Insecticide Resistance and Population Assessment in Malaria Entomology

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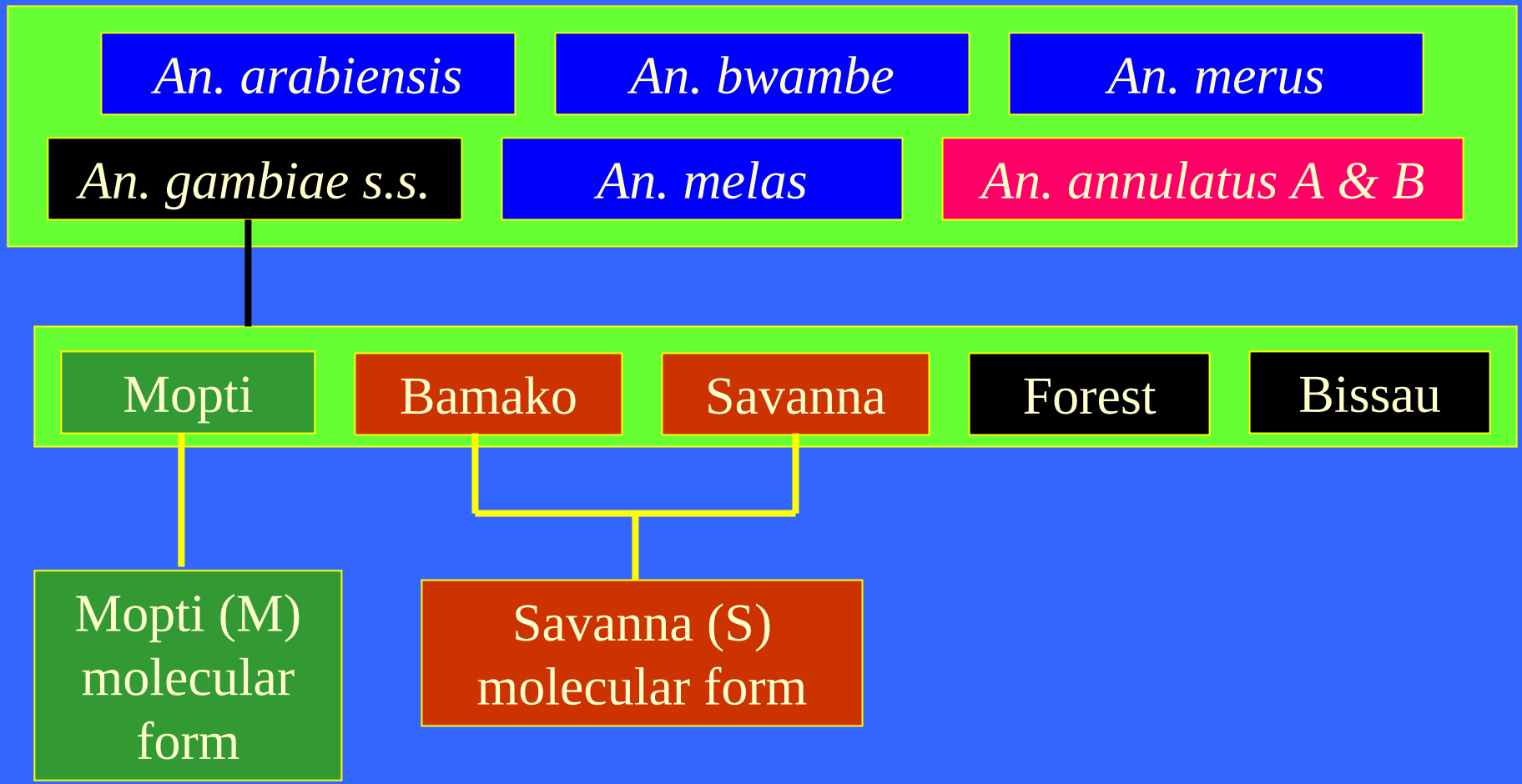
Structuring of *An. gambiae* s.s. Populations

- Potential barriers to gene flow
  - Premating - mixed swarms
  - Postmating - hybrid survival
    - Infertility
  - Polyandry - sperm competition

Levels of Speciation in *Anopheles gambiae* s.l. Complex

- *Anopheles gambiae* s.l. (1956)
  - *An. arabiensis* – more zoophilic and exophilic
  - *An. gambiae* s.s. - ***
    - Bamako, Mopti, Savanna
    - Forest, Bissau
  - *An. melas* – coastal West Africa
  - *An. merus* – coastal East Africa
  - *An. bwambae* – limited distribution
  - *An. quadriannulatus* A and B – zoophilic non-vector

Anopheles gambiae (s.l.) Complex



Complexities of Insecticide Resistance

- Metabolic Detoxification
  - Enzyme metabolism and sequestration of insecticides (Esterases, cytochrome P-450s)
  - Transcriptional regulation (GSTs)
    - Esterases in Culex
    - Monooxygenases in a variety of insects
- Target-site Mutation Derived Resistance
  - Mutations aimed at insecticide target site

Metabolic Detoxification

- Very important to mosquito control
  - Difficult to assess
  - Many potential enzymes involved
  - Mechanisms poorly understood (mutations ?)
- Detecting resistance
  - WHO bottle assays
  - Biochemical tests (color-metric assays)
  - Molecular assays (PCR-based, hybridization assays)

kdr Resistance in *An. gambiae*

- Cross resistance to pyrethroids and DDT
- Mendelian inheritance; resistant (RR), susceptible (SS), intermediate (RS)
- Single point mutation conferring amino acid change in the voltage-gated sodium channel

(Martinez-Torres et al. 1998)

kdr Resistance in *An. gambiae* and others (cont.)

- Single point mutation changing leucine TTA to phenylalanine TTT – confers high levels of resistance
- Physically mapped to 2L 20C – in range of resistance associated QTL, but unclear if GST related
- *An. gambiae* from Côte d'Ivoire and Burkino Faso
- Same mutation in *Musca domestica*, *Blatella germanica*

kdr Resistance in *An. gambiae* and Others (cont.)

- Single point mutation changing leucine TTA to serine TCA – **confers low levels of resistance**
- *An. gambiae* from Kenya
- *Culex pipiens* from China

Distribution of *kdr* Resistance

- In parts of West Africa widely distributed *An. gambiae s.s.* Savanna form (Chandre et al. 1999)
- As of 2000 only found in *An. gambiae s.s.* Mopti form in Benin (Weill et al. 2000)
- *kdr* stably transferred from Savanna to Mopti in laboratory
- Currently found in M and S forms from multiple sources
- In Bioko only found in M form, not S

Transgenic Replacement Strategy

“The goal is to replace a wild mosquito population, efficient at transmitting malaria parasites, with a population that is virtually identical in all respects except for its ability to transmit the parasites.”

- Frank Collins & Anthony James, 1996

Requirements for Strategy Implementation

- Discovery of genes that block Plasmodium development in the mosquito but do not adversely affect fitness
- Development of techniques to manipulate the mosquito genome
- Development of a genetic drive mechanism
- Elucidation of the ecology and genetic structuring of *An. gambiae*

Getting Refractory Genes or Alleles into Mosquitoes (Transformation)

- Mostly Class II transposable elements (*Hermes, mariner, Minos*)
- Insert into DNA by recognition of inverted repeat (ITR) sequence at the termini of the element, excise target DNA and integrate element
- Exogenous ITRs are engineered into system to control insertion

Where Should Integration/Expression Occur

- Integration of exogenous gene so that expression (when, where and how much) is controlled by an endogenous promoter
- Development has targeted tissues where significant pathogen / mosquito interaction occurs

Where Should Integration/Expression Occur II

- Targeted tissues: midgut, salivary glands and fat body
- Therefore target promoters of genes with specific expression in these tissues (i.e. *Apyrase* in adult salivary glands)

Refractory Genes

- Midgut mis-recognition
- Melanotic encapusulation
- Intracellular lysis
- Large variation in refractory phenotype (and transcriptional response to infection) confirm that there is a physiological cost to parasite infections in the mosquito

Antiparasite Effector Molecules

- Single-chain antibodies (i.e. made to CSP) – worked following injection
- Theory proven by flavivirus RNA*i* in mosquitoes (*Ae. Aegypti*)
- Inhibition of midgut penetration
- Melanization of oocysts

Molecular Linked Phenotypes of Interest

- Vector competence for *Plasmodium*
- Stability/movement of refractory gene in transgenic/field populations
  - Drive mechanisms

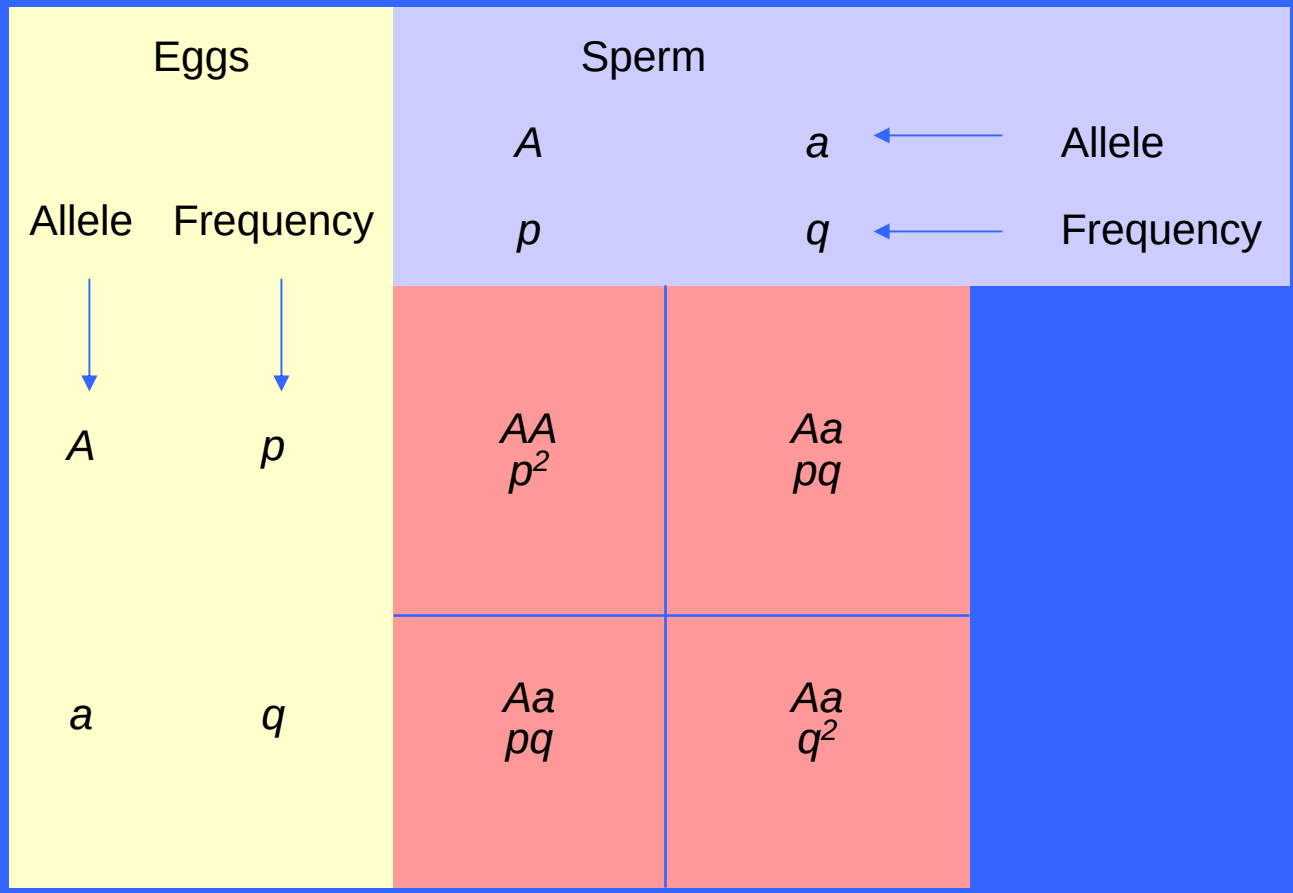
The Basics

- Mendelian genetics
- Phenotype vs. Genotype
- Homology vs. Analogy
- Hardy-Weinberg principle (1908)
  - provides a method for predicting genotype frequencies from observed allele frequencies

Hardy-Weinberg Assumptions

1. Diploid organism
2. Sexual reproduction
3. Nonoverlapping generations
4. Random mating
5. Population is very large
6. Migration is negligible
7. Mutation is negligible
8. Gene(s) under consideration are not affected by natural selection

Deriving the Hardy-Weinberg Equation



$1 = p^2 + 2pq + q^2 = \text{freq}(AA) + \text{freq}(Aa) + \text{freq}(aa)$

Evaluating for H-W Equilibrium

- Usually done using a goodness-of-fit test by means of a chi-squared value (X^2)

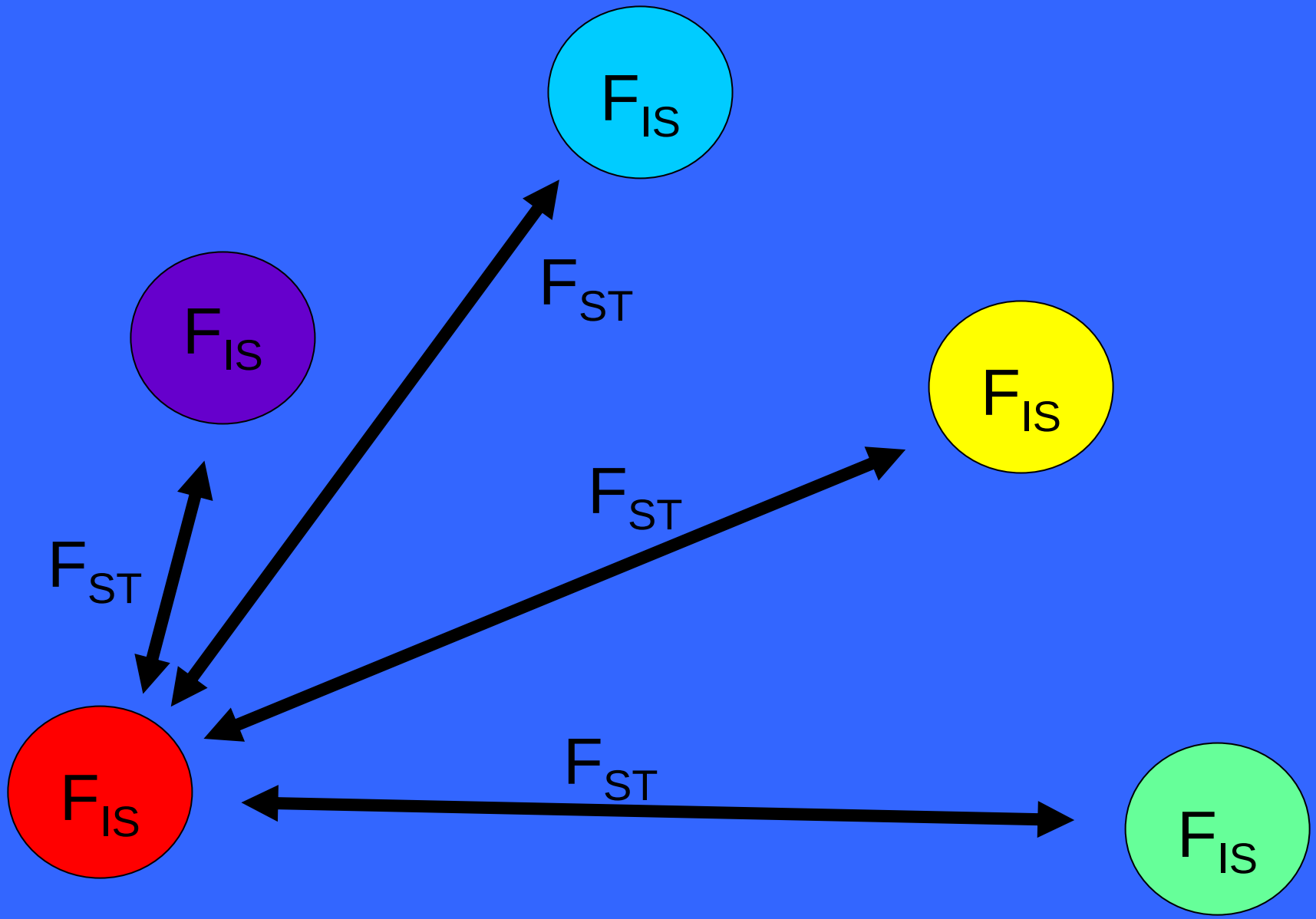
- $X^2 = \Sigma$ (observed number – expected number)^2/(expected number)

Heterozygosity (*H*)

- The frequency of heterozygous loci per individual or heterozygous individuals per locus
- *H* is relatively stable within a population for a defined set of markers independent of sample size (marker dependent)
- Often used to identify restricted gene flow among populations

Wright's F-statistics

- $(1 - F_{IT}) = (1 - F_{ST})(1 - F_{IS})$
- F_{IS} – correlation between homologous alleles within individuals with reference to a local population
- F_{IT} – correlation between homologous alleles with reference to the total population
- F_{ST} – interpreted as the variance component
- $F_I = 1 - (H_{obs}/H_{exp})$


F_{IS}
F_{IS}
F_{IS}
F_{IS}
F_{IS}
F_{ST}
F_{ST}
F_{ST}
F_{ST}

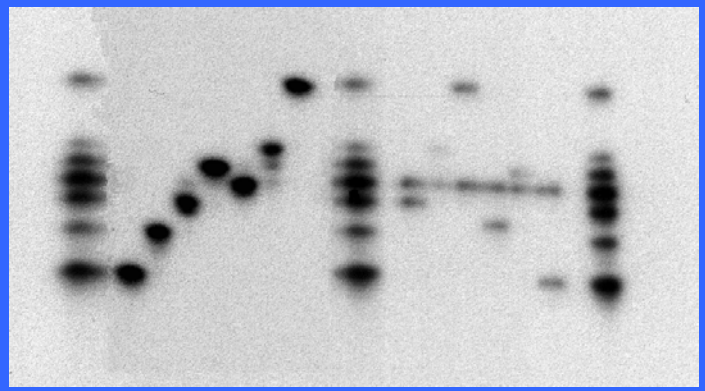
Migration

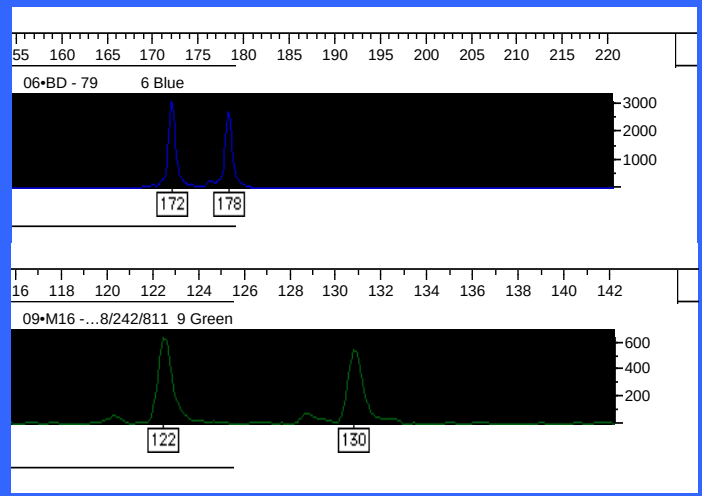
- *Nm* – is the migration frequency or more importantly the frequency of matings between populations due to migration…
- In theory as little as 1 mating between populations/generation is enough to homogenize populations

Microsatellites

DNA extraction and PCR amplify

CAAGGCGTTGACA**GTGTGTGTGTGT**CTTATCACTGCGTC
GTTCCGCAACTGT**CACACACACACA**GAATAGTGACGCAG





Mendelian Codominant Inheritance

CAAGGCGTTGACA**GTGTGTGTGTGTGTGTGTGT**CTTATCACTGCGTC
GTTCCGCAACTGT**CACACACACACACACACACA**GAATAGTGACGCAG

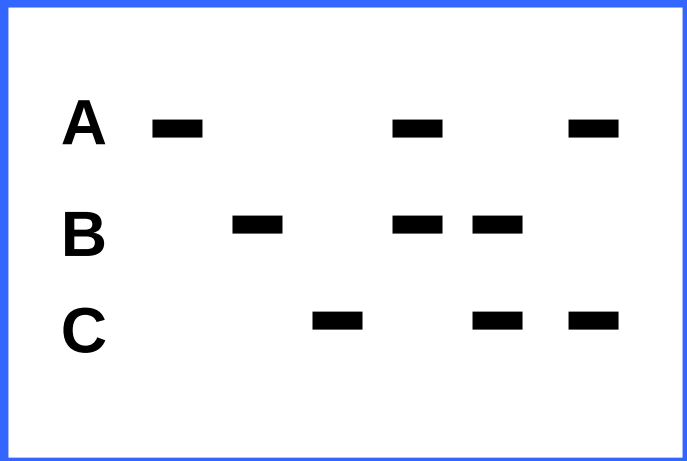
A

CAAGGCGTTGACA**GTGTGTGTGTGTGTGTGT**CTTATCACTGCGTC
GTTCCGCAACTGT**CACACACACACACACACA**GAATAGTGACGCAG

B

CAAGGCGTTGACA**GTGTGTGTGTGTGT**CTTATCACTGCGTC
GTTCCGCAACTGT**CACACACACACACA**GAATAGTGACGCAG

C



So, What About Bednets?

- Rumor is that bednets may select for resistant populations…
- Increased levels of permethrin tolerance noted in Kenyan *An. gambiae* due to selection for increased oxidase and esterase levels (Vulule et al. 1999)
- Increased levels of insecticide [DDT] resistance noted elsewhere in association with bednets… agricultural applications?

Bednets and *kdr* Resistance

- No *kdr* detected in Western Kenya despite extensive use of impregnated bednets (Ranson et al. 2000)
- Despite *kdr*, impregnated bednets may still provide protection (Chandre et al. 2000)
- *kdr* lowers sensitivity to ‘killing’ activity and also to irritant/repellent effects
- Therefore mosquitoes succumb to prolonged contact (3-18 times longer)

The Future of Insecticide Resistance Research

- Will continue to utilize cutting-edge technologies for detection and understanding (Oakeshott et al. 2003)
  - Comparative genomics for finding genes
  - Microarrays for detecting mutations in gene families (i.e. P-450s) – Detox Chip
  - Mapping of genes / traits (QTLs, positional cloning)
- Selective sweeps – no chance for recombination of resistance / susceptibility