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Review

Review of genetic diversity in malaria vectors (Culicidae: Anophelinae)

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ABSTRACT

We review previous studies on the genetic diversity of malaria vectors to highlight the major trends in population structure and demographic history. In doing so, we outline key information about molecular markers, sampling strategies and approaches to investigate the causes of genetic structure in *Anopheles* mosquitoes. Restricted gene flow due to isolation by distance and physical barriers to dispersal may explain the spatial pattern of current genetic diversity in some *Anopheles* species. Nonetheless, there is noteworthy disagreement among studies, perhaps due to variation in sampling methodologies, choice of molecular markers, and/or analytical approaches. More refined genealogical methods of population analysis allowing for the inclusion of the temporal component of genetic diversity facilitated the evaluation of the contribution of historical demographic processes to genetic structure. A common pattern of past unstable demography (*i.e.*, historical fluctuation in the effective population size) by several *Anopheles* species, regardless of methodology (DNA markers), mosquito ecology (anthropophilic vs zoophilic), vector status (primary vs secondary) and geographical distribution, suggests that Pleistocene environmental changes were major drivers of divergence at population and species levels worldwide.
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Abbreviations: ($\theta = 4N_e\mu$) θ, the population parameter for genetic diversity; N_e, the effective population size; μ, the mutation rate; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; mtDNA, mitochondrial DNA; *ITS2*, the internal transcribed spacer two; *ITS1*, the internal transcribed spacer one; *ND5*, the mitochondrial NADH dehydrogenase subunit five gene; *ND4*, the mitochondrial NADH dehydrogenase subunit four gene; RFLP, restriction fragment length polymorphism; *COI*, the mitochondrial cytochrome oxidase subunit one gene; IBD, isolation by distance; ya, years ago; km, kilometers; GRV, Great Rift Valley; PEVCH, Pleistocene environmental changes; EPA, Environmental Protection Agency; SENACYT, Secretariat for Science, Technology and Innovation of Panama.

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1. Background

Anopheles mosquitoes, of the Culicidae family (Subfamily: Anophelinae), constitute the most economically and medically important insects worldwide. They transmit filarial worms, arboviruses, and malaria parasites, causing a significant economic burden as well as human mortality (Clements, 1992; Service, 1993). Malaria control through the management of *Anopheles* populations has been a priority for the last two centuries with some qualified successes, but continues to be a challenge (WHO, 2004, 2006; Walker and Lynch, 2007). Research on the genetic diversity of malaria vectors could contribute to *Anopheles* management, but assessments of the overall patterns of *Anopheles* diversification, phylogenetic relationships and population structure are limited mainly to primary vectors (Table 1, Fig. 1) (Harbach, 2004; Hay et al., 2010; Zarowiecki et al., 2011b). Initially, genetic diversity studies in *Anopheles* were conducted using summary statistics, based on predetermined models of contemporary genetic structure that include several unrealistic assumptions. For example, the standard neutral equilibrium model assumes that the effective population size (N_e) has remained stable for at least ($\theta = 4\ N_e\mu$) generations and random mating is occurring throughout the species' range (Hey and Machado, 2003). This implies that all populations within a species have identical historical demography, with a steady rate of genetic exchange, regardless of geographic location. Although this approach is appropriate to investigate the spatial pattern of genetic diversity, it ignores demographic history (Emerson et al., 2001; Kuhner, 2008).

Recent advances in software development and the coalescent theory (Hey and Machado, 2003) have prompted more comprehensive studies of demographic history, using most of the chronological information in molecular markers (Excoffier and Heckel, 2006). Contrary to summary statistics, the non-equilibrium approach assumes that population parameters are not stable, thus there is not a steady-state pattern of genetic variation because the genetic structure differs at different points in time (Rosenberg and Nordborg, 2002; Hey and Machado, 2003). The genealogical method has resulted in a better understanding of historical demographic processes and of how past changes in N_e have influenced the history of migration events among *Anopheles* populations. It has also served to infer both past and contemporary rates of gene flow as well as to explore whether barriers to dispersal are current or historical (Walton et al., 2000; Temu and Yan, 2005; Mirabello and Conn, 2006a,b; Reiff et al., 2007; O'Loughlin et al., 2007, 2008; Morgan et al., 2010; Loaiza et al., 2010a,b; Chen et al., 2011). Ultimately, an analysis of nucleotide polymorphism based on the frequency of mutations and their genealogical relationships (*i.e.*, the combined approach) has been used to distinguish amongst genetic drift, natural selection, introgression and population history as the causes of genetic structure, thereby assessing the levels of contemporary gene flow in *Anopheles* mosquitoes more accurately (Walton et al., 2001; Onyabe and Conn, 2001b; Donnelly et al., 2004; Dusfour et al., 2004, 2007a; Bower et al., 2008; Mirabello et al., 2008; Morgan et al., 2009; Loaiza et al., 2010a,b; Neafsey et al., 2010; Zarowiecki et al., 2011a; Chen et al., 2011). The present review summarizes previous studies on the genetic diversity of malaria vectors and focuses on population structure and demographic history while also briefly touching on phylogenetic relationships, phylogeography and molecular taxonomy. In addition, we review key information about molecular markers and sampling strategies best suited to accurately investigate the spatial and temporal patterns of genetic diversity in *Anopheles* mosquitoes.

2. Rationale for exploring genetic diversity

The primary goal of such studies is to identify population demes, lineages or molecular forms that may exhibit differential involvement in malaria transmission (Donnelly et al., 2002; Michel et al., 2005b; Cohuet et al., 2010; Riehle et al., 2011). These genetic entities are defined in terms of historical genetic exchange, and their limits may vary spatially and temporally (Besansky et al., 1997; Donnelly et al., 2001, 2002; Walton et al., 2001; McKeon et al., 2010; Chen et al., 2011). Knowledge of intraspecific genetic diversity is informative about N_e, and demographic cohesiveness (*i.e.*, random mating), and can be applied to reduce malaria through vector control. Comparing estimates of N_e in *Anopheles* species before and after insecticide application can be a useful monitoring tool (Wondji et al., 2005; Czeher et al., 2010). Identification of physical or ecological barriers to gene flow can help predict the spread of genes involved in parasite refractoriness and/or insecticide resistance, thus providing practical information about the number of release points for transgenic mosquitoes (Pinto et al., 2003; Wondji et al., 2005; Catteruccia, 2007; Marshall et al., 2008; Muturi et al., 2010; Dixit et al., 2011). These studies can also shed light on the biogeographical scenario and phylogenetic relationships amongst closely related *Anopheles* species (Krzywinski and Besansky, 2003; Ma et al., 2006; Foley et al., 2007; Dusfour et al., 2007a; Sallum et al., 2007; Reidenbach et al., 2009; Bourke et al., 2010; Zarowiecki et al., 2011b). This is central for malaria control because correct vector-species identification is required for effective insecticide treatment, likely to be severely compromised by increasing numbers of *Anopheles* species complexes (Coetzee et al., 1999; Linton et al., 2001, 2003; Dusfour et al., 2004; Garros et al., 2005a; Oshaghi et al., 2007; Djadid et al., 2007; Patsoula et al., 2007; Bourke et al., 2010; Alquezar et al., 2010; Zarowiecki et al., 2011b). An understanding of the demographic history of *Anopheles* mosquitoes can provide insights into responses to past geological and climatic changes and colonization events that led to current geographic distributions. Such insights might help predict changes in mosquito biogeography due to ongoing and future climate changes worldwide (Garros et al., 2005b; Dusfour et al., 2004, 2007a; Foley and Torres, 2006; Oshaghi et al., 2006; Foley et al., 2007; O'Loughlin et al., 2008; Gutiérrez et al., 2009; Hasan et al., 2008a; Morgan et al., 2009, 2010; Pedro et al., 2010; Loaiza et al., 2010a,b; Zarowiecki et al., 2011a; Chen et al., 2011; Vicente et al., 2011). Disentangling the effects of natural selection and population history may help identify genes involved in mosquito–pathogen co-evolution, which are potential targets for malaria control intervention (Lehmann et al., 2009; White et al., 2010; Dixit et al., 2011). Lastly, molecular and genomic population analysis may, by advancing our knowledge of *Anopheles* speciation, lead to a better understanding of vector evolutionary history and malaria-transmission dynamics (Lehmann

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Definitions

Anthropophilic Mosquito species, population or molecular lineage that prefers to feed on human beings rather than on animals.
Zoophilic Mosquito species, population or molecular lineage that prefers to feed on animals rather than on human beings.
Endophilic Mosquito species, population or molecular lineage that rests mainly inside houses after taking a blood meal.
Exophilic Mosquito species, population or molecular lineage that rests mainly outside houses after taking a blood meal.
Sympatric Mosquito species, populations or molecular lineages that co-occur in the same local geographic area, but are reproductively isolated from each other.
Anthropogenic Physical or environmental changes that are driven by human activities and affect mosquito demography.

Table 1
Summary of studies on the genetic diversity of malaria vectors (*Anopheles* spp.).

Species	Markers	Likely causes of genetic structure	References
An. albimanus	*ND5*, *16S*	Restricted gene flow due to IBD	De Merida et al. (1999)
An. albimanus	msats, *ND5*	Restricted gene flow due to physical barriers (Central American cordillera)	Molina-Cruz et al. (2004)
An. albimanus	*COI*, 4 msat loci	Limited past gene flow between Pacific and Atlantic populations and expansion due to PEVCH	Gutiérrez et al. (2009)
An. albimanus	*COI*, *white*, *ITS2*	Population divergence and bottlenecks followed by demographic expansion due to PEVCH	Loaiza et al. (2010a,b)
An. darlingi	*COI*	No IBD and two colonization events in South America	Conn (1998)
An. darlingi	8 msat loci	Restricted gene flow due to IBD between locations north and south of the Amazon River	Conn et al. (2006)
An. darlingi	*COI*	Lineage divergence and demographic expansion due to PEVCH	Mirabello and Conn (2006a)
An. darlingi	*ND4*	No IBD and little genetic structure	Angêlla et al. (2007)
An. darlingi	8 msat loci	Restricted gene flow due to IBD	Scarpassa and Conn (2007)
An. darlingi	5–8 msat loci	Genetic structure due to N_e differences and IBD between locations in Amazonia	Mirabello et al. (2008)
An. darlingi	*COI*	Range expansion and restricted gene flow due to physical barriers (Andes and Amazon River)	Pedro and Sallum (2009)
An. triannulatus, *An. darlingi*	*COI*	Concordant patterns of demographic expansion due to PEVCH	Pedro et al. (2010)
An. aquasalis	*COI*	Amazon River not a barrier to gene flow	Fairley et al. (2002)
An. nuneztovari	*White*	Lineage divergence due to Miocene/Pliocene marine incursion and PEVCH	Mirabello and Conn (2008)
An. marajoara	*COI*, *white*, *ITS2*	Holocene expansion in lineage 1 due to savannah expansion or lowland extinction–recolonization of rainforest	McKeon et al. (2010)
An. marajoara	9 msat loci	Lineage divergence due to barriers to gene flow (Eastern Andean cordillera)	Brochero et al. (2010)
An. punctipennis	*COI*	No IBD and restricted gene flow due to physical barriers (Lake Champlain and the Green mountains)	Fairley et al. (2000)
An. atroparvus	8 msat loci	Pyrenees and Alps mountain ranges not barriers to gene flow	Vicente et al. (2011)
An. maculipennis	*COI*, *ITS2*	High COI homology and no ITS2 intra-specific variation	Patsoula et al. (2007)
An. messeae	*ITS2*	Nine ITS2 variants due to limited gene flow among geographical regions	Bezzhonova and Goryacheva (2008)
An. stephensi	*COI*, *COII*	No IBD and little genetic structure	Oshaghi et al. (2006)
An. dirus	*COI*	Pleistocene introgression, range expansion, or selective sweep	Walton et al. (2001)
An. dirus, *An. baimaii*	*COI*, *COII*	Lineage divergence and demographic expansion due to PEVCH	O'Loughlin et al. (2008)
An. dirus s.l.	*ITS2*	No intra-specific variation throughout species range	Prakash et al. (2010
An. dirus s.l.	msats, *COI*, *COII*, *hsp82*, *est6*, *ninaE*	PEVCH led to speciation with secondary contact and gene flow in the last 100,000 years	Morgan et al. (2010)
An. scanloni	*COI*, *COII*, *ITS2*	Lineage divergence and expansion due to PEVCH	O'Loughlin et al. (2007)
An. vagus	*COI*, *ITS2*	Pleistocene fragmentation followed by population growth due to Savanna expansion	Zarowiecki et al. (2011a)
Minimus subgroup	*COII*, *ITS1*, *ITS2*, *D3*, *gua*	Lineage divergence due to PEVCH	Sharpe et al. (2000)
An. minimus	*COII*	Lineage divergence and demographic expansion due to PEVCH	Chen et al. (2011)
An. minimus	*COI*, *ITS2*, *D3*	PEVCH drives diversification between *An. minimus* A and C	Garros et al. (2005a,b)
Neocellia Series	*COII*, *ND5*, *ITS2*, *D3*	PEVCH influenced intra-specific diversification, but did not drive speciation	Morgan et al. (2009)
An. jeyporiensis	*COII*	Demographic expansion due to PEVCH	Chen et al. (2004)
An. maculatus	7 msat loci	Restricted gene flow due to physical barriers (Phuket mountain range)	Rongnoparut et al. (2006)
An. sinensis	Control region	Restricted gene flow due to physical barriers (mountains) and recent population expansion in the last 100,000 years	Jung et al. (2007)
Barbirostris subgroup	*COI*, *ITS2*	Demographic expansion linked to human expansion in the last 100,000 years	Paredes-Esquivel et al. (2009)
An. farauti s.s.	*ND4*/*ND5*	Restricted gene flow due to physical barriers (ocean)	Reiff et al. (2007)
An. farauti s.s.	*COII*	Demographic expansion times correspond to human arrival and establishment	Hasan et al. (2008a)
An. farauti s.s., *An. irenicus*	*COII*, *ITS2*	Demographic expansion linked to human arrival for *An. farauti*, but not for *An. irenicus*	Hasan et al. (2008b)
An. farauti	*ITS1*	Concerted evolution accompanied by inter-population divergence	Bower et al. (2008)
An. flavirostris	*COI*	Deep population structure influenced more by present day than by PEVCH	Foley and Torres (2006)
An. hinesorum	*COII*	Ocean not a barrier to gene flow	Hasan et al. (2008c)
An. sundaicus s.l.	*COI*, *Cyt-b*	Lineage divergence and bottleneck followed by demographic expansion due to PEVCH	Dusfour et al. (2007a)
An. punctulatus group	*ITS1*	Concerted evolution accompanied by inter-population divergence	Bower et al. (2009)

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Table 1 (*continued*)

Species	Markers	Likely causes of genetic structure	References
An. annulipes s.l.	*COI*, *COII*, *EF-1α*	Past climate changes influenced species radiation	Foley et al. (2007)
An. gambiae	*ND5*, msats	High genetic variation and large N_e due to high dispersal capabilities	Lehmann et al. (1997)
An. gambiae	9 msat loci	Restricted gene flow across the Rift Valley despite heavy human traffic	Lehmann et al. (1999)
An. gambiae	*ND5*	Restricted gene flow due to physical barriers (Rift Valley)	Lehmann et al. (2000)
An. gambiae	11 msat loci	Restricted gene flow due to IBD with discontinuities	Lehmann et al. (2003)
An. gambiae s.s.	12 msat loci	Restricted gene flow across habitat types and demographic expansion	Pinto et al. (2003)
An. gambiae	6 msat loci	Ocean not a barrier to gene flow	Chen et al. (2004)
An. gambiae	10–17 msat loci	Restricted gene flow due to physical barriers (ocean)	Kayondo et al. (2005)
An. gambiae	11 msat loci	Restricted gene flow due to physical (ocean) and biological barriers	Moreno et al. (2007)
An. gambiae	109 genes	M and S forms expanded at different times that pre-date advent of agriculture	Crawford and Lazzaro (2010)
An. gambiae, *An. arabiensis*	*Cyt-b*, *ND5*, *ND1*	Extensive gene flow due to recent range expansion from large, stable populations	Besansky et al. (1997)
An. gambiae, *An. arabiensis*	7 msat loci	Rift Valley does not restrict gene flow between populations	Kamau et al. (1999)
An. gambiae, *An. arabiensis*	*ND5*, msats	Demographic expansion linked to human expansion in the last 100,000 years	Donnelly et al. (2001)
An. gambiae, *An. arabiensis*	*ND5*	Recent speciation or introgression	Donnelly et al. (2004)
An. gambiae, *An. arabiensis*	*COI*	Population expansion and selection shaping genetic structure	Matthews et al. (2007)
An. arabiensis	6 msat loci	Population divergence and demographic expansion	Donnelly et al. (1999)
An. arabiensis	9 msat loci	Extensive gene flow across 250 km	Simard et al. (2000)
An. arabiensis	10 msat loci	Reduced N_e due to founder effects and range expansion	Onyabe and Conn (2001a)
An. arabiensis	6 msat loci	No IBD and little population structure	Nyanjom et al. (2003)
An. arabiensis	10 msat loci	Strong genetic structure due to physical barriers	Morlais et al. (2005)
An. arabiensis	12 msat loci	Bottleneck followed by rapid expansion and diversity not significantly affected by ITNs	Wondji et al. (2005)
An. arabiensis	msats, *ND5*	Barriers to gene flow (Rift Valley), but differentiation varies among areas of genome	Temu and Yan (2005)
An. arabiensis	8 msat loci	Species occurs as single, continuous panmictic population	Muturi et al. (2010)
An. funestus	7 msat loci	Genetic structure due to higher diversity in western populations	Braginets et al. (2003)
An. funestus	6 msat loci	Restricted gene flow due to IBD across 1200 km	Temu et al. (2004)
An. funestus	10 msat loci	Significant population structure due to barriers to gene flow in Madagascar	Ayala et al. (2006)
An. funestus	msats, *ND5*	No IBD and introgression with sympatric divergences between forms	Michel et al. (2006)
An. nili	msats, *ITS2*, *D3*, *COII*, *ND4*	Extensive gene flow due to recent demographic expansion	Ndo et al. (2010)
An. moucheti	10 msat loci	Restricted gene flow due to IBD and barriers to dispersal (forest)	Antonio-Nkondjio et al. (2007, 2008)

IBD, Isolation by distance; PEVCH, Pleistocene environmental changes; N_e, the effective population size; km, Kilometers; ITNs, insect treated bed nets; msats, microsatellites; *ND5*, the mitochondrial subunit five gene; *ND4*, the mitochondrial NADH dehydrogenase subunit four gene; *ND1*, the mitochondrial NADH dehydrogenase subunit one gene; *16S*, the rRNA 16S gene; *COI*, the mitochondrial cytochrome oxidase subunit one gene; *COII*, the mitochondrial cytochrome oxidase subunit two gene; *Cyt-b*, the mitochondrial cytochrome *b* gene; *ITS2*, the internal transcribed spacer two; *D3*, the D three region of the rRNA 26S gene; *hsp82*, heat shock protein gene; *est6*, esterase gene; *ninaE*, rh1 opsin gene; *gua*, guanylate cyclase; *EF-1α* exons of elongation factor-1 alpha; PNG, Papua New Guinea.

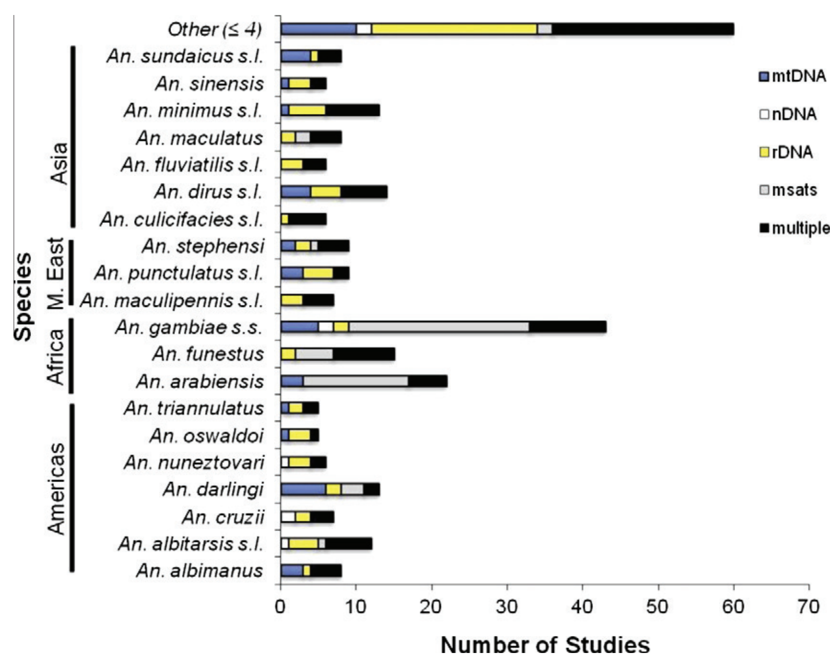

Fig. 1. The graph shows the number of previous studies conducted on the genetic diversity of primary malaria vectors (*Anopheles* spp.) around the world. The information is broken down by geographic area (in words on the left side) and type of molecular marker (in words and different colors on the right side).

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et al., 2009; Neafsey et al., 2010; Zarowiecki et al., 2011b; Costantini et al., 2009; Simard et al., 2009).

3. Sampling scheme and molecular markers

Molecular taxonomy, population structure and phylogeography all vary in scope, requiring different DNA markers and sampling schemes (Kuhner, 2008). Studies of population structure (*i.e.*, assessing patterns and rates of gene flow) generally requires many mosquitoes from several spatially distributed sites, whereas testing for monophyletic clades usually includes only a few individuals per taxon and no obligatory geographical information (Excoffier and Heckel, 2006). The phylogeographic method combines data from phylogenetics and population genetics, focusing particularly on the processes that explain geographical origins of a lineage (Beheregaray, 2008). For sampling in phylogeographic studies the optimal number of sites and mosquitoes per site is determined by the species' ecology and demographic history. Ubiquitous and widespread generalist *Anopheles* species likely maintain a higher N_e than less abundant and more unevenly distributed species; thus analysis of the former will require more sites and mosquitoes per site to achieve adequate resolution (Donnelly et al., 2002). In contrast, species that have undergone recent range expansion or bottleneck(s) generally require fewer sampling sites and individuals but need a range of different markers and more widespread sampling to more accurately determine the timing and geographic extent of these population processes (Donnelly et al., 2001, 2004; Lehmann et al., 2003; Dusfour et al., 2004; Michel et al., 2005a; Pedro et al., 2010; Morgan et al., 2010; Crawford and Lazzaro, 2010; Loaiza et al., 2010b; Zarowiecki et al., 2011a; Vicente et al., 2011). The geographic context should also be considered; some regions may require more comprehensive sampling due to a complex geological and environmental history leading to repeated isolation, greater loss of diversity and higher speciation rate (Garros et al., 2005b; Morgan et al., 2010; Loaiza et al., 2010a; Zarowiecki et al., 2011a; Chen et al., 2011). Lack of appropriate sampling in an island system may lead to tentative inferences of speciation and colonization pathway in some *Anopheles* species (Dusfour et al., 2004, 2007a,b; Foley and Torres, 2006; Hasan et al., 2008a,c), a situation that may also arise in mainland *Anopheles* species when genetic differentiation is largely due to sampling gaps rather than to a lack of genetic exchange (Molina-Cruz et al., 2004; Mirabello and Conn, 2006a; Bora et al., 2009; Pedro et al., 2010; Ndo et al., 2010).

Each molecular marker provides information on the historical demography of malaria vectors at different points in time. Mutation rate(s) for mitochondrial genes are usually higher than for nuclear genes, but slower than for microsatellite loci. Also, the recombination rate, mode of inheritance and genome location must all be considered for adequate resolution of different evolutionary processes (Rosenberg and Nordborg, 2002; Wandeler et al., 2007; Dixit et al., 2011). Agreement between different markers may provide a robust perspective on cladogenesis and population structure, especially if they operate under different evolutionary constraints (Linton et al., 2003; Garros et al., 2005b; Reidenbach et al., 2009; Neafsey et al., 2010; Alquezar et al., 2010). For instance, congruent results of demographic history in *Anopheles* mosquitoes are expected by both nuclear and mitochondrial genes when genetic divergence is caused by demographic processes, as these are genome-wide forces, whereas selection is locus specific (Lehmann et al., 1999, 2000, 2003; Michel et al., 2005a,b; Koekemoer et al., 2006; Morgan et al., 2010; Alquezar et al., 2010; Loaiza et al., 2010b; Ndo et al., 2010). Nevertheless, discrepancies can emerge when using the total-evidence approach (*i.e.*, concatenating multiple loci), as different genes may depict different molecular signals indicative of natural selection, introgression or lack of marker resolution (Sallum et al., 2007; Bourke et al., 2010; McKeon et al., 2010). Hence, the outcome of studies on genetic diversity of *Anopheles* will depend on whether markers evolved neutrally and so can unambiguously represent the relationship between genetic drift and N_e (Hey and Machado, 2003). Although the assumption of neutrality seems to hold true for most studies carried out (Reidenbach et al., 2009; Etang et al., 2009; Moreno et al., 2010; Dixit et al., 2011), there also exist instances of selective sweeps in some species (Onyabe and Conn, 2001b; Walton et al., 2001; Matthews et al., 2007; White et al., 2010; Neafsey et al., 2010).

3.1. Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been widely used to answer questions about molecular taxonomy, phylogenetic relationships and population structure in malaria vectors (Table 1, Supplementary Table 1). High copy numbers and the availability of conserved primers and PCR protocols make mtDNA an ideal starting point to investigate genetic diversity. In addition, the molecular clock, calculated using mtDNA, allows for time estimation and therefore helps to unravel the temporal component (Moreno et al., 2010). The results of mtDNA studies generally match those of others carried out with different markers (*i.e.*, microsatellites and nuclear genes) (Molina-Cruz et al., 2004; Lehmann et al., 2000, 2003; Michel et al., 2005a,b; Koekemoer et al., 2006; Vicente et al., 2011), but there are several exceptions (Di Luca et al., 2004; Oshaghi et al., 2006, 2007; Bora et al., 2009; Gutiérrez et al., 2009; McKeon et al., 2010). These exceptions are perhaps due to contemporary hybridization among previously isolated lineages, or to introgression between sister taxa (Besansky et al., 1997; Walton et al., 2000; Donnelly et al., 2004). Also, more recent restriction to gene flow might promote divergence in the mtDNA, whereas other more slowly mutating markers reveal no genetic differentiation due to incomplete lineage sorting and retention of ancestral polymorphism (O'Loughlin et al., 2007; Dusfour et al., 2007a; Bora et al., 2009; Bourke et al., 2010; McKeon et al., 2010; Vicente et al., 2011). Mitochondrial genes are inherited as a single block and may therefore provide a limited view of the species' demographic history because they are more susceptible to selective forces (Ballard and Rand, 2005; Bazin et al., 2006; Matthews et al., 2007; Dixit et al., 2011); but see also Ballard and Melvin, 2010 and Moreno et al., 2010 for more recent discussions. Furthermore, because of a strongly biased AT content, mitochondrial genes are subject to saturation leading to rapid loss of phylogenetic signal due to homoplasy (*i.e.*, multiple hits). Heterogeneity in levels of nucleotide diversity, sequence saturation and base substitution across a single mitochondrial gene such as the mtDNA *COI* barcode, might also confound the results of traditional approaches to delimit *Anopheles* species (Hebert et al., 2003; Cywinska et al., 2006; Kumar et al., 2007). This could be because fixed differences are not spread homogeneously across this gene and consequently no single DNA fragment is likely to consistently define the transition from intrapopulation to speciation processes (Roe and Sperling, 2007; Foley et al., 2007; McKeon et al., 2010).

3.2. Nuclear DNA

To complement the mtDNA history, some researchers have used nuclear loci to investigate the history of the nuclear genome and to provide independent evidence of population divergence and cladogenesis in *Anopheles* (Table 1, Fig. 1). The *white* gene, a homolog of the *Drosophila melanogaster* eye color gene, has become a gene of choice in several studies involving malaria vectors (Table 1, Supplementary Table 1). Concordant results by the *white*,

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morphological characters and some mtDNA genes indicate that this single copy gene is likely to reflect a broad spectrum of genetic diversity, from speciation events to intraspecific historical demography (Besansky and Fahey, 1997; Reidenbach et al., 2009; Loaiza et al., 2010b). Nevertheless, recombination events in some nuclear genes may compromise their use in population genetics due to non-unique signals of demographic history (Krzywinski and Besansky, 2003). Although using a nuclear gene may be a logical solution to the sex-biased representation of mtDNA, some of these genes are sex-linked; and due to a slower mutation rate, they might be too conserved to uncover genetic structure because of more recent ecological adaptations (Bora et al., 2009; McKeon et al., 2010; Dixit et al., 2011).

3.2.1. Microsatellite loci

Nuclear microsatellite loci (msats) have been developed and used primarily to estimate patterns and rates of gene flow between African malaria vectors (Table 1, Fig. 1, Supplementary Table 1). Contemporary levels of gene flow among *Anopheles* populations are generally assessed using microsatellites because, due to their high mutation rate, they can detect differentiation even in weakly structured species (Temu et al., 2004; Scarpassa and Conn, 2007; Muturi et al., 2010; Czeher et al., 2010; Vicente et al., 2011). In contrast, large-scale geographical differences are better detected using mtDNA polymorphisms because, due to maternal inheritance, N_e for mtDNA is a quarter of that of nuclear markers, and genetic drift may produce a strong signal of spatial population processes (*i.e.*, migration) (Chen et al., 2004; Jung et al., 2007; O'Loughlin et al., 2007). Nonetheless, the mutation rate for mtDNA is slower than for microsatellites, thus erroneous estimates of patterns and rates of contemporary gene flow could be obtained under the phylogeographic framework, as mtDNA may portray signals of more ancient demographic processes (Mirabello and Conn, 2006a; Foley and Torres, 2006; Reiff et al., 2007; Pedro et al., 2010; Chen et al., 2011). Several studies indicate, though, that microsatellite loci, having a larger N_e and longer coalescent time than mtDNA, also depict signals of demographic history rather than current genetic exchange between *Anopheles* populations (Lehmann et al., 1997; Onyabe and Conn, 2001a,b; Molina-Cruz et al., 2004; Gutiérrez et al., 2009; Morgan et al., 2010; Ndo et al., 2010; Czeher et al., 2010; Vicente et al., 2011).

3.2.2. The internal transcribed spacer two

The non-coding internal transcribed spacer two (ITS2) is the most commonly used source of species-specific PCR primers for uncovering cryptic *Anopheles* species (Collins and Paskewitz, 1996; Linton et al., 2001, 2003; Wilkerson et al., 2004; Li and Wilkerson, 2005, 2007; Alquezar et al., 2010) (Fig. 1, Supplementary Table 1). Length differences and fixed substitutions among ITS2 sequences are taken as proof of lineage splitting especially if the lineages are geographically codistributed (Walton et al., 2007a,b; Paredes-Esquivel et al., 2009; Alquezar et al., 2010). Despite challenging alignment issues, this marker can also be phylogenetically informative (Marrelli et al., 2005; Ma et al., 2006; Djadid et al., 2007) (Supplementary Table 1), although failure to identify putative cryptic species has been reported (Di Luca et al., 2004; Bargues et al., 2006; Bora et al., 2009). This may reflect lack of cloning (*i.e.*, overlooked intraindividual variation) and sampling gaps in some studies, or a complex, not well understood mode of evolution (Bower et al., 2008; Alquezar et al., 2010; Ndo et al., 2010; McKeon et al., 2010). The population structure of malaria vectors has also been investigated with the ITS2 marker, but to a much lesser extent than molecular taxonomy (Table 1, Supplementary Table 1). Overall, patterns of genetic divergence by the ITS2 seem to be consistent with the geographical origin of populations, and this may indicate that concerted evolution is acting intraspecifically regardless of significant intraindividual variation (Djadid et al., 2005; Patsoula et al., 2007; Bezzhonova and Goryacheva, 2008; Bower et al., 2009; Prakash et al., 2010; Loaiza et al., 2010b; Zarowiecki et al., 2011a). Also, even though natural selection and chromosomal recombination may affect the accuracy of this spacer to identify genetic discontinuities (*i.e.*, species and population boundaries), a recent study by Alquezar et al. (2010) strongly supported its evolutionary cohesion across interbreeding populations, thus reinforcing its value for taxonomic studies in malaria vectors.

4. Contemporary genetic structure

4.1. Isolation by distance

Restricted gene flow due to geographic distance, also known as isolation by distance (IBD), is an equilibrium model based on the hypothesis that gene flow is operating among neighboring populations, with the goal to identify the geographical distance that makes them genetically distinct. Under IBD, random mating is more likely to occur between mosquitoes in nearby sites than those more distantly located (Wright, 1951; Jensen et al., 2005). In general, support for IBD in malaria vectors is either contentious or weak, and it seems to vary according to species and methodologies (Table 1). De Merida et al. (1999) analyzed partial sequences of the *ND5* gene and hypothesized restricted gene flow due to geographic distance in mainland populations of *Anopheles* (*Nyssorhynchus*) *albimanus*. However, IBD was not supported by further analysis using the *COI* and *white* genes, four microsatellite loci and more comprehensive sampling across the northern Neotropics (Molina-Cruz et al., 2004; Gutiérrez et al., 2009; Loaiza et al., 2010a,b). In South America, a positive and significant relationship between genetic and geographic distance was hypothesized for *Anopheles* (*Nyssorhynchus*) *nuneztovari* s.l. using the *white* gene (Mirabello and Conn, 2008), and studies based on microsatellites demonstrated that IBD is one force shaping genetic differentiation in *Anopheles* (*Nyssorhynchus*) *darlingi*, in Amazonian Brazil (Conn et al., 2006; Scarpassa and Conn, 2007; Mirabello et al., 2008). However, in most of these cases, the correlation was weak and not detected when different lineages and geographic regions were analyzed separately. Besides, IBD was not detected in *An. nuneztovari* s.l. with analysis of mtDNA RFLPs (Conn et al., 1998) or in *An. darlingi* using the *COI* and *ND4* genes (Mirabello and Conn, 2006a; Angêlla et al., 2007).

In Africa, none of the dominant malaria vectors, *Anopheles* (*Cellia*) *gambiae* s.s., *Anopheles* (*Cellia*) *arabiensis*, and *Anopheles* (*Cellia*) *funestus* conform to the IBD pattern, despite extensive testing at the continental and regional levels, using several DNA markers (Besansky et al., 1997; Donnelly et al., 2001, 2002; Nyanjom et al., 2003; Lehmann et al., 2003; Michel et al., 2006). Likewise, no clear-cut evidence of IBD has been reported for the members of the Southeast Asian *Anopheles* (*Cellia*) *dirus* and *Anopheles* (*Cellia*) *minimus* Complexes using microsatellites and mtDNA (Walton et al., 2000; O'Loughlin et al., 2007, 2008; Morgan et al., 2010; Chen et al., 2011). The likely reasons for this trend are extensive genetic exchange due to high dispersal capabilities or a long-term and stable N_e, although these claims are hard to reconcile with a limited flight range and the strong seasonality recorded for some of these vectors (Lehmann et al., 1997; Donnelly et al., 1999; Simard et al., 2000; Pinto et al., 2003; Temu and Yan, 2005). The lack of IBD in many anophelines could be due to significant long distance human-aided dispersal or physical barriers to gene flow, but this pattern can also be the result of a recent population expansion from an already large genetic pool (Coluzzi et al., 1979; Onyabe and Conn, 2001a,b; Donnelly et al., 2001, 2002; Crawford and Lazzaro, 2010; Ndo et al., 2010). In contrast, misleading signals of IBD can

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be caused by a recent range colonization with sequential founder effect (*i.e.*, bottleneck) along a colonizing path, thus mimicking the IBD pattern (De Merida et al., 1999; Matthews et al., 2007; O'Loughlin et al., 2008; Ndo et al., 2010; Chen et al., 2011; Zarowiecki et al., 2011a; Vicente et al., 2011) or by confounding genetic information (*i.e.*, analyzing different karyotypes or lineages together) (Onyabe and Conn, 2001a,b; Michel et al., 2006; Pedro et al., 2010). Some of the seemingly contradictory IBD findings in malaria vectors could be the result of considerable differences in sampling schemes (*i.e.*, the number of sampling sites, mosquitoes per site, and the distances among sites) and the choice of DNA marker (Foley and Torres, 2006; O'Loughlin et al., 2008; Loaiza et al., 2010a; Pedro et al., 2010; Chen et al., 2011; Vicente et al., 2011). These studies are also likely to be biased because the regression analysis used to test for IBD has relied largely on straight-line distances, which probably rarely represent the effective dispersal pathways of most *Anopheles* species (Mirabello and Conn, 2006a; Pedro and Sallum, 2009; Ndo et al., 2010; Loaiza et al., 2010a; Zarowiecki et al., 2011a; Vicente et al., 2011).

4.2. Physical barriers to dispersal

Physical barriers to dispersal may impede gene flow between *Anopheles* populations, but only for those species that lack the capacity to traverse or circumnavigate them. This ability will not only depend on the species ecology (*i.e.*, flight range and the type of breeding sites), but also on the geographic extent and the shape of such barriers. Physical barriers may act in conjunction with other climatic or biological barriers and their effects might vary over time, thus making it difficult to assess their real input to genetic exchange (Fairley et al., 2002; Morlais et al., 2005; Hasan et al., 2008b; Muturi et al., 2010). Mountain ranges (cordilleras), oceans, rivers and forests can hamper gene flow for some malaria vectors according to different molecular markers, but there is also noteworthy disagreement (Table 1). Considerable evidence supports the Great Rift Valley (GRV) as a major east–west barrier for several *Anopheles* species in Africa (Kamau et al., 1999; Braginets et al., 2003; Temu and Yan, 2005). Lehmann et al. (1999, 2000) reported high mtDNA divergence among populations of *An. gambiae* s.s. located less than 700 kilometers (km) apart, but mostly for those separated by the GRV. These findings were later supported by analysis of 11 microsatellite loci and widespread continental sampling (Lehmann et al., 2003). Similarly, a clear division between eastern and western populations of *An. funestus* s.s. has been identified using microsatellites and analogous results were obtained in subsequent mtDNA and RFLPs analyses (Michel et al., 2005a,b; Koekemoer et al., 2006). In South Korea, a mitochondrial analysis suggested that the Taebaek and Sobaek mountains have promoted sub-division in *Anopheles* (*Anopheles*) *sinensis* (Jung et al., 2007), while, in Thailand, *Anopheles* (*Cellia*) *maculatus* exhibited significant microsatellite differentiation due to the Phuket mountain ranges (Rongnoparut et al., 2006). In the Americas, Molina-Cruz et al. (2004) hypothesized that mountain ranges in Costa Rica and western Panama, act as barriers to dispersal for *An. albimanus*. In the same way, the eastern Andean cordillera, in the northern Neotropics, was thought to restrict dispersal for some lineages of *An. nuneztovari* s.l. (Mirabello and Conn, 2008) and *Anopheles* (*Nyssorhynchus*) *marajora* (Brochero et al., 2010), whereas populations of *Anopheles* (*Anopheles*) *punctipennis* were partially separated by the Green Mountains in the eastern United States (Fairley et al., 2000).

In Africa, Kayondo et al. (2005) hypothesized significant microsatellite differentiation between populations of *An. gambiae* s.s. from four Lake Victoria islands and attributed it to their separation by water, higher genetic drift in smaller demes and/or local ecological adaptation. Similarly, populations of this species from Equatorial Guinea and Gabon depicted considerable microsatellite divergence in part due to an ocean barrier (Moreno et al., 2007). Ocean barriers might have also been the initial driving force causing diversification between a species from Grenada Island and two other putative species of *An. pseudopunctipennis* s.l. from the American continent (Coetzee et al., 1999). Conn et al. (2006) detected microsatellite differentiation between populations of *An. darlingi* on either side of the Amazon River near the Atlantic Ocean, but the signal was weaker for localities upriver, more distant from the mouth. In contrast, mtDNA divergence in the saltwater tolerant *Anopheles* (*Nyssorhynchus*) *aquasalis*, was not affected by this river, providing no support for a proposed fresh water barrier (Fairley et al., 2002). Populations of *An. funestus* s.s. from the east and west coasts of Madagascar were significantly differentiated based on ten microsatellite loci; although this pattern was initially thought to be due to forest-restricted dispersal, it could also be the result of adaptation to two regional bioclimates (Ayala et al., 2006). Signals of forest-restricted gene flow have also been hypothesized for *Anopheles* (*Cellia*) *moucheti* and *Anopheles* (*Cellia*) *nili* in mainland Africa. As in the case of *An. funestus* s.s. these taxa are usually restricted to woody areas along streams and rivers, so their dispersal among villages is believed to be limited by thick vegetation (Antonio-Nkondjio et al., 2007, 2008; Ndo et al., 2010).

5. Demographic history

5.1. Past unstable demography

To date, non-equilibrium populations of *Anopheles* mosquitoes have been documented worldwide, with historical bottleneck, geographic fragmentation and subsequent secondary contact via range expansion as the most likely cause of genetic structure (Table 1). In general, closely related and co-distributed *Anopheles* species have responded similarly to historical demographic processes (Walton et al., 2000; Donnelly et al., 2001, 2002; Crawford and Lazzaro, 2010; Pedro et al., 2010). Phylogenetic research in the Oriental Neocellia Series of *Anopheles* demonstrated that while speciation was almost entirely due to climatic oscillation in the Miocene most intraspecific divergence was driven by sea level changes during the Pleistocene (Morgan et al., 2009). Furthermore, speciation events giving rise to the allopatric members of the Funestus and the Minimus subgroups of the Funestus group of *Anopheles* subgenus *Cellia* was hypothesized to be due to ecological fluctuation, habitat fragmentation and genetic isolation as a result of climatic and vegetation shifts. Yet, diversification in the former group occurred earlier in Africa in the Pliocene whilst species within the latter diversified more recently in Southeast Asia during the first glaciation period (Garros et al., 2005a,b).

Most malaria vectors depict signals of population growth, albeit at different times and likely in response to different factors (Table 1). The shallow population structure in the African malaria vector *An. gambiae* s.s. was initially hypothesized to be the result of recent expanding human populations (95% Cl: 5000–10,000 ya) because of the development of agriculture (Coluzzi et al., 1979; Donnelly et al., 2001, 2002). Similarly, some members of the *Anopheles* Barbirostris subgroup have expanded demographically likely due to increased farming and cattle ranching during the Agrarian revolution, in Thailand and Indonesia (95% Cl: 13,000–20,000 ya) (Paredes-Esquivel et al., 2009). Also, a signal of protracted Pleistocene range expansion (95% Cl: 0.3–2.1 million years ago) has been detected for *Anopheles* (*Cellia*) *vagus* across the Indo-Oriental region, perhaps because of the expansion of grassland and/or the high abundance of wild ungulates at that time (Zarowiecki et al., 2011a). In contrast, the late Pleistocene population expansion of *An. darlingi* and the Holocene expansion

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in lineage 1 of *An. marajoara*, both in northeastern Brazil, were proposed to be due to changes in climatic conditions leading to either forest fragmentation and refugia isolation (Mirabello and Conn, 2006a) or savannah expansion and lowland extinction–recolonization of rainforest (McKeon et al., 2010). Forest refugia were also believed to have triggered expansions in *An. dirus* s.s. (172,202 ya) and *Anopheles* (*Cellia*) *baimaii* (234,443 ya), in Southeast Asia (O'Loughlin et al., 2008) as well as in *An. minimus* mitochondrial lineages (A) and (B) across China, Vietnam and Thailand, 208,000 and 357,000 ya, respectively (Chen et al., 2011).

5.2. Evidence for a common response of anophelines to Pleistocene environmental changes

The pattern of past unstable demography seen in many *Anopheles* species appears to be caused by fluctuation in breeding habitats; both larval habitat type and availability are key determinants of mosquito demography (Service and Townson, 2002). For example, human adapted mosquitoes are more likely to be transported via anthropogenic activities, which may promote genetic homogenization over long geographic distances despite true physical barriers to dispersal. In contrast, forest loving mosquitoes are less likely to be carried by humans because they do not breed in human-made habitats (Mirabello and Conn, 2006a; O'Loughlin et al., 2008; McKeon et al., 2010; Chen et al., 2011). Additionally, adult feeding behavior has been hypothesized as another important factor driving demographic expansion in the *An. gambiae* Complex; several members of this group preferentially feed upon human blood, thus an increase in human population is likely to trigger a corresponding increase in mosquito population (Coluzzi et al., 1979; Donnelly et al., 2001, 2002; Della Torre et al., 2005; Cohuet et al., 2010). According to these observations, anthropophilic *Anopheles* species should depict signals of population history in response to past changes in human demography, whereas non-anthropophilic species may change demographically due to historical fluctuation in environmental conditions that affected breeding habitat availability (Mirabello and Conn, 2006a; O'Loughlin et al., 2008; Chen et al., 2011). This view is consistent with the evidence of population expansion in *An. gambiae* s.s. driven by expanding human populations (Coluzzi et al., 1979; Donnelly et al., 2001, 2002; Matthews et al., 2007; Cohuet et al., 2010), and also with the signal of population growth in *An. darlingi*, *An. dirus* and *An. minimus* due to changes in environmental conditions during the Pleistocene (Mirabello and Conn, 2006a; O'Loughlin et al., 2008; Pedro and Sallum, 2009; Chen et al., 2011). This somewhat also fits with a priori predictions as the latter three species have somewhat opportunistic adult feeding behaviors and are less dependent upon humans for larval breeding (Zimmerman et al., 2006; Achee et al., 2007; O'Loughlin et al., 2008; Morgan et al., 2010; Chen et al., 2011). On the other hand, similar overlapping temporal patterns of demographic expansion may reveal a common response of *Anopheles* species due to changes in Pleistocene environmental conditions, regardless of geographic distributions, larval ecology and feeding preferences. This idea seems logical because all *Anopheles* species depend on temperature and water for larval development and survival (Service and Townson, 2002), and environmental changes are generally considered major drivers of *Anopheles* diversification (Sharpe et al., 2000; Garros et al., 2005a,b; Foley et al., 2007; Morgan et al., 2009; Chen et al., 2011; Vicente et al., 2011). Moreover, Pleistocene environmental changes (PEVCH) were substantial worldwide, triggering cyclical changes in temperature and precipitation, all of which are likely to have affected N_e of *Anopheles* mosquitoes through niche fragmentation and expansion (Costantini et al., 2009; Simard et al., 2009). To date, more than 10 dominant malaria vectors around the world, including the M and S molecular forms of *An. gambiae* s.s. have revealed patterns of genetic divergence associated with PEVCH (Table 1). Nevertheless, the expansion of *An. gambiae* s.s. may not be related to population contraction triggered by PEVCH, as the bottleneck hypothesis has not yet been supported. Instead, past changes in N_e of both M and S forms may be the result of earlier anthropogenic events such as the movement out of the ancestral East African range by early humans (Crawford and Lazzaro, 2010).

5.2.1. Primary vs secondary malaria vectors

Theoretical expectations suggest that patterns of genetic diversity are likely to differ between primary and secondary malaria vectors due to marked differences in demographic attributes (Donnelly et al., 2002). Most primary vectors are widely distributed; they have generally excellent colonizing abilities and have adapted to live under variable environmental conditions (Costantini et al., 2009; Simard et al., 2009; Cohuet et al., 2010). In contrast, due to more specific requirements in their breeding habitats, secondary vectors are generally less locally abundant and more patchily distributed (Donnelly et al., 2002; O'Loughlin et al., 2007; Mirabello and Conn, 2008; Pedro et al., 2010; McKeon et al., 2010). Based on these assumptions, secondary vectors should depict stronger signals of population size fluctuation and range fragmentation due to PEVCH than primary vectors, because they should take longer to re-colonize and recover from demographic disequilibria. Although these generalizations may appear partially supported by the outcomes of some studies cited in this review paper (Lehmann et al., 2003; O'Loughlin et al., 2007, 2008; Mirabello and Conn, 2008; Paredes-Esquivel et al., 2009; Zarowiecki et al., 2011a), the demographic attributes hypothesized for malaria vectors may vary according to different biogeographical scenarios (Foley and Torres, 2006; Zarowiecki et al., 2011a). To date, several studies have shown that both primary and secondary vectors have diverged due to historical fluctuation in N_e driven by PEVCH (Besansky et al., 1997; Donnelly et al., 2001, 2002; Hasan et al., 2008b,c; Pedro et al., 2010; McKeon et al., 2010; Zarowiecki et al., 2011a), but the paucity of secondary vectors in previous studies about genetic diversity makes it impossible to reach any firm conclusion regarding this comparison (Table 1). Future studies will have to tackle this question differently, perhaps by comparing patterns of historical demography between species with different vector status and ecologies, but overlapping geographic distributions. These studies will also need to incorporate an unbiased sampling protocol, as most *Anopheles* collections occur around human habitations and secondary vectors are not consistently anthropophilic or endophilic (Riehle et al., 2011).

5.3. Geographic patterns of demographic history

The extent of genetic divergence in *Anopheles* species due to PEVCH appears to vary depending on the geographic context. Higher population differentiation and speciation rate should be expected for island *Anopheles* species compared to continental ones. A lower N_e and higher genetic drift in smaller demes compared to broader geographic areas may explain such a pattern (Morlais et al., 2005; Kayondo et al., 2005; Pinto et al., 2003; Moreno et al., 2007; Zarowiecki et al., 2011a). This view is in agreement with the strong signals of bottleneck and isolation found in the Southeast Asian *Anopheles* (*Cellia*) *sundaicus* s.s.; a combination of cyclical island and refugium creation during the Pleistocene has been hypothesized as the cause of allopatric speciation in this coastal malaria vector (Dusfour et al., 2004, 2007a,b). A similar scenario also appears to be the cause of diversification for some members of the *Anopheles* (*Cellia*) *punctulatus* group in the Solomon Islands and Papua New Guinea (PNG) (Hasan et al., 2008a,b). However, genetic structure in some of these *Anopheles* species can also

be in response to human introduction and founder effect, because divergence and expansion times are concordant with human arrival and establishment in these islands (Hasan et al., 2008a,b; Paredes-Esquivel et al., 2009). For instance, humans are believed to have introduced *Anopheles* (*Cellia*) *farauti* s.s. into PNG and Vanuatu around 29,000 ya (Hasan et al., 2008a), but this species is not as anthropophilic as other malaria vectors (Foley and Torres, 2006). Moreover, there is uncertainty as to whether or not *An. farauti* s.s. colonized these islands via multiple introductions (Beebe and Cooper, 2002; Reiff et al., 2007; Hasan et al., 2008a). Alternatively, the historical demography of *An. farauti* s.s. could also be explained by Pleistocene range expansion (*i.e.*, re-colonization) from Australia into PNG, and then into several islands of Vanuatu (Bower et al., 2008). This demographic hypothesis is supported by sea level changes and land reconnection between Australia and PNG during most of the late Pleistocene (Beebe and Cooper, 2002), and partly concurs with the demographic history of *Anopheles* (*Cellia*) *irenicus*, a zoophilic, sympatric and closely related species of *An. farauti* s.s. that also expanded during this time frame (95% Cl: 4700–27,000 ya) (Hasan et al., 2008b).

Mainland populations of certain *Anopheles* species may also depict signals of higher genetic divergence due to PEVCH, particularly when such species inhabit narrow stretches of land. For example, despite having different larval breeding habitats and adult feeding behavior, the two most important Neotropical malaria vectors, *An. albimanus* and *An. darlingi*, expanded demographically roughly at the same time and likely due to the same PEVCH (Mirabello and Conn, 2006a; Gutiérrez et al., 2009; Loaiza et al., 2010a,b). Nonetheless, population contraction in *An. albimanus* appears to be more severe, perhaps due to its narrow geographic range across the Isthmus of Panama and Costa Rica (De Merida et al., 1999; Molina-Cruz et al., 2004; Loaiza et al., 2010a,b). In contrast, *An. darlingi* contracted and expanded across a broader geographic area, probably from the centre of its ancestral genetic pool, in Amazonian Brazil, and may have a higher long term N_e and less population differentiation than *An. albimanus* (Pedro and Sallum, 2009; Pedro et al., 2010). It is noteworthy that the geographic fragmentation hypothesized for *An. albimanus* in southern Central America (Loaiza et al., 2010a,b) coincides to some extent with the spatial discontinuity of *An. darlingi* across Costa Rica and western Panama (Mirabello and Conn, 2006a; Loaiza et al., 2009). This may suggest that both species went through the same history of population contraction, with *An. albimanus*, but not *An. darlingi* able to survive or effectively recolonize this region. In a similar fashion, PEVCH have promoted allopatric speciation between *An. dirus* s.s. and *An. baimaii* in Thailand, whereas *Anopheles* (*Cellia*) *scanloni*, restricted to the narrow Isthmus of Kra, diverged through bottleneck, isolation and rapid ecological diversification (O'Loughlin et al., 2007, 2008; Morgan et al., 2010).

6. Conclusion

A combination of genetically independent markers and adequate sampling are the best strategies for the correct identification of population demes, lineages and species relationships when working with *Anopheles* mosquitoes; this becomes a prerequisite when targeting poorly studied taxa. A multiple marker approach can also reduce bias in parameter estimation and genealogical uncertainty due to differential gene coalescence. Initially, the summary statistic or equilibrium approach was employed to study genetic diversity in *Anopheles* mosquitoes, because they are generally good flyers and have rather large N_e. As *Anopheles* are mostly distributed in lowland areas and have specific requirements in their larval breeding habitats for survival, they are more likely to be genetically structured by current geographic and ecological barriers than by historical demographic processes. Nonetheless, several studies have demonstrated that the demography of *Anopheles* species has been influenced by past fluctuation in environmental conditions. The hypotheses in this case is that *Anopheles* species adapted to humans changed demographically in response to more recent changes in their host demography, and therefore, did not retain signals of more ancient demographic processes. In contrast, less anthropophilic species may have changed in response to earlier environmental changes that affected larval habitat availability, as they do not strongly rely on humans to develop. To date, many *Anopheles* species have contracted, diverged and expanded during the Pleistocene, regardless of their levels of association with humans, larval ecology, vector status and geographic ranges. This supports the view that PEVCH were major drivers of *Anopheles* diversification worldwide. Yet, whether these changes led to speciation events or simply to population diversification appears to depend not only on the species ecology and the time since separation, but also on the geographic context. Some studies suggest that PEVCH had a more drastic effect in constricted geographic areas where oscillations in temperature, rainfall and sea levels may have all acted as drivers of bottleneck, geographic fragmentation, and subsequent population expansion. The question remains as to whether or not PEVCH have ultimately shaped the capabilities of some *Anopheles* species to vector malaria parasites regionally.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.08.004.

References

Achee, N., Grieco, J., Andre, R., Rejmankova, E., Roberts, D., 2007. A mark release–recapture study to define the flight behaviors of *Anopheles vestitipennis* and *Anopheles albimanus* in Belize, Central America. J. Am. Mosq. Control Assoc. 23, 276–282.

Alquezar, D.E., Hemmerter, S., Cooper, R.D., Beebe, N.W., 2010. Incomplete concerted evolution and reproductive isolation at the rDNA locus uncovers nine cryptic species within *Anopheles longirostris* from Papua New Guinea. BMC Evol. Biol. 10, 392.

Angêlla, A., Gil, L., Da Silva, L., Ribolla, P., 2007. Population structure of the malaria vector *Anopheles darlingi* in Rondônia, Brazilian Amazon, based on mitochondrial DNA. Mem. Inst. Oswaldo Cruz 102, 953–958.

Antonio-Nkondjio, C., Ndo, C., Awono-Ambene, P., Fontenille, D., Ngassam, P., Simard, F., 2007. Population genetic structure of the malaria vector *Anopheles moucheti* in south Cameroon forest region. Acta Trop. 101, 61–68.

Antonio-Nkondjio, C., Ndo, C., Kengne, P., Mukwaya, L., Awono-Ambene, P., Fontenille, D., Simard, F., 2008. Population structure of the malaria vector *Anopheles moucheti* in equatorial forest region of Africa. Malar. J. 7, 120.

Ayala, D., Le Goff, G., Robert, V., De Jong, P., Takken, W., 2006. Population structure of the malaria vector *Anopheles funestus* (Diptera: Culicidae) in Madagascar and Comoros. Acta Trop. 97, 292–300.

Ballard, J.W.O., Rand, D.M., 2005. The population biology of mitochondrial DNA and its phylogenetic implications. Annu. Rev. Ecol. Evol. Syst. 36, 621–642.

Ballard, J.W.O., Melvin, R.G., 2010. Linking the mitochondrial genotype to the organismal phenotype. Mol. Ecol. 19, 1523–1539.

Bargues, D.M., Latorre, M.J., Morchon, F.S., Escosa, R., Aranda, C., Sainz, S., Fuentes, M.V., Mas-Coma, S., 2006. RDNA Sequences of *Anopheles* species from the

Iberian Peninsula and an evaluation of the 18S rRNA gene as phylogenetic marker in anophelinae. J. Med. Entomol. 43, 508–517.

Bazin, E., Glemin, S., Galtier, N., 2006. Population size does not influence mitochondrial genetic diversity in animals. Science 312, 570–572.

Beebe, N.W., Cooper, R.D., 2002. Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australia and Papau New Guinea. Int. J. Parasitol. 32, 563–574.

Beheregaray, L.B., 2008. Twenty years of Phylogeography: the state of the field and the challenges for the Southern Hemisphere. Mol. Ecol. 17, 3754–3774.

Besansky, N.J., Fahey, G.T., 1997. Utility of the white gene in estimating phylogenetic relationships among mosquitoes. Mol. Biol. Evol. 14, 442–454.

Besansky, N.J., Lehmann, T., Fahey, G.T., Fontenille, D., Braak, L.E.O., Hawley, W.A., Collins, F.H., 1997. Patterns of mitochondrial variation within and between African malaria vectors, *Anopheles gambiae* and *Anopheles arabiensis*, suggest extensive gene flow. Genetics 147, 1817–1828.

Bezzhonova, O.V., Goryacheva, I.I., 2008. Intragenomic heterogeneity of rDNA internal transcribed spacer 2 in *Anopheles messeae* (Diptera: Culicidae). J. Med. Entomol. 45, 337–341.

Bora, H., Das, M.K., Ahmed, A., Sharma, Y.D., 2009. Variations in the mitochondrial DNA markers in the *Anopheles* (*Cellia*) *sundaicus* population from the Andaman and Nicobar Islands, India. Acta Trop. 112, 120–124.

Bourke, B.P., Foster, P.G., Bergo, E.S., Calado, D.C., Sallum, M.A.M., 2010. Phylogenetic relationships among species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae) based on nuclear and mitochondrial gene sequences. Acta Trop. 114, 88–96.

Bower, J.E., Dowton, M., Cooper, R.D., Beebe, N.W., 2008. Intraspecific concerted evolution of the rDNA ITS1 in *Anopheles farauti* sensu stricto (Diptera: Culicidae) reveals recent patterns of population structure. J. Mol. Biol. 67, 397–411.

Bower, J.E., Cooper, R.D., Beebe, N.W., 2009. Internal repetition and intraindividual variation in the rDNA ITS1 of the *Anopheles punctulatus* Group (Diptera: Culicidae): multiple units and rates of turnover. J. Mol. Evol. 68, 66–79.

Braginets, O.P., Minakawa, N., Mbogo, C.M., Yan, G., 2003. Population genetic structure of the African malaria mosquito *Anopheles funestus* in Kenya. Am. J. Trop. Med. Hyg. 69, 303–308.

Brochero, H., Li, C., Wilkerson, R., Conn, J.E., Ruiz-Garcia, M., 2010. Genetic structure of *Anopheles* (*Nyssorhynchus*) *marajoara* (Diptera: Culicidae) in Colombia. Am. J. Trop. Med. Hyg. 83, 585–595.

Catteruccia, F., 2007. Malaria vector control in the third millennium: progress and perspectives of molecular approaches. Pest Manag. Sci. 63, 634–640.

Chen, B., Harbach, R.E., Bultin, R.K., 2004. Genetic variation and population structure of the mosquito *Anopheles jeyporiensis* in southern China. Mol. Ecol. 13, 3051–3056.

Chen, B., Pedro, R.M., Harbach, R.E., Somboon, P., Walton, C., Butlin, R.K., 2011. Mitochondrial DNA variation in the malaria vector *Anopheles minimus* across China, Thailand and Vietnam: evolutionary hypothesis, population structure and population history. Heredity 106, 241–252.

Clements, A.N., 1992. The Biology of Mosquitoes, vol. 1. In: Clements, A.N. (Ed.). Chapman & Hall, London.

Coetzee, M., Estrada-Franco, J.G., Wunderlich, C.A., Hunt, R.H., 1999. Cytogenetic evidence for a species complex within *Anopheles pseudopunctipennis* Theobald (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 60, 649–653.

Cohuet, A., Harris, C., Robert, V., Fontenille, D., 2010. Evolutionary forces on *Anopheles* what makes a malaria vector. Trends Parasitol. 26, 130–136.

Collins, F.H., Paskewitz, S.M., 1996. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. Insect Mol. Biol. 5, 1–9.

Coluzzi, M., Sabatini, A., Petrarca, V., Di Deco, M.A., 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* Complex. Trans. R. Soc. Trop. Med. Hyg. 73, 483–497.

Conn, J.E., 1998. Systematics and population level analysis of *Anopheles darlingi*. Mem. Inst. Oswaldo Cruz 93, 647–650.

Conn, J.E., Mitchell, S.E., Cockburn, A.F., 1998. Mitochondrial DNA analysis of the Neotropical malaria vector *Anopheles nuneztovari*. Genome 41, 313–327.

Conn, J.E., Vineis, J.H., Bollback, J.P., Onyabe, D.Y., Wilkerson, R.C., Póvoa, M.M., 2006. Population structure of the malaria vector *Anopheles darlingi* in a malaria-endemic region of eastern Amazonian Brazil. Am. J. Trop. Med. Hyg. 74, 798–806.

Costantini, C., Ayala, D., Guelbeogo, W.G., Pombi, M., Some, C.Y., Bassole, I.H.N., Ose, K., Fosting, J.M., Sagnon, N.F., Fontenille, D., Besansky, N.J., Frederic, S., 2009. Living at the edge: biogreographic pattern of habitat segregation conform to speciation by niche expansion in *Anopheles gambiae*. BMC Ecol. 9, 16.

Crawford, J.E., Lazzaro, B.P., 2010. The demographic histories of the M and S molecular forms of *Anopheles gambiae* s.s.. Mol. Biol. Evol. 27, 1739–1744.

Cywinska, A., Hunter, F.F., Hebert, P.D.N., 2006. Identifying Canadian mosquito species through DNA barcodes. Med. Vet. Entomol. 20, 413–424.

Czeher, C., Labbo, R., Vieville, G., Arzika, I., Bogreau, H., Rogier, C., Diancourt, L., Brisse, S., Ariey, F., Duchemin, J.B., 2010. Population genetic structure of *Anopheles gambiae* and *Anopheles arabiensis* in Niger. J. Med. Entomol. 47, 355–366.

De Merida, A.M., Calmieri, M., Yurrita, M., Molina, A., Molina, E., Black, I.V.W.C., 1999. Mitochondrial DNA variation among *Anopheles albimanus* populations. Am. J. Trop. Med. Hyg. 6, 230–239.

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, 755–769.

Di Luca, M., Boccoloni, D., Marinucci, M., Romi, R., 2004. Intrapopulation polymorphism in *Anopheles messeae* (*Anopheles maculipennis* Complex) inferred by molecular analysis. J. Med. Entomol. 41, 582–586.

Dixit, J., Srivastava, H., Singh, O.P., Saksena, D.N., Das, A., 2011. Multilocus nuclear DNA markers and genetic parameters in an Indian *Anopheles minimus* population. Infect. Genet. Evol. 11, 572–579.

Djadid, N.D., Gholizadeh, S., Aghajari, M., Zehi, A.H., Raeisi, A., Zakeri, S., 2005. Genetic analysis of rDNA-ITS2 and RAPD loci in field populations of the malaria vector, *Anopheles stephensi* (Diptera: Culidiae): implications for the control program in Iran. Acta Trop. 97, 65–74.

Djadid, N.D., Gholizadeh, S., Tafsiri, E., Romi, R., Gordeev, M., Zakeri, S., 2007. Molecular identification of Paleartic members of *Anopheles maculipennis* in northern Iran. Malar. J. 6, 6.

Donnelly, M.J., Cuamba, N., Charlwood, J.D., Collins, F.H., Townson, H., 1999. Population structure in the malaria vector, *Anopheles arabiensis* Patton, in East Africa. Heredity 83, 408–417.

Donnelly, M.J., Licht, M.C., Lehmann, T., 2001. Evidence for recent population expansion in the evolutionary history of the malaria vectors *Anopheles arabiensis* and *Anopheles gambiae*. Mol. Biol. Evol. 18, 1353–1364.

Donnelly, M.J., Simard, F., Lehmann, T., 2002. Evolutionary studies of malaria vectors. Trends Parasitol. 18, 75–80.

Donnelly, M.J., Pinto, J., Girod, R., Besansky, N.J., Lehmann, T., 2004. Revisiting the role of introgression vs. shared ancestral polymorphisms as key processes shaping genetic diversity in the recently separated sibling species of the *Anopheles gambiae* complex. Heredity 92, 61–88.

Dusfour, I., Linton, I.M., Cohuet, A., Harbach, R.E., Baimai, V., Trung, H.D., Moh Seng, C., Matusop, A., Manguin, S., 2004. Molecular evidence of speciation between island and continental populations of *Anopheles* (*Cellia*) *sundaicus* (Diptera: Culicidae), a principal malaria vector taxon in Southeast Asia. J. Med. Entomol. 41, 287–295.

Dusfour, I., Michaux, J.R., Harbach, R.E., Manguin, S., 2007a. Speciation and phylogeography of the Southeast Asian *Anopheles sundaicus* Complex. Infect. Genet. Evol. 7, 484–493.

Dusfour, I., Blondeau, J., Harbach, R.E., Vythilingham, I., Baimai, V., Trung, H.D., Sochanta, T., Bangs, M.J., Manguin, S., 2007b. Polymerase chain reaction identification of three members of the *Anopheles sundaicus* Complex (Diptera: Culicidae), malaria vectors in Southeast Asia. J. Med. Entomol. 44, 723–731.

Emerson, B.C., Paradis, E., Thebaud, C., 2001. Revealing the demographic histories of species using DNA sequences. Trends Ecol. Evol. 16, 707–716.

Etang, J., Vicente, J.L., Nwane, P., Chouaibou, M., Morlais, I., Do Rosario, V.E., Simard, F., Awono-Ambene, P., Toto, J.C., Pinto, J., 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, 3076–3086.

Excoffier, L., Heckel, G., 2006. Computer programs for population genetics data analysis: a survival guide. Nat. Rev. Genet. 7, 745–758.

Fairley, T.L., Renaud, T.M., Conn, J.E., 2000. Effects of local geographic barriers and latitude on population structure in *Anopheles punctipennis* (Diptera: Culicidae). J. Med. Entomol. 37, 754–760.

Fairley, T.L., Póvoa, M.M., Conn, J.E., 2002. Evaluation of the Amazon River delta as a barrier to gene flow for the regional malaria vector, *Anopheles aquasalis* (Diptera: Culicidae) in northeastern Brazil. J. Med. Entomol. 39, 861–869.

Foley, D.H., Torres, E.P., 2006. Population structure of an island malaria vector. Med. Vet. Entomol. 20, 393–401.

Foley, D.H., Wilkerson, R.C., Cooper, R.D., Volovsek, M.E., Bryan, J.H., 2007. A molecular phylogeny of *Anopheles annulipes* (Diptera: Culicidae) sensu lato: the most species rich anopheline Complex. Mol. Phylogenet. Evol. 43, 283–297.

Garros, C., Harbach, R.E., Manguin, S., 2005a. Morphological assessment and molecular phylogenetics of the Funestus and Minimus Groups of *Anopheles* (*Cellia*). J. Med. Entomol. 42, 522–536.

Garros, C., Harbach, R.E., Manguin, S., 2005b. Systematics and biogeographical implication of the phylogenetic relationships between members of the Funestus and Minimus Groups of *Anopheles* (Diptera: Culicidae). J. Med. Entomol. 42, 7–8.

Gutiérrez, L.A., Naranjo, N.J., Cienfuegos, A.V., Muskus, C.E., Luckhart, S., Conn, J.E., Correa, M.M., 2009. Population structure analyses and demographic history of the malaria vector *Anopheles albimanus* Wiedemann (Diptera: Culicidae) from the Caribbean and the Pacific regions of Colombia. Malar. J. 8, 259.

Harbach, R.E., 2004. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. Bull. Entomol. Res. 95, 537–553.

Hasan, A.U., Suguri, S., Fujimoto, C., Londari Itaki, R., Harada, M., Kawabata, M., Bugoro, H., Albino, B., Tsukahara, T., Hombhanje, F., Masta, A., 2008a. Phylogeography and dispersion pattern of *Anopheles farauti* senso stricto mosquitoes in Melanesia. Mol. Phylogenet. Evol. 46, 792–800.

Hasan, A.U., Suguri, S., Fujimoto, C., Itaki, R.L., Harada, M., Kawabata, M., Bugoro, H., Albino, B., 2008b. Genetic diversity in two sibling species of the *Anopheles punctulatus* group of mosquitoes on Guadalcanal in the Soloman Islands. BMC Evol. Biol. 8, 318.

Hasan, A.U., Suguri, S., Fujimoto, C., Harada, M., Kawabata, M., Bugoro, H., Albino, B., 2008c. Population genetic structure of *Anopheles hinesorum* on Guadalcanal and Malaita of the Solomon Islands. Med. Entomol. Zool. 59, 283–295.

Hay, S.I., Sinka, M.E., Okara, R.M., Kabaria, C.W., Mbithi, P.M., Tago, C.C., Benz, D., Gething, P.W., Howes, R.E., Patil, A.P., Temperley, W.H., Bangs, M.J., Chareonviriyaphap, T., Elyazar, I.R.F., Harbach, R.E., Hemingway, J., Manguin, S., Mbogo, C.M., Rubio-Palis, Y., Godfray, H.C.J., 2010. Developing global maps of the dominant *Anopheles* vectors of human malaria. PLoS Med. 7, e209.

Hebert, P.D.N., Cywinska, A., Ball, S.L., DeWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270, 313–321.

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Hey, J., Machado, C.A., 2003. The study of structured populations – new hope for a difficult and divided science. Nat. Rev. Genet. 4, 535–543.
Jensen, J., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genet. 6, 13.
Jung, J., Jung, Y., Min, G.S., Kim, W., 2007. Analysis of the population genetic structure of the malaria vector *Anopheles sinensis* in South Korea based on mitochondrial sequences. Am. J. Trop. Med. Hyg. 77, 310–315.
Kamau, L., Mukabana, W.R., Hawley, W.A., Lehmann, T., Irungu, L.W., Orago, A.A.S., Collins, F.H., 1999. Analysis of genetic variability in *Anopheles arabiensis* and *Anopheles gambiae* using microsatellite loci. Insect Mol. Biol. 8, 287–297.
Kayondo, J.K., Mukwaya, L.G., Stump, A., Michel, A.P., Coulibaly, M.B., Besansky, N., Collins, F.H., 2005. Genetic structure of *Anopheles gambiae* populations on islands in northwestern Lake Victoria, Uganada. Malar. J. 4, 59.
Koekemoer, L.L., Kamau, L., Garros, C., Manguin, S., Hunt, R.H., Coetzee, M., 2006. Impact of the Rift Valley on Restriction Fragment Length Polymorphism typing of the major African malaria vector *Anopheles funestus* (Diptera: Culicidae). J. Med. Entomol. 43, 1178–1184.
Krzywinski, J., Besansky, N.J., 2003. Molecular systematics of *Anopheles*: from subgenera to subpopulations. Ann. Rev. Entomol. 48, 111–139.
Kuhner, M.K., 2008. Coalescent genealogy samplers: windows into population history. Trends Ecol. Evol. 24, 86–93.
Kumar, N.P., Rajavel, A.R., Natarajan, R., Jambulingam, P., 2007. DNA Barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). J. Med. Entomol. 44, 1–7.
Lehmann, T., Besansky, N.J., Hawley, W.A., Fahey, T.G., Kamau, L., Collins, F.H., 1997. Microgeographic structure of *Anopheles gambiae* in western Kenya based on mtDNA and microsatellite loci. Mol. Ecol. 6, 243–253.
Lehmann, T., Hawley, W.A., Grebert, H., Danga, M., Atieli, M., Collins, F.H., 1999. The Rift Valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya. J. Hered. 90, 613–621.
Lehmann, T., Blackston, C.R., Besansky, N.J., Escalante, A.A., Collins, F.H., Hawley, W.A., 2000. The Rift Valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya: the mtDNA perspective. J. Hered. 91, 165–168.
Lehmann, T., Licht, M., Elissa, N., Maega, B.T., Chimumbwa, J.M., Watsenga, F.T., Wondji, C.S., Simard, F., Hawley, W.A., 2003. Population structure of *Anopheles gambiae* in Africa. J. Hered. 94, 133–147.
Lehmann, T., Hume, J.C., Licht, M., Burns, C.S., Wollenberg, K., Simard, F., Ribeiro, J.M., 2009. Molecular evolution of immune genes in the malaria mosquito *Anopheles gambiae*. PLoS One 4, e4549.
Li, C., Wilkerson, R.C., 2005. Identification of *Anopheles* (*Nyssorhynchus*) *albitarsis* Complex species (Diptera: Culicidae) using rDNA internal transcribed spacer 2-based polymerase chain reaction primers. Mem. Inst. Oswaldo Cruz 100, 495–500.
Li, C., Wilkerson, R.C., 2007. Intragenomic rDNA ITS2 variation in the neotropical *Anopheles* (*Nyssorhynchus*) *albitarsis* Complex (Diptera: Culicidae). J. Hered. 98, 51–59.
Linton, Y.M., Harbach, R.E., Moh Seng, C.H., Anthony, T.G., Matusop, A., 2001. Morphological and molecular identity of *Anopheles* (*Cellia*) *sundaicus* (Diptera: Culicidae), the nominotypical member of a malaria vector species complex in Southeast Asia. Syst. Entomol. 26, 357–366.
Linton, Y.M., Smith, L., Koliopoulos, G., Samanidou-Voyadjoglou, A., Zounos, A.K., Harbach, R.E., 2003. Morphological and molecular characterization of *Anopheles* (*Anopheles*) *maculipennis* Meigen, type species of the genus and nominotypical member of the Maculipennis Complex. Syst. Entomol. 28, 39–55.
Loaiza, J., Scott, M., Bermingham, B., Rovira, J., Sanjur, O.I., Conn, J.E., 2009. Short Report: *Anopheles darlingi* (Diptera: Culicidae) in Panama. Am. J. Trop. Med. Hyg. 81, 23–26.
Loaiza, J.R., Scott, M.E., Bermingham, E., Rovira, J., Conn, J.E., 2010a. Evidence for Pleistocene population divergence and expansion of *Anopheles albimanus* in southern Central America. Am. J. Trop. Med. Hyg. 82, 156–164.
Loaiza, J.R., Scott, M.E., Bermingham, E., Sanjur, O.I., Wilkerson, R., Rovira, J., Gutiérrez, L.A., Correa, M.M., Grijalva, M.J., Birnberg, L., Bickersmith, S., Conn, J.E., 2010b. Late Pleistocene environmental changes lead to unstable demography and population divergence of *Anopheles albimanus* in the northern Neotropics. Mol. Phylogenet. Evol. 57, 1341–1346.
Ma, Y., Lia, S., Xu, J., 2006. Molecular identification and phylogeny of the Maculatus group of *Anopheles* mosquitoes (Diptera: Culicidae) based on nuclear and mitochondrial DNA sequences. Acta Trop. 99, 272–280.
Marrelli, M.T., Floeter-Winter, L.M., Malafronte, R.S., Tadei, W.P., Lourenco De-Oliverira, R., Flores-Mendoza, C., Marinotti, O., 2005. Amazonian malaria vector anopheline relationships intrepreted from ITS2 rDNA sequences. Med. Vet. Entomol. 19, 208–218.
Marshall, J.C., Pinto, J., Charlwood, J.D., Gentile, G., Santolamazza, F., Simard, F., Della Torre, A., Donnelly, M.J., Caccone, A., 2008. Exploring the origin and degree of genetic isolation of *Anopheles gambiae* from the islands of São Tomé and Príncipe, potential sites for testing transgenic-based vector control. Evol. Appl. 1, 631–644.
Matthews, S.D., Meehan, L.J., Onyabe, D.Y., Vineis, J., Nock, I., Ndams, I., Conn, J.E., 2007. Evidence for the late Pleistocene population expansion of the malarial mosquitoes, *Anopheles arabiensis* and *Anopheles gambiae* in Nigeria. J. Med. Entomol. 21, 358–369.
McKeon, S.N., Lehr, M.A., Kilpatrick, C.W., Wilkerson, R.C., Sallum, M.A., Pereira, J.B., Povoa, M.M., Conn, J.E., 2010. Lineage divergence detected in the malaria vector *Anopheles marajoara* (Diptera: Culicidae) in Amazonian Brazil. Malar. J. 9, 271.
Michel, A.P., Ingrasci, M.J., Schemerhorn, B.J., Kern, G.M., Coetzee, M., Le Goff, M., Elissa, N., Fontenille, D., Vulule, J., Lehmann, T., Sagnon, N., Costantini, C., Besansky, 2005a. Range wide population genetic structure of the African malaria vector *Anopheles funestus*. Mol. Ecol. 14, 4235–4248.
Michel, A.P., Guelbeogo, W.M., Grushko, O., Schemerhorn, B.J., Kern, M., Willard, M.B., Sagnon, N., Costantini, C., Besansky, N.J., 2005b. Molecular differentiation between chromosomally defined incipient species of *Anopheles funestus*. Insect. Mol. Biol. 14, 375–387.
Michel, A.P., Grushko, O., Guelberogo, W.M., Lobo, N.F., Sagnon, N.'.Fale., Costantini, C., Besansky, N., 2006. Divergence with gene flow in *Anopheles funestus* from the Sudan savanna of Burkina Faso, West Africa. Genetics 173, 1389–1395.
Mirabello, L., Conn, J.E., 2006a. Molecular population genetics of the malaria vector *Anopheles darlingi* in Central and South America. Heredity 96, 311–321.
Mirabello, L., Conn, J.E., 2006b. Correction to heredity (2006) 96, 311–321: molecular population genetics of the malaria vector *Anopheles darlingi* in Central and South America. Heredity 97, 438.
Mirabello, L., Conn, J.E., 2008. Population analysis using the nuclear *white* gene detects Pliocene/Pleistocene lineage divergence within *Anopheles nuneztovari* in South America. Med. Vet. Entomol. 22, 109–119.
Mirabello, L., Vineis, J.H., Yanoviak, S.P., Scarpassa, V.M., Póvoa, M.M., Padilla, N., Achee, N.L., Conn, J.E., 2008. Microsatellite data suggest significant population structure and differentiation within the malaria vector *Anopheles darlingi* in Central and South America. BMC Ecol. 8, 3.
Molina-Cruz, A., De Merida, A.M., Mills, K., Rodríguez, F., Schoua, C., Yurrita, M.M., Molina, E., Palmieri, M., Black, I.V.W.C., 2004. Gene flow among *Anopheles albimanus* populations in Central America, South America, and the Caribbean assessed by microsatellites and mitochondrial DNA. Am. J. Trop. Med. Hyg. 71, 350–359.
Moreno, M., Salgueiro, P., Vicente, J.L., Cano, J., Berzosa, P.J., De Lucio, A., Simard, F., Caccone, A., Do Rosario, V.E., Pinto, J., Benito, A., 2007. Genetic population structure of *Anopheles gambiae* in Equatorial Guinea. Malar. J. 6, 137.
Moreno, M., Marinotti, O., Krzywinski, J., Tadei, W.P., James, A.A., Achee, N.L., Conn, J.E., 2010. Complete mtDNA genomes of *Anopheles darlingi* and an approach to anopheline divergence time. Malar. J. 9, 127.
Morgan, K., O'Loughlin, S.M., Mun-Yik, F., Linton, Y.M., Somboon, P., Min, S., Htun, P.T., Nambanya, S., Weerasinghe, I., Sochantha, T., Prakash, A., Walton, C., 2009. Molecular phylogenetics and biogeography of the Neocellia Series of *Anopheles* mosquitoes in the Oriental region. Mol. Phylogenet. Evol. 52, 588–601.
Morgan, K., Linton, Y.M., Somboon, P., Saikia, P., Dev, V., Socheat, D., Walton, C., 2010. Inter-specific gene flow dynamics during the Pleistocene-dated speciation of forest-dependent mosquitoes in Southeast Asia. Mol. Ecol. 19, 2269–2285.
Morlais, I., Girod, R., Hunt, R., Simard, F., Fontenille, D., 2005. Population structure of *Anopheles arabiensis* on La Réunion Island, Indian Ocean. Am. J. Trop. Med. Hyg. 73, 1077–1082.
Muturi, E.J., Kim, C.H., Baliraine, F.N., Musani, S., Jacob, B., Githure, J., Novak, R.J., 2010. Population genetic structure of *Anopheles arabiensis* (Diptera: Culicidae) in a rice growing area of central Kenya. J. Med. Entomol. 47, 144–151.
Ndo, C., Antonio-Nkondjio, C., Cohuet, A., Ayala, D., Kengne, P., Morlais, I., Awono-Ambene, P.H., Couret, D., Ngassam, P., Fontenille, D., Simard, F., 2010. Population genetic structure of the malaria vector *Anopheles nili* in sub-Saharan Africa. Malar. J. 9, 161.
Neafsey, D.E., Lawniczak, M.K.N., Park, D.J., Redmond, S.N., Coulibaly, M.B., Traoré, S.F., Sagnon, N., Constantini, C., Johnson, C., Wiegand, R.C., Collins, F.H., Lander, E.S., Wirth, D.F., Kafatos, F.C., Besansky, N.J., Christophides, G.K., Muskavitch, M.A.T., 2010. SNP genotyping defines complex gene-flow boundaries among African malaria vector mosquitoes. Science 330, 514–517.
Nyanjom, S.R.G., Chen, H., Gebre-Michael, T., Bekele, E., Shililu, J., Githure, J., Beier, J.C., Yan, G., 2003. Population genetic structure of *Anopheles arabiensis* mosquitoes in Ethiopia and Eritrea. J. Hered. 94, 457–463.
O'Loughlin, S.M., Somboon, P., Walton, C., 2007. High levels of genetic differentiation in the malaria vector *Anopheles scanloni*. Heredity 99, 33–40.
O'Loughlin, S.M., Okabayashi, T., Honda, M., Kitazoe, Y., Kishino, H., Somboon, P., Sochantha, T., Nambanya, S., Saikia, P.K., Dev, V., Walton, C., 2008. Complex population history of two *Anopheles dirus* mosquito species in South Asia suggests the influence of Pleistocene climate change rather than human-mediated effects. J. Evol. Biol. 21, 1555–1569.
Onyabe, D.Y., Conn, J.E., 2001a. Population genetic structure of the malaria mosquito *Anopheles arabiensis* across Nigeria suggests range expansion. Mol. Ecol. 10, 2577–2591.
Onyabe, D.Y., Conn, J.E., 2001b. Genetic differentiation of the malaria vector *Anopheles gambiae* across Nigeria suggests that selection limits gene flow. Heredity 87, 647–655.
Oshaghi, M.A., Yaaghoobi, F., Abaie, M.R., 2006. Pattern of mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. Acta Trop. 99, 226–233.
Oshaghi, M.A., Shemshad, K., Yaghobi-Ershadi, M.R., Pedram, M., Vatandoost, H., Abaie, M.R., Akbarzadeh, K., Mohtarami, F., 2007. Genetic structure of the malaria vector *Anopheles superpictus* in Iran using mitochondrial cytochrome oxidase (*COI* and *COII*) and morphologic markers: a new species complex? Acta Trop. 101, 241–248.
Paredes-Esquivel, C., Donnelly, M.J., Harbach, R.E., Townson, H., 2009. A molecular phylogeny of mosquitoes in the *Anopheles barbirostris* Subgroup reveals cryptic species: implication for identification of disease vectors. Mol. Phylogenet. Evol. 50, 141–151.
Patsoula, E., Samanidou-Voyadjoglou, A., Spanakos, G., Kremastinou, J., Nasioulas, G., Vakalis, N.C., 2007. Molecular characterization of the *Anopheles maculipennis*

Complex during surveillance for the 2004 Olympic Games in Athens. Med. Vet. Entomol. 21, 36–43.
Pedro, P.M., Sallum, M.A.M., 2009. Spatial expansion and population structure of the neotropical malaria vector, *Anopheles darlingi* (Diptera: Culicidae). Biol. J. Linn. Soc. Lond. 97, 854–866.
Pedro, P.M., Uezu, A., Sallum, M.A.M., 2010. Concordant phylogeographies of two malaria vectors attest to common spatial and demographic histories. J. Hered. 101, 618–627.
Pinto, J., Donnelly, M.J., Sousa, C.A., Malta-Vacas, J., Gil, V., Ferreira, C., Petrarca, V., Do Rosario, V.E., Charlwood, J.D., 2003. An island within an island: genetic differentiation of *Anopheles gambiae* in São Tomé, West Africa, and its relevance to malaria vector control. Heredity 91, 407–414.
Prakash, A., Sarma, D.K., Bhattacharyya, D.R., Mohapatra, P.K., Bhattacharjee, K., Das, K., Mahanta, J., 2010. Spatial distribution and r-DNA second internal transcribed spacer characterization of *Anopheles dirus* Complex species (Diptera: Culicidae) in north-east India. Acta Trop. 114, 49–54.
Reidenbach, K.R., Cook, S., Bertone, M.A., Harbach, R.E., Wiegmann, B.M., Besansky, N.J., 2009. Phylogenetic analysis and temporal diversification of mosquitoes (Diptera: Culicidae) based on nuclear genes and morphology. Malar. J. 9, 298.
Reiff, D.M., Kaneko, A., Taleo, G., Amos, M., Lum, J.K., 2007. Population structure and gene flow of *Anopheles farauti* s.s. (Diptera: Culicidae) among ten sites on five islands of Vanuatu: implications for malaria control. J. Med. Entomol 44, 601–607.
Riehle, M.M., Guelbeogo, W.M., Gneme, A., Eiglmeier, K., Holm, I., Bischoff, E., Garnier, T., Snyder, G.M., Li, X., Markianos, K., Sagnon, N., Vernick, K.D., 2011. A cryptic subgroup of *Anopheles gambiae* is highly susceptible to human malaria parasites. Science 331, 596–598.
Roe, A.D., Sperling, F.A.H., 2007. Population structure and species boundary delimitation of cryptic Dioryctria moths: an integrative approach. Mol. Ecol. 16, 3617–3633.
Rongnoparut, P., Rodpradit, P., Kongsawadworakul, P., Sithiprasasna, R., Linthicum, K.J., 2006. Population genetic structure of *Anopheles maculatus* in Thailand. J. Am. Mosq. Control Assoc. 22, 192–197.
Rosenberg, N.A., Nordborg, M., 2002. Genealogical trees, coalescent theory, and the analysis of genetic polymorphisms. Nat. Rev. Genet. 3, 380–390.
Sallum, M.A.M., Foster, P.G., Li, C., Sthiprasasna, R., Wilkerson, R.C., 2007. Phylogeny of the Leucosphyrus Group of *Anopheles* (*Cellia*) (Diptera: Culicidae) based on mitochondrial gene sequences. Ann. Entomol. Soc. Am. 100, 27–35.
Scarpassa, V.M., Conn, J.E., 2007. Population genetic structure of the major malaria vector *Anopheles darlingi* (Diptera: Culicidae) from the Brazilian Amazon, using microsatellite markers. Mem. Inst. Oswaldo Cruz 102, 319–327.
Service, M.W., 1993. Mosquito Ecology: Field Sampling Methods. Chapman and Hall, London.
Service, M.W., Townson, H., 2002. The *Anopheles* vector. In: Warrel, D.A., Gilles, H.M. (Eds.), Essential Malariology, fourth ed. Arnold, London, pp. 59–84.
Sharpe, R.G., Harbach, R.E., Butlin, R.K., 2000. Molecular variation and phylogeny of members of the Minimus Group of *Anopheles* subgenus *Cellia* (Diptera: Culicidae). Syst. Entomol. 25, 263–272.
Simard, F., Lehmann, T., Lemasson, J.J., Diatta, M., Fontenille, D., 2000. Persistence of *Anopheles arabiensis* during the severe dry season conditions in Senegal: an indirect approach using microsatellite loci. Insect. Mol. Biol. 9, 467–479.
Simard, F., Ayala, D., Kamdem, G.C., Pombi, M., Etouna, J., Ose, K., Fotsing, J.M., Fontenille, D., Besansky, N.J., Costantini, C., 2009. Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. BMC Ecol. 9, 17.
Temu, E.A., Hunt, R.H., Coetzee, M., 2004. Microsatellite DNA polymorphism and heterozygosity in the malaria vector *Anopheles funestus* (Diptera: Culicidae) in east and southern Africa. Acta Trop. 90, 39–49.
Temu, E.A., Yan, G., 2005. Microsatellite and mitochondrial genetic differentiation of *Anopheles arabiensis* (Diptera: Culicidae) from western Kenya, the Great Rift Valley, and coastal Kenya. Am. J. Trop. Med. Hyg. 73, 726–733.
Vicente, J.L., Sousa, C.A., Alten, B., Caglar, S.S., Falcutá, E., Latorre, J.M., Toty, C., Barré, H., Demirci, B., Di Luca, M., Toma, L., Alves, R., Salgueiro, P., Silva, T.L., Bargues, M.D., Mas-Coma, S., Boccolini, D., Romi, R., Nicolescu, G., Do Rosário, V.E., Ozer, N., Fontenille, D., Pinto, J., 2011. Genetic and phenotypic variation of the malaria vector *Anopheles atroparvus* in southern Europe. Malar. J. 10, 5.
Walker, K., Lynch, M., 2007. Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: review of achievements and potential. Med. Vet. Entomol. 21, 2–21.
Walton, C., Handley, J.M., Tun-Lin, W., 2000. Population structure and population history of *Anopheles dirus* mosquitoes in Southeast Asia. Mol. Biol. Evol. 17, 962–974.
Walton, C., Handley, J.M., Collins, F.H., Baimai, V., Harbach, R.E., Deesin, V., Butlin, R.K., 2001. Genetic population structure and introgression in *Anopheles dirus* mosquitoes in Southeast Asia. Mol. Ecol. 10, 569–580.
Walton, C., Somboon, P., Harbach, R.E., Zhang, S., Weerasinghe, I., O'Loughlin, S.M., Phompida, S., Sochantha, T., Tun-Lin, W., Chen, B., Butlin, R.K., 2007a. Molecular identification of mosquito species in the *Anopheles annularis* Group in southern Asia. Med. Vet. Entomol. 21, 30–35.
Walton, C., Somboon, P., O'Loughlin, S.M., Zhang, S., Harbarch, R.E., Linton, Y.M., Chen, B., Nolan, K., Duongg, S., Fong, M.Y., Vythilingumi, I., Mohammed, Z.D., Trung, H.D., Butlin, R.K., 2007b. Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* Group using the ITS2 region of rDNA. Infect. Genet. Evol. 7, 93–102.
Wandeler, P., Hoeck, P.E.A., Keller, L.F., 2007. Back to the future: museum specimens in population genetics. Trends Ecol. Evol. 22, 634–642.
White, S.M., Rohani, P., Sait, S.M., 2010. Modeling pulsed releases for sterile insect techniques: fitness costs of sterile and transgenic males and the effects on mosquito dynamics. J. Appl. Ecol. 47, 1329–1339.
Wilkerson, R., Reniert, J.F., Li, C., 2004. Ribosomal DNA ITS2 sequences differentiate six species in the *Anopheles crucians* Complex (Diptera: Culicidae). J. Med. Entomol. 41, 392–401.
Wondji, C.S., Frederic, S., Petrarca, V., Etang, J., Santolomazza, F., Della Torre, A.D., Fontenille, D., 2005. Species and population 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.
World Health Organization, 2004. Global strategic framework for integrated vector management. Document WHO/CDS/CPE/PVC/2004.10, Geneva.
World Health Organization, 2006. Malaria vector control and personal protection: report of a WHO study group. WHO Technical Report Series No. 936, Geneva.
Wright, S., 1951. The genetic structure of populations. Ann. Eugen. 15, 323–354.
Zarowiecki, M., Walton, C., Torres, E., McAlister, E., Than Htun, P., Sumrandee, C., Sochanta, T., Ho Dinh, T., Ching Ng, L., Linton, Y.M., 2011a. Pleistocene genetic connectivity in a widespread, open-habitat-adapted mosquito in the Indo-oriental region. J. Biogeogr. doi:10.1111/j.1365-2699.2011.02477.x.
Zarowiecki, M., Loaiza, J.R., Conn, J.E., 2011b. Toward a new role of systematic in vector control. Parasitology. doi:10.1017/S003118201100062X.
Zimmerman, R.H., Galardo, A.K.R., Lounibos, L.P., Arruda, M., Wirtz, R., 2006. Blood meal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. J. Med. Entomol. 43, 947–956.

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