MOSQUITO EVOLUTION: A STORY WITH A BITE

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Abstract: Mosquitoes (Culicidae) are one of many families of insects that are not only important to humans, but have actually shaped the direction of human development. As vectors of pathogens that cause diseases like malaria, yellow fever, and dengue, mosquitoes are very damaging to human health and economy. Because of their importance, mosquitoes have been studied extensively, possibly more extensively than any other family of insects. The nomenclature of mosquitoes is a reflection of their taxonomy, which is a reflection of systematics studies based on evolutionary phylogeny. Much of the current debate over genus names is a result of disagreement between those who want a practical system to identify groups of mosquitoes and those who want a more precise correspondence to phylogenetic studies. The evolutionary history of mosquitoes is a single reality and our measurements of morphology, genetics, biogeography, and molecular biology are all attempts to understand that history. Studies to date have produced some consensus on the higher level evolution that has a lot of general interest, for example that the family Culicidae shared a common ancestor with Chaoboridae (fairy midges) and Corethrellidae (biting fairy midges). This trio of families, in turn, shared a common ancestor with Dixidae (dixid midges). Mosquitoes may have emerged with recognizable characteristics in the middle of the Paleozoic, though the oldest fossil is from the late Cretaceous (90 million years ago). Within Culicidae, the only strong agreement is that there are two major groups, the subfamily Anophelinae (genera: *Anopheles, Chagasia,* and *Bironella*) and the subfamily Culicinae (all other genera). There is also agreement that Anophelinae was the first to branch off from the earliest culicids and that *Chagasia* is ancestral within Anophelinae. Culicinae is much bigger and is currently divided into 13 tribes. There is agreement that the tribe Aedini (*Aedes, Psorophora, Armigeres, Haemagogus,* and other genera) and Mansoniini (*Mansonia* and *Coquillettidia*) share a common ancestor. There is also agreement that Sabethini (*Tripteroides, Sabethes, Wyeomyia,* and other genera) and Culicini (*Culex, Lutzia, Deinocerites,* and *Galindomyia*) share a common ancestor. However, conclusions about the relationships of the other seven tribes (Aedeomyiini, Culisetini, Ficalbiini, Hodgesiini, Orthopodomyiini, Toxorhynchitini, and Uranotaeniini) remain obscure, though some authors have seen an affinity between Toxorhynchitini and Sabethini. Biogeographical and morphological evidence suggest that the Old World Sabethini is ancestral to the New World Sabethini and that the New World Sabethini have a common ancestor. It has also been suggested that the *Anopheles* subgenus originated in the New World and was distributed elsewhere during times when Africa and South America were connected followed by connections between North America and Europe. Species level studies are much more common than higher level phylogenetic studies. Among the best examples are the species related to *Culex pipiens* and the *Anopheles gambiae* complex.

Mosquitoes are insects in the family Culicidae, currently defined by the form of the adult and larva (Carpenter and LaCasse 1955, Belkin 1962, Harbach 2007). The adults characteristically have elongated mouthparts that are sheathed by the labrum and that form a proboscis. In addition, portions of the wings and body have flattened scales and the legs are long. Larvae have a well-developed head, the thorax distinct and larger than the head, no legs, and a single pair of spiracles on the end of a siphon or embedded in a spiracular plate near the terminus of the abdomen. Larvae of culicids, chaoborids, and corethrellids have segment nine of the abdomen reduced, unlike members of the family Dixidae. Larvae always occupy aquatic habitats and many species of adults take blood (females only) as well as sugar. Close examination of the family shows an amazing variety of form and function, which is probably typical of most insect families.

Of course, the reason that mosquitoes receive so much attention is their medical and veterinary importance as vectors of pathogens. Among the major human diseases associated with mosquitoes are malaria, filariasis, dengue, yellow fever, West Nile disease, chikungunya disease, and a wide variety of illnesses caused by viruses. Some of the primary examples of diseases caused by pathogens transmitted by mosquitoes to animals are Rift Valley fever, Japanese encephalitis, West Nile disease, bird malaria, and dog heartworm. Most species of mosquitoes are not a problem, but the 200 or so species that are important vectors have shaped human history in many areas of the world. (Strickman et al. 2009).

The ecology of mosquitoes has a clear connection to their evolution. One way to categorize the ways organisms live is to divide them into *r* and *K* strategists (Southwood 1976). The *r* strategists have high reproductive rates (*r*), populations that have large fluctuations in response to resource availability, and a tendency to colonize new areas quickly. The *r* strategy is typical of invasive species and of many human-associated pests. The *K* strategists, in contrast, have a lower reproductive rate but a high carrying capacity (*K*) and tend toward stable populations and distributions closely coincident with constant access to resource requirements. It is easy to think of ways in which mosquitoes, especially pest or vector mosquitoes, have *r* type characteristics. The maximum reproduction by individual females is typically in the hundreds, populations are wildly variable depending on access to blood meal sources and larval habitats, and larvae are often found in marginal situations not typical of the particular species. Examples of *K* strategy elements are harder to find. Other than the display of maternal behavior by a few species, protecting eggs from rain and predators (*Trichoprosopon digitatum*) or carrying the eggs on the hind legs until they are ready to hatch (*Armigeres* subgenus *Leicesteria*) (Lounibos 1983), even the slowest reproducing, longest living species have relatively high reproductive rates and short life spans. The practical implication of mosquitoes' general tendency toward *r* strategy is that the pool of species worldwide provides many possibilities for expansion into new habitats or adaptation to existing ones.

Pressure on populations in the form of mortality factors is a major component of evolutionary mechanics. Simply put, the offspring from a mosquito producing 200 eggs will have to suffer 99% mortality to produce a stable population of that species. Such high reproductive mortality rates must produce severe selection on the population. At the very beginning of the life cycle, it is common to observe much less than 100% hatch of eggs even under the best laboratory conditions (e.g., Southwood et al. 1972). Other biotic factors include nutrition (Sumanochitrapon et al. 1998, Strickman and Kittayapong 2003), whether a shortage of carbohydrate sources for energy or blood sources for reproduction, competition from other mosquitoes for larval food (Black et al. 1989) or blood sources (Edman et al. 1972, 1974), defense by hosts during blood feeding (Edman and Kale 1971), predation (Strickman et al. 1997), and pathogens (Yan et al. 1997). Abiotic mortality factors include desiccation, habitat destruction (often carefully performed for the purpose of control), and temperature (e.g., Strickman 2006).

Selection is another important component of evolutionary mechanics. Almost any aspect of the morphology of mosquitoes could be explained by assumed selective pressures. One of the elegant examples is the highly sculptured and desiccation resistant chorion (shell) of floodwater mosquito eggs in the genera *Aedes* and *Psorophora*. These eggs are deposited in the soil and may remain in a latent state for years before flooding and hatching. Nutritional strategies of mosquitoes vary from the ability to survive in water with high organic content (e.g., *Culex pipiens*) to the ability to vary production of eggs in response to nutrition, which is probably general throughout the family. Mosquitoes respond to mammalian defensive behavior in various ways, for example by biting low on the body and making a very gentle approach (e.g., *Aedes aegypti*). In a more intricate example, animals that are sick with malaria are less able to protect themselves from bites (McCall and Kelly 2002). Larvae that experience high predation pressure may have behavioral adaptations to increase the chances of survival (Gimonneau et al. 2010). Commonly observed insecticide resistance is a fast response by populations to intense selective pressure (Ben Cheikh et al. 2009). In general, sexual selection is assumed to be a cause of the wide variation in structure of male copulatory structures (Arnqvist 1998).

Evolution would not occur if organisms did not inherit characteristics from their parents. Mosquitoes are no exception, with the usual requirements of sexual reproduction, Mendellian inheritance, chromosomal changes, etc. However, there are other mechanisms for genetic inheritance in mosquitoes, as well. For example, symbiotic bacteria including *Wolbachia* can carry genetic material between individuals (Saridaki and Bourtzis 2010). A wide variety of transposons are also responsible for horizontal gene transfer (Kidwell 2005). Hybridization between species is influenced by many factors, but it has been an important source of gene flow in at least some groups (e.g., *Culex pipiens,* Farajollahi et al. 2011). Very recently, the ability for humans to intentionally change the genome has been developed through transgenic manipulation (Allen et al. 2001).

When all of the mechanical steps of evolution are considered, the process of evolution might follow any or all of several paths. The principle contrast is between gradualism and punctuated equilibrium (Gould and Eldredge 1993). Gradualism holds that one form leads to another in a series of changes at a steady rate. Punctuated equilibrium, on the other hand, proposes that many of the major changes in phylogeny occur over relatively short time spans followed by long periods of stability. Phylogeny of mosquitoes does not really support one side or the other. On the one hand, the first mosquito fossil, *Burmaculex antiquus* (Borkent and Grimaldi 2004), has a proboscis that is not as elongated as in modern mosquitoes, suggesting a transitional form that might have developed gradually from chaoborid-like ancestors during the Mesozoic. On the other hand, the extensive radiation of sabethine species in the New World (Judd 1996) and the apparent radiation of *Anopheles* subgenus *Cellia* following the separation of South America and Africa (Krzywinski and Besansky 2003) could support a pattern of evolution consistent with punctuated equilibrium.

Although the concept of a tree of evolution with branches representing taxa as they split off from each other goes back at least to Darwin, a quantitative approach to phylogenetics was not available until it was developed by Willi Hennig, summarized in his only article in English in 1965. His concept was that the goal of an analysis was to create cladograms in which the various taxa were monophyletic, that is, derived from a common ancestor. He advocated the use of shared, derived characters (apomorphies) as the basis for determining common ancestry. That basic approach was the start of the standard cladograms that are so common in the biological literature today. Although not based on Hennig's work, the first cladogram of mosquitoes was published by Ross (1951), but that diagram actually contained little information as most of the genera emerged at the same time. It was not until Harbach and Kitching (1998) that anyone again attempted to show the relationships across the entire family, based on morphology. Updated by Harbach (2007) based on his own and others' studies, including molecular data, the firm conclusions are actually fairly simple:

1. Anophelinae and Culicinae are the two subfamilies of Culicidae, and each is monophyletic.
2. Anophelinae appeared before Culicinae, i.e., Anophelinae is basal in Culicidae.
3. The genus *Chagasia* is basal in Anophelinae.
4. Within Culicinae, tribe Aedini (*Aedes, Psorophora, Armigeres,* and other genera) and Mansoniini (*Mansonia* and *Coquillettidia*) are each monophyletic and have a common ancestor, with Aedini basal.
5. Within Culicinae, tribe Culicini (*Culex, Lutzia, Deinocerites,* and *Galindomyia*) and Sabethini are monophyletic and have a common ancestor, with Culicini basal.
6. The relationships of the other seven tribes of Culicini (Aedeomyiini, Culisetini, Ficalbiini, Hodgesiini, Orthopodomyiini, Toxorhynchitini, and Uranotaeniini) are not known with certainty, though some authors have seen an affinity between Toxorhynchitini and Sabethini (Belkin 1962).

Although Harbach (2007) disputed it, Judd (1996) proposed evidence supported by biogeography that Old World Sabethines (*Tripteroides, Malaya,* and *Maorigoeldia*) and New World Sabethines (*Sabethes, Wyeomyia, Trichoprosopon,* and others) are each monophyletic and that the Old World Sabethines are ancestral.

The timing of the development of mosquitoes was examined using many lines of evidence, including fossils, by Reidenbach et al. (2009). He calculated ranges of time for major phylogenetic events among mosquitoes. For example, the split between Anophelinae and Culicinae may have taken place between 229 and 192 million years ago (MYA), which would have been during the late Triassic or early Jurassic. Significantly, the split between Old World and New World sabethines was estimated to be between 124 and 65 MYA (early or late Cretaceous), corresponding to the hypothetical age of the separation of Asia from South America (McCarthy 2003). With the oldest fly fossil, *Permotipula patricia*, dating back to the Upper Permian (260-252 MYA), it is not hard to imagine that mosquitoes developed from chaoborid-like ancestors sometime in the Triassic (252-202 MYA). Being such an old lineage, they have experienced many different kinds of hosts and aquatic conditions, possibly accounting for the adaptability we see today in this remarkable group of insects.

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ЕВОЛЮЦІЯ МОСКІТІВ: ІСТОРІЯ УКУСУ

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Служба сільськогосподарських досліджень Міністерства сільського господарства США

Комарі є одним з багатьох сімейств комах. Як переносники збудників, які викликають такі захворювання, як малярія, жовта лихоманка і лихоманка денге, комарі дуже шкідливі для здоров'я людини та економіки. Через їх важливість для здоров'я людини, комарі були вивчені, можливо, більш широко, ніж будь-яке інше сімейство комах. Специфікація комарів є відображенням їх таксономії, яка є відображенням дослідження систематики на основі еволюційного філогенезу.

Більшість дебатів щодо назв родів комарів є наслідком розбіжностей між тими вченими, які віддають перевагу практичній системі виявлення груп комарів і тими, хто прагне більш точної відповідності філогенетичним дослідженням.

AN EXAMPLE OF MODEL OF ESTIMATING THE LEVEL OF BIOLOGICAL RISK ON FARMS BASED ON THE GAP REQUIREMENTS

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Introduction. *Biosecurity, as a technical term, describes a series of activities that must be implemented to prevent the entry of infectious organisms and their spread throughout the farm. Good biosecurity program is an effective way to reduce the risk of the outbreak and spread of infectious diseases from animal to animal and from animals to humans. To be effective it requires a careful analysis of all individual situations and individual parameters. In essence, they require the satisfaction of the previously required programs in order to achieve the desired goal, which is the highest possible level of security of the end product. Previously recognized as the most necessary programs are: GAP (Good Agriculture Practice), GVP (Good Veterinary Practice), GMP (Good Manufacturing Practice), GHP (Good Hygienic Practice), GLP (Good Laboratory Practice) and others [1].*

Systems and quality standards and farm animal breeding. Standardization of good business and management practices in the world is becoming increasingly widespread. In particular, the dominant approach to the management systems in the organization connect and integrate quality management based on business, technology and manufacturing processes. Based on this approach, today the integration of management systems is performed according to the principle: QMS (*Quality Management Systems - ISO 9000: 2000*), EMS (*Environmental Management Systems - ISO 14000: 1996*); OHSMS (*Protection And Safety Of Employees - ISO 18001: 1999*), ISO 22000, HACCP (*Monitoring And Analysis Of Critical Control Points*), GAP (*Good Agriculture Practices*), GMP (*Good Business Practice*), GLP (*Good Laboratory Practice*), GHP (*Good Hygienic Practice*) [2]. When it comes to farm animal breeding based on this concept, an initial approach to defining product safety by meeting the requirements of the HACCP system is now obsolete, making primarily GAP standards, which in itself integrate the HACCP system, ISO 9001, ISO, 14000, ISO 22000, OHSMS 18001, with the support of scientific knowledge of the field of the achieving a high level of biosecurity as a basic precondition for obtaining safe products of animal origin. It should be borne in mind that there isn't something called the ''only'' proper interpretation of any standards and does not answer the question "how" but "what" [3, 4]. The idea to develop international standards at the outset was evaluated as an unusual move, as had previously been exclusively technical standards. Today they have a much wider application. Quality policy in this area aims to: protect consumer health, protection of consumer interests and the establishment of market competence. They are regularly reviewed in line with scientific developments and customer requirements with the aim of customer satisfaction, legal requirements, survival in the market, good business practice, providing management control, reduce costs, poor quality [5].