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 arbo-viruses 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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Table 1 Comparison of the molecular markers available in *A. albopictus*

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, 6-Pgdh, Sod-1, Aat-1, Hk-1, Adk-1, Acph, Aco-1, Aco-2, Gpi, Pgm, Pks-1, Pks-2, Pgi-1, Mez-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; Žitko *et al.*, 2011; Kamgang *et al.*, 2011; Delatte *et al.*, 2011; Porretta *et al.*, 2012; Bebee *et al.*, 2013; Shaikevich 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; Shaikevich and Talbalaghi, 2013; Manni *et al.*, 2015
EPIC	RpS8, RpS9, RPS12b, Rps16, RpS20b, RpS27A, RpL30a and RpL30b	8	Phylogeography/population structure	Not used yet	White *et al.*, 2015
Microsatellites	AealbA9, AealbB51, AealbB52, AealbB6, AealbD2 and AealbF3	54	Population structure/landscape genetics/kinship	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, alb212 and alb222 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-tri44, Alb-tri-45 and Alb-tri-46				Huber *et al.*, 2001; Delatte *et al.*, 2013; Beebe *et al.*, 2013
	Aealbmic1, Aealbmic2, Aealbmic3, Aealbmic4, Aealbmic5, Aealbmic6, Aealbmic7, Aealbmic8, Aealbmic9, Aealbmic10, Aealbmic11, Aealbmic12, Aealbmic13, Aealbmic14, Aealbmic15, Aealbmic16, Aealbmic17, Aealbmic18, Aealbmic19, Aealbmic20, Aealbmic21, Aealbmic22 and Aealbmic23			With French and Vietnamese populations, we do not recommend AealbB6, AealbD2, AealbF3, and alb212 (stuttering-like profile) and 34–72 (null allele fixation) (Minard, personal communication)	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 ovitrap 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; Schlötterer, 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; Chareonviriyaphap *et al.*, 2004). Unfortunately, allozymes were soon withdrawn at the benefit of mtDNA. Besides rare exceptions (Beebe *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 bacterias (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 (Armbruster *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).

POPULATION GENETICS OF NATURAL POPULATIONS
With the 'population' issue in mind, one of the most common features of genetic studies conducted in *A. albopictus* is that the largest part of the observed genetic variation is found at the smallest hierarchical level

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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* (Rašić *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 ($N = 76$; Rašić *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 (allele 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 (Boulesteix *et al.*, 2007; Bonin *et al.*, 2007; Esnault *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

(F_{IS}, 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 heterozygotes 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 F_{IS} 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 F_{IS} 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 F_{IS} values could reflect consequences of a Whalhund effect due to the unknown population structure, the repeated presence of such cues (either high intra-individual genetic covariance or significant F_{IS}) 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
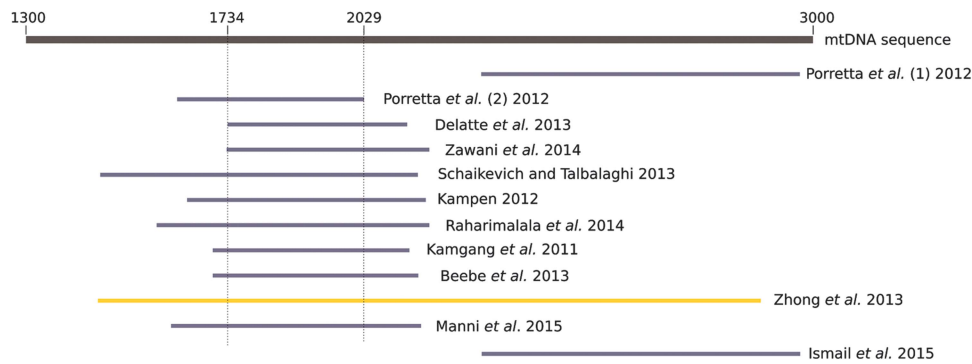
Figure 1 Comparison of the different mtDNA *COI* regions amplified for phylogeographic studies of *Aedes albopictus* (blue lines). The gray line represents the linear sequence of the *COI* gene (numbers are the number of base pairs from the origin (NCBI sequence NC_006817.1)). The yellow line is the PCR product amplified by Zhong *et al.* (2013) showing primers recommended for use.

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; Shaikevich 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 above-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. Kambampathi *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
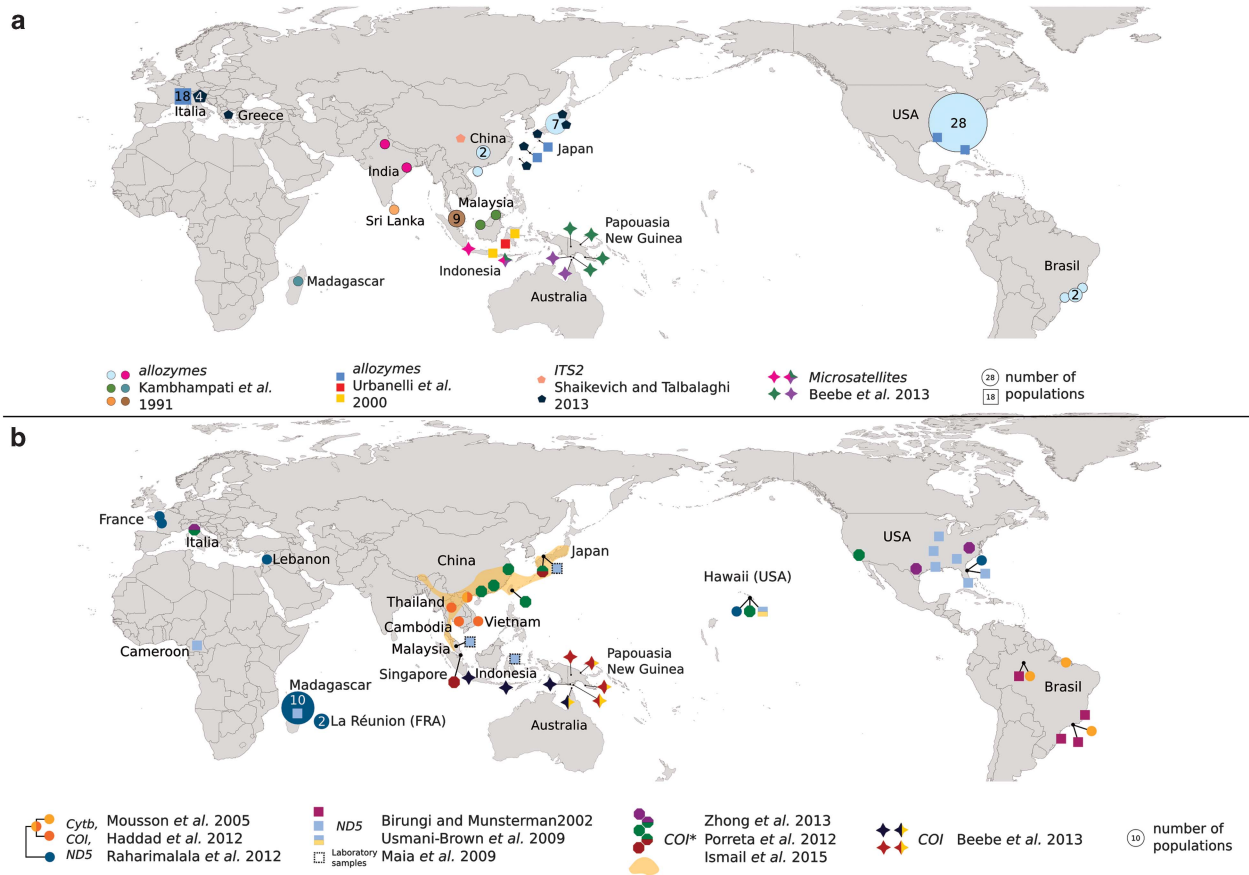
Figure 2 World maps representing homogeneous genetic groups of *Aedes albopictus* identified using (**a**) nuclear markers or (**b**) mtDNA markers. The results of comparable studies are represented with the same shape. Colors indicate genetic groups, and the types of markers are italicized. Mixed colors for mtDNA markers indicate either intermediate haplotypes (Mousson *et al.*, 2005) or mixed sampling sites. In (**b**), the orange-colored area represents the sampling of Porreta *et al.* (2012), who found no genetic structure. Asterisks refer to the new highly polymorphic *COI* sequences.

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; Usmani-Brown, 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*), Shaikevich 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 (Shaikevich 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 Africa 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 is it 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.*, 2015; 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 (Porreta *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). For *A. albopictus*, de Oliveira (2003) and Fernández *et al.* (2004) showed that the location of the strain (from USA, Caiman Island or Brazil) does not affect the competence for Dengue or Yellow Fever Virus. Artificially induced inbreeding depression did not affect also the infection rate by *Plasmodium gallinaceum* avian pathogen (O'Donnell and Armbruster, 2010). 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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