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: Culiciniae (>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 → Ookinete

5-18h

Dimopoulos
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 of 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%])