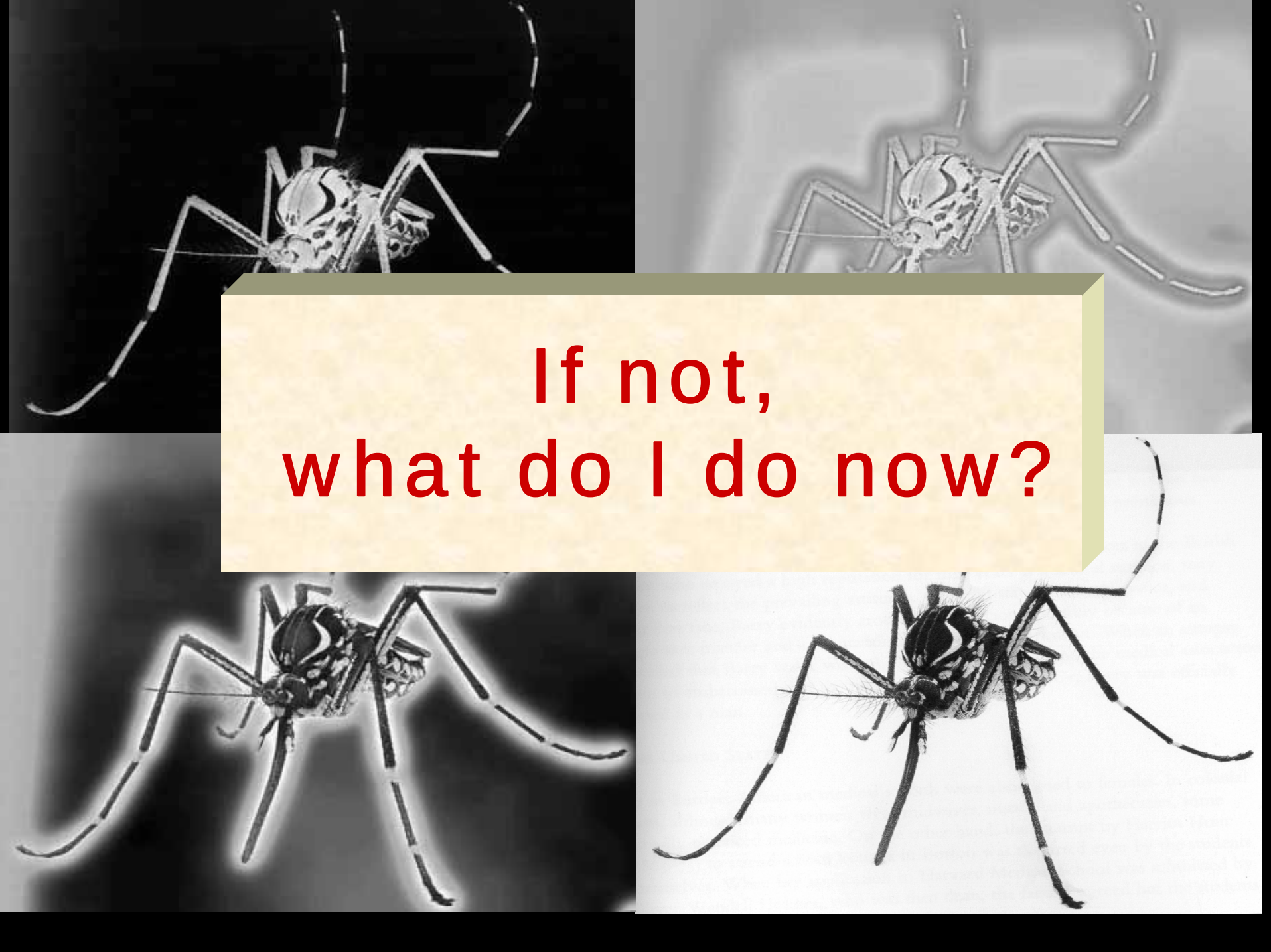
CDC Bottle Bioassay




Primary
Tactical
Question

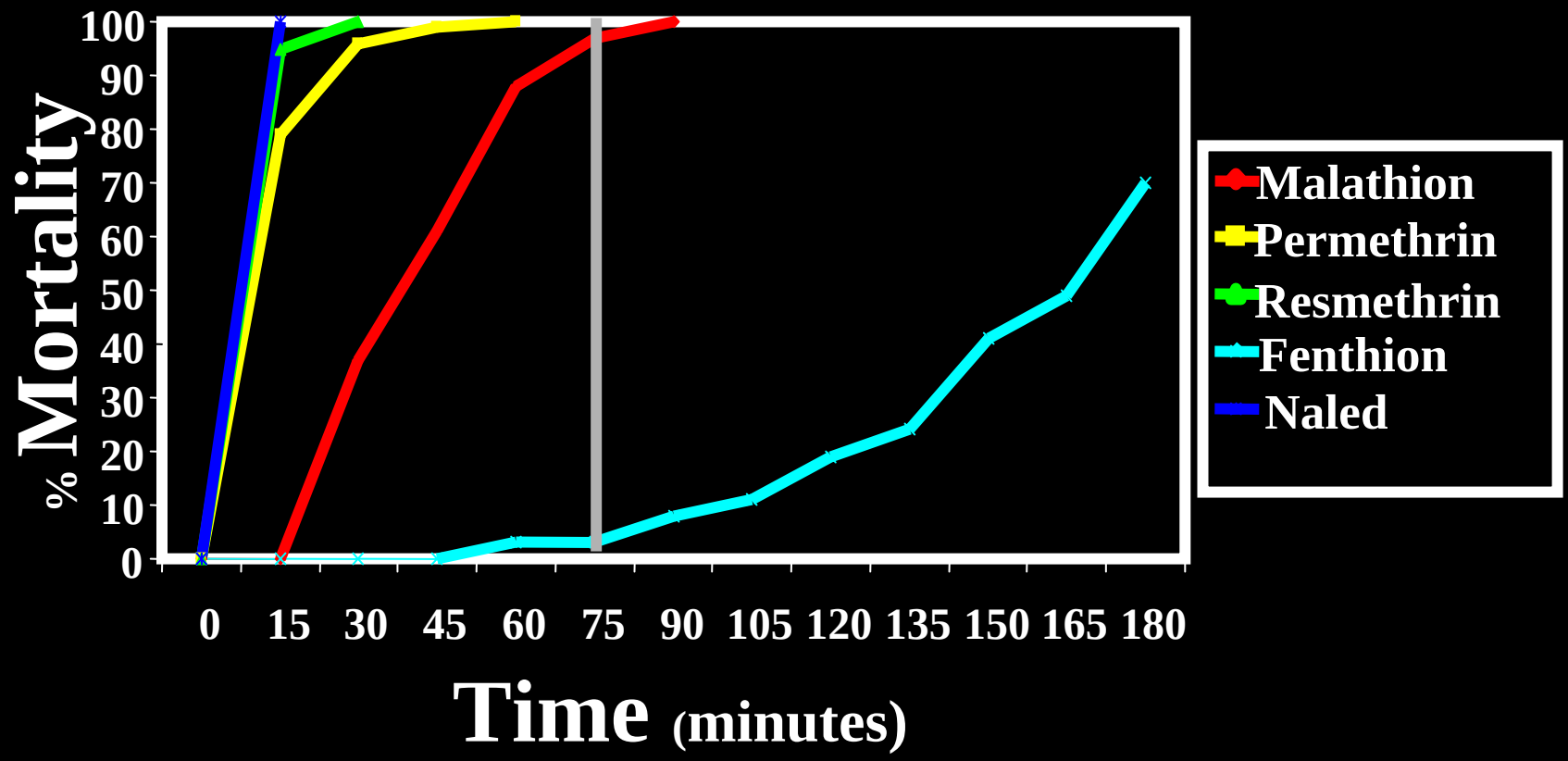


Will this formulation of this insecticide control this vector at this location at this time?

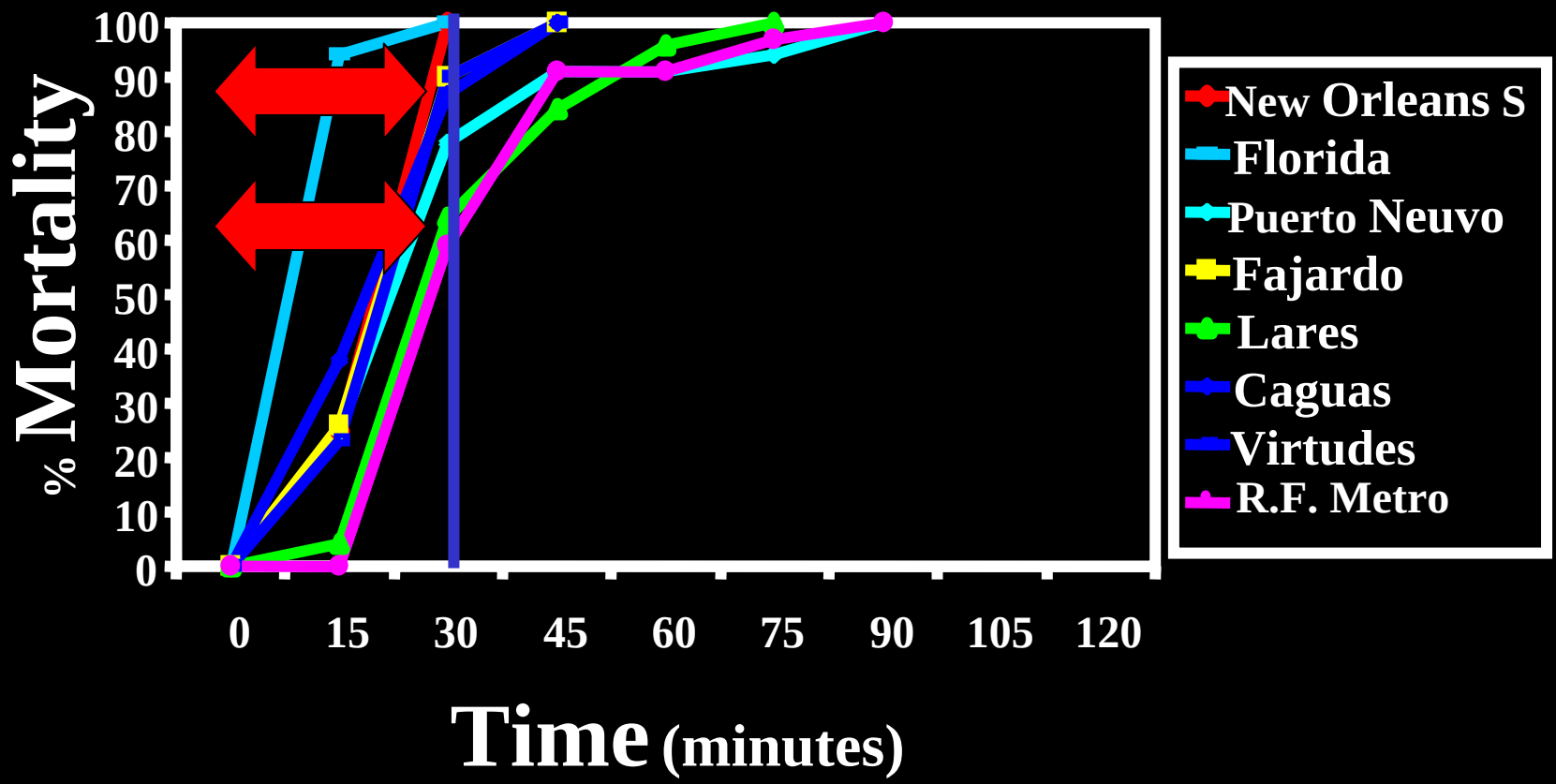


If not,
what do I do now?

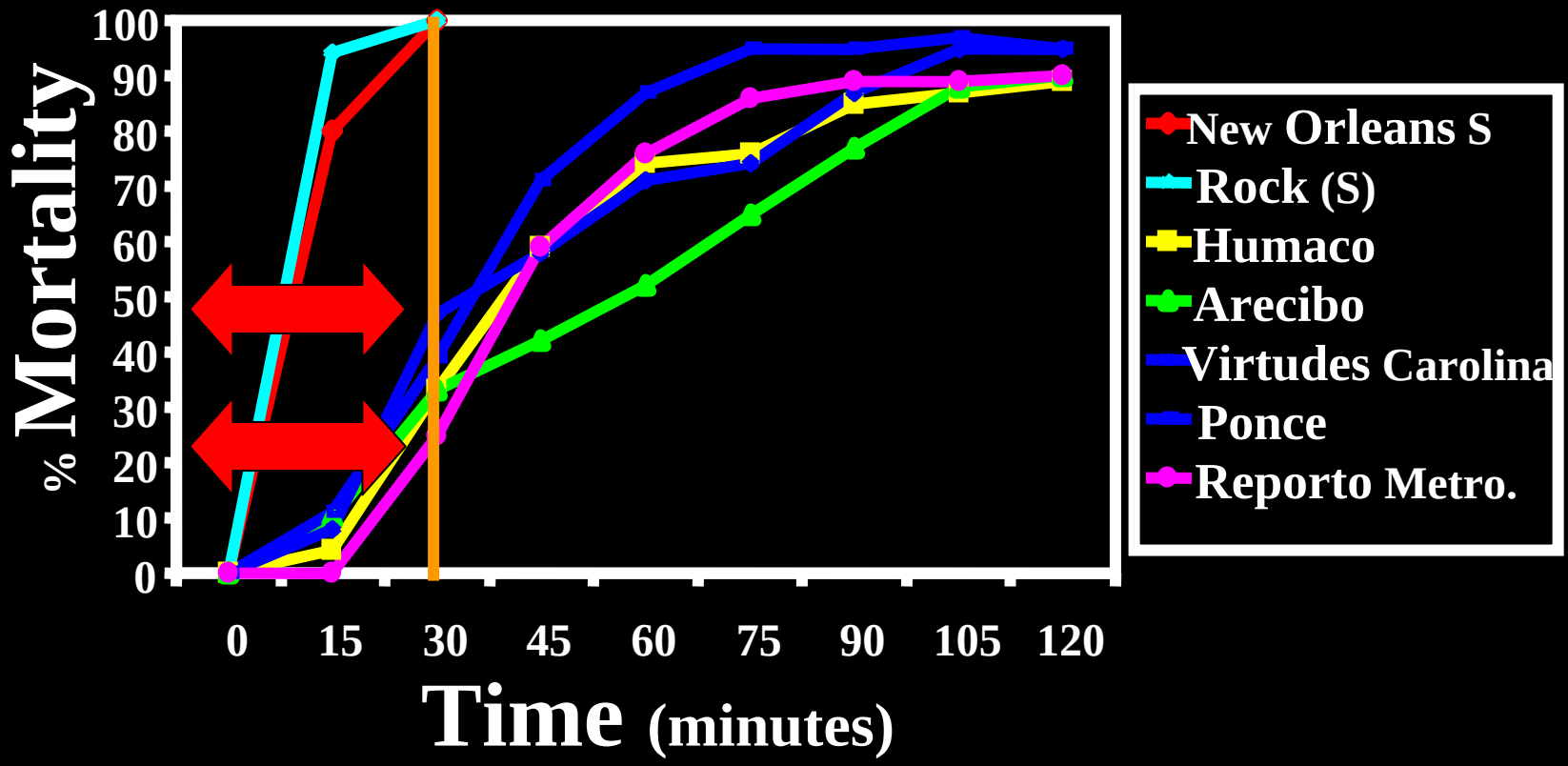
Bottle bioassays run on *Culex nigripalpus* from Indian River, Florida



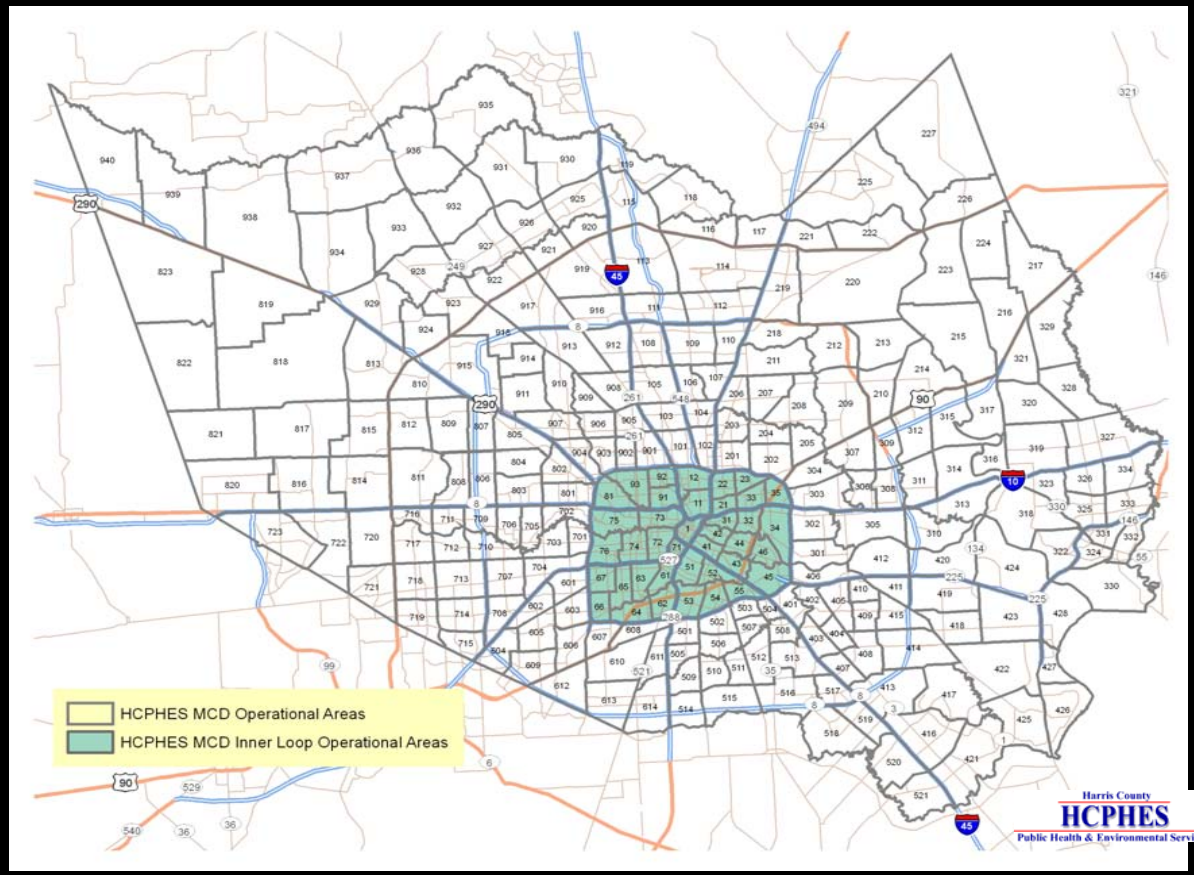
1996 Puerto Rico *Aedes aegypti* assayed with Permethrin



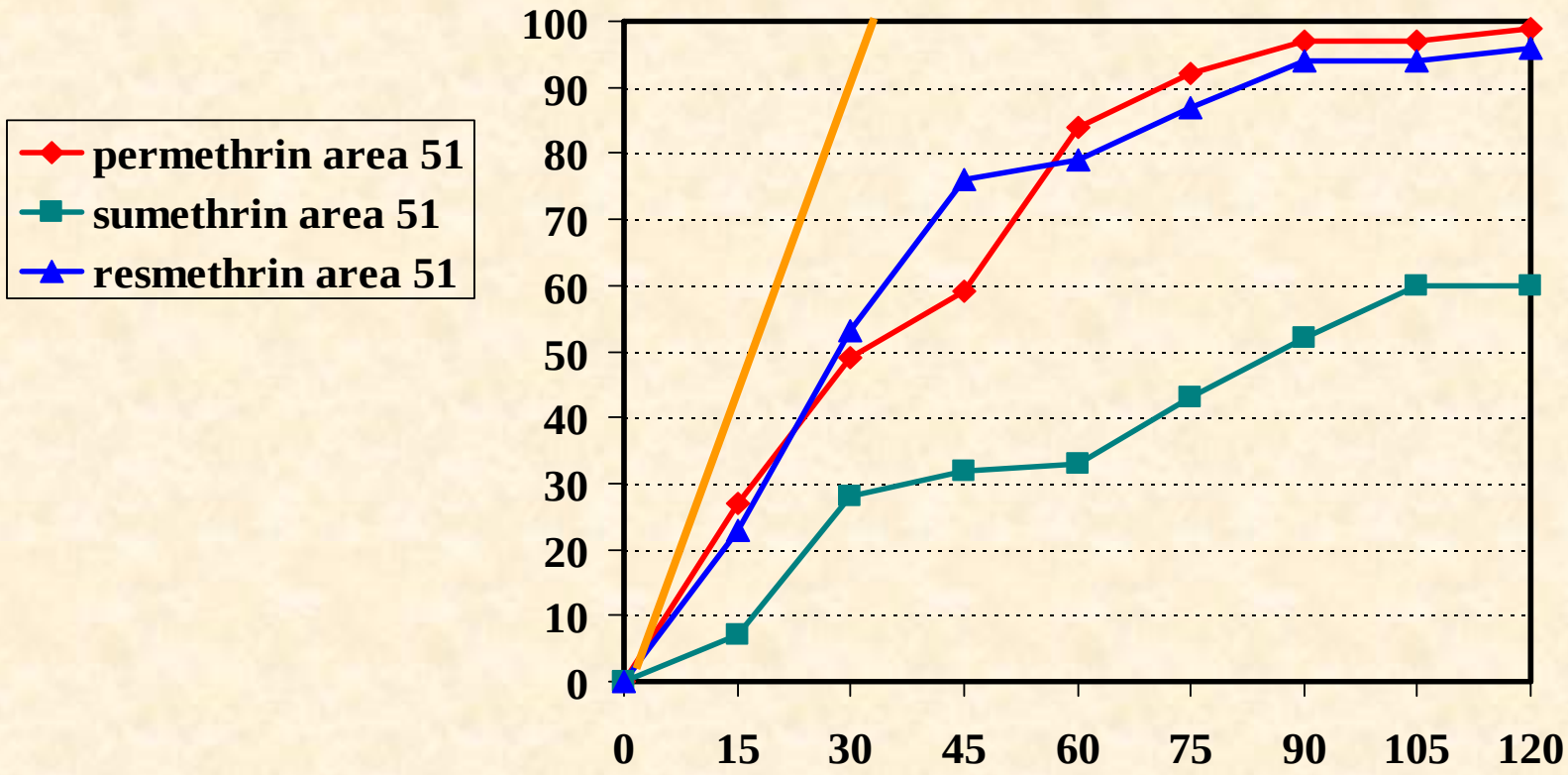
1999 Puerto Rico *Aedes aegypti* assayed with Permethrin

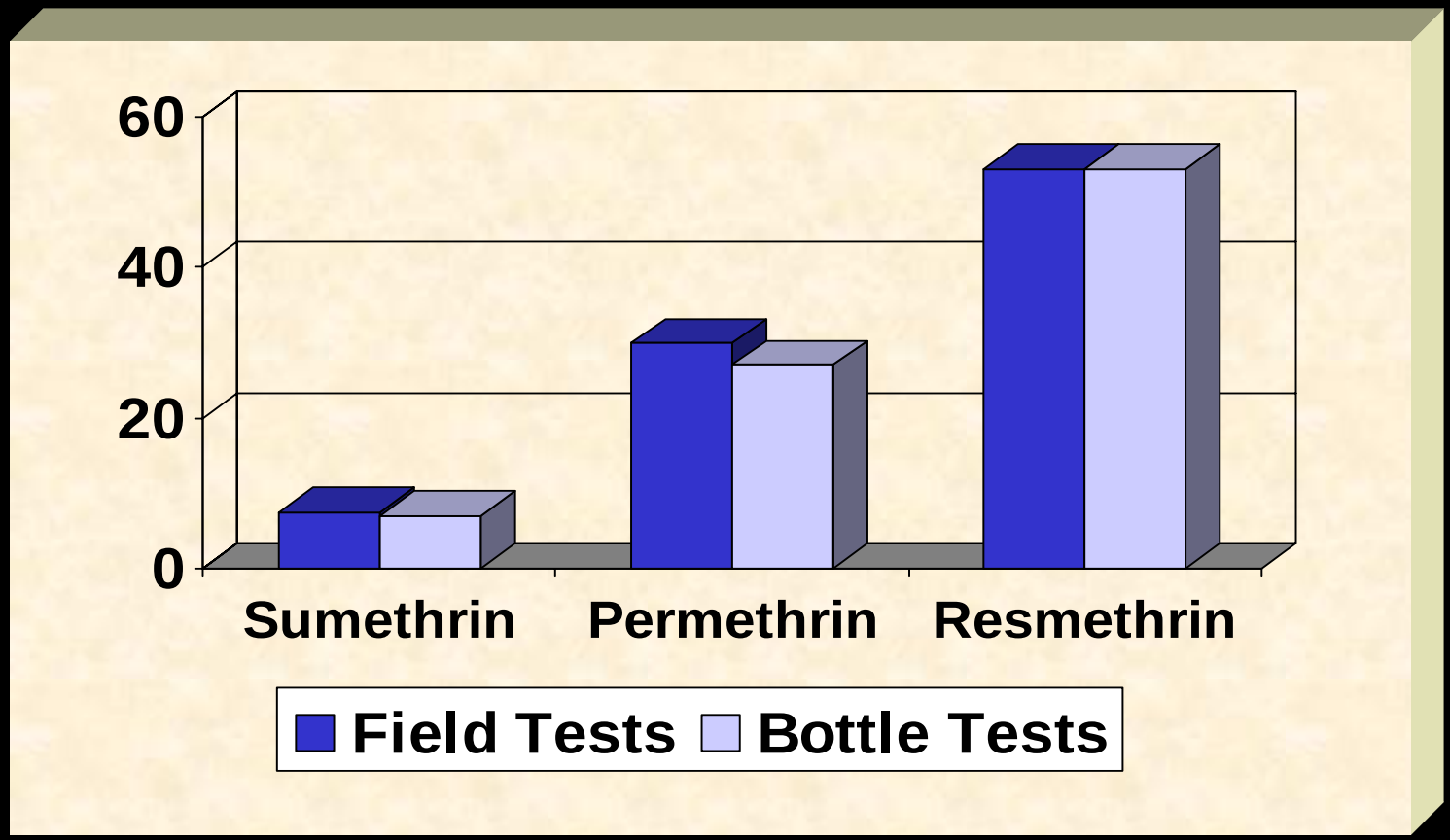


Mosquito Control Operational Areas

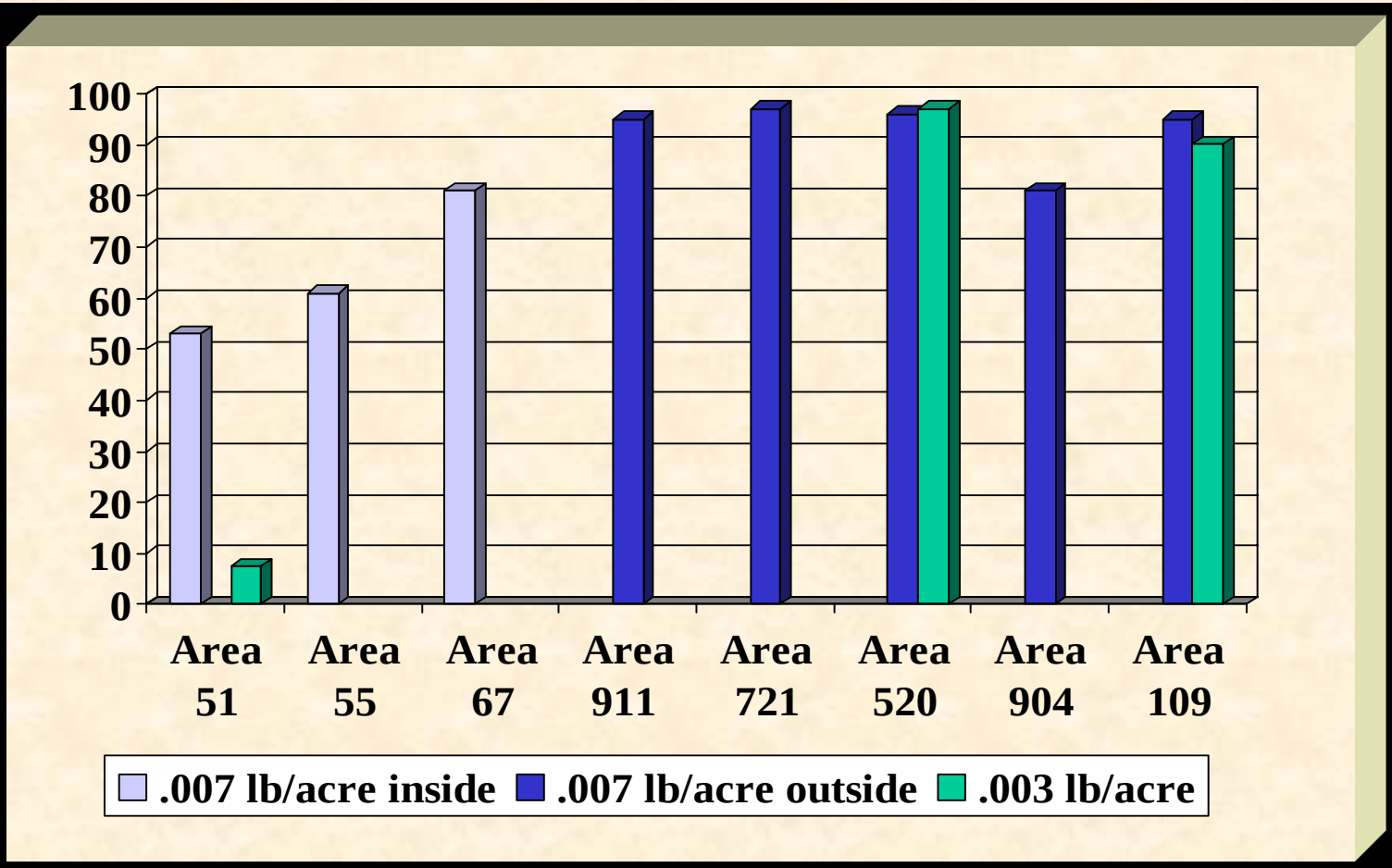


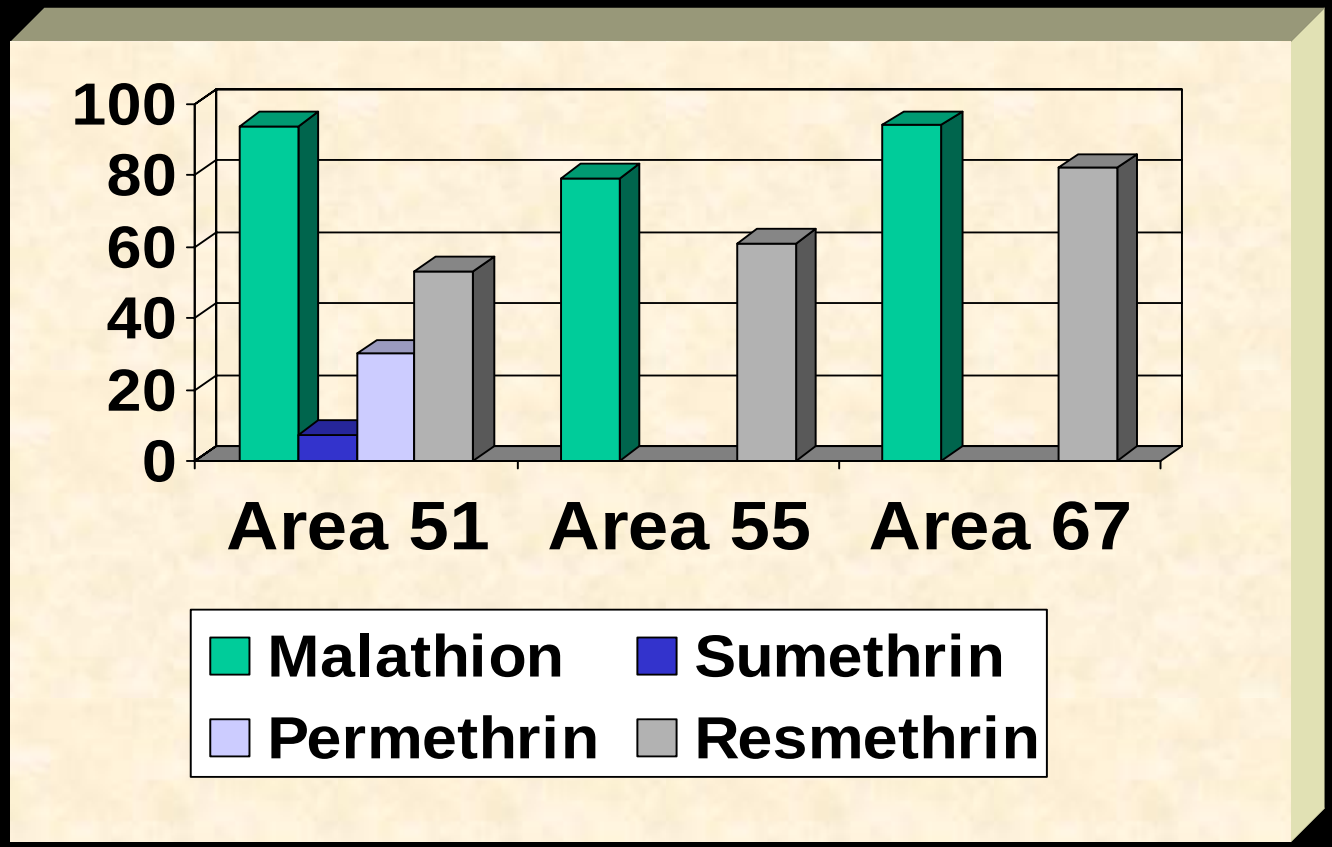
Area 51 bottle assay results



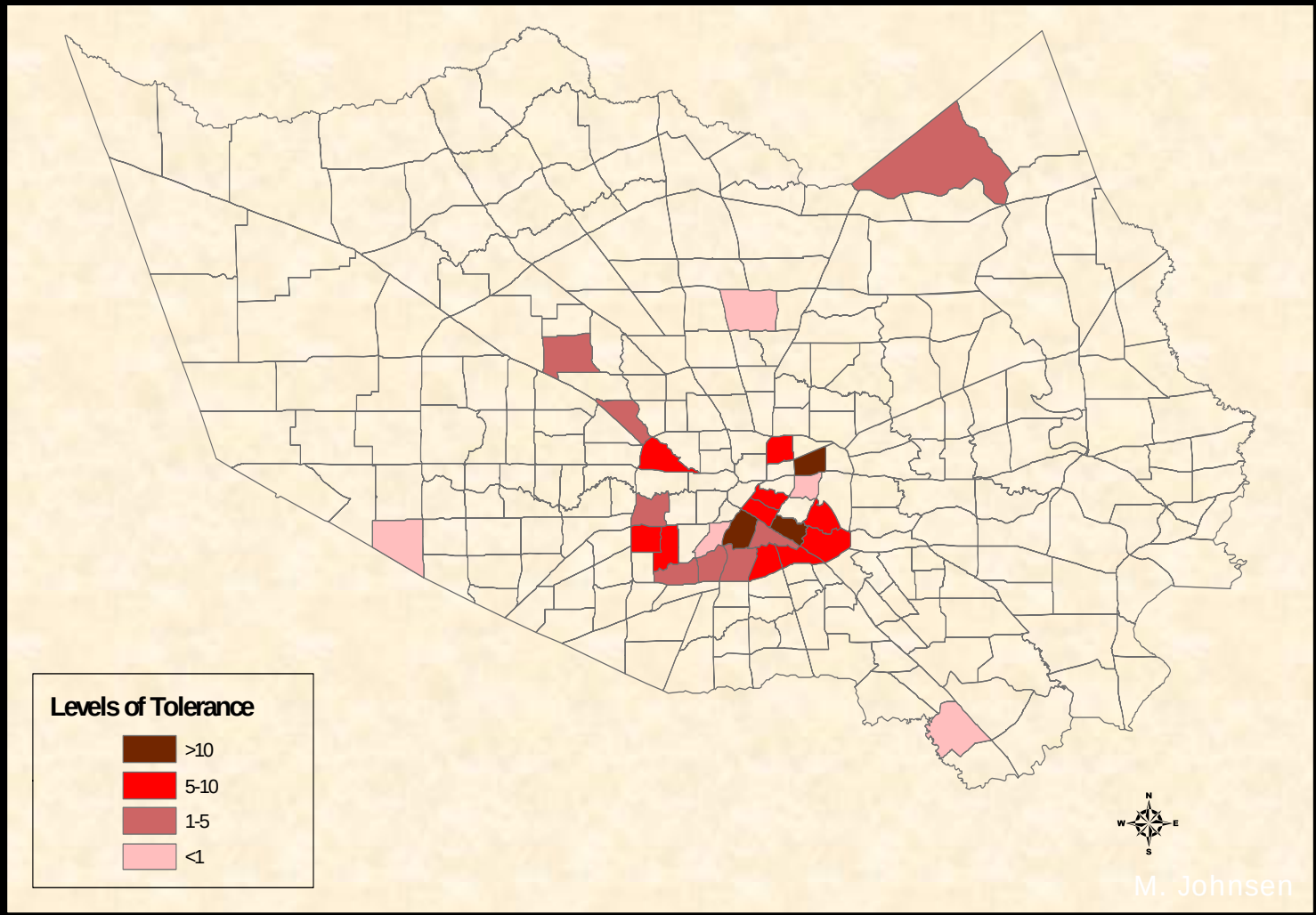

60
40
20
0
Sumethrin
Permethrin
Resmethrin
Field Tests
Bottle Tests

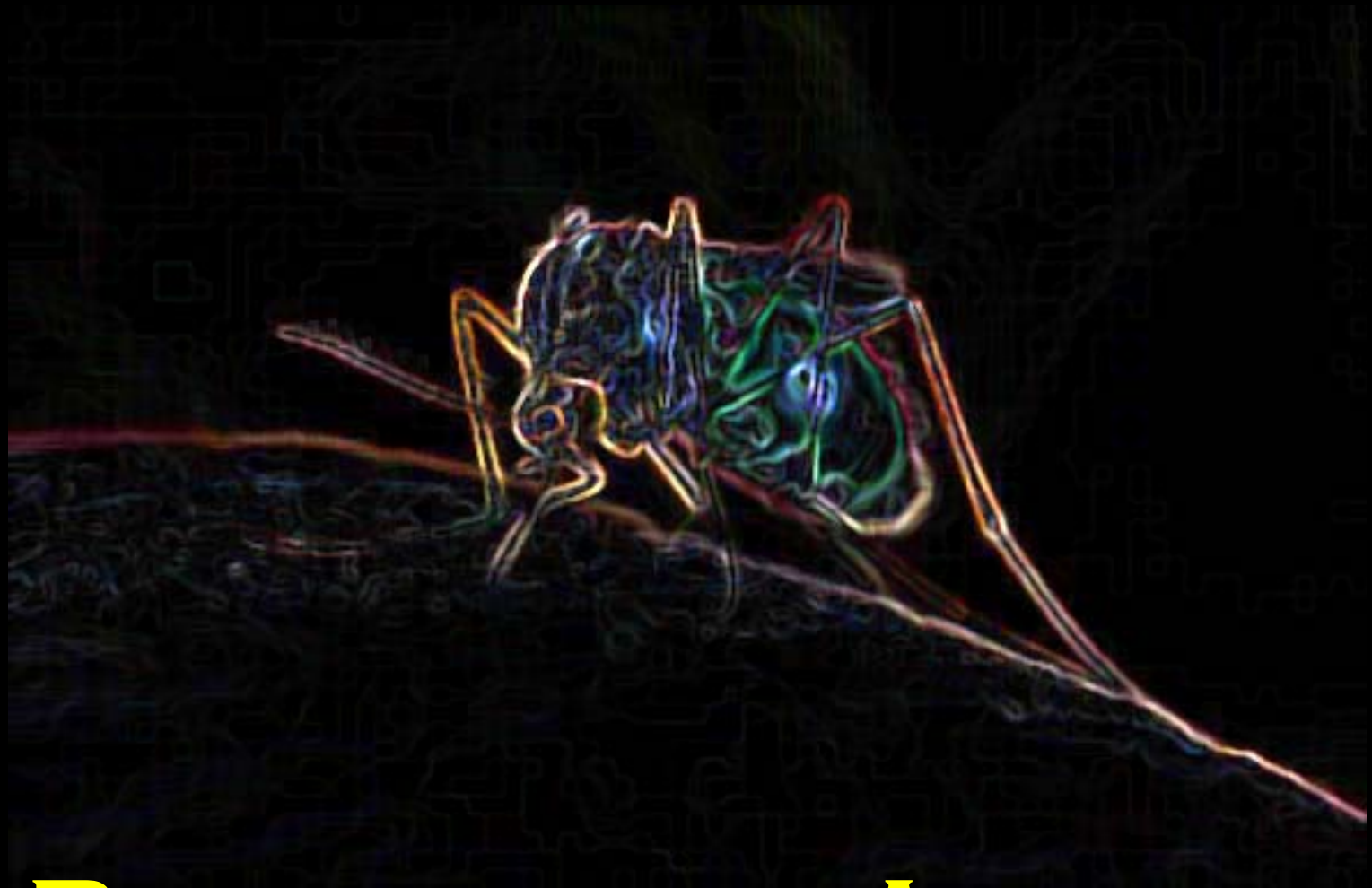
Percent mortality in feral *Culex quinquefasciatus* exposed to .003 or .007 lb/acre Resmethrin




100
80
60
40
20
0
Area 51
Area 55
Area 67
Malathion
Sumethrin
Permethrin
Resmethrin

Levels of Resistance to resmethrin in *Culex quinquefasciatus* from Selected Operational Areas in Harris County, Texas



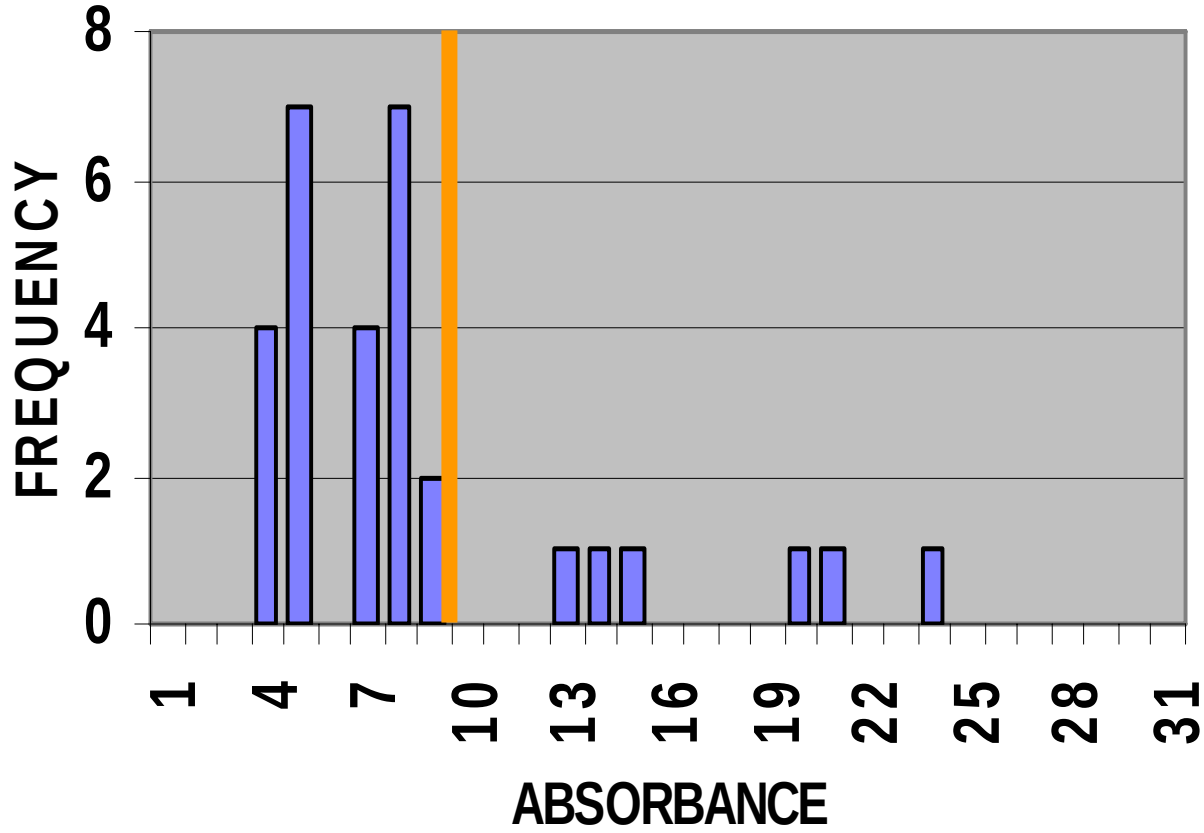


Biochemical Assays
Molecular Assays

Measure expressed protein levels
Multiplexed
Five resistance enzymes / insect

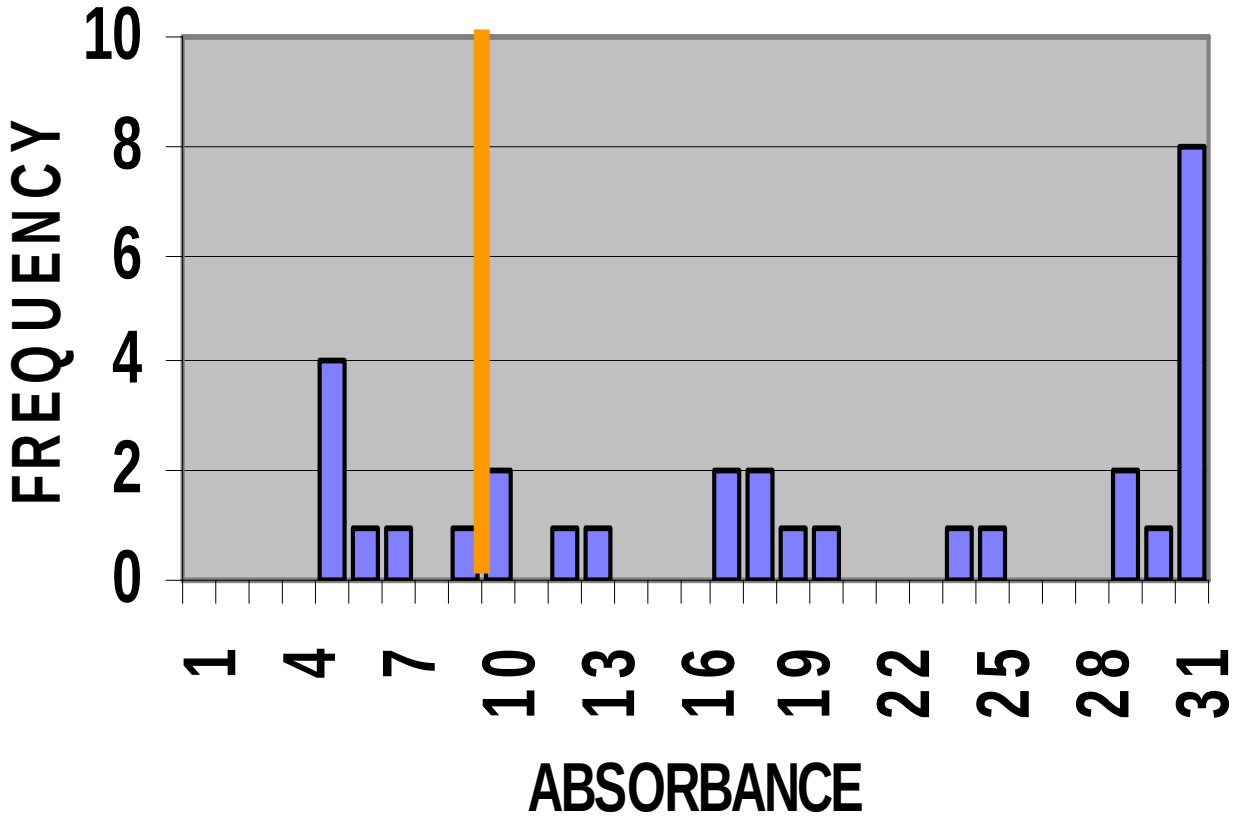
Culex pipiens Esterase

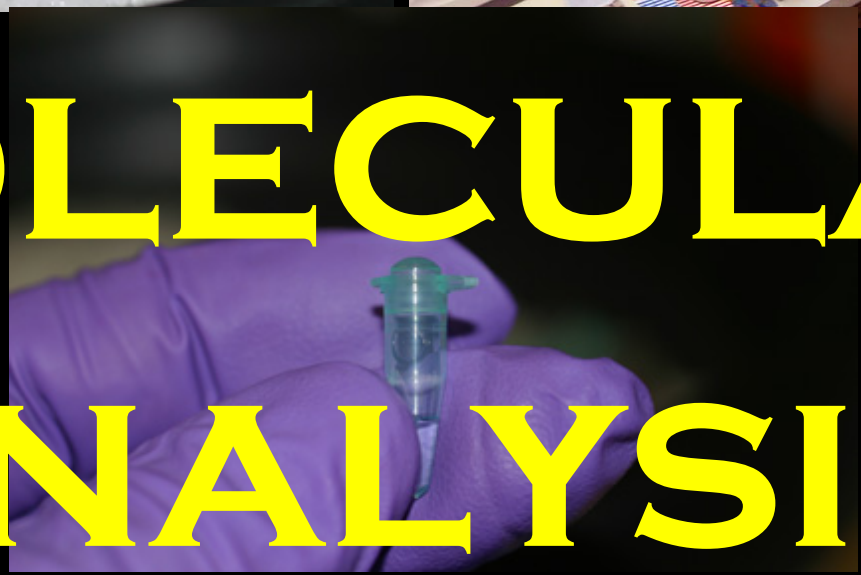
NORTON, PORTAGE COUNTY, OHIO



CULEX PIPIENS ESTERASE

COVENTRY, SUMMIT COUNTY, OHIO





Molecular Analysis

Because of the expense, molecular assays must be multiplexed to allow the required information to be obtained from each insect.

The overwhelming challenge is to make this Practical.

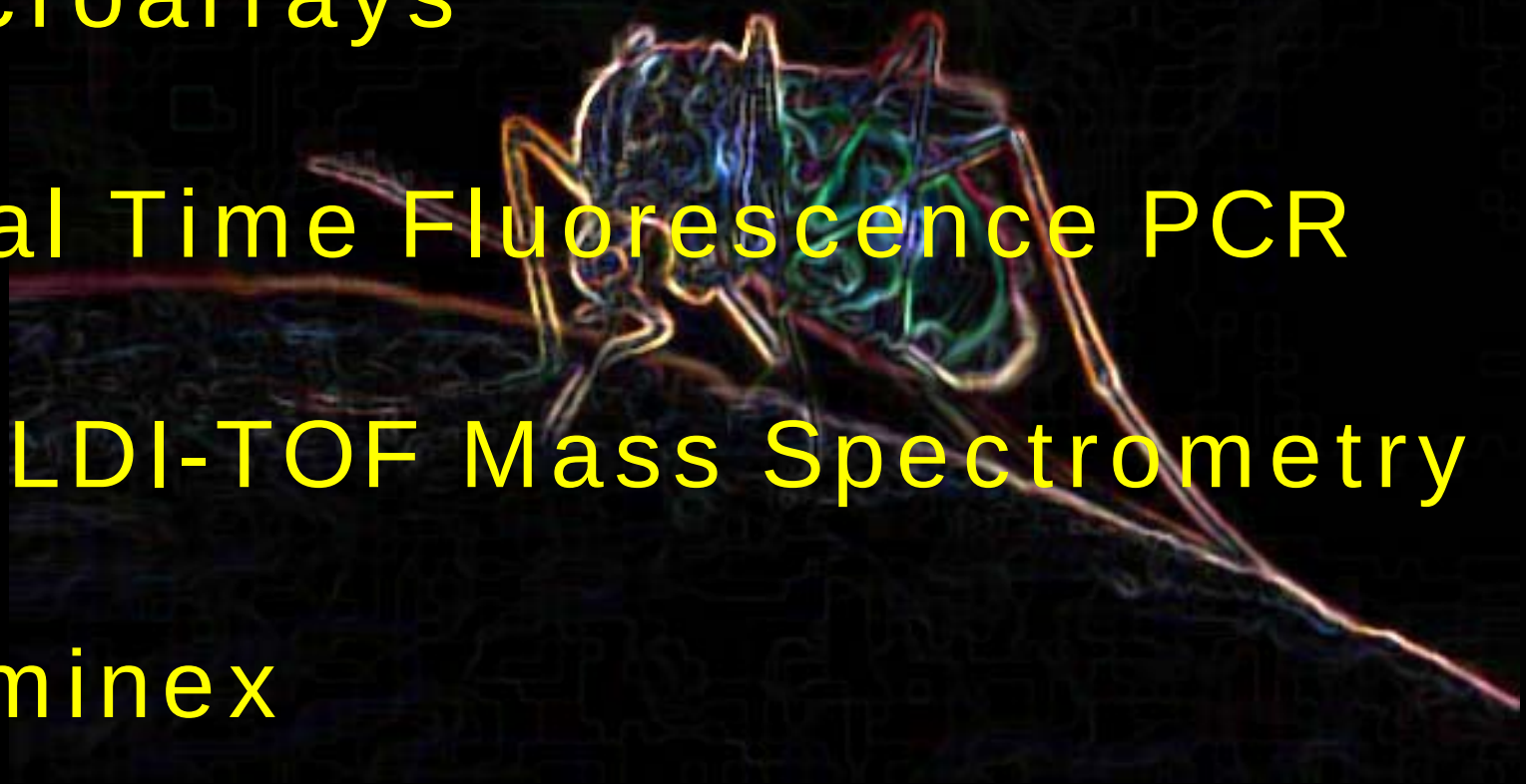
Direct Sequencing of Cloned Genes

Microarrays

Real Time Fluorescence PCR

MALDI-TOF Mass Spectrometry

Luminex



Fluorescence PCR allows measurement of :

Gene Expression - Quantitative RTPCR.

Oxidase resistance

Copy Number - Quantitative PCR.

Esterase gene copy numbers

Point Mutations – kdr point mutations

Melting Point Analysis.


BIO-RAD
Bio-Plex System

Extreme Multiplexing (x 100)

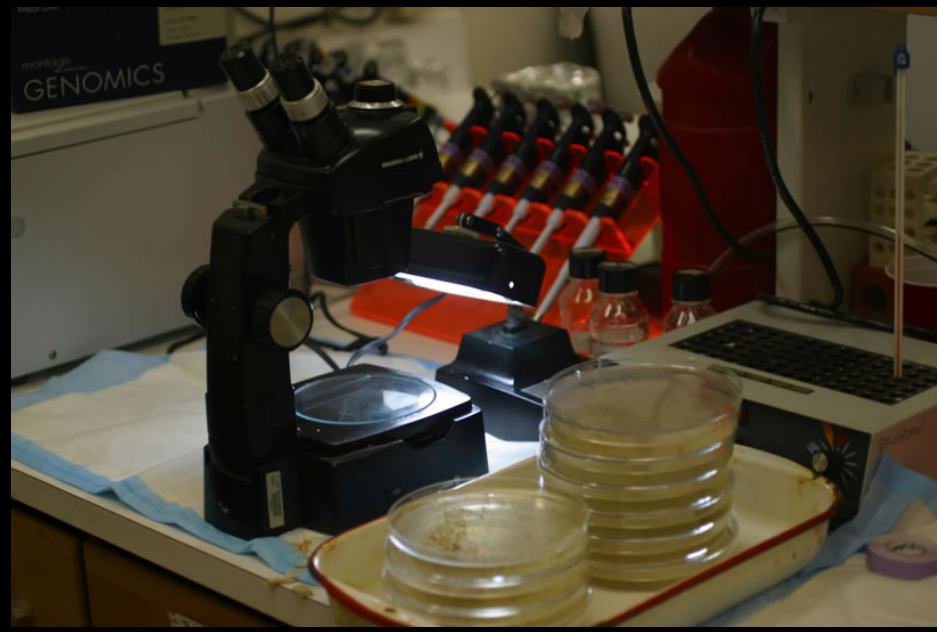
All resistance mechanisms

Species ID – vector

Species/type –parasite or virus

Multiple SNP data – vector

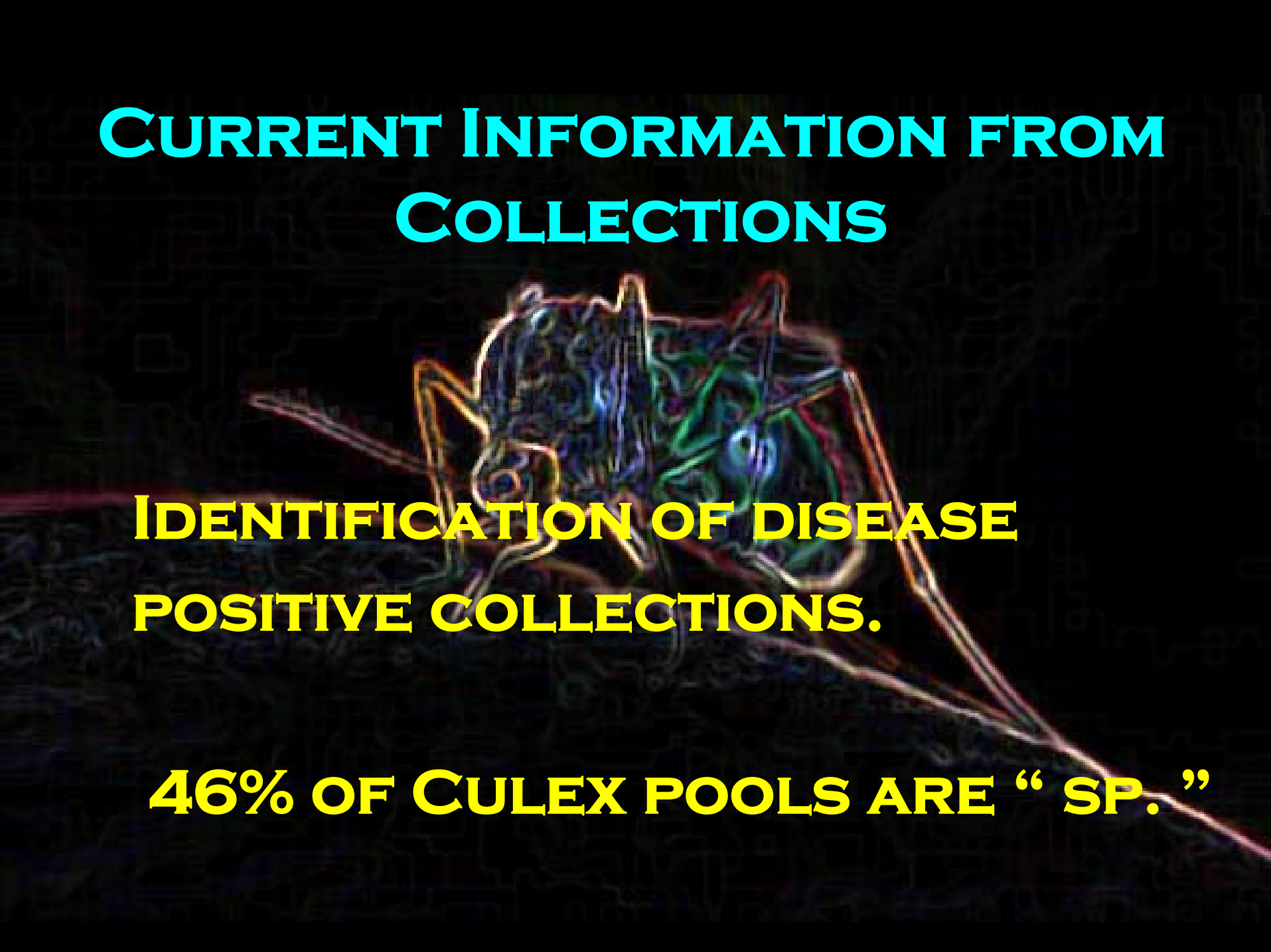
Multiple SNP data – pathogen


GENOMICS

Mosquito Collections


1/24/2002 12:27pm

Current Information from Collections



Identification of disease positive collections.

46% of Culex pools are “ sp. ”

Potential Additional Information from Collections

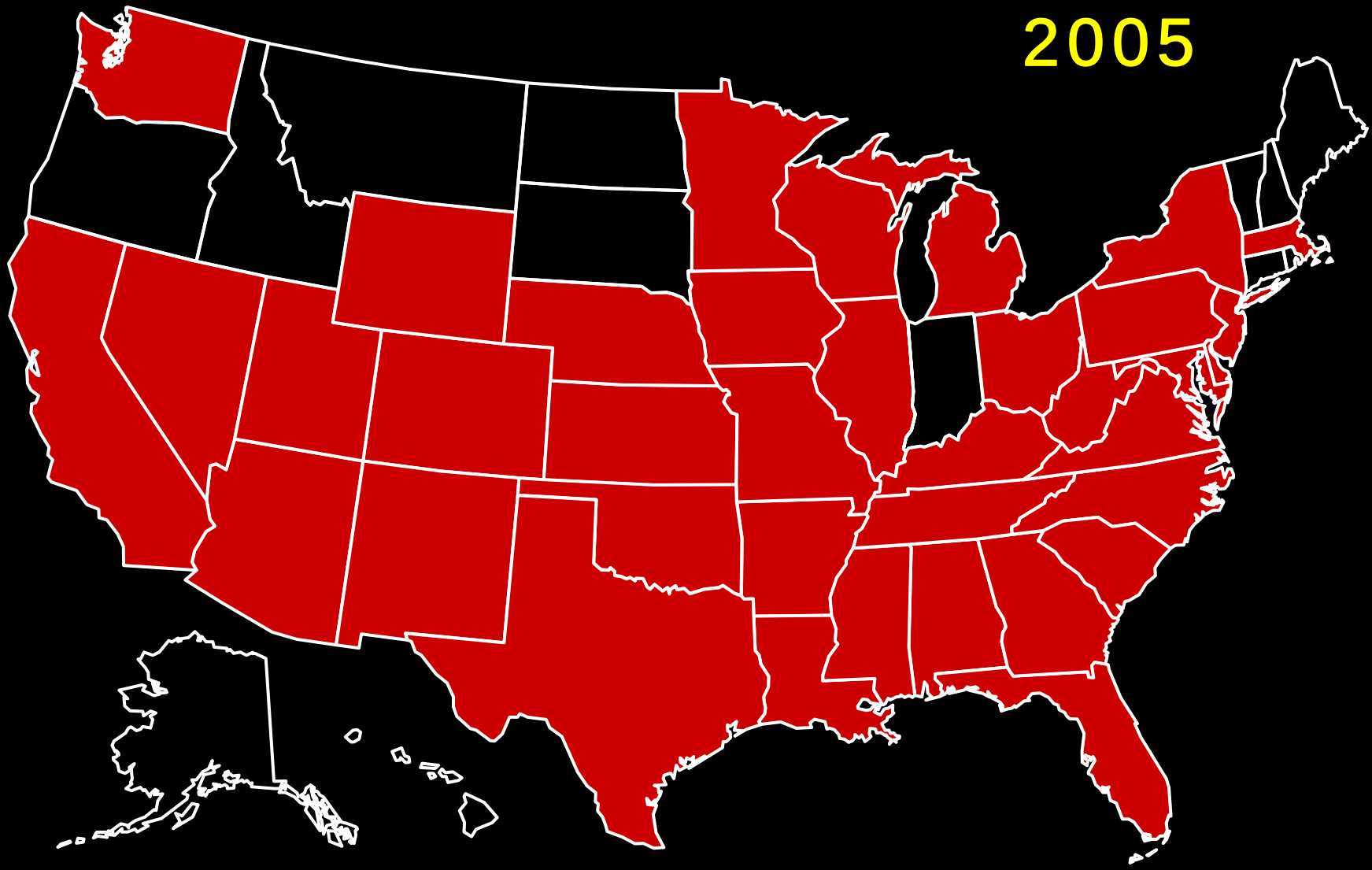
- Species Identification
- Resistance mechanism(s)
- Correlation of ID, resistance data, disease positives
- Population Markers (SNPs)

U.S. Army Center for Health Promotion and Preventative Medicine (CHPPM)

CHPPM – North	Fort Meade Alexandra Spring
CHPPM – South	Fort McPherson Denny Kuhr
CHPPM – West	Fort Lewis Miguel Quintana

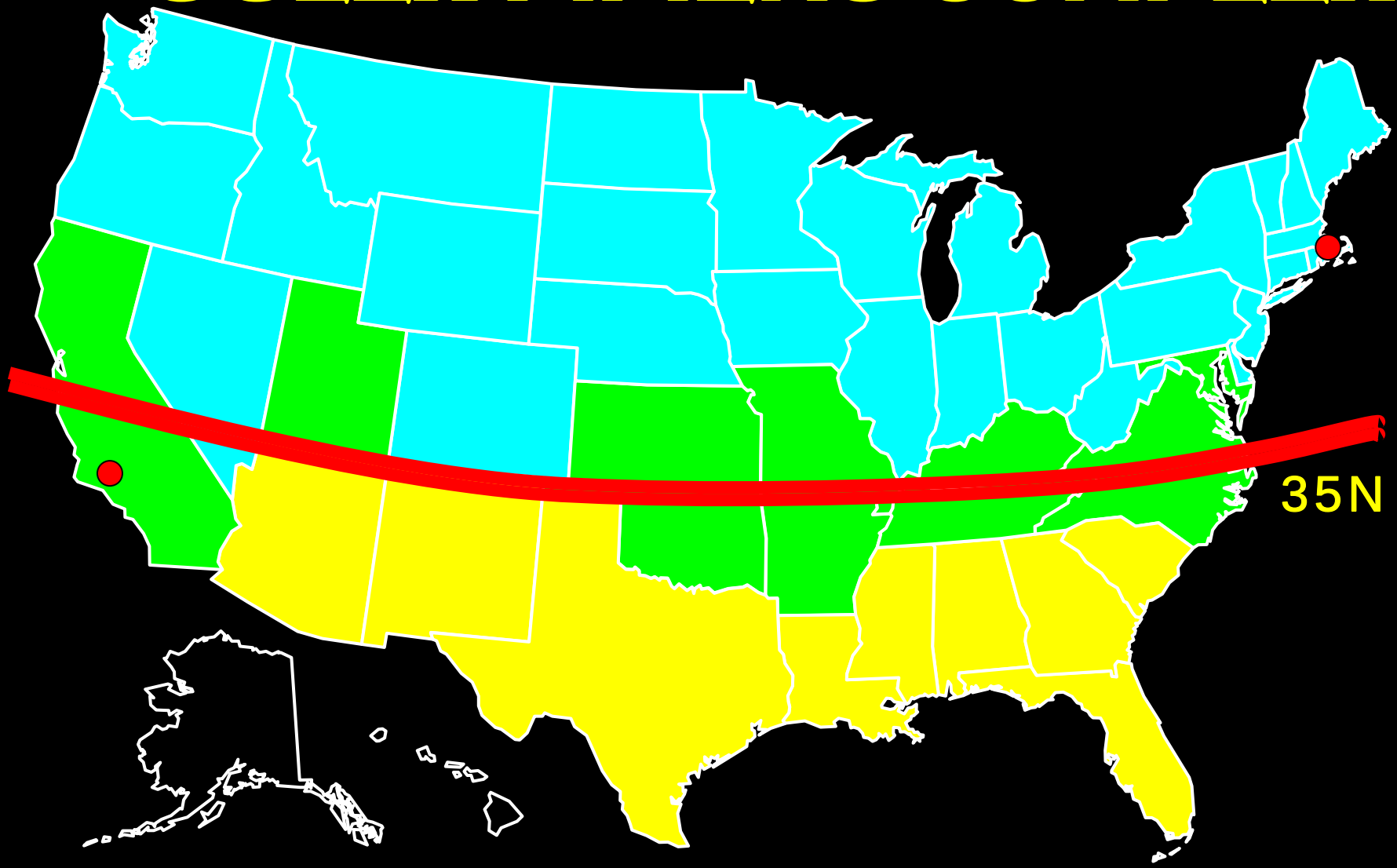
Collections are stored in our freezer facility

At present: >55,000 collections
5-12 year series for many sites


2005

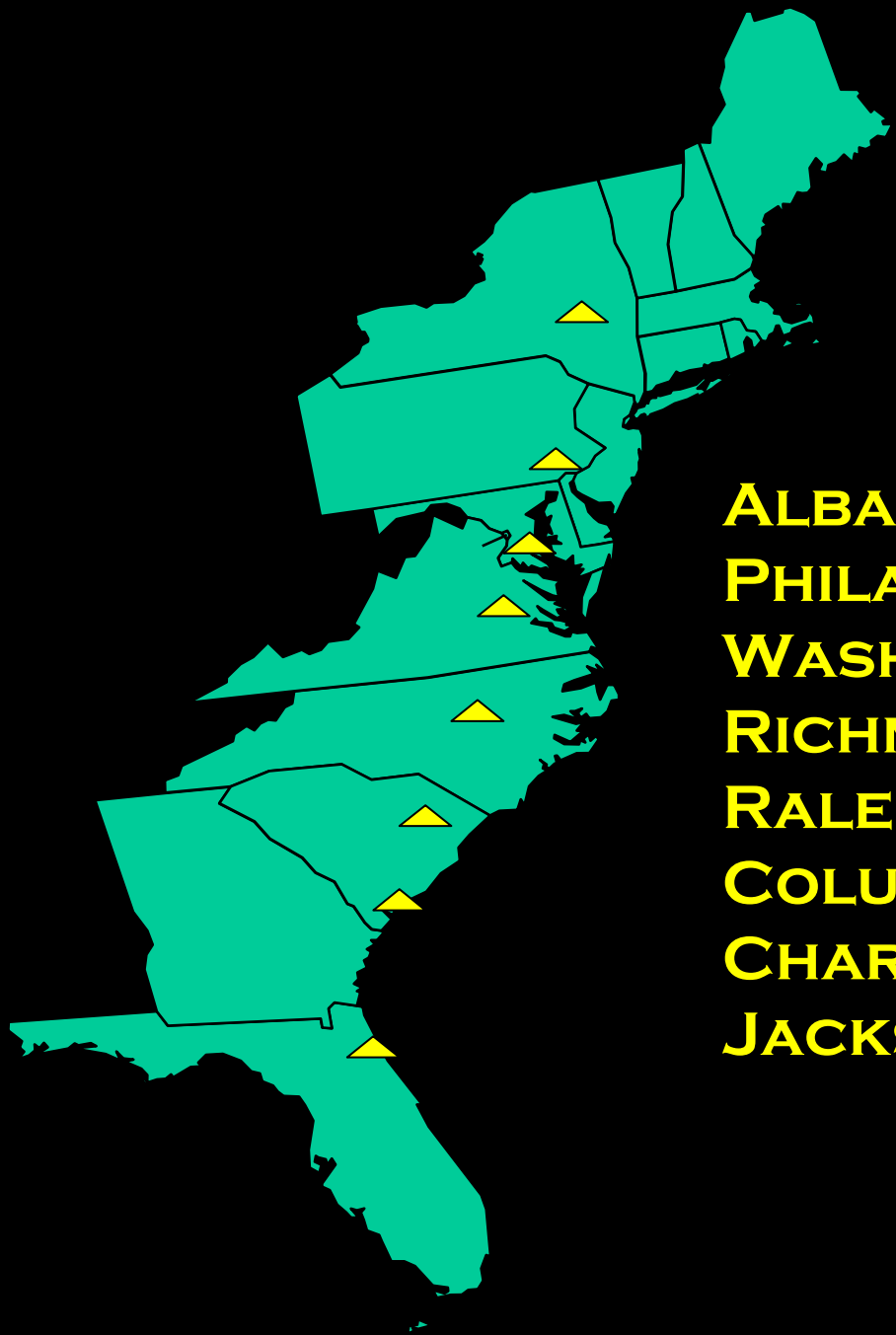
Culex pipiens
Culex quinquefasciatus
Culex molestus
Culex restuans
Culex salinarius
Culex nigripalpus
Culex tarsalis

Culex pipiens complex



Laura Kramer, Joe Vineis
State University of New York, Albany

Dennis White
New York Department of Health



Albany, New York
Philadelphia, Pennsylvania
Washington, D.C.
Richmond, Virginia
Raleigh, North Carolina
Columbia, South Carolina
Charleston, South Carolina
Jacksonville, Florida

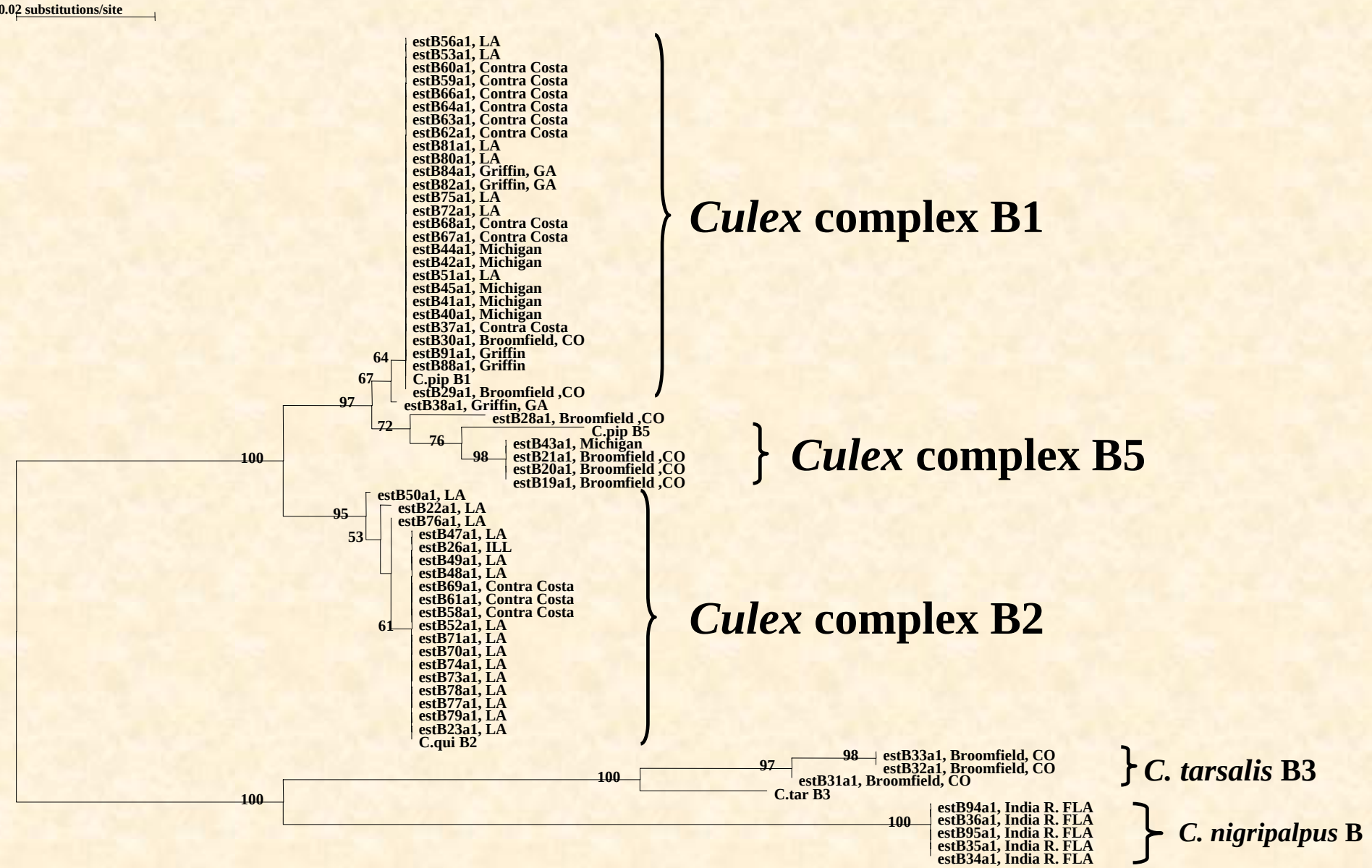
Three types of resistance genes in Culex.

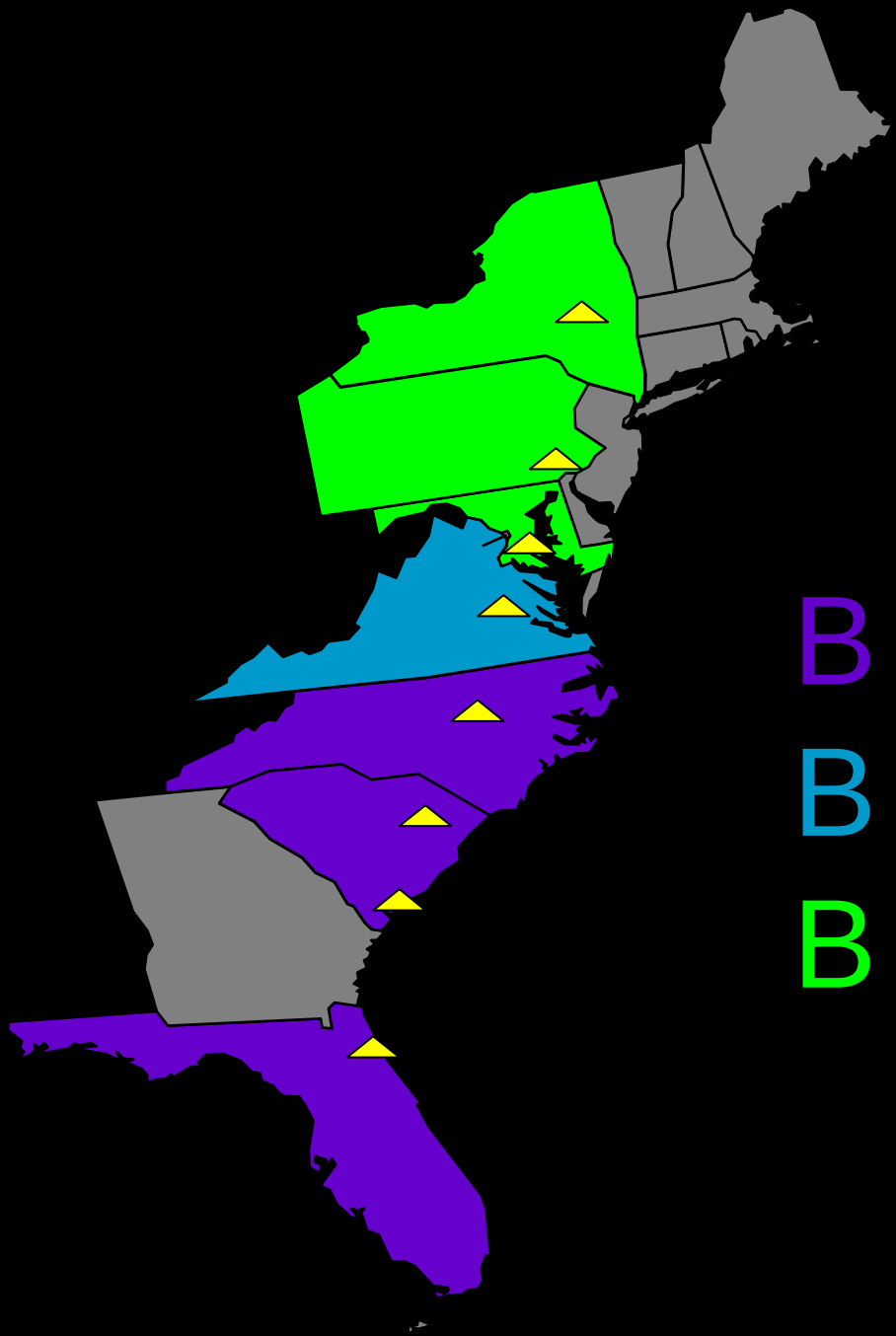
Multiple copy genes (esterases)

Upregulated genes (oxidases,GSTs)

Point mutations (kdr)

Culex spp. WGB19-95 *est* B Study

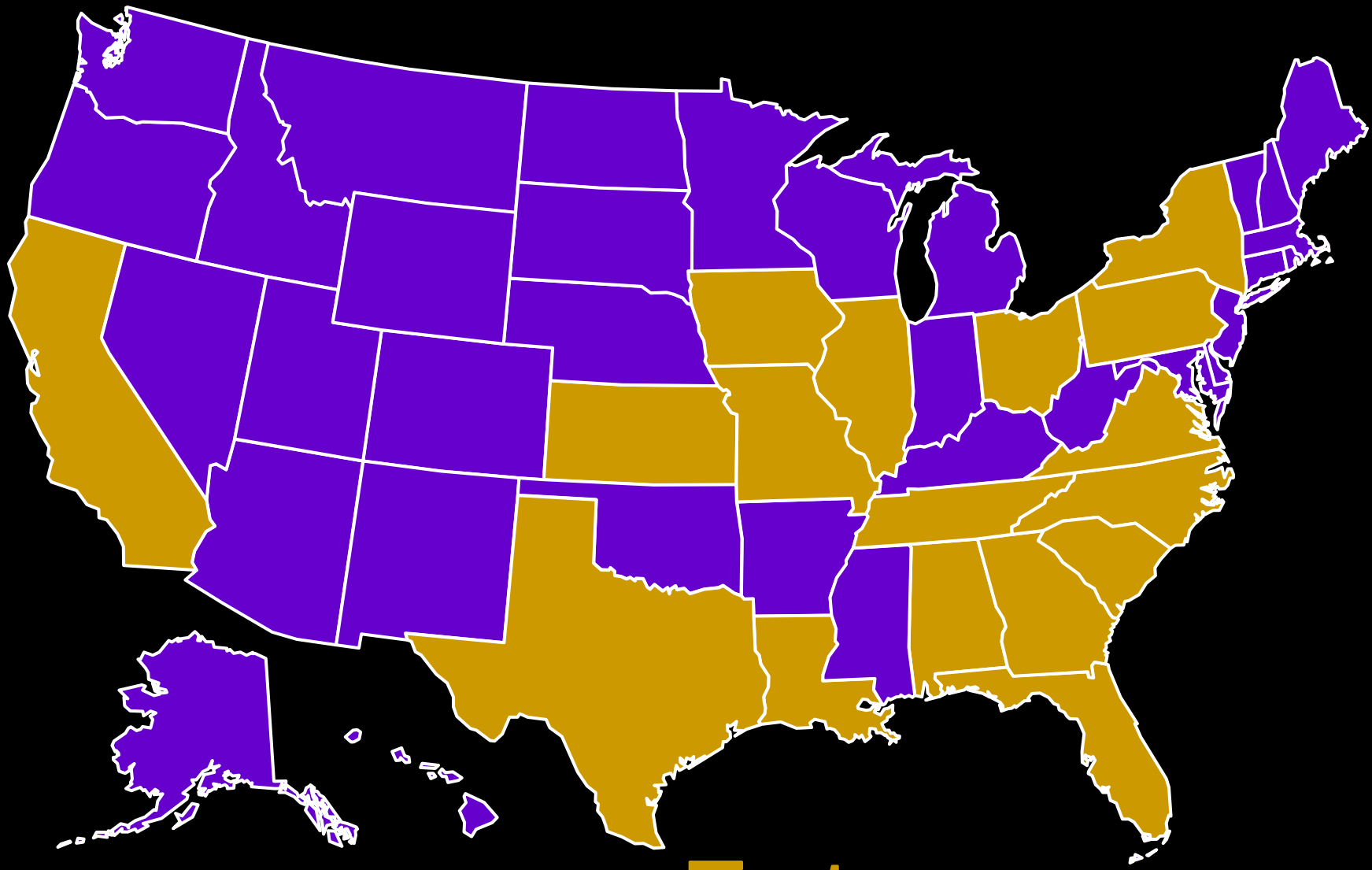




B1,B2

B1,B2,B5

B1,B2,B5,BP


Esterases

LightCycler Data Analysis (wgb1) - [Quantification: BU080201; F1; BUL-BUR seg 3]

File Quantification Report Window Help

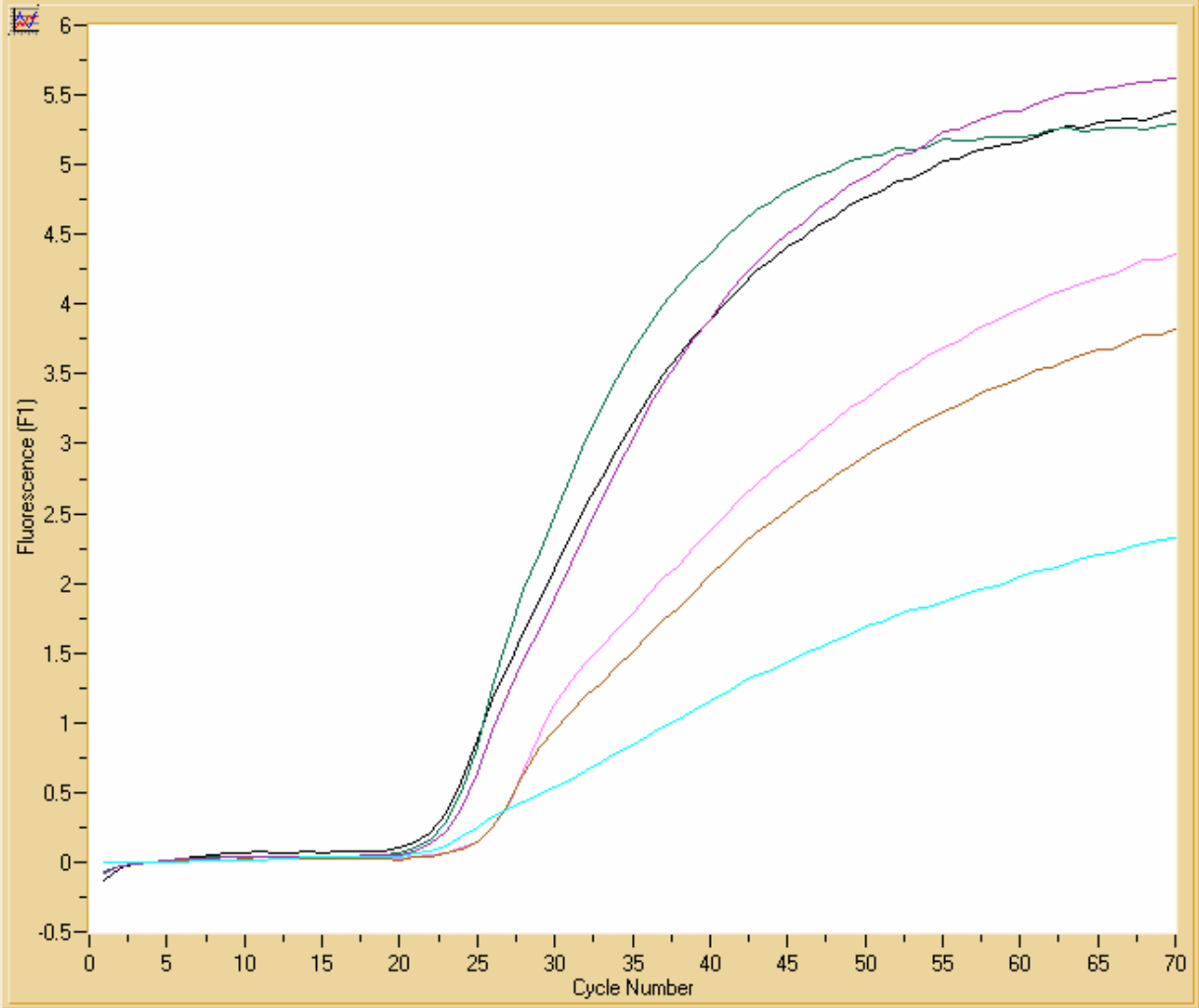
Analysis
- Fit Points
- Second Derivative Maximum

Baseline Adjustment
- None
- Arithmetic
- Proportional
- Normalized

Analysis Notes

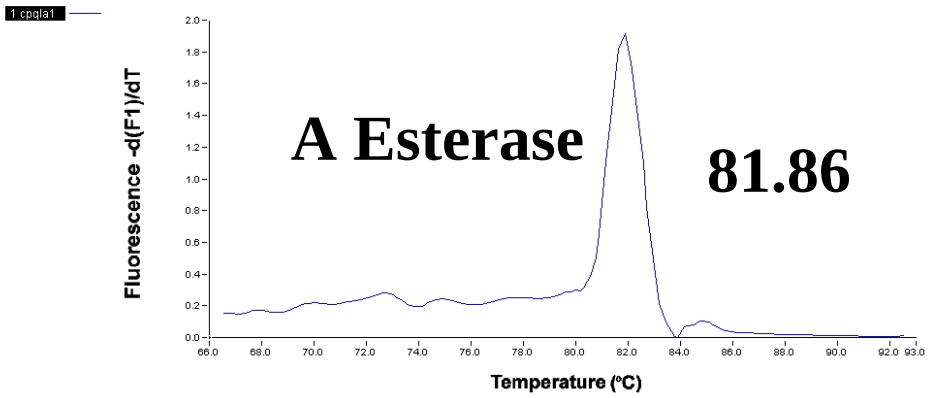
P...	Name	Calculat...	Cro...
1	BRTAR1	0.000E+00	22.76
2	BRTAR2	0.000E+00	23.20
3	BRTAR3	0.000E+00	23.06
4	BRTAR4	0.000E+00	22.13
5	BRTAR5	0.000E+00	23.49
6	BRTAR6	0.000E+00	22.83
7	BRTAR7	0.000E+00	23.14
8	BRTAR8	0.000E+00	23.49
9	BRTAR9	0.000E+00	22.87
10	BRTAR10	0.000E+00	22.79
11	CTL1	0.000E+00	22.94
12	CTL2	0.000E+00	22.49
13	CTL3	0.000E+00	22.89
14	CTL4	0.000E+00	23.28
15	CTL5	0.000E+00	25.24
16	CTL6	0.000E+00	24.10
17	CTL7		
18	CTL8	0.000E+00	21.29
19	CTL9	0.000E+00	23.17
20	CTL10	0.000E+00	24.71
21	NEG	0.000E+00	21.83

Step 1: Baseline | Step 2: Analysis



Start | Microsoft ... | Front | LCDA | 6:23 PM

File: K:\GROUP\WGBDATA\LightCycler Data\AU072502\AU072502.ABT Program: MELT Run By: Administrator
Run Date: Jul 25, 2002 10:58 Print Date: September 12, 2002

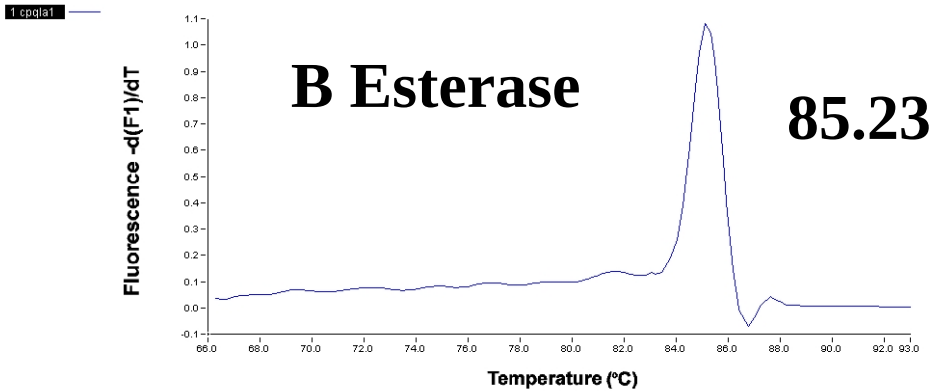


Digital Filter: Enabled Calculation Method: Polynomial

Degrees to Average: 4.0 Peaks to Fit: 0 Weighted Fit: Enabled

Color Compensation: Off

File: K:\GROUP\WGBDATA\LightCycler Data\Bu072402\BU072402.ABT Program: MELT Run By: Administrator
Run Date: Jul 24, 2002 15:35 Print Date: September 12, 2002

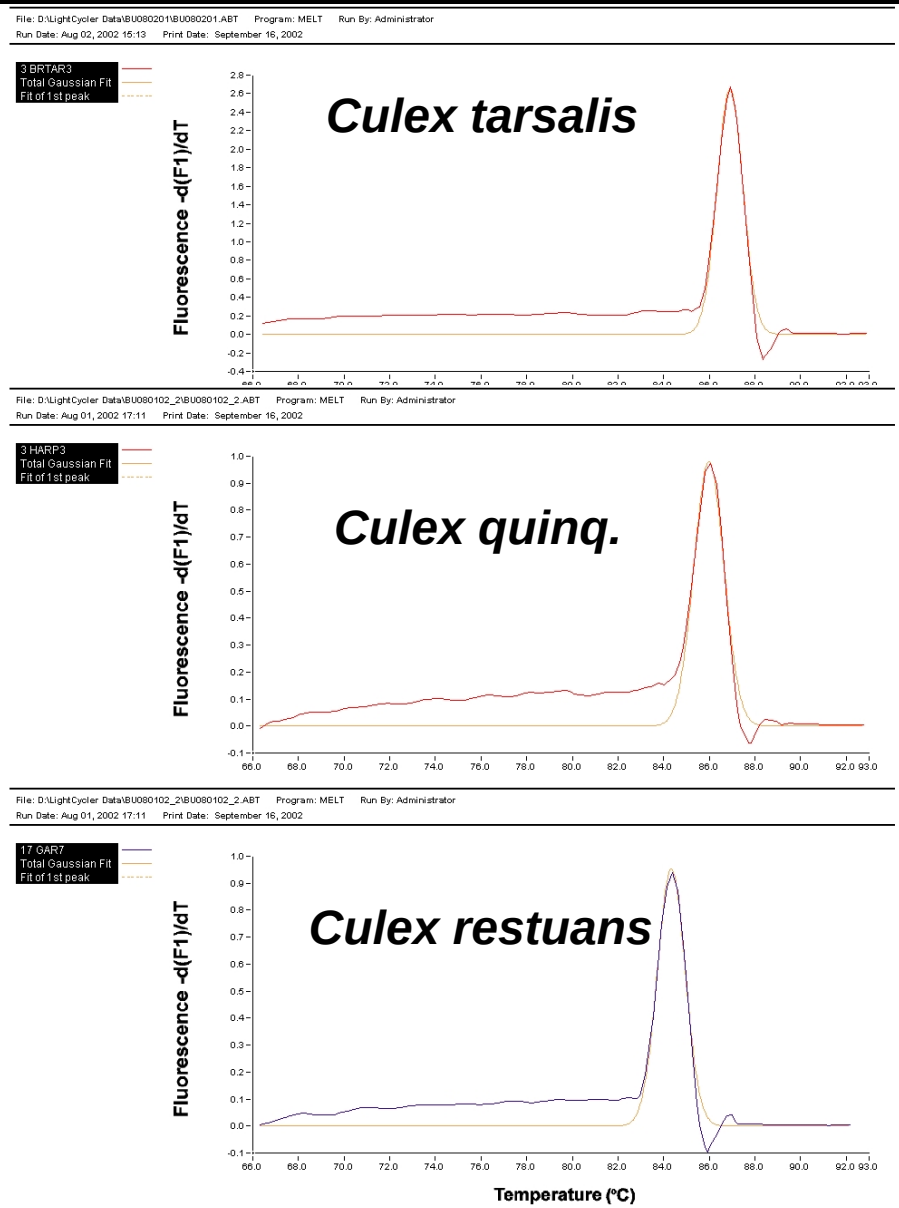


Digital Filter: Enabled Calculation Method: Polynomial

Degrees to Average: 4.0 Peaks to Fit: 0 Weighted Fit: Enabled

Color Compensation: Off

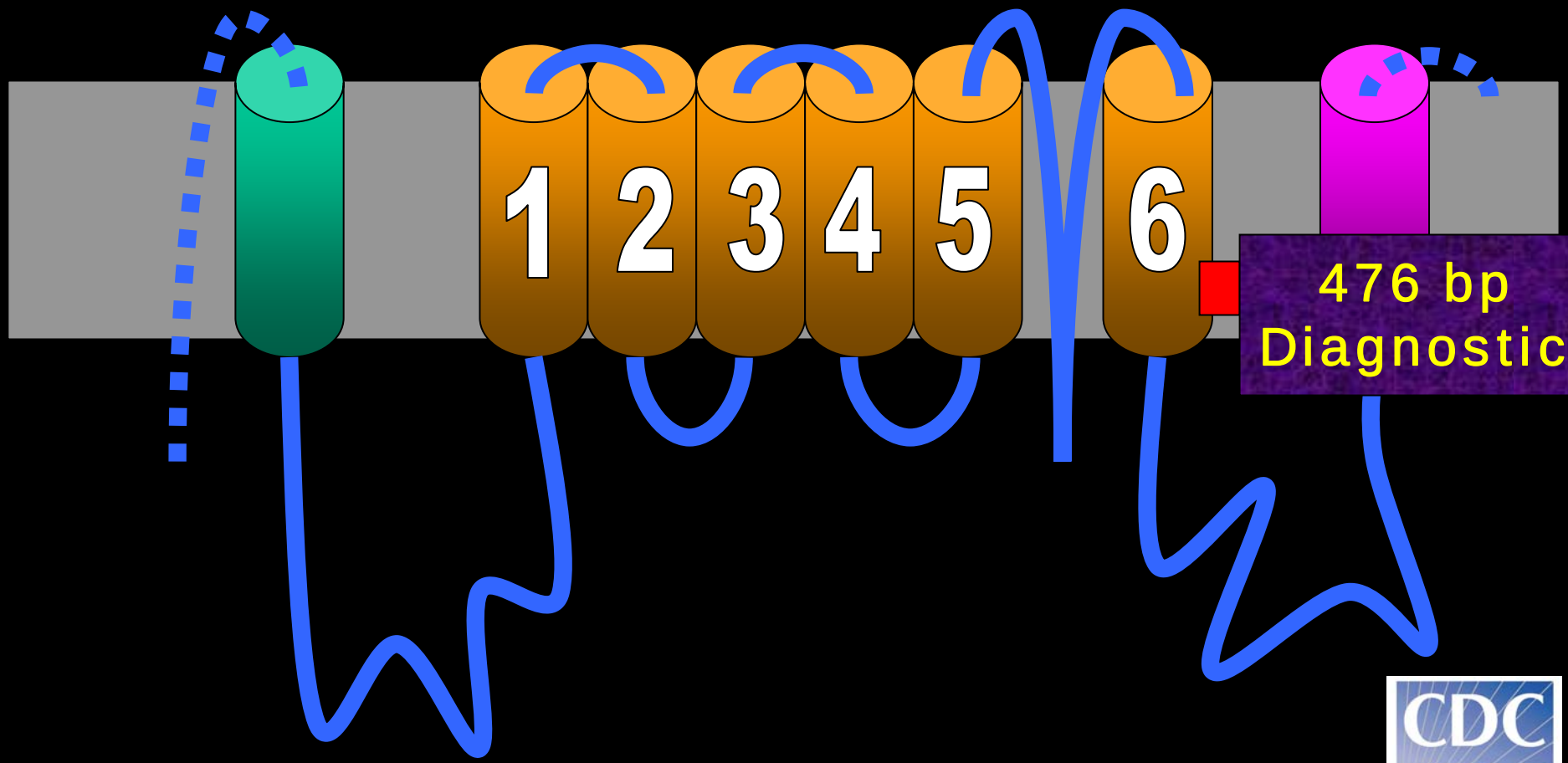
CDC
SAFER·HEALTHIER·PEOPLE™



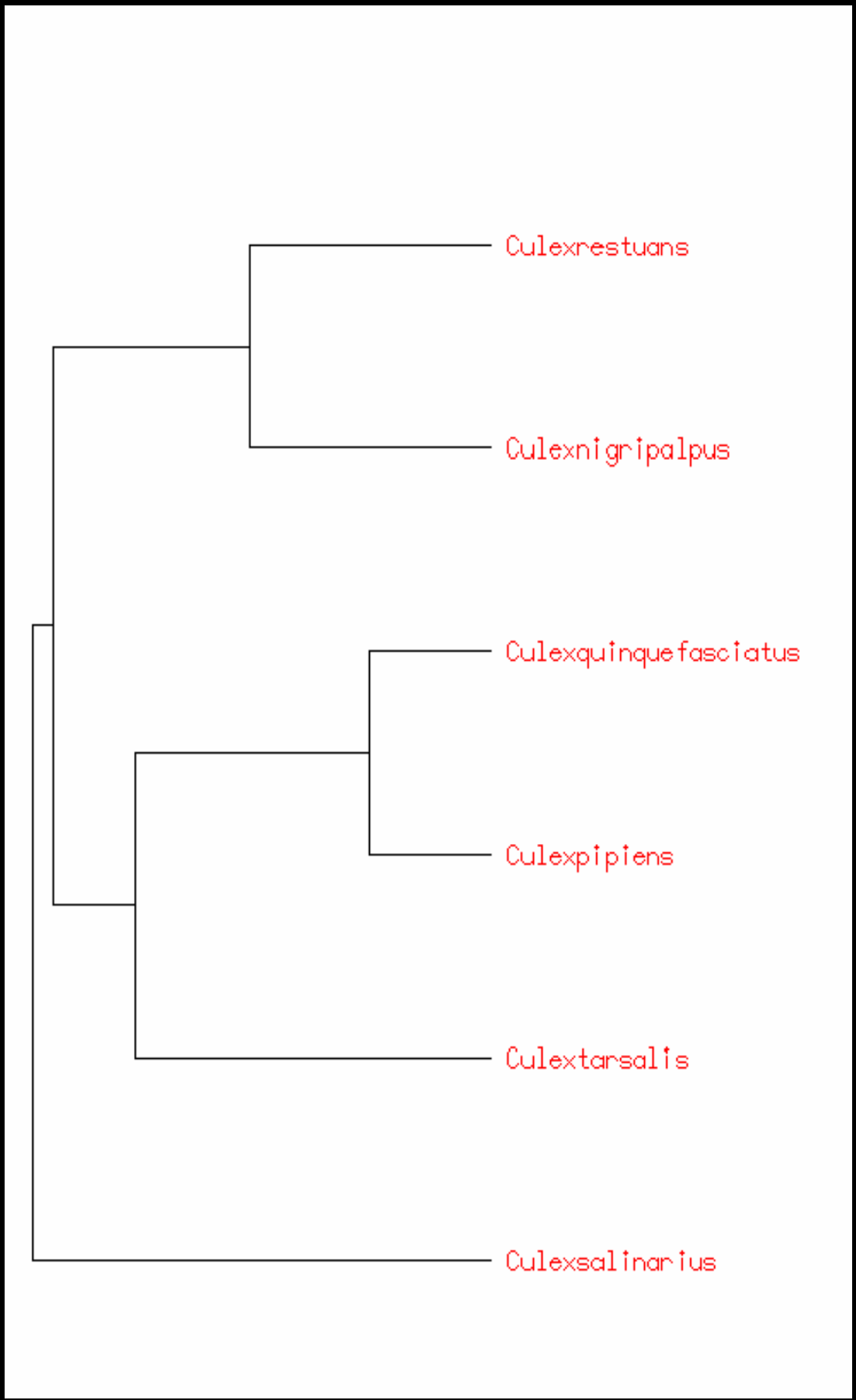
Esterase B

CDC
SAFER·HEALTHIER·PEOPLE™

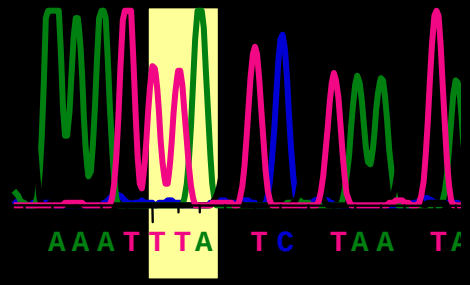
Diagnostic region for *Culex* point mutations in the sodium channel gene :



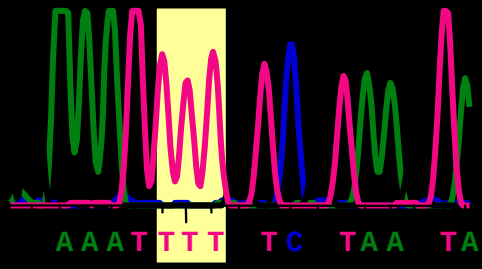
```
     1                                                        50
res   TTAGTCGTGA GTAT...... .......... .TCCAGCG.. ........TG
pip   TTAGTCGTGA GTAT...... .......... .TCCAGTG.. ........TG
qnq   TTAGTCGTGA GTAT...... .......... .TTCACC... .........G
nig   TTAGTCGTAA GTAA...... .......... .TCAAGC... ........AG
sal   TTAGTCGTAA GTATTGCTAG TGGGGCCAAG ATCCAACGGC CCGGAAGAAG

      51                                                      100
CK30  AAGTCT.... .TCGTGATTG A...TCTAGT GTGCGCGCTA GAGCTGTCAA
CK02  AAATCT.... .GAGGGATTG A...TCTAGT GTGCACATTA GAGCTGTCAA
CK29  GAGTCT.... .TGGAAA... .......... .TGAACATAA GAACTGTCAA
CK13  GAATCT.... ...GAGAT.. ....TCAAGT TTGTATAGTA GAACTGTCAA
CK09  GAGTCTGTAT GGAGGGGGTG AAATTCAAGT GTGAATTGTT GAACTGTCAA

      101                                                     150
CK30  AACATCGCCA ACAGCATGCA AGAAAAGGTG GGAACGAAAA ACTTTAAGGT
CK02  AACATCGCCA AGAGCATGCA A.TAAAGGTA AGCACGAAAA ACTCTAAGGT
CK29  AACATCGCCA AGAGCATG.T AGGAAAGGTG GGAACGAAAA ACTTTTAGGT
CK13  AACAG.GTCA A.AACTTCTG AAATTCCGAA ATTTCAAAAT TCCAAAATTC
CK09  AATAACGTCA AGATTGTGTA AG.AAAGGTG AGC...AGAA TCATGAAACT

      151                                             193
CK30  CACATTTGTA CCTTTGATGT AAACAAACAG TTC~~~~~~~ ~~~
CK02  AACATTTGCA CCTTTGATGT AAACAAACAG TTC~~~~~~~ ~~~
CK29  AACATTTGAT CTTTGGATGT AAACAAACAG TCA~~~~~~~ ~~~
CK13  CAAAATTCCA AAATTCCAAA ATTCCAAAAT TCCAAAATTC CAA
CK09  CGCA~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
```


Culexrestuans
Culexnigripalpus
Culexquinquefasciatus
Culexpipiens
Culextarsalis
Culexsalinarius

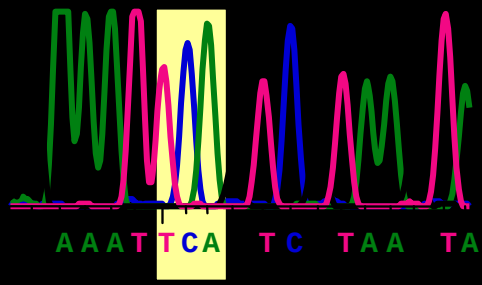
Leucine TTA - S

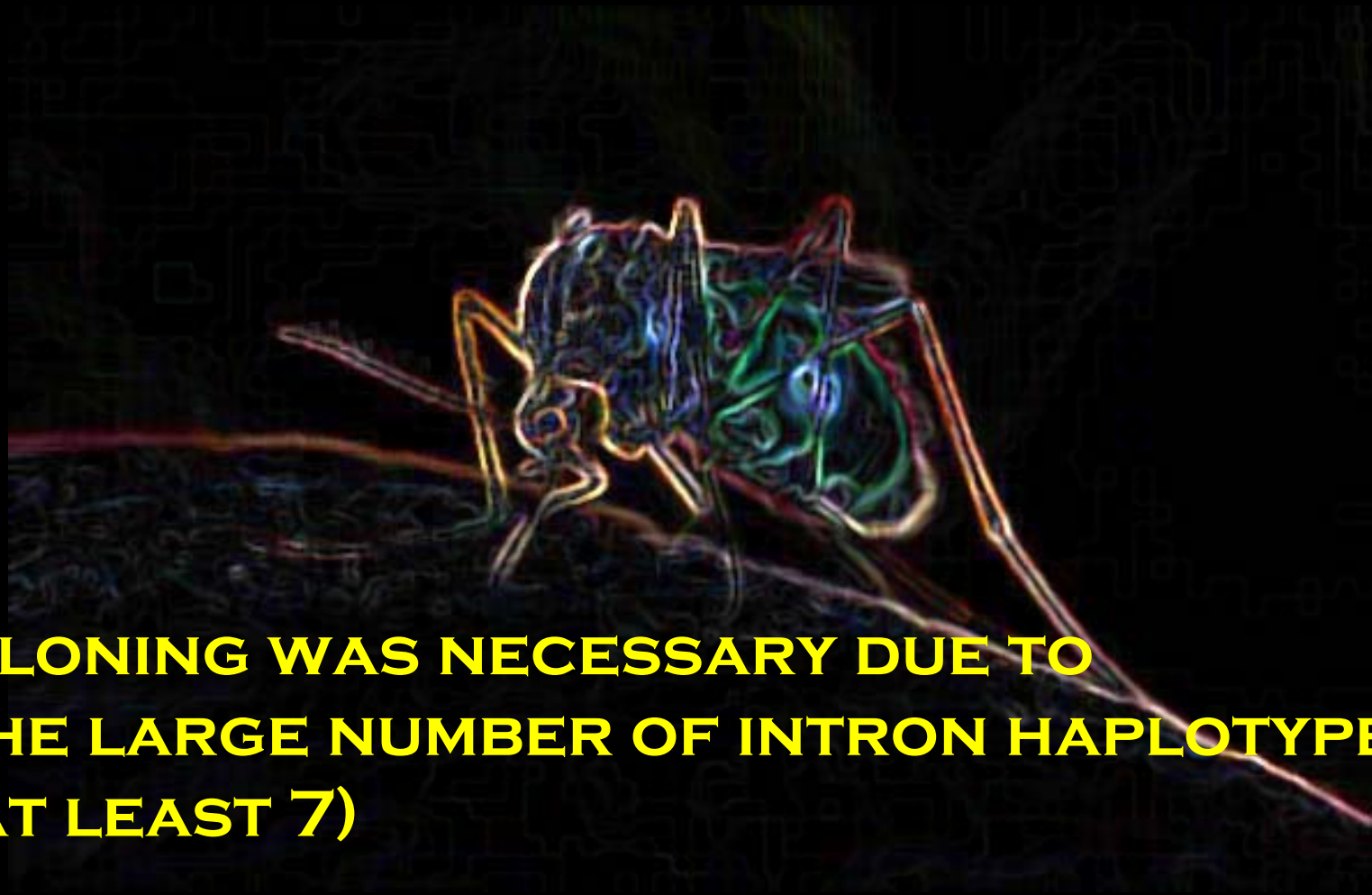


Phenylalanine TTT – R1



Serine TCA – R2





Cloning was necessary due to
the large number of intron haplotypes.
(at least 7)

The kdr mutations in different
populations are associated with
different intron haplotypes.

-TAGCGA-

-TCGTGA-

-TTTTAA-

-AAGAGA-

-TGGAAA-

-GAGGGA-

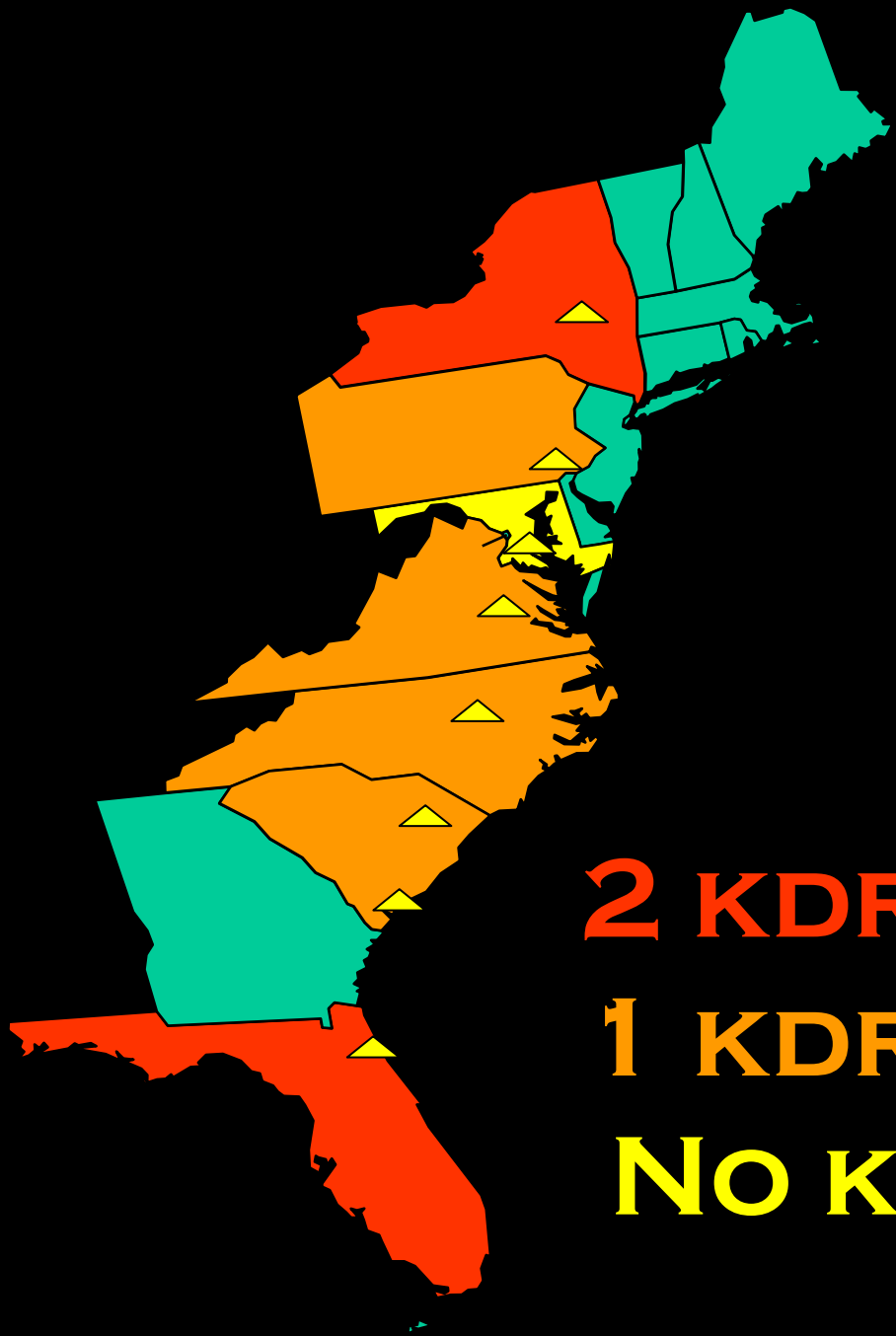
-TTGAGT-

-TAGCGA-

	TTT	TCA
Albany	100%	100%
Columbia	100%	--
Jacksonville	20%	90%

-TCGTGA-

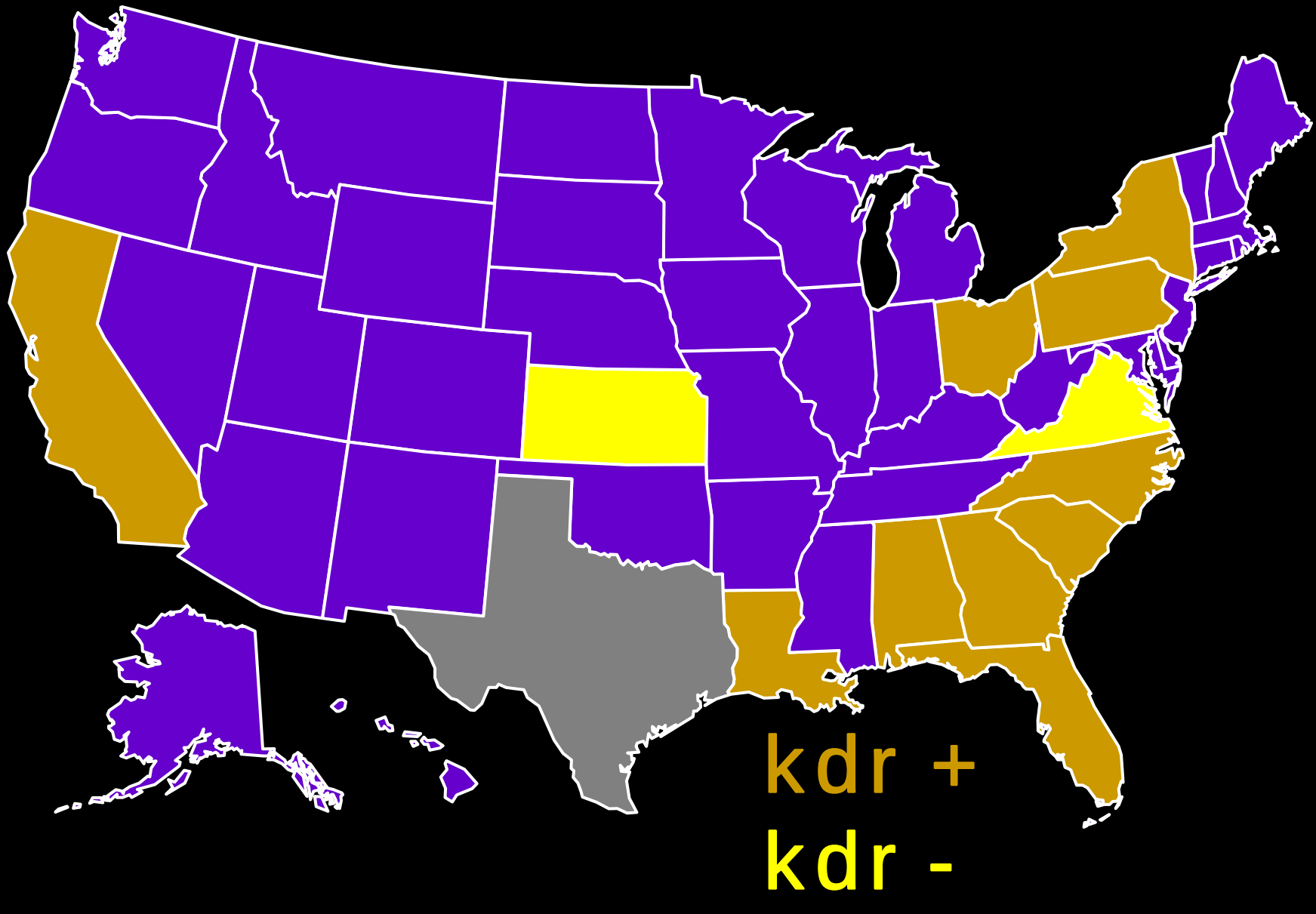
Albany	0%	0%
Columbia	0%	--
Jacksonville	80%	10%



2 kdr mechanisms

1 kdr mechanism

No kdr

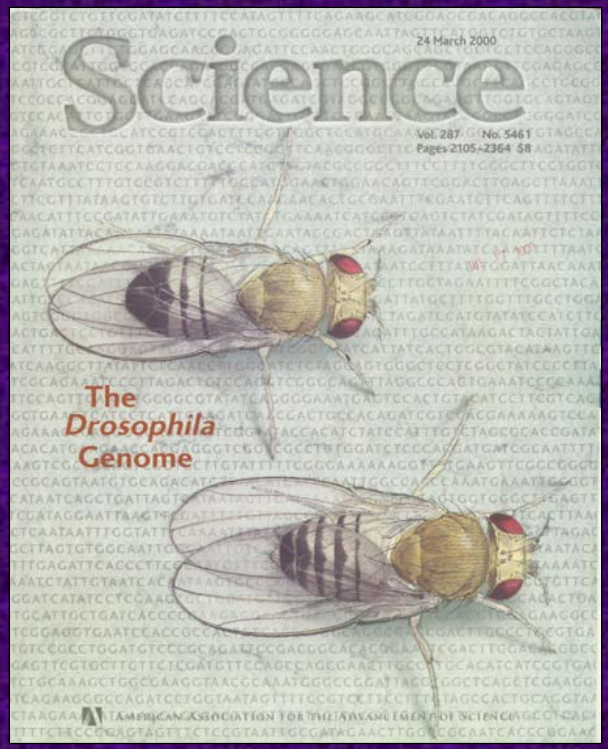

kdr +
kdr -

Upregulated Genes

Oxidases
Glutathione s-transferases

Enhanced Oxidase Activity

P450 Oxidase Polymorphism



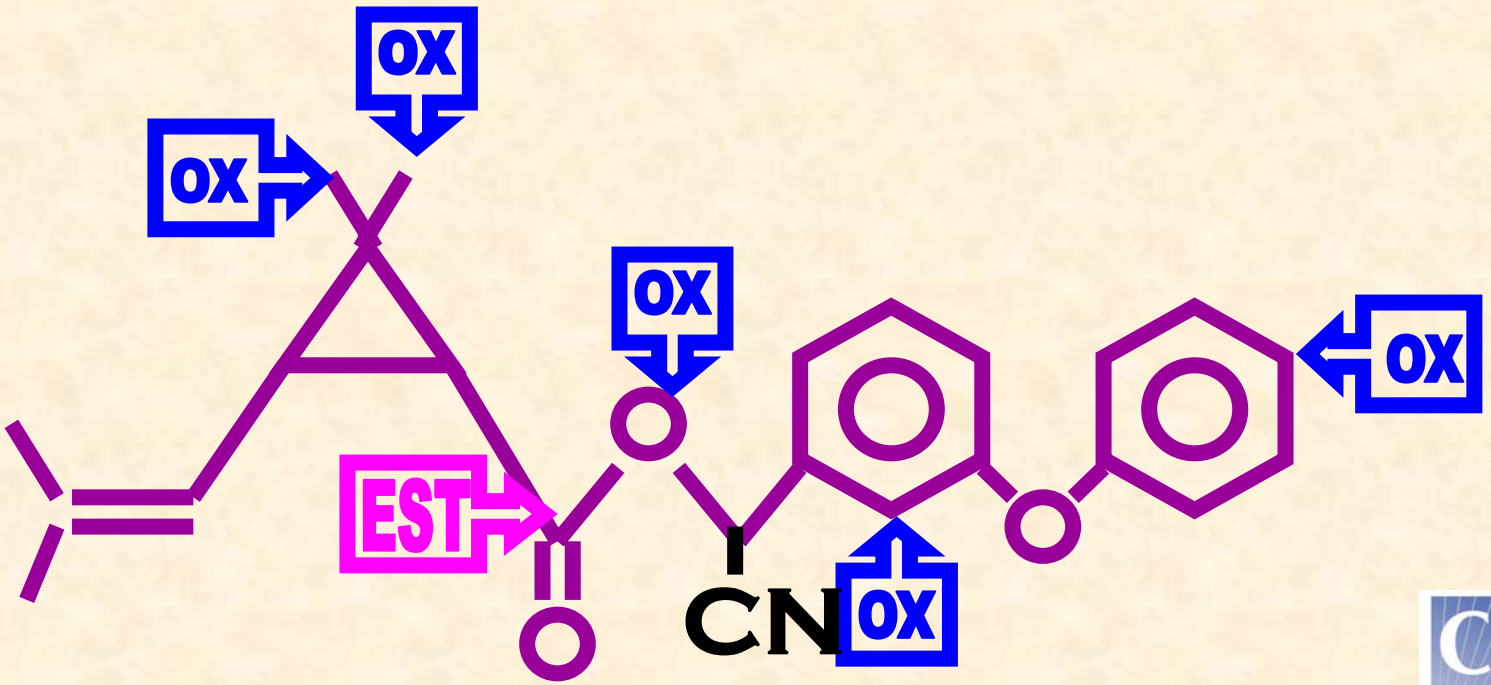
Drosophila
80 P450 genes

Anopheles gambiae
111 P450 genes

Culex pipiens **– 52 P450 genes thus far.**

CDC
SAFER · HEALTHIER · PEOPLE

Detoxification enzyme attack on a synthetic pyrethroid:



```
  1  MFAWIICAAA AVPLVYFLIV YQFSYWKRRG ITQLTPSFPF GDLGPFFRQR
 51  SSLGVVYADV YRLCKRLPFV GIYLSLRPML VVNDPELIKN VLVRDFDHFH
101  DRGLYVNEEK DPLSGHLFAL GGEQWRHHRS KLTPTFTSGR LKEMFTNLVQ
151  IGRVLQDHVA KRAGEDIEIR DVMARYTTDI IASVGFGIEN DSINEKGNIF
201  REMGTKVFSP DLKTILRLTS TFFTPKLNAL FGFKFIAQEI EDFIMNVVRE
251  TLEYRESNKV VRKDMMQLLM QLRNSGTVSI DDRWDIEVST NKKKLSLEQV
301  TAHAFVFFIA AYET SSTTIS FCLFELARNP EIQKKVQQEI DQVLASHNGE
351  ITYDNINEMK YLENCIDETL RKYPAVPFLN RECSKDYKIP GTDTTIEKGT
401  SLVIPVLGLH RDPDHYPEPD RFIPERFSNF EDISTKPYLP FGAGPRNCIG
451  LRLGKLQTKA GLVMMLSKFN VRLADETYAS KELALDARSV VLMPVGGIKV
501  SISERRAS*Y KLK*LVI*YL NITINDTKIN YL
```

Anopheles gambiae

D P E F Y P E P D Q F N P D R F M P E E
D P E Y Y P E P D Q F N P D R F L P E E
D P E F Y P E P D Q F D P D R F L P E E
D P E Y Y P E P E R F D P D R F L P E E
D P E F Y P E P D R F D P D R F L P E E
D P E Y Y P E P D R F D P D R F L P E E
D P E Y Y P E P D R F N P D R F L P E E
D P E Y Y P E P E R F D P E R F L P E E
D P E Y Y P E P D Q F D P D R F L P E E
D P D Y Y P E P D R F D P D R F V P E E
D Q E Y Y P E P D R F N P D R F L P E E
D Q E Y Y P D P E R F D P D R F L P E E
D P E Y Y P D P E R F D P D R F L P E E

Culex quinquefasciatus

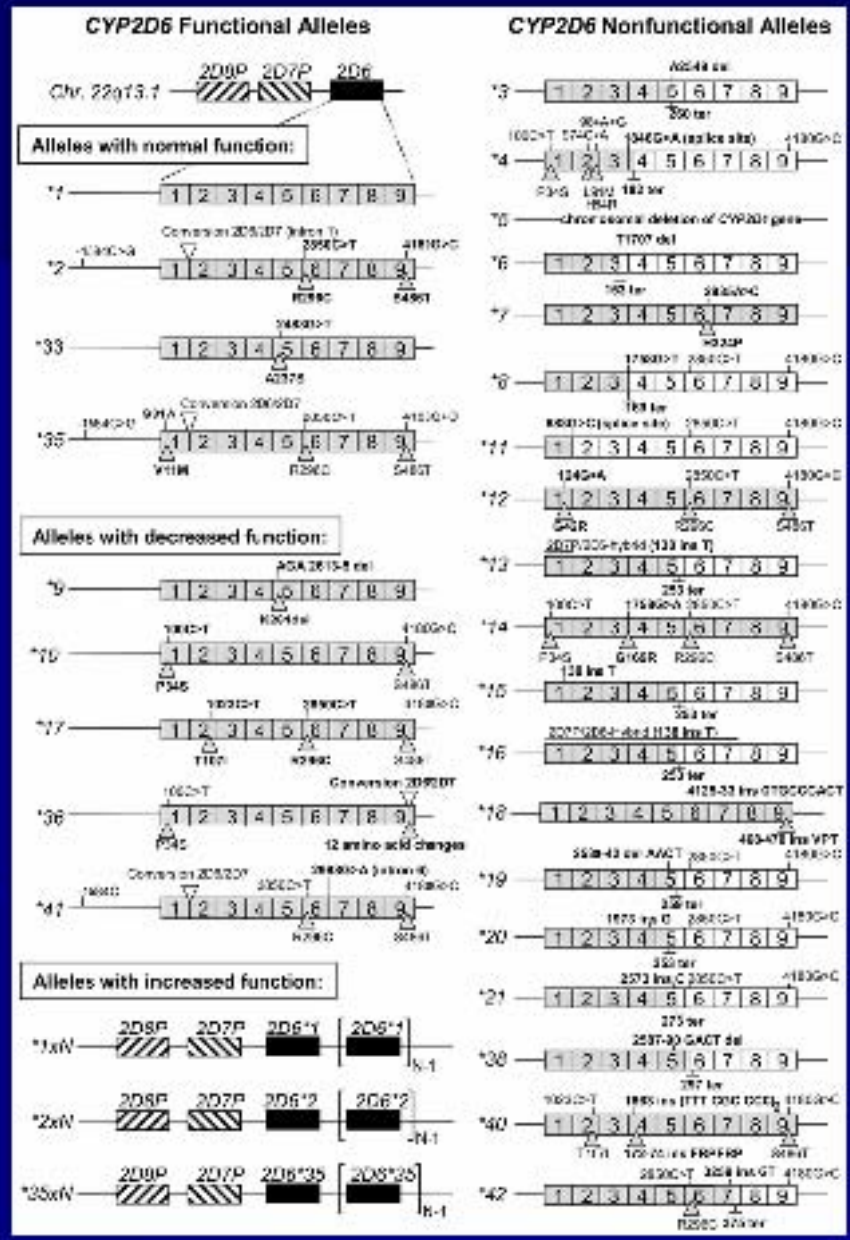
D P D H Y P E P D R F I P E R F S N F E
D P D I Y P N P E V F N P E R F I P E L

Culex pipiens

D P E Q F P D P E R F D P D R F L P E H
D P D Q F P D P E R F D P D R F L P E S

Alleles of *CYP2D6*

- More than 80 known
  - SNPs
  - INDELs
  - Complete gene deletion or duplications
- Clinical significance of most unclear



Zanger UM,et al. Naunyn Schmiedebergs Arch Pharmacol 2004

Cytochrome P450s (CYPs)

Genetic variants are associated with altered drug levels, but not with disease

- CYP2D6: estimated to metabolize 25% of all prescribed drugs
- CYP2C9: 5%
- CYP2C19: 15%
- CYP3A4/5: 50%

Examples of drugs metabolized by

CYP2D6

- Antiarrhythmics
- Antipsychotics
- Antidepressants
- Opiates
- Selective serotonin reuptake inhibitor (SSRI)

CYP2C9

- Anticonvulsants
- Antidepressants
- Hypoglycemics
- Anticoagulants
- Antibacterial
- Cancer Chemotherapy
- Nonsteroidal anti-inflammatory drugs

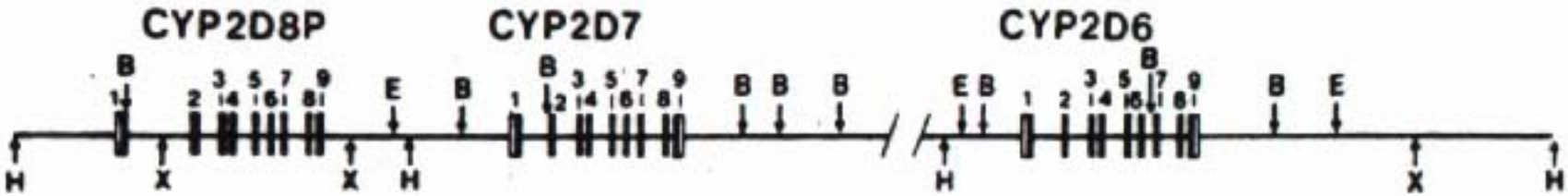
CYP2C19

- Anticonvulsants
- Antidepressants
- Anti-ulcer
- Antimalaria
- Cancer Chemotherapy
- Proton pump inhibitors

Example: Antidepressants and CYPs

Brand Name	*Generic Name*	*Mechanism*	*CYP Metabolism*
Effexor	Venlafaxine	*mixed*	*CYP3A4, CYP2D6, CYP2C9, CYP2C19*
Wellbutrin	Bupropion HCL	*mixed*	*CYP2B6, CYP2D6, CYP1A2, CYP3A4 +*
Desyrel	Trazodone	*mixed*	*CYP3A4, CYP2D6*
Zoloft	Sertraline	*SSRI*	*CYP3A4, CYP2D6, CYP2C9, CYP2C19*
Prozac	Fluoxetine	*SSRI*	*CYP2B6, CYP2C9, CYP2C19, CYP3A4+*
Celexa	Citalopram	*SSRI*	*CYP3A4, CYP2C19, CYP2D6*
Lexapro	Escitalopram	*SSRI*	*CYP3A4, CYP2C19*
Elavil	Amitriptyline	*TCA*	*CYP3A4, CYP2D6, CYP2C19, CYP2B6+*
Norpramin	Desipramine	*TCA*	*CYP2D6, CYP1A2*
Sinequan	Doxepine	*TCA*	*CYP2D6, CYP1A2, CYP3A4*
Pamelor	Nortriptyline	*TCA*	*CYP1A2, CYP2D6, CYP2C19, CYP3A4*
Anafranil	Clomipramine	*TCA*	*CYP2D6, CYP1A2, CYP2C19, CYP3A4*

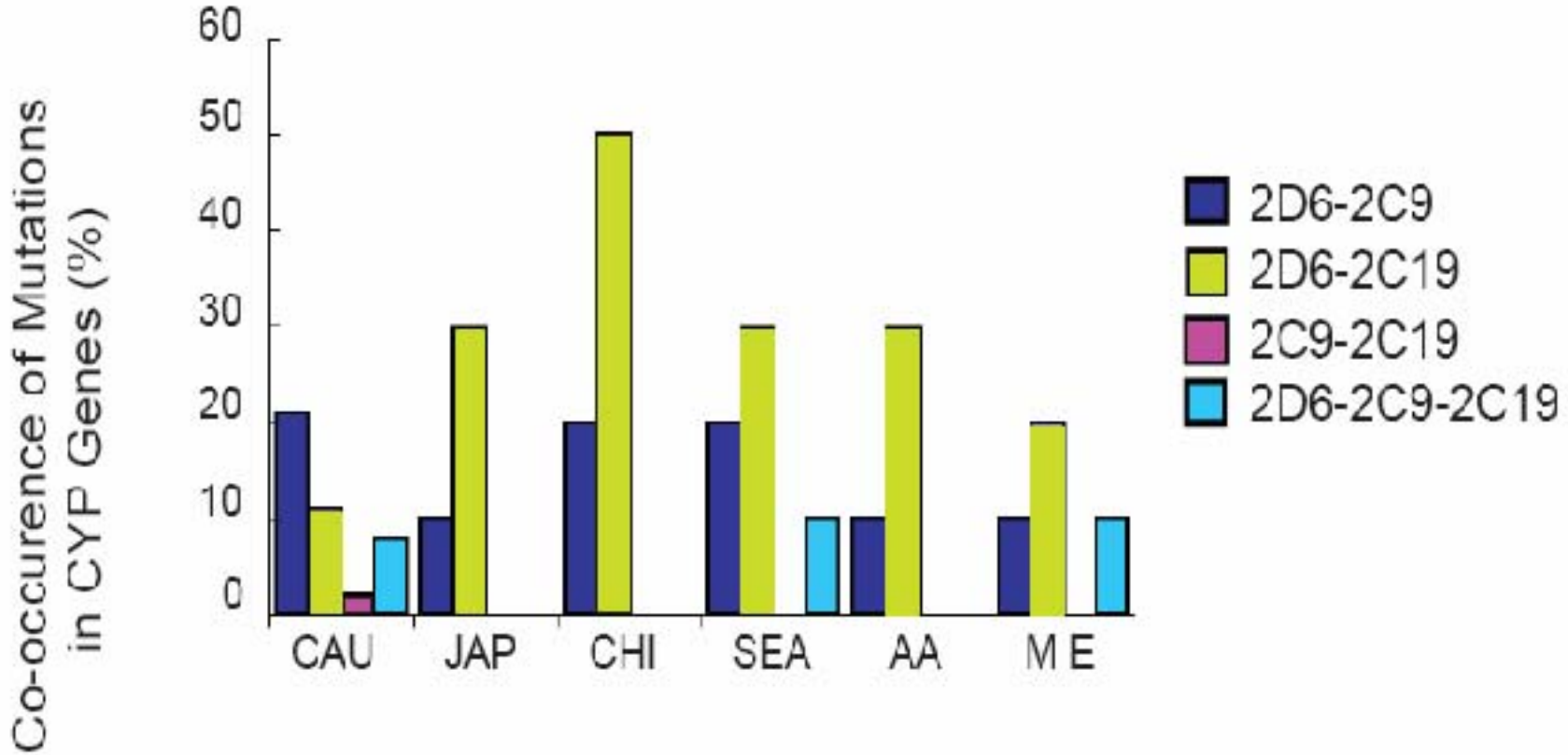
CYPs: Analytical challenges



Lancet 1990; 336: 529-32

- Superfamily/Pseudogenes:
  - 57 genes, 33 pseudogenes
- Gene dose important
- Many SNPs are common among alleles
- Many rare variants
- Importance of haplotypes not known

Co-occurrence of mutations in 2D6, 2C9, 2C19



GSTs

Insect specific

Delta, epsilon classes

gstE2 DDT, Permethrin resistance in *Aedes aegypti*

More widely distributed

Theta, sigma classes


QUESTIONS ?