REVIEW

Population genetics of the Asian tiger mosquito
Aedes albopictus, an invasive vector of human diseases

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The Asian tiger mosquito Aedes albopictus is currently one of the most threatening invasive species in the world. Native to Southeast Asia, the species has spread throughout the world in the past 30 years and is now present in every continent but Antarctica. Because it was the main vector of recent Dengue and Chikungunya outbreaks, and because of its competency for numerous other viruses and pathogens such as the Zika virus, A. albopictus stands out as a model species for invasive diseases vector studies. A synthesis of the current knowledge about the genetic diversity of A. albopictus is needed, knowing the interplays between the vector, the pathogens, the environment and their epidemiological consequences. Such resources are also valuable for assessing the role of genetic diversity in the invasive success. We review here the large but sometimes dispersed literature about the population genetics of A. albopictus. We first debate about the experimental design of these studies and present an up-to-date assessment of the available molecular markers. We then summarize the main genetic characteristics of natural populations and synthesize the available data regarding the worldwide structuring of the vector. Finally, we pinpoint the gaps that remain to be addressed and suggest possible research directions.

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INTRODUCTION

Biological invasions, in spite of their critical impacts on native biodiversity and human societies, represent a special opportunity for population geneticists to observe in naturae phenomena that have otherwise remained mostly theoretical (Bock et al., 2015). For example, the study of a recently installed alien species represents an occasion to investigate the link between genetic diversity, whether neutral or not, and invasion success (Dlugosch and Parker, 2008; Handley et al., 2011). When such a species is also a threat to human health, as disease vectors are, the collection of empirical knowledge about population dynamics and gene flow can be used to anticipate risks (for example, modeling spread and epidemiological implications) and develop control strategies.

Indeed, the existence of multiple locally adapted vector populations could enhance the spread of parasites or arboviruses through space and time (McCoy, 2008). This has been well documented, for example, for malaria transmission in Africa: the vector system consists of several anopheline species and populations within species (for example, chromosomal forms in Anopheles gambiae sensu stricto), each of which adapted to specific bioclimatic conditions, that coexist in certain places but can also replace each other geographically or seasonally (Fontenille and Simard, 2004). The genetic variation among vector populations or individuals also diversifies their interactions with viruses and parasites, their environments and can thus alter their transmission dynamics (Barrett et al., 2008; Harris et al., 2010; Zouache et al., 2014). The intensity of gene flow among host populations is also an important point to take into account because it could influence the diffusion of key alleles such as those involved in insecticide resistance (Caprio and Tabashnik, 1992; Lenormand et al., 1999).

The Asian tiger mosquito Aedes (Stegomyia) albopictus (Skuse, 1894) has been described as one of the 100 worst invasive species in the world (Global Invasive Species Database, http://www.issg.org/database/). Originating from South and East Asia, this species has spread throughout the world mostly since the second half of the twentieth century, and it is now found on every continent except Antarctica (Kraemer et al., 2015). The new areas colonized by A. albopictus include such disparate environments as tropical South America, Africa and the mostly temperate areas of Northern America and Europe.

Although A. albopictus has long been considered a vector of secondary importance, its involvement in recent Dengue and Chikungunya outbreaks and its competence for numerous other arboviruses and nematode parasites including the threatening emerging Zika virus (Paupy et al., 2009; Grard et al., 2014) emphasizes the need to more extensively study the biology of this species (Bonizzoni et al., 2013). A. albopictus had an important role in the re-emergence of Dengue and Chikungunya in some of the recently invaded regions (Teixeira et al., 2009; Paupy et al., 2010), and it has also been implicated in new outbreaks such as the 2007 Chikungunya episode in Italy (Rezza et al., 2007). Thus, it represents a relevant case study in which invasion genetics could bring substantial improvement in vector survey and management activities.

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| Type of marker | Markers/primers name | Locus in genome | Recommended usage | Comment/advice | References |
|----------------|----------------------|-----------------|-------------------|----------------|------------|
| Allozymes      | G-3-pdh, Ldh, Hbdh, Mdh-1, Mdh-2, Mdhp-1, Mdhp-2, Idh-1, Idh-2, E-Fgdh, Sod-1, Aat-1, Acp-1, Aco-1, E-Cyp, Pgm, Pks-1, Pks-2, Pgi-1, Mos-1, Got-1, Got-2, Fum-1, Est-1, Aks-2, Aox-1 and Agk-2 | 30 | Population structure | Investigation of breeding structure | Discriminate populations at the worldwide scale | Black et al., 1988a; Black et al., 1988b; Kambhampati et al., 1991; Urbanelli et al., 2000; Chareonviriyaphap et al., 2004 |
| RAPD           | G06, G08, G09, G10, OPA11, OPAB01, OPB04, OPC15, OPM11, OPS01 and OPS10 | 188 | Strongly discouraged | Strongly discouraged because of the reproducibility issues with RAPD (Jones et al., 1997; Perez et al., 1998; Isabel et al., 1999) | Mutebi et al., 1997; Ayres and Romão, 2002; Gupta and Preet, 2014 |
| mtDNA          | COI, ND5 and Cytb    | 3   | Phylogeography | Most surveys discovered few haplotypes separated by short evolutionary distances | Birungi and Munstermann, 2002; Mousson et al., 2005; Usmani-Brown, 2009; Zitko et al., 2011; Kamgang et al., 2011; Delatte et al., 2011; Porretta et al., 2012; Bebee et al., 2013; Shaievich and Talbalaghi, 2013; Zhong et al., 2013; Zawani et al., 2014 |
|                | COI (longer)        | 1   | Phylogeography/population structure | Highly polymorphic with hundreds of haplotypes identified both in native and colonized areas. We would recommend for the use of COI, using the PCR primers from Zhong et al. (2013), which encompasses a large (1433 bp) sequence that contains the portions used in most of the published studies | Porretta et al., 2012; Zhong et al., 2013; Ismail et al., 2015 |
| rDNA           | ITS2                | —   | Phylogeography/population structure | High polymorphism and codominant data | ITS2 is multi copied, thus genotyping needs several cloning and sequencing runs, and some intra-individual variation should be expected and taken into account | Higa et al., 2010; Shaievich and Talbalaghi, 2013; Manni et al., 2015 |
| EPIC           | RpS8, RpS9, RPS12b, Rps16, RpS20b, RpS27A, Rpl.30a and Rpl.30b | 8   | Phylogeography/population structure | Not used yet | White et al., 2015 |
| Microsatellites | AedB4, AedB51, AedB52, AedB6, AedB2 and AedB3 | 54  | Population structure | Allow intra-population discrimination of genetic membership. Genetic clustering, genetic variance partition among and within sampling sites, and landscape genetics | Porretta et al., 2006 |
|                | AedC, 34–72, ab212 and ab222 |  | | | Huber et al., 2001; Delatte et al., 2013 |
|                | Alb-di-4, Alb-di-6, Alb-tri-3, Alb-tri-6, Alb-tri-18, Alb-tri-20, Alb-tri-21, Alb-tri-25, Alb-tri-33, Alb-tri-41, Alb-tri-44, Alb-tri-45 and Alb-tri-46 |  | | | Beebe et al., 2013 |
|                | Aedalbmic1, Aedalbmic2, Aedalbmic3, Aedalbmic4, Aedalbmic5, Aedalbmic6, Aedalbmic7, Aedalbmic8, Aedalbmic9, Aedalbmic10, Aedalbmic11, Aedalbmic12, Aedalbmic13, Aedalbmic14, Aedalbmic15, Aedalbmic16, Aedalbmic17, Aedalbmic18, Aedalbmic19, Aedalbmic20, Aedalbmic21, Aedalbmic22 and Aedalbmic23 |  | | | Manni et al., 2015 |
|                | Ap1, Ap2, Ap3, Ap5 and AC2 |  | | | Medley et al., 2015 |
In addition, knowledge about the genetic diversity and the genetic structure of the Asian tiger mosquito could help researchers evaluate the risk of disease and insecticide resistance spread, as well as identify the origins and frequencies of introductions. Such knowledge is also required to search for loci that could be involved in environmental adaptation.

The genetic structure of a substantial number of native and invasive populations of this species has been investigated throughout the world, multiplying the number of population genetics reports that are available. They are, however, highly disparate and range from local reports to worldwide genotype comparisons.

Regarding the increasing interest in such data about *A. albopictus*, we present here a comprehensive synthesis of what is known and the questions that remain to be investigated. In the following review, we first propose some guidelines to characterize the key features of the genetic structure of natural populations and exhaustively describe the genetic markers previously developed for *A. albopictus*. We then examine different attempts to resolve the geographical origin of invasive populations and finally suggest directions for future studies of *A. albopictus*.

**A GUIDELINE FOR POPULATION GENETICS IN MOSQUITOES**

**The sampling issue**

First, it is important to notice that the boundaries used to describe ‘populations’ in genetic studies are generally fuzzy, which is particularly true with *A. albopictus*. Most often, the term ‘population’ refers to one specific sampling site (such as a point in a city or a GPS coordinate). However, it is not always clear whether this ‘sampling site’ encompasses one or multiple discrete sampling points, such as one or several traps or artificial containers, and if so the average distances between them and the number of specimens sampled in each (for example, Urbanelli et al., 2000; Zhong et al., 2013 and Manni et al., 2015). In the following discussion, we will use ‘sampling site’ to refer to the location in which a population is sampled (for example, neighborhood, cemetery and park) and ‘sampling point’ to mean the actual unit of sampling within the sampling site (for example, a trap or an artificial container). In addition, it is crucial to clearly establish whether the collected samples are eggs, larvae or flying adults. This is important because in *A. albopictus*, larvae or eggs collected from the same breeding site are likely to belong to the same progeny. This potential drawback could be easily avoided by collecting only a few (ideally one) individuals per ovitraps and setting multiple traps throughout the sampling site, such as in the study by Delatte et al. (2013). Then, even if adults have been shown to have low dispersal capabilities (200–500 m on average (Liew and Curtis, 2004; Lacroix et al., 2009; Marini et al., 2010)), we could confidently expect that flying adults collected at a sample point are more likely to represent the sampling site diversity than eggs or larvae from the same breeding site. If flying adults are collected, it will also be informative to report whether one or more traps were used (or if aspirator/human sampling was performed at a precise point or in multiple places in the sampling site). Indeed, a comprehensive description of the sampling strategy is needed for the sake of data interpretation, especially for calculating the inbreeding coefficient ($F_{IS}$) and for partitioning the genetic variance (analysis of molecular variance).

The genetic markers issue

Useful genetic markers are expected to have several key features: selective neutrality, ease of scoring in all specimens of the species and sufficient variability to allow for measures of genetic differentiation, and genetic clustering of individuals. They also should be robust to re-genotyping and/or allow comparison with genotypes from new samples (see for instance Lowe et al. (2004) for comprehensive guidelines about genetic markers in the field of molecular ecology). Depending on the purpose, the selected markers should be suitable for phylogeography analyses (such as mitochondrial DNA (mtDNA)), allow interpretation about the breeding structure (such as codominant allozymes or microsatellites) or be sufficiently numerous in the genome to tease apart the effects of demographic history from those of natural selection (Lowe et al., 2004; Schlotterer, 2004; Selkoe and Toonen, 2006; Avise, 2009; Garvin et al., 2010; Stapley et al., 2010).

A critical comparison of the genetic markers available in *A. albopictus* is reported in Table 1. In addition, a discussion about the yet-to-be-applied dense markers can be found in Box 1. The first population genetics studies in this species were conducted in the late 1980s, using polymorphic enzymes. These markers have remarkable resolution and allowed to investigate genetic relationship between individuals and populations around the world using principal component analyses; they also allowed analyses of genetic variance between and within populations (Black et al., 1988a, b; Kambhampati et al., 1991; Urbanelli et al., 2000; Charoev variantsqahap et al., 2004). Unfortunately, allozymes were soon withdrawn at the benefit of mtDNA. Besides rare exceptions (Bebee et al., 2013), these last showed a low level of genetic variation among samples (few haplotypes separated by only one or two mutation steps). To allow a good comparison of the studies based on mtDNA, the sequences available from data banks should be of similar size and from the same region of the gene(s). That is not the case in *A. albopictus*, in which a survey of the available sequences in GenBank for COI shows that only a small region of 295 bp is shared (Figure 1). Recently, extensive sequencing at the 5’ and 3’ ends of the COI gene displayed more polymorphism than previously sequenced parts (Table 1). However, in species with such complicated demographic histories, the use of mtDNA markers may not be the most relevant choice. Indeed, variation at mtDNA only reflect the demography in terms of the maternal line; it could be also considered as an unique locus, so the accuracy of the information given is thus more sensitive to stochastic events such as population bottlenecks. In addition, it could be affected by either direct selection on mitochondrial genes or by linkage disequilibrium with any other selected cytoplasm component such as symbiotic or parasitic bacteria (Hurst and Jiggins, 2005). In *A. albopictus*, the low diversity found at certain mtDNA markers, which first had been attributed to small effective population sizes of the founder populations, was later suggested to be a consequence of such a cytoplasmic sweep induced by Wolbachia infection (Armbuster et al., 2003). Phylogeographic analyses in *A. albopictus* may thus benefit from combining these mtDNA markers to a new one recently developed from the ribosomal DNA internal transcribed spacer 2 (ITS2, Table 1), a nuclear locus that should not be affected by this kind of issue.

Finally, and although they are considered as marker of choice for population genetics analysis, the development of microsatellites in the Asian tiger mosquito has been winding. Indeed, the reproducibility and efficiency of certain microsatellites could be questioned, given the fact that most authors developed their own markers for their studies. However, recent publications have provided new sets of such loci in which both their number and resolution allowed various fine-scale population genetics analysis (Table 1).
Box 1 Toward dense markers in Aedes albopictus?

Genome-wide analyses such as genomic scan for selection will require the development of dense markers in A. albopictus. Restriction site-associated DNA sequencing (RADseq) is currently one of the most popular methods used to study large numbers of single-nucleotide polymorphisms in the genomes of non-model species. No study using this method has been published in A. albopictus yet, but some of us have tested its feasibility in a pilot experiment (Goubert et al., unpublished data). Briefly, we used the method described by Henri et al. (2015) to genotype eight individuals from two distinct populations from La Réunion Island, France. We used the SbfI enzyme, which has an 8 bp recognition site and is thus usually considered to be a ‘low’ frequency cutter. Nevertheless, we observed a very large number of sites, producing a total amount of 149 795 RAD loci (using the Stacks pipeline (Catchen et al., 2011)). This number is almost 10 times higher than expected by in silico prediction using the genome sequence of the related species Aedes aegypti. This high number of loci implies that RADseq, at least in this simple version, cannot be performed for many individuals in most labs due to high cost. Interestingly, a RADseq experiment conducted in A. aegypti (Brown et al., 2014) also using SbfI discovered a very similar number of RAD loci (184 178) using 128 individuals from around the world. In the end, only 1504 single-nucleotide polymorphisms were effectively used in this study; this number is enough for most genetic structure analysis purposes but remains limited in the context of genomic scans, especially considering the investment needed to produce these genotypes. Investigation on other restriction enzymes or the development of double-digest RAD-seq (Peterson et al., 2012) could help us to further decrease the complexity of A. albopictus genome. The latter approach, recently developed in A. aegypti (Rasić et al., 2014), proved efficient and increased the number of single-nucleotide polymorphisms that could be genotyped up to 9369 single-nucleotide polymorphisms per individual on average (N = 76; Rasić et al., 2015).

In addition, the development of such methods in A. albopictus should thus focus on the sequencing coverage issue and should be aware of several biases that have been identified in RADseq (alleles drop out, uneven sequencing depending on restriction fragment length) that could lead to false estimates of critical population parameters, even with double-digest RAD-seq, such as the effective population size (Davey et al., 2012; Gautier et al., 2012; Arnold et al., 2013) and that could be thus problematic for any demographic inference made in this species.

As an alternative to RADseq, monitoring the insertion polymorphisms created by transposable elements (TEs) is a way to obtain a very large number of markers in the genome of A. albopictus, as it has been demonstrated that some TE families have several thousand well-conserved copies in this species (Goubert et al., 2015). The transposon display technique involves an enzymatic digestion of the total DNA followed by amplification of insertions of a whole TE family using a single pair of primers. TE insertion polymorphism has successfully been used in several studies involving mosquitoes (Boulestex et al., 2007; Bonin et al., 2007; Ensnault et al., 2008). Recently, transposon display using five TE families led us to amplify more than 120 000 insertion loci in 140 A. albopictus females from Vietnamese and European populations (Goubert et al., unpublished data).

considered, which has been often referred as ‘within population’ (that is, within the sampling site; Black et al., 1988a, b; Kambhampati et al., 1991; Zhong et al., 2013; Gupta and Preet, 2014). The amount of variance attributed to this level, also called ‘between individuals in population’, is remarkably high compared with the upper levels (among groups or among populations in groups) and represents more than half of the total genetic variation, regardless of the marker used (allozymes, mtDNA, microsatellites, RAPD or ITS2) and regardless of the population being invasive or native. Recently, new studies have revealed that what has also been called a ‘high local differentiation’ could be caused by a lack of variation at the intra-individual level (FIS, Delatte et al., 2013; Manni et al., 2015). In the analysis of molecular variance context (Excoffier, 2004), this level would represent the covariance of alleles of a given locus within individuals within populations. For example, Manni et al. (2015) using ITS2 polymorphism showed that if the lower hierarchical level was ‘within population’, the genetic variance attributed to that level was 84.78%; when they added the ‘within individuals’ level to the analysis of molecular variance, the first level fell to 17.51%, and individual level represented 74.36% of the genetic (co)variance. Equivalent results were found by Delatte et al. (2013) in La Réunion Island, in which 80.766% of the variation was estimated to be at the individual level using microsatellites. It is important to highlight that these studies encompass populations from the native (Thailand) or old (La Réunion) and recent (Italy) invaded areas and thus suggest that A. albopictus populations could share this pattern globally.

The first studies that investigated the genetic structure of natural populations concluded that such a pattern of variation within the sampling sites was likely due to high genetic drift accompanying the establishment of the local population (that is, individuals found in a given sampling site), which implies that such ‘populations’ were founded by small numbers of adult individuals and had low dispersion rates that restricted gene flow (Black et al., 1988a; b; Kambhampati et al., 1991). This explanation makes sense in light of recent findings because a high level of genetic covariance within individuals would mean that they could have a high level of inbreeding, which is suggested by their breeding structure.

It is interesting to confront this hypothesis with the results from codominant data, such as allozyme and microsatellite data. For allozymes, most studies reported significant deviation from Hardy–Weinberg equilibrium for several loci and populations toward a heterozygote deficit (Black et al., 1988a; Kambhampati et al., 1991; Urbanelli et al., 2000; Paupy et al., 2001; Vazeille et al., 2001; de Oliveira, 2003). Using microsatellites, the FIS has been shown to be positive, ranging from 0.1 to 0.2, supporting a high rate of inbreeding (Delatte et al., 2013; Manni et al., 2015; Minard et al., 2015). The homozygote excess found with microsatellites has sometimes been attributed to the presence of null alleles (Kamgang et al., 2011 (no FIS data), Delatte et al., 2013), but this should be interpreted with caution because estimation of null allele frequencies is more difficult in populations that are not actually at Hardy–Weinberg equilibrium and if no independent prior of the actual inbreeding level is given (Van Oosterhout et al., 2006; Chybicki and Burczyk, 2009). However, Manni et al. (2015) obtained similar results with new markers with an apparently low level of null alleles. Even if we cannot exclude the possibility that significant FIS values could reflect consequences of a Wahlund effect due to the unknown population structure, the repeated presence of such cues (either high intra-individual genetic covariance or significant FIS) among the studies considered here highly support the inbreeding hypothesis.

Thus, a credible portrait of a ‘typical’ population of A. albopictus would be a network of interconnected breeding sites that each have a high level of inbreeding. Kinship analysis among individuals in sampling points and sampling sites would be very useful to test this hypothesis. This would allow to understand this original genetic structure that is also found in Aedes aegypti, the yellow fever mosquito that is also a mostly human-dispersed and invasive mosquito (Gonçalves da Silva et al., 2012; Damal et al., 2013). However, in A. aegypti, probably because its spread from Africa to America and then Asia is older than the worldwide spread of A. albopictus, there is genetic evidence for a progressive invasion along with a reduction of genetic diversity from the first to the last colonized continents (Powell...
and Tabachnick, 2013). This has, to the best of our knowledge, not been shown in A. albopictus. In addition, the results of two studies (Paupy et al., 2001; Vazeille et al., 2001) suggest that the urbanization gradient could positively influence the local genetic structuring (for instance at the scale of a city), but this assumption has not been formally tested yet.

Filling the gap between genetic structure and knowledge from behavioral ecology would also be extremely valuable, especially regarding the mating behavior of A. albopictus. Such studies would help to define the boundaries of what should be considered a ‘population’ and could suggest a uniform sampling strategy for use for future studies. Meanwhile, as remarked earlier, it is important to carefully report the sampling strategy, especially the distance between breeding sites if sites are used as the sampling unit.

WORLDWIDE GENETIC STRUCTURE OF NATIVE AND INVASIVE POPULATIONS

It is often assumed that invasive populations are subject to founder events that leads to a reduced genetic diversity in the invaded range (Handley et al., 2011). However, the influence of such events on genetic diversity is modulated and sometimes counterbalanced by several factors such as the amount of invaders and the frequency of introduction (that is, the propagule pressure) (Handley et al., 2011; Bock et al., 2015). In A. albopictus, few studies have specifically focused on these aspects, and there is no evidence that invasions have been followed by a reduction of genetic variability. On the contrary, there are several indications of repeated and possibly massive introductions (detailed below). In addition, the colonization pattern of A. albopictus is characterized by an absence of a natural progressive wave front (that could be accompanied by allele ‘surfing’ at the edge of the invasive range), but is well explained by human-mediated dissemination via the transportation infrastructure (Medlock et al., 2012; Roche et al., 2015).

Genetic diversity in the native area

To determine the origins of invasive populations, it is important to look at both genetic diversity and structure in the native area. This ranges from Southern (including India) and tropical Eastern Asia (including Indonesia) to East China and Japan (Hawley, 1988; Bonizzoni et al., 2013). One weakness of several studies looking at the origins of invasive populations is the use of a restricted sample from the native range (Birungi and Munstermann, 2002; Mousson et al., 2005; Usmani-Brown, 2009) or even the use of laboratory samples that have low genetic variation (Birungi and Munstermann, 2002). Second, most of these studies used markers with low variation such as certain mtDNA sequences (Birungi and Munstermann, 2002; Mousson et al., 2005; Usmani-Brown, 2009; Haddad et al., 2012; Shaivechiv and Talbalaghi, 2013; Zawani et al., 2014). Recently, and since the development of new COI primers, two studies offered a more comprehensive view of the genetic diversity in native areas. Porretta et al. (2012) and Zhong et al. (2013) showed that the expected native area, including Japan, shows high genetic diversity (62 haplotypes found in 174 individuals, and 66/346, respectively) with little or no genetic structure. Combining phylogeography and species distribution modeling (using climate data), Porretta et al. (2012) argue that the current distribution of A. albopictus in Southeast continental Asia, Japan and the Indochinese peninsula is the result of recolonization from a single but large and ecologically diverse area, the Sundaland, which existed during the last glacial maximum (LGM, ~21 000 years BP) as an area connecting Sumatra, Java and Borneo to the Indochinese peninsula. The authors suggest that a progressive range expansion of genetically different but connected populations could be at the origin of the current genetic structure in the continental native area.

Even if no clear genetic structure has been reported, the aboven-mentioned studies did not include populations from the southern range of A. albopictus (such as Sumatra, Java or Borneo), with the exception of Zhong et al. (2013), who included a single sampling site from Singapore and a recent study of Ismail et al. (2015) who studied two populations sampled near Kuala Lumpur in Malaysia (Figure 2a). Thus, it would be interesting to investigate the genetic diversity and structure of these populations and compare them with continental ones. Furthermore, it is worth mentioning that allozyme studies have shown evidence for differentiation between the southern insular populations and northern ones, as well as between western (India, Sri Lanka) and eastern populations of the native area (Kambhampati et al., 1991; Urbanelli et al., 2000; Figure 2).

Southwest islands of the Indian Ocean (~1500 years BP to −)

SWIO (Southwest islands of the Indian Ocean) includes Madagascar, La Réunion and other smaller islands such as Mayotte, Mauritius, Rodrigue, Glorieuse Comoros and Seychelles. On the basis of anthropological research, Delatte et al. (2011) hypothesized that the first colonization of SWIO by A. albopictus could have begun 1500–2000 years ago; later SWIO were frequented by spice traders in the seventeenth to eighteenth centuries, probably favoring the spread of A. albopictus in this area. Kambhampati et al. (1991) showed that 24 individuals from a single sampling site collected in 1988 in Madagascar and genotyped at enzymatic loci formed a distinct cluster from samples of the native area including Sri Lanka, China, Japan, India, Borneo and

Figure 1 Comparison of the different mtDNA COI regions amplified for phylogeographic studies of Aedes albopictus (blue lines). The gray line represents the linear sequence of the COI gene (numbers are the number of base pairs from the origin (NCBI sequence NC_006817.1)). The yellow line is the PCR product amplified by Zhong et al. (2013) showing primers recommended for use.
Malaysia (Figure 2a). Using a COI polymorphism, Delatte et al. (2011) identified two distinct genetic groups in this area. The first includes numerous widespread haplotypes from samplings performed in 2006–2007, whereas the second contains much older samples (1956 and 1992) from Madagascar and La Réunion suggesting distinct waves of invasion. Attempt to identify the origin of populations sampled from 2000 and earlier in SWIO barely failed with mtDNA markers, as the lack of variation gave little statistical support about the structure reported in Figure 2b (Mousson et al., 2005; Usmani-Brown, 2009; Raharimalala et al., 2012).

Hawaii (~nineteenth century to ~)

The Hawaiian islands are a location of interest in the biogeography of A. albopictus because they represent a possible source of introduction for trans-Pacific territories, especially the USA. Zhong et al. (2013) showed that the genetic diversity found in Hawaii is very similar to that of samples from continental Southeast Asia, but is well differentiated from that found in Singapore. Usmani-Brown (2009) found the highest haplotype diversity in their worldwide survey in a ND5 mtDNA fragment in Hawaii, and they hypothesized according to the low variability of this marker elsewhere that such a pattern should be the result of a longer presence time of older invasive populations in Hawaii compared with the other introduced populations.

The Americas (1985 to ~)

Colonization of the Americas, and especially the USA, by A. albopictus in the 1980s was the starting point for studies of the population genetics of this species. The first invasive populations settled in Texas, and the species was quickly found in many important cities of the Southwest and Midwest United States (Sprenger and Wuithiranyagool, 1986; Black et al., 1988a); At the same time, A. albopictus was also found in Brazil (Forattini, 1986). An early enzymatic survey related the Brazilian samples to Japanese and Chinese ones (Kambhampati et al., 1991). However, using mtDNA polymorphism, Birungi and Munstermann (2002) showed that one mutation step discriminates US and Brazilian populations at the ND5 marker, and the marker later showed no variation between A. albopictus populations anywhere in the world except Brazil (Maia et al., 2009; Mousson et al., 2005) also found evidence that Brazilian populations could belong to a separate genetic group because they formed a slightly different phylogenetic group with populations of the Indochinese peninsula at mtDNA markers (Cytb – ND5 – COI; Figure 2b). However, these results are tenuous, and further investigations about the origin of Brazilian populations are needed.

In the USA, surveys suggested that a large number of individuals were repeatedly introduced due to intense maritime exchanges, which allowed A. albopictus to settle (Reiter and Sprenger, 1987; Moore, 1999; Zhong et al., 2013). For example, early after the first reports of invasion, Kambhampati et al. (1990) performed a 5-year survey of allele frequencies in several localities in the country, and they found no
reduction of heterozygosity with occasional increases in certain cities and a rapid increase in effective population sizes. Most of the surveys acknowledged that US populations were mostly related to the northernmost Asian populations including Japan (Kambhampati et al., 1991; Urbanelli et al., 2000; Birungi and Munstermann, 2002; Mousson et al., 2005; Morales Vargas et al., 2013). Interestingly, some individuals sampled in Los Angeles in 2001 were related to Singapore haplotypes and were not found in 2011, suggesting that only genotypes from temperate climates could have settled successfully in the USA (Zhong et al., 2013).

Europe (1979 to –)

The first invasive population was reported in 1979 in Albania (Adhami and Murati, 1987), but the spread of the Asian tiger mosquito in this continent seems to have dramatically increased since the 1990s. Italy is probably the country of most concern because of the high density of mosquito populations, which contributed to the 2007 Chikungunya outbreak. Surveys using various types of genetic markers link the Italian samples to Japanese and the US ones (Urbanelli et al., 2000 (allozymes), Zhong et al., 2013 (COI), Shaivech and Talbalaghi, 2013 (ITS2)), but this picture could be biased: in two of these studies, the group from which Italian populations were discriminated are either represented by one population (Zhong et al., 2013) or one genotype (Shaivech and Talbalaghi, 2013). A recent survey by Manni et al. (2015) using microsatellites and ITS2 polymorphism compared populations from Italy, La Réunion Island and Thailand; it revealed that in spite of a high polymorphism found with those markers, no continental structuring of the populations could be found. Recent work comparing French and Vietnamese populations with microsatellites showed that a significant but small difference exists between the continents (Minard et al., 2015). Finally, the most informative study could be the older one, in which Urbanelli et al. (2000) using allozymes, found evidence for structure between populations from Italy, US and Japan vs populations from several Indonesian islands (Figure 2a). This underscores the need to include both North and Southeast Asian populations. Due to climatic similarities, it seems important to also compare European populations with those in northern and continental locations of the native range, such as China, that have been far less studied but could be a credible source of European invaders.

Africa

In central Africa, A. albopictus has been reported in Nigeria (first record in 1991), Cameroon (2000), Equatorial Guinea (2001), Gabon (2007) and Central African Republic (2009) (Savage et al., 1992; Fontenille and Toto, 2001; Toto et al., 2003; Coffinet et al., 2007; Diallo et al., 2010). Using COI and microsatellites, Kamgang et al. (2011) identified two distinct genetic clusters present in the same locations (microsatellites) that led the authors suspect the occurrence of multiple invasion events. In addition, COI analyses revealed a stronger identity of the Cameroonian haplotypes with tropical populations (India, Thailand, Vietnam and Brazil) than with temperate (France, Greece and USA) or Hawaii and SWIO sources. This contrasts with the findings of Usmani-Brown (2009), who found at the ND5 loci that the individuals sampled in Cameroon were mostly related to several US, Hawaii, Rome (Italy) and SWIO populations. Furthermore, a recent study in the Central African Republic highlighted the relatedness of samples with both the tropical and temperate haplotypes cited earlier (Kamgang et al., 2013). These results suggest multiple sources of introductions for Western African populations.

Other locations

On the basis of same combination of mtDNA markers used by Mousson et al. (2005), the geographical origin of individuals found in Lebanon (first record in 2003) was attributed to temperate areas (Haddad et al., 2012; Figure 2b). However, the low variation of the mtDNA markers used suggests that these results should be interpreted with caution. The geographic origin of Australian invaders found in the Torres Strait Islands region since 2004 (North Australia) was investigated using microsatellites and mtDNA, and it was found that this region was not settled from nearby Papua New Guinea (as previously thought) but rather by individuals related to Timor-Leste and Jakarta, highlighting the role of humans (probably illegal fishing) in the spread of A. albopictus (Beebe et al., 2013).

CONCLUSIONS AND SUGGESTED RESEARCH DIRECTIONS

This compilation of 30 years of research illuminates the key features of the population genetics of A. albopictus. The most striking results are the apparent lack of genetic structure according to geography and the high variability found within sampling sites, independently of whether the populations are native or invasive. The lack of isolation by distance seems to be due to a combination of low natural dispersion capabilities and a high level of human-mediated spread as recently demonstrated by Medley et al. (2015) using a landscape genomics framework. The ‘populations’ of A. albopictus exhibit apparent high levels of inbreeding, which could lead to high contrasts between genotypes from different sampling points in the same site. To validate this hypothesis, more care should be taken to report the sampling strategies, and kinship analysis should be undertaken to study the genetic relationships within and between the sampling points of a sampling site.

Analysis of the available data stresses that future population genetics studies in A. albopictus should include a more exhaustive worldwide sampling from temperate (including China), sub-tropical and tropical native areas in order to infer the origin of invasive populations. It is important, in particular, to know whether restricted gene flow really exists between tropical non-diapausing and temperate-diapausing populations in the native area, and if phenotypically similar populations share a genetic kinship. Particularly, the photoperiodical diapause, which has a demonstrated genetic basis (Urbanski et al., 2012; Poelchau et al., 2013), seems to be an important component of climatic adaptation, favoring the invasive success of A. albopictus. Thus, the remaining questions in this field include how much of the genome has a low level of differentiation and if it is possible to find highly differentiated regions that could be involved in this adaptation? Are there other traits involved in the local adaptation for which we can identify a genetic basis? To what extent could phenotypic plasticity be responsible for both ecological polymorphism and genetic homogeneity? In addition, another unknown is whether urbanization could also contribute to the local genetic structuring of this species, as it has been suggested in a few studies (Paupy et al., 2001; Vazeille et al., 2001).

The current availability of more informative markers (new microsatellites, ITS2) and large collections of samples including different time periods would, however, make possible the investigation and modeling of invasion routes (Cristescu, 2015), and the comprehensive study of the genetic structuring of the vector. The recent publication of genome assemblies and their forthcoming annotation for two strains of A. albopictus (Chen et al., 2013; Dritsou et al., 2015) will provide valuable information about the physical location and environment of existant markers, and will make possible the design of new markers or to investigate population structure, or detect the footprint of natural selection in candidate regions.
Another field of investigations is the evolutionary impact of the endosymbiotic bacteria Wolbachia—whose two strains are co-infecting almost all populations of A. albopictus studied (Werren et al., 1995; Kittayapong et al., 2002; De Albuquerque et al., 2011; Zouache et al., 2011; Bourtzis et al., 2014; Minard et al., 2015)—over the mtDNA polymorphism. Indeed, the hypothesis of a mitochondrial hitchhiking—and thus a reduced mtDNA diversity—due to Wolbachia could be questioned, since the discovery of a new highly polymorphic region of the COI gene in recent studies (Porretta et al., 2012; Zhong et al., 2013).

Knowledge about the genetic structure will also be valuable for the study of vector/pathogen interactions. Indeed, the genetic variability of vectors would affect the outcomes of close relationships with viruses or pathogens. In A. aegypti, the host genotype modulates the transcriptional response during infection with a strain of Dengue virus (Behura et al., 2014), and the way the mosquitoes can acquire the virus when it is present at low levels during feeding (Pongsiri et al., 2014). However, Zouache et al. (2014) demonstrated a three-level genotype X genotype X environment interaction between A. albopictus strain, CHKV strain and temperature on CHKV transmission potential, suggesting the need for research in this field.

We suggest that future studies should involve multiple strains per geographic location because most of the genetic variability in A. albopictus is observed at a local scale.

The case of A. albopictus represents a concrete example of a fast and successful invasion sustained by high propagule pressure and high genetic diversity. Its expansion into the invaded areas is strongly driven by human activities that are thus actively involved in the shape of the current genetic structure. Because of its epidemiological importance, and also because of its status as an invasive species, the Asian tiger mosquito should be considered a model species for which an increase of knowledge would benefit a large community of researchers. However, standardization of sampling and genotyping methods is also recommended in order to avoid the dispersion of data that would become irrelevant for global inference.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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