Review in Advance first posted online on August 15, 2011. (Changes may still occur before final publication online and in print.)

Evolution of *Anopheles gambiae* in Relation to Humans and Malaria

Bradley J. White, Frank H. Collins, and Nora J. Besansky

Eck Institute for Global Health and Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556; email: nbesansk@nd.edu

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

Annu. Rev. Ecol. Evol. Syst. 2011. 42:111–32

The *Annual Review of Ecology, Evolution, and Systematics* is online at ecolsys.annualreviews.org

This article's doi:
10.1146/annurev-ecolsys-102710-145028

Copyright © 2011 by Annual Reviews.
All rights reserved

1543-592X/11/1201-0111$20.00

Keywords

adaptation, coevolution, ecological speciation, chromosomal inversions, heterogeneous genomic divergence, mosquito, sibling species complex

Abstract

The closely related and morphologically indistinguishable mosquito species in the Afrotropical *Anopheles gambiae* complex differ dramatically in their contribution to malaria transmission, ranging from major vectors through minor or locally important vectors and nonvectors. Radiation of the *A. gambiae* complex and ongoing diversification within its nominal species appears to be a product of recent and rapid adaptation to environmental heterogeneities, notably those of anthropogenic origin. Polytene chromosome and genomic analyses suggest that paracentric chromosomal inversions and possibly other low-recombination regions have played instrumental roles in this process by facilitating ecotypic differentiation both within and across semipermeable species boundaries. Forthcoming complete genome sequences from several members of the *A. gambiae* complex will provide powerful tools to accelerate ongoing investigation of how genetic diversification of populations and species has shaped behavioral and physiological traits, such as vector competence, that bear on vectorial importance.

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

INTRODUCTION

Female *Anopheles* mosquitoes are mammalian ectoparasites that are dependent on a proteinaceous blood meal for egg production. Similar to their mammalian hosts, the basal lineages of *Anopheles* appear to have diversified rapidly in association with the separation of South America, Africa, and Laurasia ~120–95 Mya (Krzywinski et al. 2001, Nishihara et al. 2009). Efforts to reconstruct *Anopheles* phylogeny have been complicated not only by these older bursts of radiation but also by much more recent bursts leading to the many clusters (complexes) of closely related and isomorphic sibling species typical of this taxon (Harbach 2004).

Anopheles mosquitoes also are the exclusive vectors of mammalian malaria. However, of ~500 recognized species, only approximately two to three dozen are important vectors of human malaria, and the vast majority of anophelines are of little or no consequence to public health (Collins & Paskewitz 1995). In principle, the four traits needed to make a highly efficient human malaria vector are known: (*a*) strong preference for human blood, (*b*) physiological competence to parasite infection, (*c*) long life, and (*d*) high population density. Notably, the rare species that possess all four of these traits are not clustered phylogenetically but rather are interdigitated with nonvector species in four of six subgenera and even in sibling species complexes. This pattern implies recent, rapid, and repeated evolution of traits conferring vectorial efficiency. A trigger for the repeated emergence of human malaria vectors in different anopheline lineages may have been the growth of Neolithic human populations following the development of agriculture, and the resulting environmental changes.

Identifying the yet unknown genetic differences that have allowed a handful of species to become major malaria vectors is the ultimate goal motivating the complete genome sequencing and assembly of fifteen *Anopheles* species, a project set to launch in 2011 (Besansky 2008). Powerful insights about the nature of key evolutionary innovations of vectors can be gained by careful comparison within species complexes—closely related sibling species that differ in vector status—and across these replicate systems of vector evolution. Although many if not most malaria vectors belong to sibling species complexes, nearly all are severely understudied. A notable exception is the Afrotropical *Anopheles gambiae* complex, whose nominal species was the first, and to date remains the only, anopheline with a completely sequenced reference genome (Holt et al. 2002). The morphologically indistinguishable members of this complex span the vectorial capacity gradient from major vectors through minor or locally important vectors and nonvectors. Taking the *A. gambiae* complex as a case study and potential model, this review aims to synthesize the substantial progress made in understanding, at both the genetic and phenotypic levels, the diversification of these species in relation to humans, malaria, and a heterogeneous environment.

THE *ANOPHELES GAMBIAE* SPECIES COMPLEX

Discovery of the role of anopheline species in malaria transmission before the turn of the past century solved one mystery but raised another. "Anophelism without malaria" was a phrase coined to describe regions in southern Europe where the presumed vector *Anopheles maculipennis* was abundant but where disease transmission was sparse or nonexistent (Hackett 1937). In the early 1920s, several researchers hypothesized that this phenomenon could be explained by the existence of divergent "races" of *A. maculipennis* that were nearly identical morphologically but that differed in behavior, ecology, and/or physiology. Research over the next two decades confirmed this hypothesis, and today it is known that what was once called *A. maculipennis* is actually a group of at least ten Palearctic species (Harbach 2004). These species show variation in oviposition sites, host-feeding preferences, and vector competence, which help to explain the substantial geographic

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

variation in historical rates of malaria transmission across Europe. This first discovery of sibling species in *Anopheles* not only resolved the paradox of anophelism without malaria but also influenced subsequent investigations of other vectors, particularly in cases in which the assumed vector species was unusually widespread and apparently plastic in breeding habits and other behaviors. Such was the case for *A. gambiae*, which was not recognized as a species complex until the second half of the twentieth century. Below, we revisit the discovery of the complex and more recent attempts to understand the genetic and phenotypic differences between these species.

Discovery and Distribution of the Saltwater Species

Early indications of possible subdivision within the normally freshwater breeding *A. gambiae* came from observations of saltwater breeding populations along the western and eastern coasts of Africa (e.g., Evans 1931). Quantitative differences in egg morphology and adult cuticle color between saltwater and freshwater populations existed but did not prove diagnostic (Chwatt 1945, Muirhead Thompson 1951). However, when western and eastern saltwater populations were reciprocally crossed to each other or to freshwater populations, all male progeny were completely sterile (the corresponding females were fertile) (Davidson 1962, Paterson 1962). On the basis of this evidence, western and eastern saltwater *A. gambiae* were eventually elevated to full species status and named *Anopheles melas* and *Anopheles merus*, respectively. A third salt-tolerant population of uncertain taxonomic status was found breeding in brackish pools near hot springs in the Semliki Forest of Uganda (White 1973). Again, crossing studies were instrumental in its recognition as a full species, which was eventually named *Anopheles bwambae*.

The ranges of *A. merus* and *A. melas* are primarily restricted by the availability of brackish, coastal breeding sites (Caputo et al. 2008, Coetzee et al. 2000, Okara et al. 2010; see also maps of Sinka et al. 2010). Both species can complete development in fresh water and occasionally can be collected from such breeding sites, but they have been unable to penetrate far into the interior of the continent, presumably owing to competition with *A. gambiae*. To this point, *A. merus* is abundant in inland southern Africa where *A. gambiae* is often absent or rare (Coetzee et al. 2000, Cuamba & Mendis 2009). *A. bwambae* is highly endemic to Ugandan thermal springs, and unlike the other two saline species it appears to actively avoid ovipositing in normal freshwater habitats; its larvae cannot complete development in such sites (Harbach et al. 1997, White 1973). Although adult females from all three of the saltwater siblings have been found infected with malaria parasites on numerous occasions, they are generally considered only minor/local vectors owing to their limited distribution.

Discovery and Distribution of the Freshwater Species

In contrast to the saltwater species, discovery of multiple freshwater species within the *A. gambiae* complex was unanticipated and accidental. During the course of genetic studies of dieldrin resistance, Davidson & Jackson (1962) crossed resistant and susceptible strains of what was presumed to be *A. gambiae* and were surprised to find that many of their crosses produced fully sterile male progeny. After ruling out linkage between sterility and dieldrin resistance, they identified two distinct mosquito mating types, which were termed A and B. Matings between (but not within) types produced completely sterile males with no viable sperm, which caused Davidson & Jackson to conclude that they were dealing with two distinct species; these later became known as *A. gambiae sensu stricto* (hereafter *A. gambiae*) and *Anopheles arabiensis*. A third freshwater sibling, initially called mating type C and eventually named *Anopheles quadriannulatus*, also was discovered by crossing experiments, which were prompted by the observance of a persistently outdoor resting

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

and zoophilic vector population during the course of indoor antimalarial spraying (Paterson & van Eeden 1963). More recently, *A. quadriannulatus* has been subdivided into two allopatric species provisionally named *A. quadriannulatus* A and B on the basis of crossing experiments (Hunt et al. 1998).

A. gambiae and *A. arabiensis* have widespread, extensively overlapping distributions extending throughout most of sub-Saharan Africa (see Sinka et al. 2010). On the basis of their anthropophilic tendencies, they are two of the three most important malaria vectors on the African continent. *A. quadriannulatus* B in Ethiopia and A in southeastern Africa appear to have relictual distributions, and given their zoophilic behavior they are considered nonvectors.

Species Identification

Although morphologically indistinguishable, the sibling species can be discriminated by fixed genetic differences. These were first discovered at the cytological level through studies of the banding pattern of polytene chromosomes obtained from larval salivary glands (Coluzzi & Sabatini 1967, 1968, 1969) and, later, ovarian nurse cells. Most siblings are distinguished by at least one fixed chromosomal inversion difference (all except the homosequential *A. quadriannulatus* A and B) (Coluzzi et al. 1979, 2002). Cytotaxonomy allowed for intensive field studies, which had been severely hampered by cumbersome crossing experiments. This method also quickly revealed the potential for genetic introgression, as rare F1 hybrid females were found in nature between *A. gambiae* and *A. arabiensis*, *A. gambiae* and *A. melas*, and *A. arabiensis* and *A. quadriannulatus* (Coluzzi et al. 1979, White 1971). Although F1 hybrid males show sterility in all pairwise crosses in the *A. gambiae* complex, the F1 females are fertile and could be conduits for interspecific gene flow.

The development of a DNA-based diagnostic assay abolished the requirement for particular developmental stages and tissue preservation methods. The PCR assay in routine use (Scott et al. 1993) is based on fixed nucleotide differences identified in the intergenic spacer of the ribosomal DNA (rDNA) gene (Collins et al. 1987). The rDNA proved a particularly useful marker not only owing to its rapid, concerted evolution, but also because its high copy number (>500/genome) allows amplification from small amounts of and/or poor quality genomic DNA. Although it fails to differentiate *A. quadriannulatus* A and B (Hunt et al. 1998) and the saltwater species *A. merus* and *A. melas*, this is of no practical importance, as both of these species pairs are allopatric. There is some indication that rDNA may have introgressed between *A. gambiae* and *A. arabiensis* (Besansky et al. 1994) as well as between incipient species of *A. gambiae* (Caputo et al. 2011), and there is one report of more than one rDNA locus per genome (Wilkins et al. 2006), but because these occurrences are relatively rare in most localities, none has proved problematic in the routine application of rDNA-based species identification.

Reproductive Isolation

Most species in the *A. gambiae* complex are not separated by physical (geographic) barriers. Range overlap may extend to the microspatial scale, where different species can be synchronously breeding. Reproductive isolation is not absolute, although hybrids are exceedingly rare, and is considered to have arisen as a by-product of ecologically based divergent selection associated with alternative larval aquatic habitats (Ayala & Coluzzi 2005, Costantini et al. 2009).

Prezygotic isolation. Mating occurs in variably sized swarms formed by males around dusk or dawn; females fly into these swarms, and couples depart (Charlwood et al. 2002, Manoukis et al. 2009, Marchand 1984). Females generally mate only once, although the specific mechanism

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

underlying mating refractoriness in inseminated females is unknown (Klowden 2001). Prezygotic isolation between sibling species could have three different explanations: (*a*) males from each species form physically separate swarms, and females do not enter heterospecific swarms (swarm segregation); (*b*) females enter mixed swarms but copulate only with conspecific males (intraswarm mate recognition); or (*c*) some combination of these two mechanisms. Data from incipient species of *A. gambiae* suggest that both mechanisms play at least some role in prezygotic isolation but that swarm segregation is the primary isolation mechanism (Diabate et al. 2009, Pennetier et al. 2009). It would be useful to learn if swarm segregation is the main mechanism of prezygotic isolation between the other sibling species and how females use sensory cues (pheromonal, auditory, and visual) or physical differences (e.g., height) to identify conspecific swarms. Such behavioral differences are presumably more distinct between full sibling species than between the more recently diverged *A. gambiae* incipient species, thus making them easier to discern.

Postzygotic isolation. Only two studies have examined the genetic basis of hybrid sterility between sibling species, which is expressed in F1 males and backcross females (Slotman et al. 2004, 2005a). Using *A. gambiae* and *A. arabiensis*, both studies demonstrated that the X chromosome plays a major role in postzygotic isolation, as small regions of the autosomes are incompatible with the X chromosome from the opposite species. These two species differ by large independent rearrangements of the X chromosome, which prevent recombination of this chromosome in female hybrids. Similar studies using species pairs with the same X arrangement (e.g., *A. gambiae–A. merus* and *A. quadriannulatus–A. melas*) and higher-resolution mapping would shed light on the number of sterility/inviability factors on the X that account for its large effect on intrinsic isolating barriers.

Evolutionary Relationships Among the Species

The discovery of fixed differences among species in the banding pattern of polytene chromosomes provided more than a practical tool for cytotaxonomy. Paracentric chromosomal rearrangements differentiating species also offered the first insights into phylogenetic relationships in the *A. gambiae* complex. In their pioneering cytogenetic studies, Coluzzi & Sabatini (1967, 1968, 1969) examined mitotic, meiotic, and salivary polytene chromosomes not only in each of the five species known at the time (three freshwater and two saltwater species) but also in hybrids from all pairwise crosses in both directions. This approach made possible a precise assessment of (*a*) homologies between chromosomes in each species pair, (*b*) differences in their banding pattern, and (*c*) the degree of synapsis between homologous chromosomes in hybrids.

Overall, these studies revealed close genetic affinities among all species pairs in the complex, as expected: clear homology of the chromosome complement (heteromorphic sex chromosomes and two autosomes) and close correspondence of the polytene banding pattern along the length of homologs. Any changes in banding pattern were primarily owing to paracentric chromosomal inversions. By assuming the unique origin of each fixed inversion and adopting the principle of parsimony, Coluzzi & Sabatini (1969) deduced the relationships illustrated in **Figure 1*a*** (without the evolutionary directionality implied by the *Anopheles christyi* root, unknown at the time). Remarkably, these relationships contradict the expectation that *A. gambiae* and *A. arabiensis* might be sister taxa on the basis of their strong association with humans, as well as the expectation that the saltwater species *A. melas* and *A. merus* would be sister taxa on the basis of their ecological and biometrical similarities. Instead, cytogenetic evidence suggested that these physiological, behavioral, and ecological similarities resulted from evolutionary convergence (Coluzzi & Sabatini 1969). Indeed, although *A. gambiae* and *A. arabiensis* were shown to be similar with respect to

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

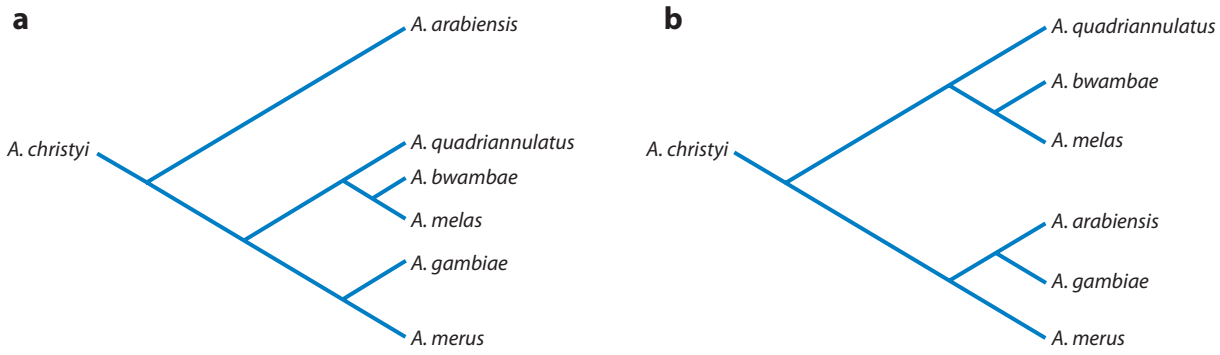

Figure 1

Evolutionary relationships within the *Anopheles gambiae* complex inferred from (*a*) X-linked sequences and fixed inversion differences or (*b*) autosomal sequences and mtDNA. Conflict hinges on whether the sister species to *A. gambiae* is *Anopheles merus* (panel *a*) or *Anopheles arabiensis* (panel *b*). Tentative placement of the root is based on evidence from the outgroup species *Anopheles christyi* (Caccone et al. 1996) and the ancestral status of the 2La arrangement in the *A. gambiae* complex (Ayala & Coluzzi 2005, Sharakhov et al. 2006).

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

autosomal banding pattern—even to the extent of apparent sharing of some of the same polymorphic paracentric inversions on chromosome 2—they differed by extensive fixed rearrangements of the X chromosome. In *A. gambiae–A. arabiensis* hybrids, almost complete asynapsis of the X chromosome was observed, in distinction to nearly complete synapsis in hybrids between either of these species and *A. quadriannulatus*. The *A. quadriannulatus* X chromosome was presumed to be an intermediate or ancestral step between the X chromosomes of *A. gambiae* and *A. arabiensis* not only owing to the greater intimacy of pairing but also because the latter species differ from *A. quadriannulatus* by only two (or three) different overlapping inversions, whereas they differ from each other by five.

In contrast to the freshwater species whose fixed inversion differences are X-linked, differences between the saltwater and freshwater species result from fixed autosomal inversions. *A. merus*, considered the sister to *A. gambiae* on the basis of two shared overlapping fixed inversions on the X, differs from other taxa owing to two unique overlapping inversions fixed on chromosome 2R. *A. melas*, which is allied with *A. quadriannulatus* on the basis of a shared X chromosome banding pattern, nonetheless appears to be the most differentiated species cytologically. One long inversion difference is fixed on chromosome 2R, extensive areas of asynapsis are generally observed in hybrids between this species and other members of the *A. gambiae* complex, and the size as well as the shape of the X chromosome is unique in *A. melas*, presumably owing to rearrangements involving heterochromatin (unseen in polytene chromosomes, as heterochromatin does not polytenize).

Subsequent molecular phylogenetic investigations based on mitochondrial DNA, rDNA, and other nuclear gene markers largely recapitulated the species relationships deduced from cytogenetics, with a few exceptions (Besansky et al. 1994, 2003; Caccone et al. 1996). The exceptions have two likely and nonexclusive explanations. First, radiation of the *A. gambiae* complex is assumed to have occurred both recently and rapidly (Ayala & Coluzzi 2005), which means that few phylogenetically informative characters may exist for resolving affinities among lineages. Accordingly, the differences in tree topology among studies employing small numbers of loci may be more apparent than real, as suggested by low confidence in many of the inferred relationships (Besansky et al. 1994, 2003; Caccone et al. 1996). Second and arguably more important, real and strong conflict actually does exist between alternative topologies in terms of the relationship inferred between the two most important malaria vector species: *A. gambiae*

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

and *A. arabiensis* (compare **Figure 1*a*,*b***). Consistent with the original cytogenetic observations suggesting shared polymorphic autosomal inversions between this species pair, laboratory crossing experiments (della Torre et al. 1997) and increasingly higher-resolution molecular genetic studies lend compelling evidence for introgression of inversions and other genome regions between this species pair (Besansky et al. 1994, 2003; Caccone et al. 1996; della Torre et al. 1997; Donnelly et al. 2004; White et al. 2007, 2009). These and other studies (Slotman et al. 2005b) indicate a higher capacity for introgression on the autosomes than on the X chromosome, in agreement with much lower levels of sequence divergence on the autosomes relative to the X (Besansky et al. 2003, Neafsey et al. 2010, Wang-Sattler et al. 2007). Of particular note, molecular cloning and characterization of the breakpoints of cytologically identical paracentric inversions shared by *A. gambiae* and *A. arabiensis* on chromosome 2 (the 22-Mb 2La and 8-Mb 2Rb inversions) revealed that the breakpoints also are the same (Sharakhov et al. 2006). On the basis of sequences determined from loci inside the rearranged regions, same-arrangement divergence is smaller than between-arrangement divergence regardless of taxonomic origin (White et al. 2007, 2009). Moreover, despite generally low levels of linkage disequilibrium characteristic of *A. gambiae* populations (Harris et al. 2010b, Weetman et al. 2010), sequences determined from autosomal loci in natural populations of *A. gambiae* and *A. arabiensis* revealed sharing of full-length haplotypes, which strongly implicate recent interspecific introgression (Besansky et al. 2003). However, in the same populations, sequences sampled from the X chromosome showed high genetic divergence and no shared polymorphism between this species pair.

The frequency of genetic introgression between *A. gambiae* and *A. arabiensis*, and its extent along the autosomes, has yet to be investigated in detail. However, a speculative argument has been proposed to account for these observations (Ayala & Coluzzi 2005). *A. gambiae* is envisioned to have arisen in the African rainforest with a karyotype resembling that of *A. quadriannulatus*—largely devoid of inversion polymorphism and carrying the standard (uninverted) arrangements characteristic of present-day forest populations of *A. gambiae*. With deforestation accompanying the rise of agricultural settlements during the Neolithic period, *A. gambiae* may have come into secondary contact with *A. arabiensis*, and hybridization could have resulted in the introgression from *A. arabiensis* into *A. gambiae* of chromosomal inversions (2Rb, 2La) that are beneficial in arid environments, facilitating the spread of *A. gambiae* from rainforest to drier savannas.

Prospects for a Phylogeny

As a result of genetic introgression, phylogenetic inference in the *A. gambiae* complex is strongly dependent on the genomic regions in which the markers are located (**Figure 1**), and it is not clear that simplistic attempts to reconstruct evolutionary relationships on the basis of a strict consensus among multiple dispersed loci would be an informative strategy. Moreover, although not reported to date, genetic introgression between other species pairs is entirely possible and should be considered as more sequence data from natural populations become available for phylogenetic reconstruction. Unambiguously distinguishing genetic introgression from retained ancestral polymorphism among recently radiated species is a notoriously difficult problem, particularly in the case of inversion polymorphisms preserved by long-term balancing selection. Assuming little or no recombination between the sex chromosomes, the combination of Y-chromosome sequence data with an appropriate outgroup species seems to be a promising approach to future phylogenetic reconstruction in this complex. As males are sterile in all interspecific crosses, no recent introgression should have occurred on the Y chromosome. Moreover, its low nucleotide diversity mitigates the problem of shared ancestral polymorphism. Unfortunately, informative single-copy sequences have yet to be isolated from the Y chromosome in these mosquitoes (Krzywinski et al. 2006).

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

GENETIC HETEROGENEITY WITHIN *ANOPHELES GAMBIAE SENSU STRICTO*

A. gambiae is a highly polymorphic mosquito capable of thriving in a wide diversity of ecoclimatic conditions, but only in association with humans. The degree of specialization on humans at all stages of the life cycle suggests a very recent and human-driven origin subsequent to the Neolithic revolution (Ayala & Coluzzi 2005, Coluzzi et al. 2002). Major human population expansion in sub-Saharan Africa probably began in the fifth millennium BC in west and central Africa with the introduction of sedentary agriculture and domestic animals, particularly cattle. The introduction of iron smelting from North Africa at approximately 500 BC probably greatly expanded the spread of agriculture and associated deforestation. These expansions likely facilitated adaptation of *A. gambiae* to human environments and specialization on human blood meals. Moreover, polytene chromosome and genomic analyses point to ongoing diversification within this species involving ecotypic differentiation and ecological speciation driven by anthropogenic environmental change.

Chromosomal Inversions and Ecotypic Differentiation

A. gambiae carries an exceptional number of inversion polymorphisms (Coluzzi et al. 1979, 2002; Pombi et al. 2008). These chromosomal rearrangements are nonrandomly distributed on the polytene complement and are disproportionately abundant on the right arm of chromosome 2. They are also nonrandomly distributed geographically, microspatially, and seasonally with respect to ecological and climatic variables, especially aridity (Coluzzi et al. 1979, Costantini et al. 2009, Lee et al. 2009, Simard et al. 2009, Toure et al. 1998). At least four different arrangements form latitudinal clines in western and central Africa from near absence in the humid southern rainforests to fixation in the xeric Sudan savannas to the north (Coluzzi et al. 1979, Wondji et al. 2005). In the middle of the clines, the frequency of these polymorphic inversions fluctuates temporally according to seasonal rainfall (Toure et al. 1998). Significant differences in inversion frequency also occur at a local level; these are correlated with differences in degree of aridity in the indoor versus outdoor nocturnal microclimate within a given village (Coluzzi et al. 1977, 1979). That these patterns persist and are replicated in different parts of Africa argues for an environment-dependent adaptive value of inversions in *A. gambiae.* Because inversions protect against recombination when heterozygous, they can stabilize sets of locally adapted alleles and promote ecotypic differentiation as well as speciation (Coluzzi 1982, Kirkpatrick & Barton 2006, Manoukis et al. 2008).

Efforts to identify specific physiological or behavioral traits conferred by inversions in *A. gambiae* have been few. In accord with an expectation based on climatic associations, inversion 2La confers greater larval heat tolerance and adult resistance to desiccation (Gray et al. 2009, Rocca et al. 2009). The genetic basis of adaptive traits inside inversions remains wholly unknown. From a logistical standpoint, traditional quantitative trait locus mapping is difficult owing to suppressed recombination between rearranged regions. However, genetic exchange between alternative arrangements does occur at a low but nontrivial frequency (Stump et al. 2007). Over time in large natural populations with heterokaryotypes, such exchange has the potential to homogenize genetic polymorphism between arrangements unless countered by selection (Feder & Nosil 2009). On the basis of this principle, divergence mapping has been applied to scan for candidate targets of selection within inversions.

Using 150,000 markers on a gene-based microarray, White and colleagues (2007, 2009) identified two ~1-Mb candidate regions implicated in the selective maintenance of the ~22-Mb 2La inversion. However, only one such region of elevated divergence was uncovered on the basis of similar scans of four 2R inversions. In the future, higher-resolution (e.g., resequencing) approaches may elucidate small signatures of selection missed by coarser genome scans. When coupled with

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

appropriate phenotypic assays, association mapping in field-collected mosquitoes holds promise for identifying candidate genes.

Incipient Speciation

Extensive studies of chromosomal inversion polymorphisms and their relationship to environmental heterogeneities led to the discovery of cryptic reproductive units within *A. gambiae* (Bryan et al. 1982, Coluzzi et al. 1985, Toure et al. 1998). In sympatric population samples from Mali and Senegambia, inversion frequencies on chromosome 2R showed significant and temporally stable departures from Hardy-Weinberg equilibrium, including large heterozygote deficits. The heterozygote deficits were proposed to be an artifact of combining nonpanmictic populations carrying different inversion frequencies. Indeed, equilibrium was restored if assortatively mating chromosomally differentiated taxa (termed chromosomal forms) were hypothesized: In Senegambia, these were termed "Bissau" and "Savanna" (Bryan et al. 1982, Coluzzi et al. 1985); in Mali, "Savanna," "Mopti," and "Bamako" (Toure et al. 1998). The forms were characterized not only by different combinations and frequencies of inversions but also by different larval ecologies, although neither diagnostic (fixed) inversion differences nor intrinsic postzygotic barriers were found.

Evidence for the genome-wide differentiation expected of reproductively isolated taxa was sought outside of the chromosomal inversions, initially with poor success (e.g., Lanzaro et al. 1998), presumably owing to retained ancestral polymorphism and/or residual gene flow that was exacerbated by low resolution from a limited number of genetic markers. However, sequence variants identified in the rDNA near the centromere of the X chromosome revealed two nonpanmictic *A. gambiae* populations in various parts of Africa, which were designated the M and S molecular forms (della Torre et al. 2001). To the present day, these molecular forms are routinely recognized by fixed single-nucleotide polymorphism (SNP) differences in the rDNA (Fanello et al. 2002) that are unlinked and independent of autosomal inversion polymorphisms. This development eased the burden of taxonomic identification but raised the question of how well the new molecular method agreed with the previous chromosomal definitions across Africa. In Mali and Burkina Faso, there appeared to be good (though not precise) correspondence between the molecularly defined M taxon and the chromosomally defined Mopti taxon. However, the rDNA marker failed to distinguish the Savanna and Bamako chromosomal forms in Mali (both of which are detected as the S molecular form), and on closer inspection in Burkina Faso and elsewhere in Africa, the correlation between molecular and chromosomal form definitions deteriorates (Costantini et al. 2009, della Torre et al. 2001, Gentile et al. 2002, Simard et al. 2009). Moreover, the same rDNA-based M and S molecular subdivisions were uncovered within chromosomally undifferentiated (monomorphic) populations in forested areas of Cameroon (Wondji et al. 2002). This observation, together with the fact that inversions are shared across molecular (and chromosomal) forms, indicated that the inversions themselves do not contain the genes responsible for reproductive isolation. On the contrary, they appear to confer similar ecological adaptations across incipient species boundaries (Costantini et al. 2009). The M and S molecular forms are now widely recognized as the biologically relevant, assortatively mating reproductive units. The chromosomal definitions are of uncertain taxonomic value and are rarely invoked. However, the evolutionary significance of the chromosomally defined Bamako and Bissau forms remains unclear and requires study, and further subdivisions within the M and S forms would not be surprising.

The M and S forms appear to be the products of an ongoing ecological speciation process potentially triggered by competition at the larval stage. The ancestral S form characteristically breeds in rain-dependent ephemeral puddles. The M form is proposed to have arisen following the invasion of a new ecological niche characterized by larger and more stable anthropogenic larval

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

habitats (Costantini et al. 2009). In the savannas of West Africa, the M form breeds in habitats associated with irrigated agriculture (e.g., artificial lakes and rice paddies; Gimonneau et al. 2011). Longer-lived habitats extend breeding opportunities geographically and seasonally but also impose higher predator pressures. Consistent with a taxon adapted to more permanent breeding sites, the M form develops more slowly than the S form and is outcompeted in the absence of predators, but it is superior at predator avoidance (Diabate et al. 2005, 2008; Gimonneau et al. 2010). In the humid south of Cameroon in Central Africa, the M form also occupies long-lived anthropogenic breeding sites, but these are associated with urbanization (C. Kamdem, B.T. Fossog, F. Simard, J. Etouna, C. Ndo, P. Kengne, P. Boussès, F.-X. Etoa, P. Awono-Ambene, D. Fontenille, C. Antonio-Nkondjio, N.J. Besansky, & C. Costantini, in review), which suggests that population structure and local adaptation contribute to important phenotypic differences between geographic locations. Reinforcing this point, genetic studies have revealed subdivision between West and Central African M-form populations (Lee et al. 2009, Slotman et al. 2007b, Turner & Hahn 2007).

Application of the rDNA marker on thousands of M- and S-form mosquitoes sampled across sub-Saharan Africa revealed that hybridization rates between M and S forms are geographically variable, ranging from nil in Cameroon to >20% in westernmost Africa (Caputo et al. 2008, Oliveira et al. 2008), but are generally low (~1%; Costantini et al. 2009, della Torre et al. 2005, Simard et al. 2009, Tripet et al. 2001). Laboratory crossing experiments demonstrate that hybrids suffer no intrinsic (genetic) reduction in viability or fertility (Diabate et al. 2007), but premating behavioral barriers certainly exist in nature (Diabate et al. 2009, Pennetier et al. 2009), and postmating extrinsic isolating barriers are likely to exist in the form of reduced larval survival and mating success of M-S hybrids, although this has yet to be formally demonstrated. Nevertheless, although difficult to quantify, some amount of realized gene flow (i.e., genetic introgression) must occur, as indicated by the transfer of insecticide resistance alleles between forms (e.g., Etang et al. 2009, Weill et al. 2000).

The Speciation Island Model and Pericentromeric Divergence

Recently, Turner et al. (2005) used a gene-based microarray to scan for regions of elevated divergence between M- and S-form populations sampled from Cameroon. This and a subsequent array-based scan of Malian M and S samples revealed a paucity of differentiation across the genome except for the pericentromeric regions of all three chromosomes (Turner et al. 2005, White et al. 2010a). Under the assumption of substantial realized gene flow that homogenized most of the genetic variation between forms, these regions—termed speciation islands—were expected to contain alleles directly responsible for reproductive and ecological isolation. These alleles would be held together by reduced recombination near the centromeres and protected from introgressive gene flow by selection (Turner et al. 2005). Coalescent simulations suggested that reduced recombination alone could not account for the observed pattern of pericentromeric differentiation between forms, which supports the role of selection acting on these regions. However, a crucial parameter in the simulations was migration (gene flow) between forms; if gene flow was significantly overestimated, the pattern need not be explained by divergent ecological selection, and the pericentromeric genes need not bear directly on the speciation process (White et al. 2010a).

Quantifying the amount of realized gene flow between M and S forms is not straightforward. Until recently, available tools allowed detection of hybrid females only at a single X-linked marker. Because a single marker cannot distinguish between F1 and later-generation hybrids, it was theoretically possible that all hybrids sampled were F1s that failed to reproduce owing to ecological or behavioral maladaptation (Turner & Hahn 2010). To shed light on this issue, >500 M- and S-form mosquitoes sampled from West, Central, and East Africa were genotyped at diagnostic

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

markers developed from the centromere-proximal regions of chromosomes 2 and 3 in addition to the X-linked rDNA (White et al. 2010a). All except five multilocus genotypes revealed perfect genetic association across three independent chromosomes throughout the geographic samples, thus emphasizing the rarity of introgression between forms. However, among the five recombinant genotypes were two putative backcross progeny, which suggests that realized gene flow is nonzero. Improved estimates of gene flow between M and S forms, based on expanded application of multilocus markers and complemented by simulation studies, should improve our understanding of the speciation process and its genetic underpinnings, including the role of centromere-linked regions.

Assuming that centromere-linked M-S divergence is due to ecologically based divergent selection, a major question remains: How could pericentromeric regions alone, depauperate of genetic variation (save repetitive elements), provide the phenotypic diversity necessary for ecological speciation? In fact, recent comparative whole-genome sequencing and SNP genotyping of M and S forms revealed that divergence is not confined to the centromeres but rather is widespread (Lawniczak et al. 2010, Neafsey et al. 2010). Previous microarray-assisted genome scans likely missed this genome-wide pattern and captured only centromere-proximal divergence not merely because of lower resolution and statistical power, but also because the experimental design was geared to detect fixed rather than frequency differences. Although fixed differences between M and S forms are relatively uncommon in regions of normal recombination, such regions often bear the signature of divergent selection and have similar levels of absolute difference when compared with low-recombination regions.

Currently, a paucity of information is available about specific phenotypes relevant to ecological adaptation and reproductive isolation of M and S forms, and there is an attendant lack of genome-wide association studies, both of which are crucial guides to understanding the genetic architecture of *A. gambiae* speciation. Available evidence does not allow a direct assessment of the importance of pericentromeric regions to the speciation process. However, even if their importance is unambiguously established, these regions are unlikely to be the exclusive harbor of genes responsible for M-S isolation. Undue emphasis has been placed on pericentromeric regions owing to the expectation that genes underlying M-S isolation should be distinguished by fixed differences driven by selective sweeps. Although this expectation is reasonable for traits controlled by one or few genes and for alleles with large effects, there is a growing appreciation, based on theoretical and empirical work, that substantial and rapid adaptive shifts can also occur by modest allelic frequency changes distributed across many loci (known as polygenic adaptation; Pritchard & Di Rienzo 2010). Because polygenic adaptation can occur without dramatic changes in allele frequency, it may have been overlooked in *A. gambiae* owing to limitations in the methodological approach (e.g., microarray-assisted mapping; see above) or statistical approach (e.g., tests aimed at detecting only the signatures of strong selective sweeps) (Pritchard & Di Rienzo 2010). The sweep and polygenic models of adaptation are not mutually exclusive; both may play a role, just as both pericentromeric and noncentromeric regions may contribute to the ecological speciation process. Historical and ongoing anthropogenic environmental modifications such as climate change and urbanization represent nonequilibrium forces acting in spatially heterogeneous ways on M- and S-form populations, which complicates efforts to develop appropriate null models. Ultimately, definitive answers on the genetic basis of *A. gambiae* speciation will depend upon directly linking genotypes to phenotypes instrumental in M-S isolation.

EVOLUTIONARY DIVERSIFICATION AND VECTORIAL CAPACITY

Vectorial capacity can be loosely defined as the number of potential new malaria cases originating per day from each existing malaria case, owing to transmission by a particular vector species

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

(Garrett-Jones 1964). It is dependent upon the traits mentioned earlier: host preference, population density relative to the host, longevity, and vector competence, a term formally used to characterize the physiological ability for a given vector species to support the development of a parasite from its initial acquisition in a blood meal through transmission to a new host. Adaptive shifts of vector populations into new larval habitats and the potentially ensuing process of ecological speciation can have a profound, if indirect, impact on these parameters (Coluzzi et al. 2002). Expansion into new aquatic niches may increase population density and longevity (Coluzzi et al. 2002) but may also impose new immune challenges that could alter vector competence (White et al. 2010b). Variation among members of the *A. gambiae* complex exists for many traits that bear on vectorial capacity, although an understanding of their physiological and genetic basis is generally lacking, with the notable exception of vector competence.

Host Preference

Preference for human blood is a trait with obvious implications for vector status. Traditionally, *A. gambiae* has been considered the most anthropophilic species, followed by *A. arabiensis*, *A. melas*, and *A. merus*; *A. quadriannulatus* has been considered zoophilic. However, host preference likely varies considerably within species owing to population structure and environmental conditions, and reports of host preference also vary depending upon the methods used to measure this poorly defined behavior (Besansky et al. 2004, Takken & Knols 1999, Torr et al. 2008). Future studies pinpointing the neuromolecular and genetic basis of inherent host preference within anophelines may allow for the manipulation of this trait in nature as a means of malaria control, but progress will depend upon more precise behavioral definitions and assays.

Adult Biting and Resting Behavior

Members of the *A. gambiae* complex differ in their tendency to enter houses to obtain blood meals and to rest indoors afterward. Contributing to the overall anthropophilic behavior of *A. gambiae* are the indoor biting (endophagic) and indoor resting (endophilic) habits characteristic of this species. At the other end of the spectrum, the zoophilic *A. quadriannulatus* is considered to exhibit both exophagic and exophilic behavior. However, as with host preference, these tendencies may vary owing to local environmental conditions and genetically based differences between and even within populations. The Garki Project—a massive study on the epidemiology and control of malaria in the African savanna—failed to interrupt malaria transmission because a significant portion of the resident *A. gambiae* and *A. arabiensis* populations avoided contact with insecticide-treated interiors by biting and resting outdoors (Molineaux & Grammicia 1980). Polytene chromosome analysis of parallel indoor/outdoor mosquito collections within savanna villages revealed a nonrandom distribution of polymorphic inversion karyotypes: chromosomal arrangements associated with arid climates at larger geographic scales also were more frequent in the indoor microclimate, where the nocturnal saturation deficit is higher than it is outdoors (Coluzzi et al. 1977, 1979). These observations suggest that chromosomal inversions condition divergent behavioral responses with respect to microclimatic heterogeneities in the degree of aridity, although this hypothesis has yet to be tested directly.

Larval Habitat Selection

Saltwater tolerance is a trait that involves ionic regulation at the aquatic larval stage, and it appears to have been a factor in the adaptive radiation of the *A. gambiae* complex into diverse larval

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

habitats. Both the rectum and anal papillae are important in the physiology of this trait (Coetzee & le Sueur 1988, Smith et al. 2008). A mechanistic understanding of the physiology and genetics of ion regulation could open up new classes of larvicide and may also help to predict the potential of the *A. gambiae* complex to invade emerging urban habitats with highly polluted water.

Vector Competence

One of the most obvious and better-studied phenotypes that influence vector status is susceptibility to *Plasmodium* infection. At the species level, each sibling in the *A. gambiae* complex has been found naturally infected with *Plasmodium falciparum* except for *A. quadriannulatus*, which was shown to be competent in the laboratory (Takken et al. 1999). Although differences may exist in the degree of susceptibility between species (Habtewold et al. 2008), most variation seems to occur within species—between populations and individual mosquitoes.

Natural genetic variation in *Plasmodium* susceptibility has been observed in both laboratory colonies and field populations of *A. gambiae* (Collins et al. 1986, Niare et al. 2002, Riehle et al. 2006, Vernick et al. 1995). Despite the variety of *Plasmodium-Anopheles* interactions that are involved in the sporogonic phase of parasite development (i.e., the portion of the life cycle in the mosquito), all well-characterized instances of natural variation in vector competence can be attributed to polymorphisms in mosquito immune genes.

TEP1. Some of the first evidence of the effect of mosquito innate immunity on *Plasmodium* sporogony came from studies of mosquito strains selected to be refractory to parasite development. In 1986, two mosquito lines were selected from a single parent colony of *A. gambiae* that were susceptible and refractory to the simian parasite *Plasmodium cynomolgi* (Collins et al. 1986). The refractory strain killed and encapsulated nearly all early-stage ookinete parasites of most *Plasmodium* species tested, including human, simian, avian, and rodent malaria species. The only species against which this phenotype was significantly diminished upon infection was the human malaria parasite *P. falciparum*, and strains most resistant to encapsulation were those of African origin. This observation suggested that the *A. gambiae-Plasmodium* host-parasite system may be an example of a coevolutionary arms race. Under this hypothesis, *A. gambiae* has evolved resistance to *Plasmodium* species that naturally infect it, and in response, the parasites evolved the ability to evade the new immune defenses. The non-African *Plasmodium* species, which have not encountered this *Anopheles* defensive phenotype, have not evolved counter-defenses and are thus much more sensitive to the killing and encapsulation mechanisms.

Using genetic crosses between the selected strains, Blandin et al. (2009) mapped the genomic region controlling the parasite-killing component of this refractory phenotype to a fairly large interval on the left arm of *A. gambiae* chromosome 3. Within this region is a gene encoding a thioester-containing protein (TEP1), an innate immune system gene previously known to have both antiparasitic activity and high levels of sequence polymorphism (Blandin et al. 2004, Levashina et al. 2001, Obbard et al. 2008). Elevated diversity at this gene is primarily due to the segregation of two highly divergent alleles named *TEP1r* and *TEP1s*, which are associated with colonies either refractory or susceptible to malaria (Blandin et al. 2004). On the basis of this evidence, the divergent alleles of *TEP1* were proposed as the candidate genetic polymorphisms responsible for the refractory/susceptible phenotypes. Blandin et al. (2009) elegantly confirmed this prediction using reciprocal RNA interference to target the specific *TEP1* alleles in F1 hybrids.

Understanding how these two divergent *TEP1* alleles evolved and the current selective pressures controlling their maintenance and distribution in contemporary populations of *A. gambiae* can shed light on the evolution of vector competence. Both *TEP1s* and *TEP1r* alleles have been

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

sampled across large geographic distances and species boundaries, from *A. gambiae* populations in West, Central, and East Africa and in *A. arabiensis* (Obbard et al. 2008). Sequence analysis suggests that gene conversion from a neighboring and paralogous *TEP* gene likely was involved in the generation of these highly divergent alleles and may continue to shape their evolution (Obbard et al. 2008). Consistent with this suggestion, a recent study showed that the *TEP1r* class actually contains two distinct suballelic classes termed $TEP1r^A$ and $TEP1r^B$ (White et al. 2010b). $TEP1r^B$, which appears to be the derived allele, was recently swept to fixation in West African M-form populations of *A. gambiae*, but it is absent from all S-form populations and from *A. arabiensis*, both of which are polymorphic for *TEP1s* and $TEP1r^A$.

Although *TEP1* is clearly evolving under nonneutral evolutionary pressures, and resistance alleles of both subclasses confer increased resistance to malaria infection by *A. gambiae* in the laboratory, it remains to be seen if the malaria resistance phenotype is manifested in natural populations. Whatever the case, *Plasmodium* is just one of many pathogens that may be mediating the evolution of *TEP1*, and—in light of low mosquito infection prevalence and intensities in adult populations—it may exert negligible selective pressure relative to pathogens encountered in the aquatic larval habitat. TEP1 has a broad spectrum of activity against both bacteria and fungi (Dong et al. 2006). The genetic differentiation of *TEP1* between M- and S-form mosquitoes undoubtedly reflects the substantial reproductive isolation between these emerging species, but selection pressures on this gene may also differ between M and S forms. If so, *Plasmodium* infection of adult mosquitoes is unlikely to exert the differential pressure, as M and S forms have similar human-biting behavior. Instead, differential exposure of M and S larvae to waterborne pathogens in alternative aquatic habitats could be the cause of the M-form-specific selective sweep (Lehmann & Diabate 2008, White et al. 2010b). Field studies aimed at identifying larval pathogens in the breeding sites, elucidating their impact on larval fitness, and dissecting the role of innate immunity in their suppression (including the relative contributions of different *TEP1* alleles) could illuminate this question and open up a neglected area of vector competence research.

APL1. The genetic mapping approach that implicated *TEP1* in the refractory phenotype was based on *A. gambiae* laboratory colonies infected with cultured rodent malaria parasites, which makes it difficult to translate the importance of these findings for natural malaria transmission systems. This issue was bypassed in a series of experiments in which the progeny of wild-collected female *A. gambiae* were infected with locally transmitted strains of *P. falciparum* (Menge et al. 2006, Niare et al. 2002, Riehle et al. 2006). Together these studies identified a major effect region on the left arm of chromosome 2 controlling *P. falciparum* infection intensity in both Malian and Kenyan populations of *A. gambiae* (Riehle et al. 2007). Using bioinformatic and functional screens, Riehle et al. (2007) identified a single candidate gene named *Anopheles Plasmodium*-responsive leucine-rich repeat 1 (*APL1*). Later studies showed that *APL1* was not a single gene but a cluster of three very closely related paralogous genes termed *APL1A*, *APL1B*, and *APL1C* (Riehle et al. 2008). When each of these three genes were individually knocked down, only silencing of *APL1A* resulted in increased *P. falciparum* infection prevalence, which suggests that it is the best candidate of the three paralogs (Mitri et al. 2009). However, experiments have yet to connect nucleotide variation at this gene directly to *P. falciparum* infection intensity.

Strong parallels exist between patterns of molecular variation observed within and between M and S forms at the *TEP1* and *APL1* genes (Rottschaefer et al. 2011). Each of the three *APL1* paralogs exhibit exceptionally high diversity, and *APL1A* is polymorphic for two highly divergent alleles ($APL1A^1$ and $APL1A^2$). Although the *APL1* locus resides within the 2La inversion, there is only moderate differentiation between *APL1* sequences on alternative arrangements of 2La. Instead, most variation is partitioned between sympatric M- and S-form populations from West

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Africa. Moreover, there is strong evidence for a recent selective sweep affecting all three *APL1* genes in the M form but not in the S form. As TEP1 and APL1C physically interact (Fraiture et al. 2009, Povelones et al. 2009), it is tempting to suggest that this could be a case of parallel epistatic sweeps. As with the *TEP1* alleles, there is no clear evidence that *APL1C* polymorphism is actually an adaptive response to *Plasmodium*-mediated selection.

Scans for natural selection at immune genes. Key genes controlling mosquito immune defenses are expected to have undergone repeated selective sweeps or otherwise show signatures of nonneutral evolution. With this expectation, molecular evolutionary studies of more than 80 *A. gambiae* immune genes have been conducted (Cohuet et al. 2008, Lehmann et al. 2009, Obbard et al. 2007, Parmakelis et al. 2008, Simard et al. 2007, Slotman et al. 2007a). Aside from *TEP1* and *APL1*, these screens have identified only two additional genes that show evidence of selection, but in both cases the signature of adaptive protein evolution is weak (Parmakelis et al. 2010, Slotman et al. 2007a).

Direct evidence that *Plasmodium* infections impose a fitness cost to *A. gambiae* is quite limited. Although laboratory studies of *Plasmodium*-infected *Anopheles* species have documented a variety of fitness effects on the mosquito host, including increased mortality, behavioral abnormalities (e.g., increased probing time, decreased flying ability), and reductions in fecundity (reviewed in Hurd 2003), most have involved parasite-vector combinations that never occur in nature and have employed strains adapted to (long-term) laboratory culture. Furthermore, mosquito infection levels in these studies often exceed natural levels by one or two orders of magnitude. However, a study by Hogg & Hurd (1997) convincingly demonstrated that natural *P. falciparum* infections cause a significant, albeit slight, decrease in *A. gambiae* fecundity.

If *P. falciparum* does not impose strong selective pressure on *An. gambiae*, molecular evidence of an arms race between the parasite and mosquito immune genes may be difficult to come by. Mosquito immune genes must attack a broad range of pathogens, presumably including all four species of human *Plasmodium* parasites transmitted by *A. gambiae*. Moreover, significant and complex genotype-genotype interactions between mosquito and parasite may prevent the rapid selection of any single parasite refractory mechanism (Harris et al. 2010a, Lambrechts et al. 2005). Much of the relevant adaptive evolution may be occurring in *Plasmodium* as it continually finds new ways to circumvent the *A. gambiae* immune system, which is also evolving in response to other selective pressures (Cohuet et al. 2010).

Even if no exclusively anti-*Plasmodium* immune genes exist, one might expect to find a large number of mosquito immune genes to exhibit adaptive evolution as seen, e.g., in *Drosophila* (Schlenke & Begun 2003). It may be inherently difficult to detect selection in the *A. gambiae* complex, particularly in the two primary vectors *A. gambiae* and *A. arabiensis* (Obbard et al. 2009). High intraspecific diversity, low interspecific divergence, and substantial shared polymorphism limit the application of many standard tests of selection that require a closely related but reproductively isolated outgroup, which has not been firmly established. In addition, failing to account for historical population growth, migration, and differences in effective population size can weaken population genetic tests of natural selection in *A. gambiae* (Crawford & Lazzaro 2010). The forthcoming complete genome sequences of the *A. gambiae* species complex and its potentially closely related outgroup species *A. christyi* may help address both problems. As proof of principle, the use of more demographically correct null models was shown to increase the power of a relatively simple gene-based statistic (Tajima's *D*) to detect selection, but such models may prove even more powerful when applied at a whole-genome scale (Crawford & Lazzaro 2010).

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

FUTURE DIRECTIONS

Interest in *A. gambiae* and other species in the *A. gambiae* complex derives from two motives: (*a*) the role of species in this complex as vectors of human malaria parasites, particularly *P. falciparum*, and (*b*) the evident rapid pace of diversification as these species adapt to heterogeneous natural and human-created environments. *A. gambiae* provides a particularly rich subject for investigating and testing hypotheses about both sympatric speciation and the role of chromosome inversions in speciation and local adaptation. To some significant extent, the outcomes of these two motivating goals may converge. Ultimately, many of the important local adaptations that underlie selection for chromosomal inversions and even the speciation process itself will be found to be attributable to genotypes and phenotypes that themselves can constitute the targets for more efficiently implemented malaria control tools targeted at vectors. Below, we list some key questions whose investigation is likely to advance our understanding of the genotypic and phenotypic determinants of vectorial capacity in *A. gambiae* and other anopheline vectors when genomic resources become available.

1. What are the genetic and physiological determinants of ecological traits (e.g., larval habitat selection, blood meal host choice, resting behavior) that characterize and distinguish important vector species and populations (e.g., ecotypes)?
2. What are the genetic and physiological determinants of species-isolating mechanisms?
3. What is the role of chromosomal inversions and other low-recombination regions (e.g., pericentromeric regions) in local adaptation and reproductive isolation?
4. What are the evolutionary relationships among species and ecotypes in the *A. gambiae* complex, and can Y-chromosome sequences be used to resolve these relationships?
5. What are the important microbial pathogens of larval mosquitoes, and are these key drivers of immune system evolution in *Anopheles*?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited owing to space constraints. We thank the National Institutes of Health (grants R01AI063508 and R01AI076584; VectorBase contract HHSN 272200900039C) and the Bill and Melinda Gates Foundation (grant 45114) for funding.

LITERATURE CITED

Ayala FJ, Coluzzi M. 2005. Chromosome speciation: humans, *Drosophila*, and mosquitoes. *Proc. Natl. Acad. Sci. USA* 102(Suppl. 1):6535–42

Besansky NJ. 2008. *Genome analysis of vectorial capacity in major* Anopheles *vectors of malaria parasites.* **http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/AnophelesGenomesProposal_Aug3.pdf**.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Besansky NJ, Hill CA, Costantini C. 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol.* 20:249–51

Besansky NJ, Krzywinski J, Lehmann T, Simard F, Kern M, et al. 2003. Semipermeable species boundaries between *Anopheles gambiae* and *Anopheles arabiensis*: evidence from multilocus DNA sequence variation. *Proc. Natl. Acad. Sci. USA* 100:10818–23

Besansky NJ, Powell JR, Caccone A, Hamm DM, Scott JA, Collins FH. 1994. Molecular phylogeny of the *Anopheles gambiae* complex suggests genetic introgression between principal malaria vectors. *Proc. Natl. Acad. Sci. USA* 91:6885–88

Blandin S, Shiao SH, Moita LF, Janse CJ, Waters AP, et al. 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* 116:661–70

Blandin SA, Wang-Sattler R, Lamacchia M, Gagneur J, Lycett G, et al. 2009. Dissecting the genetic basis of resistance to malaria parasites in *Anopheles gambiae*. *Science* 326:147–50

Bryan JH, Di Deco MA, Petrarca V, Coluzzi M. 1982. Inversion polymorphism and incipient speciation in *Anopheles gambiae* s.str. in The Gambia, West Africa. *Genetica* 59:167–76

Caccone A, Garcia BA, Powell JR. 1996. Evolution of the mitochondrial DNA control region in the *Anopheles gambiae* complex. *Insect Mol. Biol.* 5:51–59

Caputo B, Nwakanma D, Jawara M, Adiamoh M, Dia I, et al. 2008. *Anopheles gambiae* complex along The Gambia river, with particular reference to the molecular forms of *An. gambiae* s.s. *Malar. J.* 7:182

Caputo B, Santolamazza F, Vicente JL, Nwakanma DC, Jawara M, et al. 2011. The "far-west" of *Anopheles gambiae* molecular forms. *PLoS ONE* 6:e16415

Charlwood JD, Pinto J, Sousa CA, Madsen H, Ferreira C, do Rosario VE. 2002. The swarming and mating behaviour of *Anopheles gambiae s.s.* (Diptera: Culicidae) from Sao Tome Island. *J. Vector Ecol.* 27:178–83

Chwatt LJ. 1945. Studies on the melanic variety of *Anopheles gambiae* in southern Nigeria. *Trop. Med. Hyg.* 48:22–30

Coetzee M, Craig M, le Sueur D. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol. Today* 16:74–77

Coetzee M, le Sueur D. 1988. Effects of salinity on the larvae of some Afrotropical anopheline mosquitoes. *Med. Vet. Entomol.* 2:385–90

Cohuet A, Harris C, Robert V, Fontenille D. 2010. Evolutionary forces on *Anopheles*: What makes a malaria vector? *Trends Parasitol.* 26:130–36

Cohuet A, Krishnakumar S, Simard F, Morlais I, Koutsos A, et al. 2008. SNP discovery and molecular evolution in *Anopheles gambiae*, with special emphasis on innate immune system. *BMC Genomics* 9:227

Collins FH, Mendez MA, Rasmussen MO, Mehaffey PC, Besansky NJ, Finnerty V. 1987. A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. *Am. J. Trop. Med. Hyg.* 37:37–41

Collins FH, Paskewitz SM. 1995. Malaria: current and future prospects for control. *Annu. Rev. Entomol.* 40:195–219

Collins FH, Sakai RK, Vernick KD, Paskewitz S, Seeley DC, et al. 1986. Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science* 234:607–10

Coluzzi M. 1982. Spatial distribution of chromosomal inversions and speciation in anopheline mosquitoes. In *Mechanisms of Speciation*, ed. C Barigozzi, pp. 143–53. New York: Alan R. Liss

Coluzzi M, Petrarca V, DiDeco MA. 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Boll. Zool.* 52:45–63

Coluzzi M, Sabatini A. 1967. Cytogenetic observations on species A and B of the *Anopheles gambiae* complex. *Parassitologia* 9:73–88

Coluzzi M, Sabatini A. 1968. Cytogenetic observations on species C of the *Anopheles gambiae* complex. *Parassitologia* 10:155–65

Coluzzi M, Sabatini A. 1969. Cytogenetic observations on the salt water species, *Anopheles merus* and *Anopheles melas*, of the *gambiae* complex. *Parassitologia* 11:177–86

Coluzzi M, Sabatini A, della Torre A, Di Deco MA, Petrarca V. 2002. A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Science* 298:1415–18

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

Coluzzi M, Sabatini A, Petrarca V, Di Deco MA. 1977. Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex. *Nature* 266:832–3

Coluzzi M, Sabatini A, Petrarca V, Di Deco MA. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. R. Soc. Trop. Med. Hyg.* 73:483–97

Costantini C, Ayala D, Guelbeogo WM, Pombi M, Some CY, et al. 2009. Living at the edge: Biogeographic patterns of habitat segregation conform to speciation by niche expansion in *Anopheles gambiae*. *BMC Ecol.* 9:16

Crawford JE, Lazzaro BP. 2010. The demographic histories of the M and S molecular forms of *Anopheles gambiae s.s*. *Mol. Biol. Evol.* 27:1739–44

Cuamba N, Mendis C. 2009. The role of *Anopheles merus* in malaria transmission in an area of southern Mozambique. *J. Vector Borne Dis.* 46:157–9

Davidson G. 1962. *Anopheles gambiae* complex. *Nature* 196:907

Davidson G, Jackson CE. 1962. Incipient speciation in *Anopheles gambiae* Giles. *Bull. World Health Organ.* 27:303–5

della Torre A, Fanello C, Akogbeto M, Dossou-yovo J, Favia G, et al. 2001. Molecular evidence of incipient speciation within *Anopheles gambiae* s.s. in West Africa. *Insect Mol. Biol.* 10:9–18

della Torre A, Merzagora L, Powell JR, Coluzzi M. 1997. Selective introgression of paracentric inversions between two sibling species of the *Anopheles gambiae* complex. *Genetics* 146:239–44

della Torre A, Tu Z, Petrarca V. 2005. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. *Insect Biochem. Mol. Biol.* 35:755–69

Diabate A, Dabire KR, Heidenberger K, Crawford J, Lamp W, et al. 2008. Evidence for divergent selection between the molecular forms of *Anopheles gambiae*: role of predation. *BMC Evol. Biol.* 8:5

Diabate A, Dabire RK, Kim EH, Dalton R, Millogo N, et al. 2005. Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: a transplantation experiment. *J. Med. Entomol.* 42:548–53

Diabate A, Dabire RK, Millogo N, Lehmann T. 2007. Evaluating the effect of postmating isolation between molecular forms of *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* 44:60–64

Diabate A, Dao A, Yaro AS, Adamou A, Gonzalez R, et al. 2009. Spatial swarm segregation and reproductive isolation between the molecular forms of *Anopheles gambiae*. *Proc. Biol. Sci.* 276:4215–22

Dong Y, Aguilar R, Xi Z, Warr E, Mongin E, Dimopoulos G. 2006. *Anopheles gambiae* immune responses to human and rodent *Plasmodium* parasite species. *PLoS Pathog.* 2:e52

Donnelly MJ, Pinto J, Girod R, Besansky NJ, Lehmann T. 2004. Revisiting the role of introgression versus shared ancestral polymorphisms as key processes shaping genetic diversity in the recently separated sibling species of the *Anopheles gambiae* complex. *Heredity* 92:61–68

Etang J, Vicente JL, Nwane P, Chouaibou M, Morlais I, et al. 2009. Polymorphism of intron-1 in the voltage-gated sodium channel gene of *Anopheles gambiae* s.s. populations from Cameroon with emphasis on insecticide knockdown resistance mutations. *Mol. Ecol.* 18:3076–86

Evans AM. 1931. Observations made by Dr. M.A. Barber on a melanic, coastal race of *Anopheles costalis*, Giles (*gambiae*) in southern Nigeria. *Ann. Trop. Med. Parasitol.* 25:443–53

Fanello C, Santolamazza F, della Torre A. 2002. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Med. Vet. Entomol.* 16:461–64

Feder JL, Nosil P. 2009. Chromosomal inversions and species differences: When are genes affecting adaptive divergence and reproductive isolation expected to reside within inversions? *Evolution* 63:3061–75

Fraiture M, Baxter RH, Steinert S, Chelliah Y, Frolet C, et al. 2009. Two mosquito LRR proteins function as complement control factors in the TEP1-mediated killing of *Plasmodium*. *Cell Host. Microbe* 5:273–84

Garrett-Jones C. 1964. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature* 204:1173–75

Gentile G, Della Torre A, Maegga B, Powell JR, Caccone A. 2002. Genetic differentiation in the African malaria vector, *Anopheles gambiae* s.s., and the problem of taxonomic status. *Genetics* 161:1561–78

Gimonneau G, Bouyer J, Morand S, Besansky NJ, Diabate A, Simard F. 2010. A behavioral mechanism underlying ecological divergence in the malaria mosquito *Anopheles gambiae*. *Behav. Ecol.* 21:1087–92

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Gimonneau G, Pombi M, Choisy M, Morand S, Dabire RK, Simard F. 2011. Larval habitat segregation between the molecular forms of the mosquito *Anopheles gambiae* in a rice field area of Burkina Faso, West Africa. *Med. Vet. Entomol.* In press

Gray EM, Rocca KA, Costantini C, Besansky NJ. 2009. Inversion 2La is associated with enhanced desiccation resistance in *Anopheles gambiae*. *Malar J.* 8:215

Habtewold T, Povelones M, Blagborough AM, Christophides GK. 2008. Transmission blocking immunity in the malaria non-vector mosquito *Anopheles quadriannulatus* species A. *PLoS Pathog.* 4:e1000070

Hackett LW. 1937. *Malaria in Europe, an Ecological Study*. London: Oxford Univ. Press

Harbach RE. 2004. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bull. Entomol. Res.* 94:537–53

Harbach RE, Townson H, Mukwaya LG, Adeniran T. 1997. Use of rDNA-PCR to investigate the ecological distribution of *Anopheles bwambae* in relation to other members of the *An. gambiae* complex of mosquitoes in Bwamba County, Uganda. *Med. Vet. Entomol.* 11:329–34

Harris C, Lambrechts L, Rousset F, Abate L, Nsango SE, et al. 2010a. Polymorphisms in *Anopheles gambiae* immune genes associated with natural resistance to *Plasmodium falciparum*. *PLoS Pathog*. 6:e1001112

Harris C, Rousset F, Morlais I, Fontenille D, Cohuet A. 2010b. Low linkage disequilibrium in wild *Anopheles gambiae s.l.* populations. *BMC Genet.* 11:81

Hogg JC, Hurd H. 1997. The effects of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae s. l.* in north east Tanzania. *Parasitol* 114:325–31

Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, et al. 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 298:129–49

Hunt RH, Coetzee M, Fettene M. 1998. The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.* 92:231–35

Hurd H. 2003. Manipulation of medically important insect vectors by their parasites. *Annu. Rev. Entomol.* 48:141–61

Kirkpatrick M, Barton N. 2006. Chromosome inversions, local adaptation and speciation. *Genetics* 173:419–34

Klowden MJ. 2001. Sexual receptivity in *Anopheles gambiae* mosquitoes: absence of control by male accessory gland substances. *J. Insect Physiol.* 47:661–66

Krzywinski J, Chrystal MA, Besansky NJ. 2006. Gene finding on the Y: Fruitful strategy in *Drosophila* does not deliver in *Anopheles*. *Genetica* 126:369–75

Krzywinski J, Wilkerson RC, Besansky NJ. 2001. Toward understanding Anophelinae (Diptera, Culicidae) phylogeny: insights from nuclear single-copy genes and the weight of evidence. *Syst. Biol.* 50:540–56

Lambrechts L, Halbert J, Durand P, Gouagna LC, Koella JC. 2005. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to *Plasmodium falciparum*. *Malar. J.* 4:3

Lanzaro GC, Toure YT, Carnahan J, Zheng L, Dolo G, et al. 1998. Complexities in the genetic structure of *Anopheles gambiae* populations in west Africa as revealed by microsatellite DNA analysis. *Proc. Natl. Acad. Sci. USA* 95:14260–65

Lawniczak MK, Emrich SJ, Holloway AK, Regier AP, Olson M, et al. 2010. Widespread divergence between incipient *Anopheles gambiae* species revealed by whole genome sequences. *Science* 330:512–14

Lee Y, Cornel AJ, Meneses CR, Fofana A, Andrianarivo AG, et al. 2009. Ecological and genetic relationships of the Forest-M form among chromosomal and molecular forms of the malaria vector *Anopheles gambiae sensu stricto*. *Malar. J.* 8:75

Lehmann T, Diabate A. 2008. The molecular forms of *Anopheles gambiae*: a phenotypic perspective. *Infect. Genet. Evol.* 8:737–46

Lehmann T, Hume JC, Licht M, Burns CS, Wollenberg K, et al. 2009. Molecular evolution of immune genes in the malaria mosquito *Anopheles gambiae*. *PLoS ONE* 4:e4549

Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC. 2001. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, *Anopheles gambiae*. *Cell* 104:709–18

Manoukis NC, Diabate A, Abdoulaye A, Diallo M, Dao A, et al. 2009. Structure and dynamics of male swarms of *Anopheles gambiae*. *J. Med. Entomol.* 46:227–35

Manoukis NC, Powell JR, Touré MB, Sacko A, Edillo FE, et al. 2008. A test of the chromosomal theory of ecotypic speciation in *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* 105:2940–45

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Marchand RP. 1984. Field observations on swarming and mating in *Anopheles gambiae* mosquitos in Tanzania. *Netherlands J. Zool.* 34:367–87

Menge DM, Zhong D, Guda T, Gouagna L, Githure J, et al. 2006. Quantitative trait loci controlling refractoriness to *Plasmodium falciparum* in natural *Anopheles gambiae* mosquitoes from a malaria-endemic region in western Kenya. *Genetics* 173:235–41

Mitri C, Jacques JC, Thiery I, Riehle MM, Xu J, et al. 2009. Fine pathogen discrimination within the APL1 gene family protects *Anopheles gambiae* against human and rodent malaria species. *PLoS Pathog.* 5:e1000576

Molineaux L, Grammicia G. 1980. *The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa.* Geneva, Switzerland: World Health Organ.

Muirhead Thompson RC. 1951. Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast. *Bull. Entomol. Res.* 41:487–503

Neafsey DE, Lawniczak MK, Park DJ, Redmond SN, Coulibaly MB, et al. 2010. SNP genotyping defines complex gene-flow boundaries among African malaria vector mosquitoes. *Science* 330:514–17

Niare O, Markianos K, Volz J, Oduol F, Toure A, et al. 2002. Genetic loci affecting resistance to human malaria parasites in a West African mosquito vector population. *Science* 298:213–16

Nishihara H, Maruyama S, Okada N. 2009. Retroposon analysis and recent geological data suggest near-simultaneous divergence of the three superorders of mammals. *Proc. Natl. Acad. Sci. USA* 106:5235–40

Obbard DJ, Callister DM, Jiggins FM, Soares DC, Yan G, Little TJ. 2008. The evolution of *TEP1*, an exceptionally polymorphic immunity gene in *Anopheles gambiae*. *BMC Evol. Biol.* 8:274

Obbard DJ, Linton YM, Jiggins FM, Yan G, Little TJ. 2007. Population genetics of *Plasmodium* resistance genes in *Anopheles gambiae*: no evidence for strong selection. *Mol. Ecol.* 16:3497–510

Obbard DJ, Welch JJ, Little TJ. 2009. Inferring selection in the *Anopheles gambiae* species complex: an example from immune-related serine protease inhibitors. *Malar. J.* 8:117

Okara RM, Sinka ME, Minakawa N, Mbogo CM, Hay SI, Snow RW. 2010. Distribution of the main malaria vectors in Kenya. *Malar. J.* 9:69

Oliveira E, Salgueiro P, Palsson K, Vicente JL, Arez AP, et al. 2008. High levels of hybridization between molecular forms of *Anopheles gambiae* from Guinea Bissau. *J. Med. Entomol.* 45:1057–63

Parmakelis A, Moustaka M, Poulakakis N, Louis C, Slotman MA, et al. 2010. *Anopheles* immune genes and amino acid sites evolving under the effect of positive selection. *PLoS ONE* 5:e8885

Parmakelis A, Slotman MA, Marshall JC, Awono-Ambene PH, Antonio-Nkondjio C, et al. 2008. The molecular evolution of four anti-malarial immune genes in the *Anopheles gambiae* species complex. *BMC Evol. Biol.* 8:79

Paterson HE. 1962. Status of East African salt-water-breeding variant of *Anopheles gambiae* Giles. *Nature* 195:469–70

Paterson JS, van Eeden GJ. 1963. A new member of the *Anopheles gambiae* complex: a prelimary report. *Med. Proc.* 9:414–18

Pennetier C, Warren B, Dabire KR, Russell IJ, Gibson G. 2009. "Singing on the wing" as a mechanism for species recognition in the malarial mosquito *Anopheles gambiae*. *Curr. Biol.* 20:131–36

Pombi M, Caputo B, Simard F, Di Deco MA, Coluzzi M, et al. 2008. Chromosomal plasticity and evolutionary potential in the malaria vector *Anopheles gambiae* sensu stricto: insights from three decades of rare paracentric inversions. *BMC Evol. Biol.* 8:309

Povelones M, Waterhouse RM, Kafatos FC, Christophides GK. 2009. Leucine-rich repeat protein complex activates mosquito complement in defense against *Plasmodium* parasites. *Science* 324:258–61

Pritchard JK, Di Rienzo A. 2010. Adaptation—not by sweeps alone. *Nat. Rev. Genet.* 11:665–67

Riehle MM, Markianos K, Lambrechts L, Xia A, Sharakhov I, et al. 2007. A major genetic locus controlling natural *Plasmodium falciparum* infection is shared by East and West African *Anopheles gambiae*. *Malar. J.* 6:87

Riehle MM, Markianos K, Niare O, Xu J, Li J, et al. 2006. Natural malaria infection in *Anopheles gambiae* is regulated by a single genomic control region. *Science* 312:577–79

Riehle MM, Xu J, Lazzaro BP, Rottschaefer SM, Coulibaly B, et al. 2008. *Anopheles gambiae* APL1 is a family of variable LRR proteins required for Rel1-mediated protection from the malaria parasite, *Plasmodium berghei*. *PLoS ONE* 3:e3672

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Rocca KA, Gray EM, Costantini C, Besansky NJ. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malar. J.* 8:147

Rottschaefer SM, Riehle MM, Coulibaly B, Sacko M, Niaré O, et al. 2011. Exceptional diversity, maintenance of polymorphism, and recent directional selection on the *APL1* malaria resistance genes of *Anopheles gambiae*. *PLoS Biol.* 9:e1000600

Schlenke TA, Begun DJ. 2003. Natural selection drives *Drosophila* immune system evolution. *Genetics* 164:1471–80

Scott JA, Brogdon WG, Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 49:520–29

Sharakhov IV, White BJ, Sharakhova MV, Kayondo J, Lobo NF, et al. 2006. Breakpoint structure reveals the unique origin of an interspecific chromosomal inversion (2La) in the *Anopheles gambiae* complex. *Proc. Natl. Acad. Sci. USA* 103:6258–62

Simard F, Ayala D, Kamdem GC, Etouna J, Ose K, et al. 2009. Ecological niche partitioning between the M and S molecular forms of *Anopheles gambiae* in Cameroon: the ecological side of speciation. *BMC Ecol.* 9:17

Simard F, Licht M, Besansky NJ, Lehmann T. 2007. Polymorphism at the *defensin* gene in the *Anopheles gambiae* complex: testing different selection hypotheses. *Infect. Genet. Evol.* 7:285–92

Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, et al. 2010. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic precis. *Parasit. Vectors* 3:117

Slotman M, Della Torre A, Powell JR. 2004. The genetics of inviability and male sterility in hybrids between *Anopheles gambiae* and *An. arabiensis*. *Genetics* 167:275–87

Slotman M, della Torre A, Powell JR. 2005a. Female sterility in hybrids between *Anopheles gambiae* and *A. arabiensis*, and the causes of Haldane's rule. *Evolution* 59:1016–26

Slotman MA, della Torre A, Calzetta M, Powell JR. 2005b. Differential introgression of chromsomal regions between *Anopheles gambiae* and *An. arabiensis*. *Am. J. Trop. Med. Hyg.* 73:326–35

Slotman MA, Parmakelis A, Marshall JC, Awono-Ambene PH, Antonio-Nkondjo C, et al. 2007a. Patterns of selection in anti-malarial immune genes in malaria vectors: evidence for adaptive evolution in *LRIM1* in *Anopheles arabiensis*. *PLoS ONE* 2:e793

Slotman MA, Tripet F, Cornel AJ, Meneses CR, Lee Y, et al. 2007b. Evidence for subdivision within the M molecular form of *Anopheles gambiae*. *Mol. Ecol.* 16:639–49

Smith KE, VanEkeris LA, Okech BA, Harvey WR, Linser PJ. 2008. Larval anopheline mosquito recta exhibit a dramatic change in localization patterns of ion transport proteins in response to shifting salinity: a comparison between anopheline and culicine larvae. *J. Exp. Biol.* 211:3067–76

Stump AD, Pombi M, Goeddel L, Ribeiro JMC, Wilder JA, et al. 2007. Genetic exchange in 2La inversion heterokaryotypes of *Anopheles gambiae*. *Insect Mol. Biol.* 16:703–09

Takken W, Eling W, Hooghof J, Dekker T, Hunt R, Coetzee M. 1999. Susceptibility of *Anopheles quadriannulatus* Theobald (Diptera: Culicidae) to *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* 93:578–80

Takken W, Knols BG. 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annu. Rev. Entomol.* 44:131–57

Torr SJ, Della Torre A, Calzetta M, Costantini C, Vale GA. 2008. Towards a fuller understanding of mosquito behaviour: use of electrocuting grids to compare the odour-orientated responses of *Anopheles arabiensis* and *An. quadriannulatus* in the field. *Med. Vet. Entomol.* 22:93–108

Toure YT, Petrarca V, Traore SF, Coulibaly A, Maiga HM, et al. 1998. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia* 40:477–511

Tripet F, Toure YT, Taylor CE, Norris DE, Dolo G, Lanzaro GC. 2001. DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae*. *Mol. Ecol.* 10:1725–32

Turner TL, Hahn MW. 2007. Locus- and population-specific selection and differentiation between incipient species of *Anopheles gambiae*. *Mol. Biol. Evol.* 24:2132–38

Turner TL, Hahn MW. 2010. Genomic islands of speciation or genomic islands and speciation? *Mol. Ecol.* 19:848–50

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print

Turner TL, Hahn MW, Nuzhdin SV. 2005. Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biol.* 3:e285

Vernick KD, Fujioka H, Seeley DC, Tandler B, Aikawa M, Miller LH. 1995. *Plasmodium gallinaceum*: a refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. *Exp. Parasitol.* 80:583–95

Wang-Sattler R, Blandin S, Ning Y, Blass C, Dolo G, et al. 2007. Mosaic genome architecture of the *Anopheles gambiae* species complex. *PLoS ONE* 2:e1249

Weetman D, Wilding CS, Steen K, Morgan JC, Simard F, Donnelly MJ. 2010. Association mapping of insecticide resistance in wild *Anopheles gambiae* populations: major variants identified in a low-linkage disequilbrium genome. *PLoS ONE* 5:e13140

Weill M, Chandre F, Brengues C, Manguin S, Akogbeto M, et al. 2000. The *kdr* mutation occurs in the Mopti form of *Anopheles gambiae* s.s. through introgression. *Insect Mol. Biol.* 9:451–55

White BJ, Cheng C, Sangare D, Lobo NF, Collins FH, Besansky NJ. 2009. The population genomics of trans-specific inversion polymorphisms in *Anopheles gambiae*. *Genetics* 183:275–88

White BJ, Cheng C, Simard F, Costantini C, Besansky NJ. 2010a. Genetic association of physically unlinked islands of genomic divergence in incipient species of *Anopheles gambiae*. *Mol. Ecol.* 19:925–39

White BJ, Hahn MW, Pombi M, Cassone BJ, Lobo NF, et al. 2007. Localization of candidate regions maintaining a common polymorphic inversion (2La) in *Anopheles gambiae*. *PLoS Genet.* 3:e217

White BJ, Lawniczak MK, Cheng C, Coulibaly MB, Wilson MD, et al. 2010b. Adaptive divergence between incipient species of *Anopheles gambiae* increases resistance to *Plasmodium*. *Proc. Natl. Acad. Sci. USA* 108:244–49

White GB. 1971. Chromosomal evidence for natural interspecific hybridization by mosquitoes of the *Anopheles gambiae* complex. *Nature* 231:184–85

White GB. 1973. Comparative studies on sibling species of *Anopheles gambiae* Giles complex (Dipt., Culicidae) III. The distribution, ecology, behavior and vectorial importance of species D in Bwamba County, Uganda, with an analysis of biological, ecological, morphological and cytogenetical relationships of Ugandan species D. *Bull. Entomol. Res.* 63:65–97

Wilkins E, Howell PI, Benedict MQ. 2006. Observations of *Anopheles gambiae* with mixed rDNA arrays containing both Mopti and Savanna types. *Am. J. Trop. Med. Hyg.* 75:170–70

Wondji C, Frederic S, Petrarca V, Etang J, Santolamazza F, et al. 2005. Species and populations of the *Anopheles gambiae* complex in Cameroon with special emphasis on chromosomal and molecular forms of *Anopheles gambiae* s.s. *J. Med. Entomol.* 42:998–1005

Wondji C, Simard F, Fontenille D. 2002. Evidence for genetic differentiation between the molecular forms M and S within the Forest chromosomal form of *Anopheles gambiae* in an area of sympatry. *Insect Mol. Biol.* 11:11–19

Annu. Rev. Ecol. Evol. Syst. 2011.42. Downloaded from www.annualreviews.org
by University of California - Riverside on 10/21/11. For personal use only.

REVIEWS IN ADVANCE

Changes may still occur before final publication online and in print