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# Malaria Entomology

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# Malaria Facts

- Humans are the only reservoir for the human malarias
- Malaria is exclusively vectored by mosquitoes in the genus *Anopheles*
- 300-500 million cases are but a fraction of the disease burden we would see if all anophelines could vector *Plasmodium*

# Binomial Nomenclature

- Linnaeus 1758

**Kingdom: Animal**

**Phylum: Arthropoda**

**Class: Insecta or Hexapoda**

**Order: Diptera**

**Family: Culicidae**

**Subfamily: Culicinae (>3000 spp)**

**Genus: *Anopheles***

**Species:**

**Subspecies:**

**Tribe:**

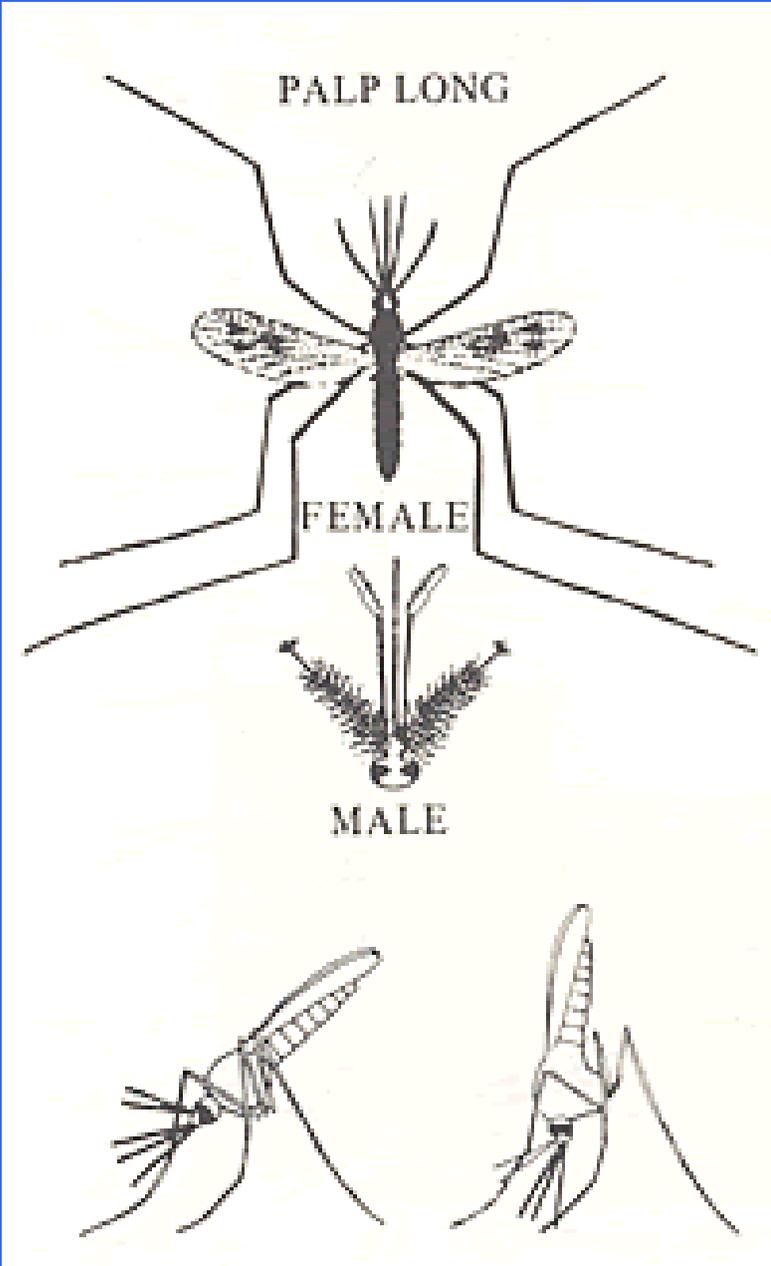
**(C - Forms)**



*Anopheles freeborni*

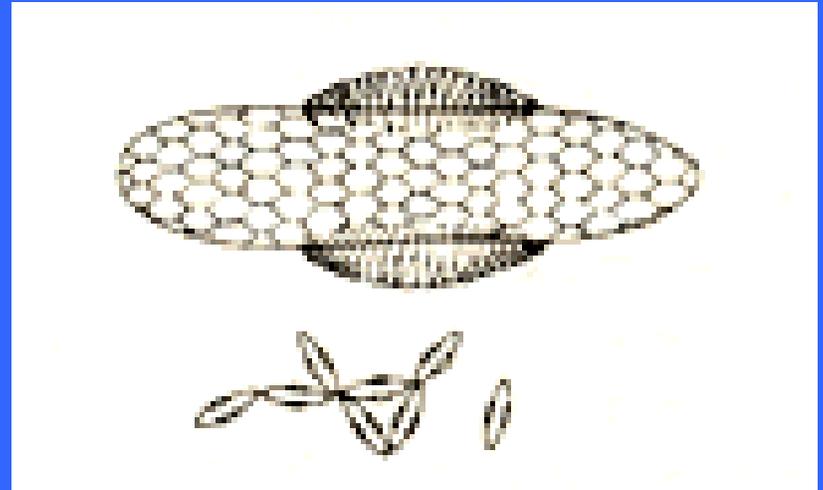
# *Anopheles* Mosquitoes

- 422 species worldwide
  - ~70 vector *Plasmodium*, ~40 considered important
  - Most common in the tropics and subtropics, but also distributed in temperate climates and may extend to summer arctic distributions
  - Phylogenetically distinct from the culicines



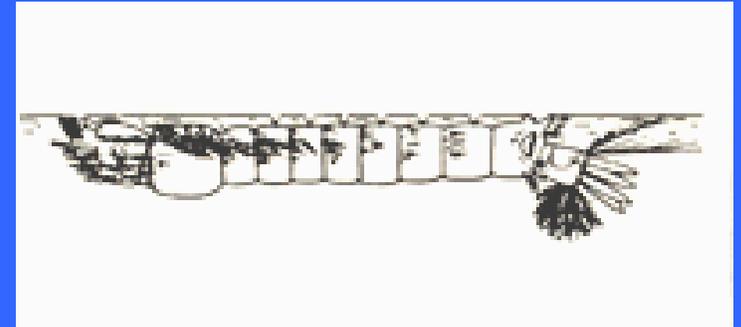
# Immature *Anopheles*

- Eggs deposited on water's surface, eggs float, larval embryogenesis (72 hr) and hatching must occur within 4 days of oviposition, no diapause in anopheline eggs



# Immature *Anopheles*

- 4 larval stages (instars), same general morphology, typified by increase in size
- Larval stage: one week to months, temp dependent
- Pupae – quiescent developmental stage (~24 hrs)



# Larval Anopheles

## Movement

- Suspended beneath water surface by water tension
- Spiracles closed for diving, movement over mud

## Feeding

- Head rotates through 180°, mouth brushes sweep
- Sweep from biofilm containing bacteria, protozoa, pollen grains, fungal spores, etc...
- Occasional cannibalism of smaller larvae

# Adult *Anopheles*

- Emerge through dorsal longitudinal slit of pupa
- Males often emerge first and form swarms, cannot copulate until genitalia rotate 180°
- Females emerge, enter swarm, copulate in the air
- Females may mate more than once
- Sperm is stored in the spermatheca for lifetime
- Males feed on nectar, females primarily on blood
- Aestivation in adult females

# Adult Dispersal

## Active dispersal

- Somewhat variable in the literature
- Up to 11 km documented in *An. gambiae*
- Majority of anophelines have limited dispersal

## Passive dispersal

- Wind blown
- Human transport (*An. gambiae* to Brazil)

# Mosquito Collection

- **Dippers for larvae**
- **Traps (light and CO2 baited) for adults**
- **Oviposition traps for eggs**
- **Aspiration for adults**
- **Landing/biting collections**

If most mosquitoes have the same basic biology and anatomy, what characteristics differentiate 422 species of anophelines?

# The Concept of Species Complex

- Biological and morphological variation in *Anopheles* was recognized <1900
- After WWI troops returning to Europe carried malaria, causing indigenous outbreaks
- Localized distribution of outbreaks was surprising with known broad distribution of *An. maculipennis*
- ‘anophelism without malaria’

# Species Complex (cont.)

- Investigations of the behavior, ecology and reproductive compatibility of morphological variants of *An. maculipennis* revealed existence of sibling species
- Gave rise to concept of species complex
- At least 14 species are now recognized in the *An. maculipennis* complex (or *An. maculipennis sensu lato*)
- Species complexes are common among anophelines

# Cytology of *Anopheles gambiae* s.l.

- Studies of polytene chromosome inversions polymorphism in *An. gambiae* s.l. began in the 1950s
- Coluzzi and colleagues started to publish chromosomal based investigations in the late 1960s
- By 1979 enough data had accumulated to allow Coluzzi to publish and solidify the species status of the *An. gambiae* complex in the context of polytene chromosome inversions (6 species)

# Cytology of *An. gambiae* s.l.

## Key Assumptions

- Morphology read in terms of banding patterns of polytene chromosome inversions represent genetic composition of individual
- Comparisons of banding patterns represent genetic relationships between individuals with shared morphological polymorphisms reflecting shared ancestry

# Basis for Delineation of Species in *An. gambiae* s.l. Species Complex

- Polytene chromosome inversions
- Mosquito biology and ecology
  - Geographic distribution
  - Habitat characteristics (salinity, aridity)
- Behavior
  - Host preference
  - Feeding behavior
- Investigations of insecticide resistance reveal reproductive incompatibilities

# Behavioral Terminology

- **Zoophilic** - prefers non-human animals
- **Anthropophilic** - prefers humans
- **Exophily** - prefers to live outdoors
- **Endophily** - prefers to live indoors
- **Exophagy** - prefers to feed outdoors
- **Endophagy** - prefers to feed indoors
- **“domestic”** - endophilic and rests there afterwards
- **“wild”** - exophilic and never go indoors
- **“intermediate”** - endophilic but leaves after feeding

# 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 & B** – zoophilic non-vector

Why is it valuable to differentiate  
between mosquito species or  
chromosomal forms or populations?

# Climate/Habitat Adaptations?

- Several researchers have illustrated clines in inversion frequencies with climate characteristics (i.e. aridity)
- Also population density differences in time and space

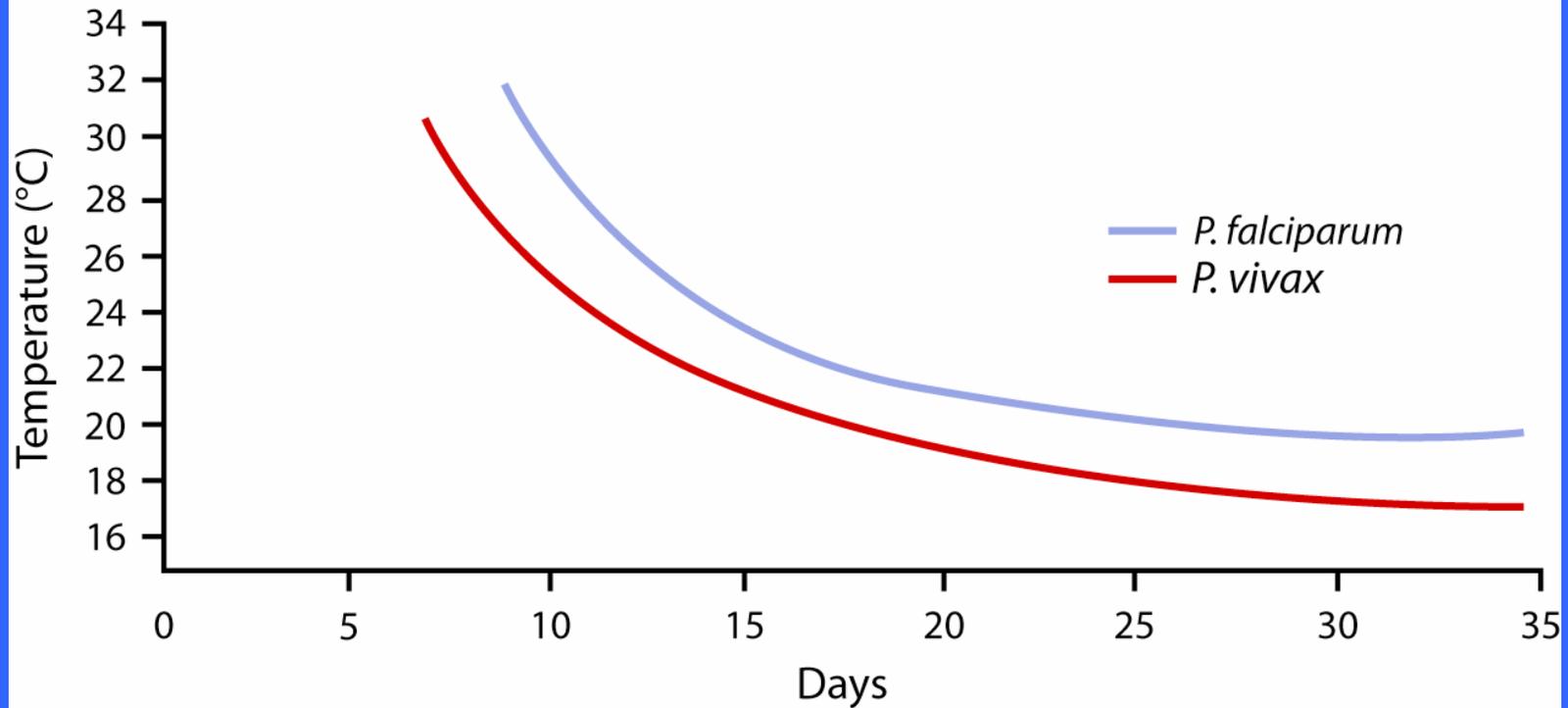
# Differentiating Between Members of the *An. gambiae s.l.* Species Complex

- Morphology/behavior/habitat
- Karyotyping of polytene chromosomes
- Molecular methods
  - rDNA, mtDNA
  - Microsatellites
  - RAPDs
  - Molecular linked phenotypes

# Endemic Malaria

- **Hypoendemicity** – denotes areas with little transmission and no effects of malaria on the general population
- **Mesoendemicity** – typical among small rural communities in the subtropical zones, variable transmission intensity depending on local circumstances
- **Hyperendemicity** – in areas with intense but seasonal transmission, immunity is insufficient to prevent the effects of malaria on all age groups
- **Holoendemicity** – high perennial transmission resulting in a considerable degree of immune response in all age groups, particularly adults

## Duration of Sporogonic (Extrinsic) Development of Malaria Parasites in *Anopheles* in Relation to the Environmental Temperature

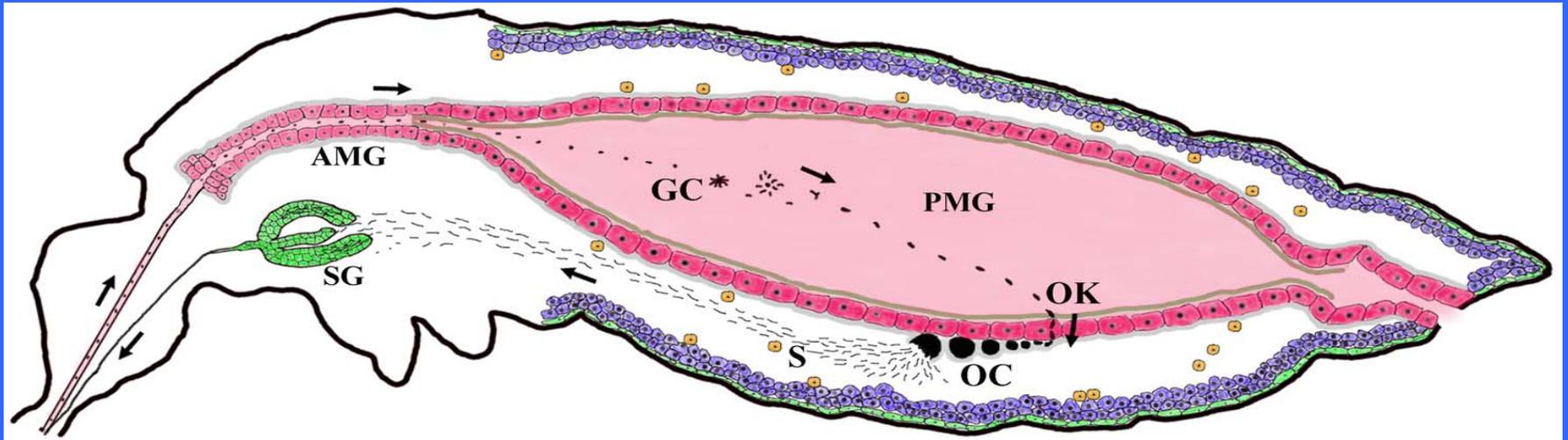


Adapted by CTLT from Macdonald, 1957

# Malaria Transmission in Mosquitoes

- **Horizontal** - transmission of a pathogen from one vector to another through host sharing, etc...
- **Cyclo-propagative** - pathogen undergoes essential development and multiplies in vector (*Babesia*, malaria)
  - *Plasmodium* has 5 developmental steps in the mosquito

Zygote  $\xrightarrow{5-18h}$  Ookinete



# Molecular Linked Phenotypes of Interest

- Vector competence for *Plasmodium*
- Vector competence is an evaluation of the vector's capability (mechanical or biological) to transmit a pathogen
- Two well studied barriers to *Plasmodium* development in *Anopheles*
  - Midgut penetration barrier
  - Oocyst melanization

# Vectorial Capacity vs. Vector Competence

- **Vectorial capacity** is a measurement of the efficiency of vector-borne disease transmission
- **Vector competence** is an evaluation of the vector's capability (mechanical or biological) to transmit a pathogen
- Therefore, vector competence is actually an additional component of vectorial capacity

# Vectorial capacity is a measurement of the efficiency of vector-borne disease transmission

$$C = \frac{ma^2VP^n}{-\log_e P}$$

C = Vectorial capacity, the number of infective bites received daily by a single host

m = Density of vectors in relation to density of hosts

a = Proportion of vectors feeding on a host divided by the length of gonotrophic cycle in days

V = Vector competence

P = Daily survival of vectors

n = Extrinsic incubation period

Where the vectorial capacity is a measure efficiency of pathogen transmission, EIR is a more direct measure of risk.

Entomologic inoculation rates (EIR) = the product of the mosquito biting-rate times the proportion of infected mosquitoes (sporozoite rates [1-20%])