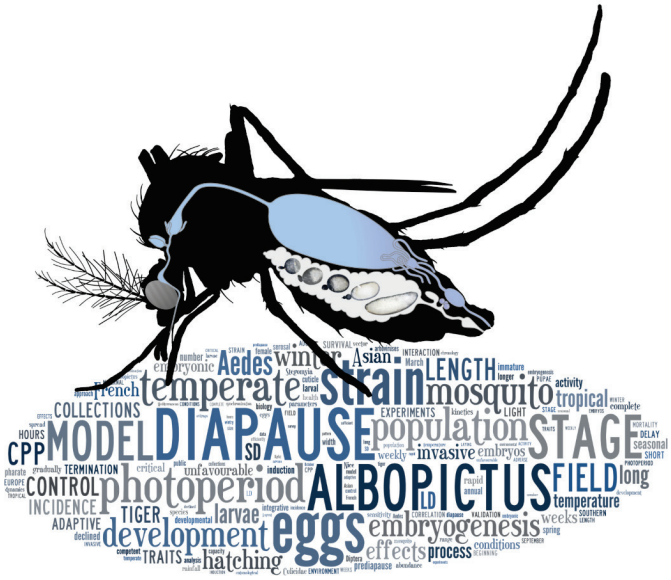
Eco-physiological mechanisms and adaptive value of egg diapause in the invasive mosquito *Aedes albopictus* (Diptera: Culicidae)

A dissertation by

GUILLAUME LACOUR



Prof. Thierry Hance, UCL, Belgium (thesis supervisor)

Prof. Marie-Laurence De Keersmaecker, UCL, Belgium (president of jury)

Dr. Didier Fontenille, Institut Pasteur du Cambodge, Cambogia (jury member)

Dr. Patrick Grootaert, Royal Belgian Institute of Natural Sciences, Belgium (jury member)

Prof. Hans Van Dyck, UCL, Belgium (jury member)

Dr. Philippe Vernon, Rennes 1 University, France (jury member)

Abstract

The success of widespread invasive species depends of their ability to support the selection pressures of the colonized environments. The climatic determinant is a key barrier for naturalization and spread of invasive species, especially climate seasonality in temperate area. Diapause is the prime component of the winter response in insects, and a convenient model of phenotypic plasticity. The role of diapause in the adaptive capacity of an invasive species was investigated in the Asian tiger mosquito *Aedes albopictus* in French Mediterranean area. We show phenotypic differentiations between tropical and temperate strains (in embryogenesis kinetics, egg width and volume, and wing length) likely linked to genotypes. The diapause preparation phase *per se* increased the timing of embryogenesis, while photoperiod is responsible of egg volume variability, both findings of importance for molecular research. The role plays by diapause on the *Ae. albopictus* temperate population dynamics was demonstrated through an integrated approach. Life history traits (egg and larval survival, adult wing length and shape) of non-diapausing individuals were not differently affected by winter than diapausing individuals, likely due to the cold acclimation capacity. Diapause initiation and termination in field occurred at constant timing despite different winter conditions. The modeling of diapause shutdown demonstrated that diapause is not strictly obligatory to the species persistence, although it procured serious temporal and abundance advantages in the Southern margins of the temperate area. It highlights that the prime role of diapause is the seasonal synchronization and the prevention of egg hatching under fluctuating winter conditions. The suitability of the Mediterranean area to the establishment of diapausing or tropical invasive mosquito species is confirmed. Diapause is a plastic response with rapid evolution, which has implications for Northward spread of *Ae. albopictus*, and the future expansion of its active period in the Mediterranean basin.

Résumé

Le succès des espèces envahissantes dépend de leur capacité d'adaptation pour surmonter les pressions sélectives de leur nouvel environnement. Le facteur climatique, tel que la saisonnalité, est un obstacle majeur pour la survie et l'expansion des espèces envahissantes. La diapause est le paramètre essentiel de la réponse hivernale des insectes et constitue un modèle d'étude de la plasticité phénotypique. Cette thèse s'intéresse à la valeur adaptative de la diapause du moustique tigre *Aedes albopictus* sous le climat méditerranéen. La phase de préparation de la diapause allonge l'embryogenèse tandis que la photopériode maternelle modifie le volume des œufs. Une approche intégrée a permis de mettre en évidence l'influence de la diapause sur la dynamique de population d'*Ae. albopictus.* Les traits d'histoire de vie (survie des œufs et des larves, morphométrie alaire) des individus non diapausants ne diffèrent pas de ceux des individus diapausants après un hiver, probablement grâce à une capacité d'acclimatation au froid. L'initiation et la levée de la diapause se produisent à date quasi-constante malgré des conditions hivernales différentes. La modélisation de la suppression de la diapause démontre que la diapause n'est pas strictement obligatoire sous le climat méditerranéen mais avantage fortement l'abondance et la distribution saisonnière du moustique tigre. Le rôle fondamental de la diapause est de synchroniser l'espèce avec les saisons et d'empêcher les éclosions sous des conditions hivernales fluctuantes. Les régions méditerranéennes sont propices à l'installation d'espèces envahissantes de moustiques, capables ou non de diapause. La diapause étant une réponse plastique à évolution rapide, ce processus sera déterminant pour l'expansion septentrionnale et l'extension de la période d'activité en Méditerranée du moustique tigre.

« Tandem aliquando, invasores fiunt vernaculi »

Proverbe romain.

A mes parents,
A mes mentors, Daniel & Christiane ROUGON et Alexandre CARRON.

Cette thèse a débuté en Octobre 2009 et a été réalisée à l'Entente Interdépartementale de Démoustication du littoral méditerranéen (EID Méditerranée), au sein de l'agence des Alpes-Maritimes jusqu'en juillet 2010 puis à la direction générale de Montpellier. Une assistance aux expérimentations a été fournie par les laboratoires du MIVEGEC (UMR IRD224-CNRS5290, Université de Montpellier) et du Centre de recherche sur la biodiversité (BDIV, Université catholique de Louvain).

Les travaux présentés dans cette thèse ont été financés par l'EID Méditerranée, le ministère de la santé et la Direction Générale de la Santé, le programme européen LIFE+ ("Integrated Mosquito Control Management" LIFE08/ENV/F/000488), et le BDIV.

Remerciements

Ce mémoire célèbre l'aboutissement d'un long périple intellectuel, et d'un acharnement mental et parfois même physique: "Delenda Carthago"!

Merci à l'EID Méditerranée, Mr le président Christian Jean, Mr le directeur général Didier Amadou, Mr le directeur technique Didier Moulis et Mr le directeur scientifique Christophe Lagneau pour m'avoir fait confiance pour mener ce travail de recherche de A à Z et avoir assuré le financement de la thèse et sa réussite. Merci à Thierry Hance pour avoir accepté de m'encadrer malgré les quelques 800 km de distance, sa confiance et ses conseils au cours de ces années de thèse. Merci bien sûr aux membres de mon comité d'accompagnement (Gérard Duvallet, Paul Reiter, Frédéric Simard) et aux membres de mon jury de thèse pour leurs précieux conseils et leurs appréciations.

Merci aux collègues de l'EID Méditerranée qui m'ont apporté leur soutien de façon continue ou occasionnelle dans l'accomplissement des travaux: l'équipe opérationnelle des Alpes-Maritimes (Bernard Cadiou, Lionel Chanaud, Marc Raselli, Romain Benoît, Marion Viano, etc.) tout d'abord, puis la direction générale de Montpellier bien sûr: Marie Torres, Bruno Gaven, Magali Grondin, Philippe Tourrier, dont le travail au laboratoire de confinement a été essentiel pour le bon déroulement des expérimentations; Bethsabée Scheid, Myriam Cros, Jean-Baptiste Ferré, Grégory l'Ambert, Marie-Laure Setier-Rio, Charles Tizon, Yvon Perrin, Sandie Bellec, Maxime Duhayon, Stéphanie DiMéglio, Réda Tounsi, Christine Bousquet pour le travail abattu côte à côte, vos conseils et votre aide; la team des "filles du littoral" (Camille y compris) et Mathilde pour ces moments précieux de détente autour d'un café. Thanks Nathalie Barras, pour ton travail de relecture acharné et minutieux, sans lequel j'aurais été mis à l'amende pour viol de la langue de Shakespeare. Bien le bonjour à Jean-Michel Penuélas et l'équipe batiment, avec qui j'ai œuvré et bataillé pour la construction, la maintenance et la perpétuation du laboratoire de confinement. Salutations à Laurent Micheli, qui m'aura vu déboulé jour, nuit et WE à l'EID Méditerranée. Merci aux autres collègues que je n'ai pas cité, qu'ils ne m'en tiennent pas rigueur! Merci à mes stagiaires Florian Vernichon, Nicolas Cadilhac, Lucie Marquereau, Renaud Zanino, Maëliss Gion et Adeline Toro qui ont travaillé dans la joie et quelques larmes sur nombre de recherches en lien plus ou moins étroit avec ma thèse. Je jure que tous ont survécu aux méthodes de management et de motivation dictées par "la Science".

Salutations et remerciements bien sûr aux collègues, confrères, thésards et scientifiques rencontrés au cours de ma thèse; j'ai plaisir à dire que nos différents échanges m'ont ouvert l'esprit! Puisqu'il faut bien donner quelques noms, je citerai ainsi Bulent Alten, Diego Ayala, Philippe Boussès, Sébastien Boyer, Jean Sébastien Dehecq (et les précieuses souches de la Réunion), Jean-Pierre Dujardin, Roger Eritja, Albin Fontaine (un véritable arracheur de glandes), Didier Fontenille, Filiz Günay, Frédéric Jourdain, Gilbert Le Goff, Aurélien Mercier, Nicolas Pocquet (réfugié scientifique), Jorian Prud'Homme (un véritable arracheur d'ailes), David Roiz, Ada Shukullari (et les souches albanaises si réticentes à l'élevage), Frédéric Simard, Annelise Tran, Enkelejda Velo, et tant d'autres que j'oublie momentanément (les noms et moi…).

Abbreviation list

Ae.: Aedes

An.: Anopheles

AS: abdominal segmentation

BI: Breteau index

CHIKV: chikungunya virus

CPP: critical photoperiod

Cx.: Culex

DENV: dengue virus

EB: egg burster

ELP: egg laying period

HAE: hour after egg laying

HI: house index

IMS: Invasive mosquito species

LD: long daylight

Oc: ocelli

RI: reluctance index

SC: serosal cuticle

SD: short daylight

SPAM: strain Provence-Alpes-Maritimes

WNV: West Nile virus

Definitions

Diapause - A form of dormancy that is hormonally programmed in advance of its onset and is not immediately terminated in response of favourable conditions.

Quiescence - An immediate response to a decline of any limiting environmental factor below the physiological thresholds, with immediate resumption of the processes if the factor rise above them.

Photoperiodism - The ability to determine day length with precision and to react to changes.

Propagule - Any of various structures capable of being propagated or acting as an agent of reproduction.

Skip oviposition - The distribution of eggs from the same batch among multiple containers.

Vector competence - The physiological ability of a vector organism to acquire, maintain and transmit an infectious agent, as described by susceptibility to a pathogen, immune response, and sustaining infection long enough for disease transmission to occur.

List of thesis papers

This thesis is based on the following articles, which are either published, submitted or in preparation.

PAPER I. **Lacour, G.**, Vernichon, F., Cadilhac, N., Boyer, S., Lagneau, C., Hance, T., 2014. When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Insect Physiology 71, 87-96.

PAPER II. **Lacour, G.**, Chanaud, L., L'Ambert, G., Hance, T., 2015. Seasonal Synchronization of Diapause Phases in *Aedes albopictus* (Diptera: Culicidae). PLoS ONE 10(12).

PAPER III. **Lacour, G.**, Jean-Baptiste Ferré, J.-B., Jeannin, C., L'Ambert, G., Le Monnier, E., Leparc-Goffart, I., Prat, C., Roiz, D., Tizon, C., Vernichon, F., Lagneau, C., Hance, T. Entomological Investigation on the Chikungunya Outbreak in Montpellier, 2014: vector Abundances, Viral Detection and Viral Overwintering. *In prep.*

PAPER IV. Tran, A.*, L'Ambert, G.*, **Lacour, G.***, Benoît, R., Demarchi, M., Cros, M., Cailly, P., Aubry-Kientz, M., Balenghien, T., Ezanno, P., 2013. A Rainfall- and Temperature-Driven Abundance Model for *Aedes albopictus* Populations. International Journal of Environmental Research and Public Health 10(5), 1698-1719. (* for equal contributions of authors)

PAPER V. **Lacour, G.**, Tran, A., Prudhomme, J., Lagneau, C., Hance, T. Why Bothered About Winter? The Adaptive Advantage of Diapause for *Aedes albopictus* in Mediterranean Area. *In prep.*

PAPER VI. **Lacour, G.**, Tran, A., L'Ambert, G., Lagneau, C., Hance, T. Invaders in Tires: the Diapause Photo-Inhibition Tool Against Introduced Invasive Mosquito Species. *In prep.*

For ease of reading, the references cited in these papers were all grouped in a single section at the end of this thesis manuscript.

Table of contents

Preface

A brief introduction to the long mosquito history

The mosquito issue

The mosquito nuisance has always been suffered by mankind, to a point where this painful hematophagous characteristic was sometimes used. Some Siberian tribes used mosquitoes as a mean of torture and killing: the victim was attached to a tree and was left for food to flying hematophagous insects until death by exsanguination (Lockwood 2012). As a vector, mosquitoes played a significant –and sometimes major- role in history. Paludism, *i.e.* malaria, is suspected to have participated to the fall of great Athenian and Roman empires. Napoleon's battle plans were often counteracted by vector-borne disease mortality in his army. Vectors are also responsible of significant migratory fluxes; *Anopheles* and paludism caused movements from countryside to cities during centuries, whereas urban population took flight and massively abandoned the infested cities infested during yellow fever outbreaks in Americas (Gillett 1971).

The responsibility of mosquitoes in epidemic diseases was often suggested in history but gone always unheeded and forgotten. Things changed since 1878, when Sir Patrick Manson demonstrated the development of the filarial worm-parasite *Wuchereria bancrofti* in the night-feeding mosquitoes. In 1881 Carlos Finlay demonstrated that Yellow fever was transmitted by *Aedes aegypti* (Gillett 1971). The vectorial role of mosquitoes was finally proven and the war against mosquitoes could start. It should be noted that a part of mankind still ignores the vector status of mosquitoes today, even in developed countries; from 23 to 35% of inhabitants of Mayotte and La Reunion islands did not believe that mosquitoes were responsible of the chikungunya outbreak in 2006-07 (Setbon and Rode 2008). On the contrary, some countries developed research on mosquitoes as biological weapons since the dawn of the World War II (Lockwood 2012).

The mosquito control issue

Successful eradication examples of a mosquito species in a country-scale are rare. *Anopheles gambiae* was eradicated from Brazil in 1940, 10 years after its introduction by mail planes from Africa; this success has to be credited to the good organization of the campaign, but also to the low

genetic diversity of the invasive population. In the meantime, the mosquito had been responsible of a malaria outbreak causing 14 000 deaths (Vinogradova 2012).

Mosquito control is based on the diminution of the level of nuisance, but the eradication of vector species has always been expected. We can illustrate this fact by citing the “New Jersey Mosquito *Extermination* Association” (the oldest association of United States of America, founded in 1912) which was renamed in the “New Jersey Mosquito *Control* Association” in 1974, 2 years after the ban of DDT in USA. The economic benefit of vector eradication is obvious; the estimated cost of malaria in infested regions of Africa vary from 7 to 32% of the annual income for a medium-size family (Chima *et al.* 2003). Far from ethical considerations, the vector specicide (*i.e.* the extinction of a species) was limited by financial and technical parameters. Nowadays, with the development of genetic and new control methods, the specicide principle is increasingly considered by researchers against some vectors of high medical importance (Fang 2010, Koplan 2012).

The mosquito control in French Mediterranean area

The French Mediterranean area is suitable to mosquito proliferation, due to the warm climate and wetlands. It was the theatre of some epidemics during History: the last outbreak of paludism in Camargue occurred in 1942-43. The economic development of the Mediterranean littoral was dependent of the disappearance of the insalubrities issue and the reduction of the mosquito’s nuisance. The mosquito control public organism “EID Méditerranée” was created in 1958 by departments to assist the land settlement planning and the development of touristic activities. The swamp mosquitoes *Ae. caspius* and *Ae. detritus* and the urban mosquito *Culex pipiens* have been the main target species (Boisson 1967).

The spread of the invasive species *Ae. albopictus* from Italy since 2004 (presented in chapter I) create a new public health issue and a massive source of nuisance (Delaunay *et al.* 2007). The mosquito control organisms are challenged to manage this species, from technical to political dimensions. As the Asian tiger mosquito ecology differed from native species, its biology need to be better studied to adapt the mosquito control strategies and management policies.

Chapter I. Bio-ecology of the invasive mosquito *Aedes albopictus* in link with the diapause syndrome

I. A note on the biological invasion phenomenon

Each invasive process can be modeled in 5 stages based on the “propagule pressure” concept (Colautti and McIsaac 2004). The passage from a stage to the next is determined by the propagule ability to prevent or to adapt to ecological filters, including survival to transport conditions or new environment (figure I.1). This model refer to population and their biogeographically characteristics, not to the entire species. The process can be simplified in three phases: initial dispersal far from endemic area, establishment of self-sustaining populations, and spread (Puth and Post 2005).

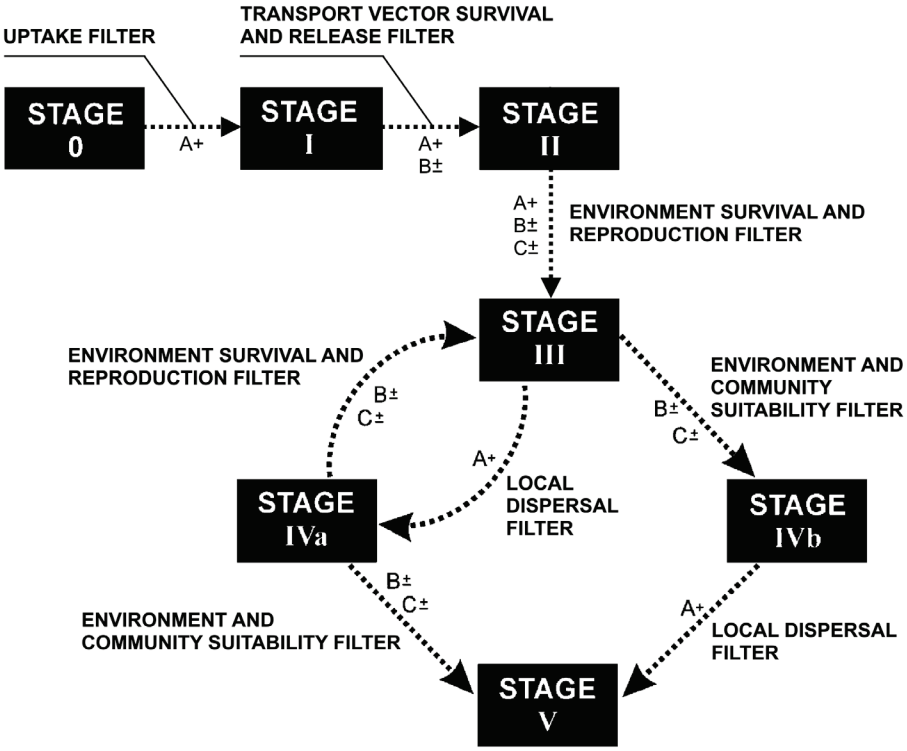


Figure I.1. General framework of the invasion process. Potential invaders begins as propagules residing in a donor region (stage 0), and pass through a series of filters that may preclude transition to subsequent stages. Under this framework, a nonindigenous species may be localized and numerically rare (stage III), widespread but rare (stage IVa), localized but dominant (stage IVb) or widespread and dominant (stage V). Three classes of determinants affect the probability that a potential invader will pass through each filter: (A) propagules pressure; (B) physico-chemical requirements of the potential invader; and (C) community interactions. Determinants may positively (+) or negatively (-) affect the number of propagules that successfully pass through each filter (adapted from Colautti and MacIsaac 2004).

The mechanistic process of the biological invasion is defined as (Valéry *et al.* 2008):

> *« A biological invasion consists of a species' acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation, which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population. »*

In the case of the colonization of the temperate area by *Ae. albopictus*, it appeared that the species was partially pre-adapted in its native area. Indeed, the breeding preference shift from sylvian to urban areas (see the following subchapter on the biology of *Ae. albopictus*) provided access to a larger diversity and quantity of breeding sites. The invasive species continued to adapt after its introduction in alien areas, as demonstrated by niche modeling of successive invasions (Medley 2010).

Other factors can also play a role in the invasive success of the Asian tiger mosquito in the temperate area. For example, the ecological niche of immature stages of *Ae. albopictus* is largely not overlapped by other mosquito species in Europe; it corresponds to the *Darwin's Naturalization Hypothesis*, arguing that the naturalization of an invasive species is facilitated in absence of other native species phylogenetically closely related (Darwin 1859).

The ability to survive to winter through diapause likely constitutes the major competitive advantage to the proliferation of *Ae. albopictus* in temperate area. The study of the invasive process and its determining factors is not the purpose of the thesis. The study will only focus on the adaptive role of the diapause process in the invasive strain in temperate area. The results will evidently refer to the ongoing invasion in Europe, which allows following the spatio-temporal responses of the Asian tiger mosquito to evolutionary processes.

Aedes albopictus is an interesting biological model for our research aims, because of its invasive status and adaptability, and its diapause biology: *i)* the diapause ability is clearly differentiated between tropical strains (likely genetically unable of diapause) and diapausing temperate strains, and *ii)* the parameters of diapause induction are known and easily controlled in laboratory. The next chapters will describe the biology of *Ae. albopictus* and its diapause process.

II. The Asian tiger mosquito *Aedes albopictus*

Complete reviews on the morphology (figure I.2) and biology of *Aedes albopictus* are available in scientific literature (Estrada-Franco *et al.* 1995). This subchapter focuses on aspects of its biology and ecology which are influenced by diapause.

Figure I.2. Oviposition of a female of *Aedes albopictus* on a moist surface (photo credit: EID Méditerranée/J-B Ferré).

2.1. Systematic

The systematic assignment of the Asian tiger mosquito is described below:

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Diptera
Taxon: Nematocera
Infraorder: Culicomorpha
Family: Culicidae
Tribe: Aedini
Genus: *Aedes*
Subgenus: Stegomyia
Species: *albopictus*

Its belongs to the *Aedes* genus, a specialized mosquito genus in which all species laid their eggs on moist surfaces above the water level instead of on water surface. Originally named *Culex albopictus* (Skuse, 1894), it was renamed *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894). It is sometimes referred as *Stegomyia albopicta*, a name from the classification of Reinert (Reinert *et al.* 2004). This new classification was controversial and not universally accepted. It is now proposed to maintain the usage of the traditional aedine genera names (Wilkerson *et al.* 2015). Thus we will refer to the Asian tiger mosquito as *Aedes* (*stg*) *albopictus*, shortened to *Ae. albopictus* in this memory.

2.2. Geographic distribution

The Asian tiger mosquito is native to South-East Asia forests (figure I.3). It became a widely invasive vector species, extending its distribution range faster than any mosquito species during the last 30 years (Bonizzoni *et al.* 2013). The first effective establishment of *Ae. albopictus* in a temperate country outside its native area was recorded in Albania in 1979 (Adhami and Reiter 1998). In 1985 it started to invade the United States of America from Japan (Kuno 2012). In 1990 it was introduced in Northern Italy and spread since to Western Europe (Dalla Pozza *et al.* 1994), especially around the Mediterranean basin (table I.1). Introductions in United States and Italy were done as dormant egg through the international trade of used tires. The

species is currently established in 19 countries and microstates of geographical Europe (figure I.4). *Aedes albopictus* is the most widely distributed mosquito invasive species in Europe, but some others are introduced in Europe and succeed to establish.

Some accidental importations of *Ae. albopictus* in Europe did not succeed to establish an invasive population. The invasive success depends mainly on the size of the introduced population and the ability to face to new ecological conditions. The diapause inability or the bad synchronization with winter are probably the major reason of the failure of persistence of these strains in France (Schaffner and Karch 2000), Belgium (Schaffner *et al.* 2004, Deblauwe *et al.* 2015), The Netherlands (Scholte *et al.* 2012), Czech Republic (Šebesta *et al.* 2012), Bosnia and Herzegovina, Bulgaria, Austria (Seidel *et al.* 2012) and Germany (Becker *et al.* 2013, Kampen *et al.* 2013, Werner and Kampen 2015). The rapid application of mosquito control measures can enhance or cause the successful eradication in some areas, but introduced populations disappeared also in some European countries as Belgium which did not implement standard mosquito control measures (Deblauwe *et al.* 2015).

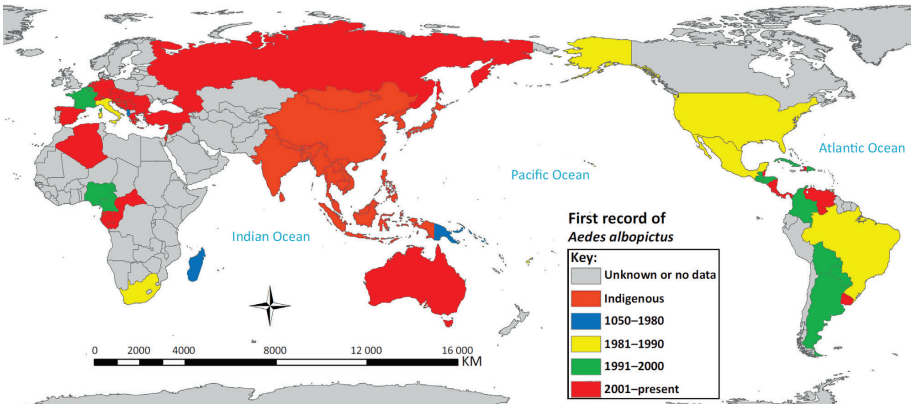


Figure I.3. World distribution range of *Aedes albopictus* introductions. Map indicating the first reports (interception on imports, local captures, and documented endemic populations) of *Ae. albopictus* by country (corrected and actualized from Bonizzoni *et al.* 2013).

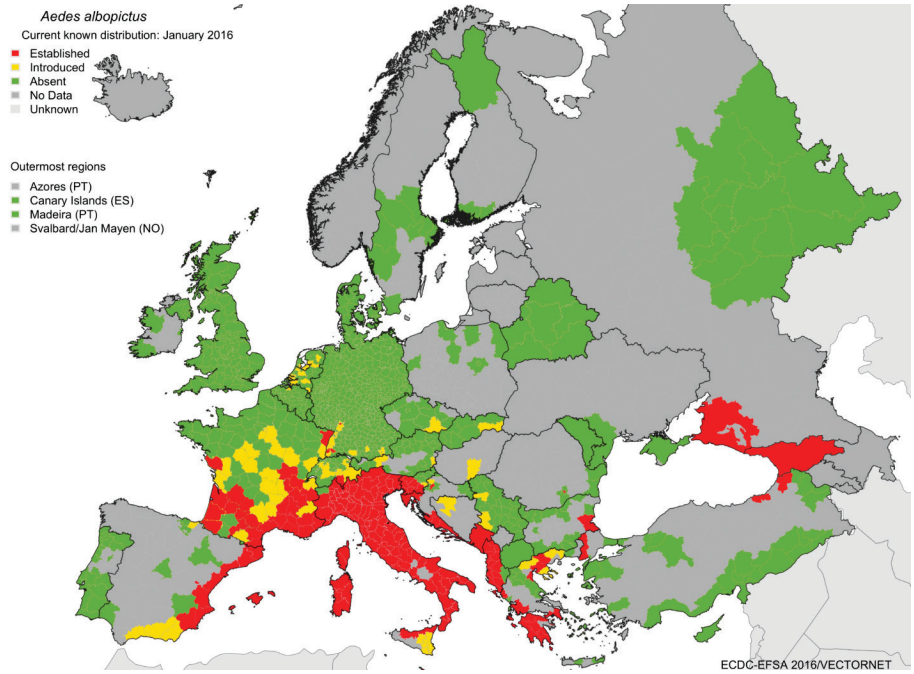


Figure I.4. Distribution range of *Aedes albopictus* in Europe in January 2016 (Map ECDC VBORNET, actualized from table I.1). Red areas correspond to established populations (evidence of reproduction and overwintering) of the species in at least one municipality, and yellow areas correspond to introductions of the species (without confirmed establishment) within the last 5 years of the distribution status date.

Table I.1. Chronology of Europe invasion by the Asian tiger mosquito (Adapted and actualized from ECDC 2009).

***Aedes albopictus* invasion**			**Other IS present**
Country (city of discovery)	**First year of report***	**Reference**	**Species* (reference)**
Albania (Laç)	1979	Adhami & Murati 1987; Adhami & Reiter 1998	-
Italy (Genoa)	1990	Sabatini *et al.* 1990	*Ae. koreicus* (Capelli *et al.* 2011)
Montenegro (Podgorica)	2001	Petric *et al.* 2001	-
Greece (Corfu & Igoumenidsa)	2003	Samanidou-Voyadjoglou *et al.* 2005	-
Switzerland (Ticino)	2003	Flacio *et al.* 2004	*Ae. japonicus*
Croatia (Zagreb)	2004	Klobučar *et al.* 2006	-
France (Menton)	2004	Delaunay *et al.* 2007	*Ae. japonicus*
Spain (Barcelona)	2004	Aranda *et al.* 2006	-
Monaco	2006	Schaffner, in ECDC 2009	-
San Marino	2007	Mignani, in ECDC 2009	-
Slovenia (Nova Gorica)	2007	Kalan *et al.* 2011	*Ae. japonicus* (Seidel *et al.* 2012 ; Kalan *et al.* 2014)
Vatican city	2007	Venturelli in ECDC 2009	-
Malta (Mellieha & Marsascala)	2009	Gatt *et al.* 2009; Buhagiar 2009	-
Bulgaria	2011	Mikov, in Oter *et al.* 2013	-
Turkey (Kesan & Ipsala)	2011	Oter *et al.* 2013	-
Russia (Sotchi)	2012	Ganushkina *et al.* 2016	*Ae. aegypti* + *Ae. koreicus* (Ganushkina *et al.* 2016)
Georgia	2012	Ganushkina *et al.* 2016	*Ae. aegypti* (Ganushkina *et al.* 2016)
Romania (Bucharest)	2012	Prioteasa *et al.* 2015	
Germany (Freiburg/Breisgau & Heidelberg)	2015	Pluskota *et al.* 2016	*Ae. japonicus* (Becker *et al.* 2011)

* Successfull establishment only. Failed introductions are not mentioned in this table.

The climate suitability for establishment of *Ae. albopictus* was quite well defined by the analysis of distribution range in Japan and United States (Nawrocki and Hawley 1987, Kobayashi *et al.* 2002). The 10°C coldest-month isotherm delimits the area of continuously breeding populations of *Ae. albopictus* and those that must undergo a period of dormancy during

cold winter (Mitchell 1995). The species distribution in Japan and USA is limited by the 11°C mean annual temperature (Kobayashi *et al.* 2002). However, in all the models, the report of climatic conditions corresponding to the species distribution in one continent did not accurately predict the distributions for other continents (Medley 2010). First modeling of the potential distribution in Europe corresponded roughly to the current observed distribution. Each continental invasion, from Asia to North America and from North America to Europe, corresponds to niche divergence, potentially explained by reduction of the genetic diversity caused by founders effect (Medley 2010). Recent climatic suitability and distribution models integrated continental specificities and European knowledge on the diapause of *Ae. albopictus* (Caminade *et al.* 2012, Erguler *et al.* 2016).

In terms of altitude, The Asian tiger mosquito was found in villages up to 1,700-1,800 m in Thailand, reaching 2,100 m in Nepal (Estrada-Franco *et al.* 1995, Dhimal *et al.* 2015). In Europe, it was found in villages up to 1,200 m in Albanian mountains. However, in Albania the presence of the mosquito at this high altitude could result from an annual passive introduction by anthropogenic transport (cars or cable cars) and not be permanently established (Mersini *et al.* 2015). Similar observations are regularly done in France, but as in Albania, mosquito populations are well established until 600-800 m, with population being probably univoltine at higher altitude (EID Méditerranée unpublished data).

2.3. *Aedes albopictus* biology

The Asian tiger mosquito has a great ecological plasticity and adaptability.

> *"If a single theme emerges from a broad view of the biology of Aedes albopictus it is that of variability"* (Hawley 1988).

The following section summarizes the knowledge on the invasive populations in temperate area, and focus on biological parameters which are likely to be more or less influenced by the diapause process.

2.3.1.Breeding sites

In its native area, *Ae. albopictus* was a forest species. The species laid eggs in tree holes, sectioned bamboo, rock holes and small ground depressions.

Observations suggested that the species adapted to domestic and artificial breeding sites during the 20th century (Hawley 1988). This major ecological shift allowed the spread of the Asian tiger mosquito in vegetated suburban and urban area. The species can be still found in forest environment but in much lower density than in periurban area (Suter *et al.* 2016). *Aedes albopictus* has a behavioral preference to oviposit in breeding sites with a small or medium volume of water with decaying vegetation (Unlu *et al.* 2013). This species made skip oviposition, distributing the eggs from the same batch among a broad range of breeding sites. The majority of eggs are laid in containers placed at ground level (<1 m), even though in tropical area eggs were found in tree-holes at the height of 15 m (Estrada-Franco *et al.* 1995, Unlu *et al.* 2013). *Aedes albopictus* used commonly diverse containers in Europe, such as barrels, buckets, plant pots, flowerpot trays, plastic containers, used tires and others (Carrieri *et al.* 2011b, Bonizzoni *et al.* 2013). Catch basins are some very productive breeding sites in Italy, but not yet in France (Carrieri *et al.* 2011b).

2.3.2. Interspecific competitivity

The concept of ecological niche consists of the conditions necessary to support the vital activities (including breeding, feeding and mating conditions) of a type of organism (Alley 1982). Some stages of the biological cycle of a species are more exposed to perturbations. In Culicidae, the larval stage is commonly the most sensitive to interspecific (and in a lesser extend intraspecific) interactions, and can end to a competitive exclusion, also called Gause's principle or Grinnell's axiome. The competition between species that seek the same ecological niche should lead to the survival of one species while the other disappears under a given set of environmental conditions (Alley 1982).

In Europe, *Ae. albopictus* interacts with *Culex pipiens* in medium- and big-sized containers such as big buckets, catch basins and water tanks. It was demonstrated in laboratory in Italian strains that *Ae. albopictus* was more competitive than *Cx. pipiens* to exploit larval food resources at summer temperature (around 25°C). This competitive advantage was not evident with cooler temperature, found in breeding sites at the beginning and the end of the favorable season (Carrieri *et al.* 2003). Similar results were observed when the Asian tiger mosquito was put in competition with *Ae. j. japonicus* (Kaufman and Fonseca 2014). *Aedes albopictus* is a superior competitor, with a superior growth rate under larval competition for food resources or in presence of pesticide in water (Kaufman and Fonseca 2014). A competitive equivalence may exist for few species, such as *Ae. triseriatus* which seems

to be not impacted by larval competition, even if studies are still rare to confirm these observations (Juliano 2010).

In Europe, the ecological niche situation agrees with the *Darwin's Naturalization Hypothesis*: none of the native or alien species is phylogenetically closely related (Darwin 1859). However in its native area, the Asian tiger mosquito is submitted to interspecific competition with other Stegomyia species as *Ae. aegypti*. The ecological niches of both species are very similar, which Gause's principle. Up to the last quarter of the 20th century, laboratory experiments demonstrated that *Ae. aegypti* was a better larval competitor than *Ae. albopictus* (Estrada-Franco *et al.* 1995). Nowadays all the recent studies found that *Ae. albopictus* is a better competitor than *Ae. aegypti*; this shift suggesting a rapid adaptation or population replacement of *Ae. albopictus*. Finally, the phenomenon of competitive exclusion between these both species was confirmed by a meta-analysis of laboratory studies, consistent with field observations (Juliano 2010). The competitive exclusion is also reinforced by the capacity of sterilization of *Ae. aegypti* females during interspecific cross-mating by *Ae. albopictus* males (Estrada-Franco *et al.* 1995).

The competitive exclusion concept predicts the extinction for one of the competing populations under the condition of stability in the environment and genetic composition. The seasonal variations in environmental conditions can permit an indefinite coexistence of true competing species (Alley 1982). A seasonal shift was demonstrated in temperate area in *Cx. pipiens* competiting with *Ae. albopictus*. The adult abundance of *Cx. pipiens* greatly decreased in mid summer when the density of Asian tiger mosquito increased (Carrieri *et al.* 2003, Unlu *et al.* 2013). The seasonality and the adaptation of competitive species in temperate area are likely to be achieved in a stable equilibrium.

In the near future, competitive interactions between different invasive mosquito species will be observed in Europe: *Ae. albopictus*, *Ae. aegypti* and *Ae. koreicus* are established in Sochi Coastline (Ganushkina *et al.* 2016), and *Ae. albopictus* and *Ae. aegypti* are both found in Georgia and north-eastern Turkey (Akiner *et al.* 2016). In Western Europe, *Ae. albopictus* and *Ae. japonicus* are both present in Switzerland and in Alsace, France (EID Méditerranée, unpublished data).

2.4. Vectorial status of *Aedes albopictus*

2.4.1. Vectorial competence

The vector competence of the Asian tiger mosquito, *i.e.* the ability of a vector to transmit a disease, was experimentally demonstrated for 25 arboviruses (table I.2). Some are relevant to Europe: Chikungunya (CHIKV), Dengue virus (DENV), Zika virus (ZIKV), West Nile Virus (WNV), Yellow fever virus, Sindbis virus, Rift Valley Fever virus, etc. (Gratz 2004, Paupy *et al.* 2009, Wong *et al.* 2013). The Asian tiger mosquito could be competent for other viruses. For example, the vectorial competence of *Ae. albopictus* for Zika virus was still unknown until few years ago (Medlock *et al.* 2012, Wong *et al.* 2013). The Asian tiger mosquito generates veterinary issue too, by transmitting Dirofilaria (nematodes) to dogs and sometimes to humans (Paupy *et al.* 2009).

The competence of temperate populations of *Ae. albopictus* for viral strains was determined under different temperatures (Vega-Rua *et al.* 2013; Zouache *et al.* 2014). Under temperate conditions, a strong interaction was observed between French *Ae. albopictus* populations and the ECSA CHIKV genotype, especially with the E1-A226V adaptive mutation (Zouache *et al.* 2014). The CHIKV is detectable in the salivary glands as soon as 2 days after the mosquito infection (Talbalaghi *et al.* 2010).

Humans co-infections with CHIKV and DENV-1 or DENV-2 were reported in outbreaks in Madagascar in 2006 (Ratsitorahina *et al.* 2008) and in Gabon in 2007 (Leroy *et al.* 2009), where the Asian tiger mosquito was the main vector. Although co-infections in mosquitoes have never been demonstrated in the field, laboratory tests demonstrated that *Ae. albopictus* can be orally infected with both CHIKV and DENV-1 and remained concomitantly infectious for both (Vazeille *et al.* 2010).

Nowadays Zika virus is a hot topic. Vectorial competence was tested on three strains of *Ae. albopictus* from Singapore, Rio de Janeiro, Brazil, and Vero Beach, USA. A low level of transmission of ZIKV was observed between 10 to 14 days at 28-29°C (Wong *et al.* 2013, Chouin-Carneiro *et al.* 2016). The interspecific variability of ZIKV infection is evident among strains, as the Asian strain was the more susceptible to infection and the Brazilian the less susceptible. The North-American strain -genetically closed to European populations (Urbanelli *et al.* 2000)- had an intermediate infectious susceptibility but data lack about vector competence under a more temperate environment.

Table I.2. Overview of vectorial competence and capacity of *Aedes albopictus* (Adapted and completed for field outbreaks from Koch *et al.* 2016)

Pathogenic agent	Laboratory competence		Field data	
	Infection of mosquito	Transmission to host	Trapped infected mosquito	Main vector of outbreak
Flaviviridae				
Dengue fever virus	+	+	+	+[a]
Japanese encephalitis virus	+	+	+	
St. Louis encephalitis virus	+	+		
West Nile virus	+	+	+	
Yellow fever virus	+	+	+	
Zika virus	+	+	+	+
Togaviridae				
Chikungunya virus	+	+	+	+[a]
Eastern equine encephalitis virus	+	+	+	
Getah virus	+	+		
Mayaro virus	+	+		
Ross-River virus	+	+		
Sindbis virus	+	+		
Venezuelan equine encephalitis virus	+	+		
Western equine encephalitis virus	+	+		
Bunyaviridae				
Cache Valley virus			+	
Jamestown Canyon virus	+	+	+	
Keystone virus	+		+	
La Crosse virus	+	+	+	
Oropouche virus	+			
Potosi virus	+	+	+	
Rift Valley fever virus	+	+		
San Angelo virus	+	+		
Tensaw virus			+	
Trivittatus virus	+			
Nodaviridae				
Nodamura virus	+			
Reoviridae				
Orungo virus	+	+		
Onchocercidae				
Dirofiliaria immitis			+	
Dirofiliaria repens			+	

[a] in both tropical and temperate area

2.4.2.Field outbreaks

The risk of emergence or re-emergence of these arboviruses is relevant to the European outbreak history. The most explosive and virulent dengue outbreak was recorded in 1927-28 in Greece, and was transmitted by another urban and anthropophilic vector, *Ae. aegypti* (Halstead 2008). The vectorial role of *Ae. albopictus* in European outbreaks was proven for dengue and chikungunya:

- Numerous autochthonous cases of dengue fever were reported in Europe repeatedly since 2010, in France (La Ruche *et al.* 2010, Marchand *et al.* 2013, Giron *et al.* 2015) and in Croatia in 2010 (Gjenero-Margan *et al.* 2011).
- The CHIKV outbreak of 2005-2007 in the Indian Ocean alerted public health authorities of the threat represented by *Ae. albopictus*. This disease, poorly documented at that time, affected an estimated range of 312,500 people out of 757,000 inhabitants of the island of La Reunion (Brouard *et al.* 2008). The first CHIKV outbreak of the temperate area occurred in Emilia-Romagna province in Italy in 2007, with more than 205 cases (Rezza *et al.* 2007). This outbreak is still the most important occurred in Europe, but indigenous transmissions are repeatedly reported in France in 2010 (Grandadam *et al.* 2011) and in 2014 (Delisle *et al.* 2015).

Other temperate countries were recently concerned by epidemics with *Ae. albopictus* as the only vector. For example, a DENV-1 outbreak occurred in Tokyo, Japan, in 2014, with 160 cases registered (Kutsuna *et al.* 2015). The health issue in 2016 concerned particularly the Zika virus, as *Ae. albopictus* was the main vector of a ZIKV outbreak in Gabon in 2007 (Grard *et al.* 2014).

With at least 60 human cases recorded, the prevalence of dirofilarioses increased in Italy for the decade following the introduction of *Ae. albopictus* (Pampiglione *et al.* 2001). The guilt of this latter is reasonably certain, as adult mosquitoes were found contaminated with filarial nematodes (Cancrini *et al.* 2003).

Despite the winter in the temperate countries colonized by *Ae. albopictus,* the Mediterranean regions -located under the 20°C isotherm in summer- would be particularly exposed to the vector-borne disease risk, as climatic conditions are favorable to the virus-vector combining (Mitchell 1995). The Asian tiger mosquito faces other favorable conditions for transmission of

arboviruses, specifically a non-immune human population in densely populated cities.

2.4.3. Transovarial infections

Transovarial infections are not rare among Culicids, and play sometimes a role in the persistence of viruses from year to year. In temperate climate of North-Eastern USA, St. Louis encephalitis virus was found in overwintering *Cx. pipiens* adults collected in Maryland and Pennsylvania in January-February 1977 (Bailey *et al.* 1978), and WNV was isolated in 2000 in New-York city in hibernating adults of *Cx. pipiens* (Nasci *et al.* 2001). The rate of infected *Cx. pipiens* for these Flaviviruses in Northern America was quite similar, from 0.1 to 0.3%. (Bailey *et al.* 1978, Nasci *et al.* 2001). It is clear that vertical transmission of WNV in overwintering *Culex* mosquitoes contributes to the maintenance of this virus during winter.

The possibility of vertical transmission in *Ae. albopicus* was shown in laboratory for Flavivirus as WNV (Baqar *et al.* 1993). For Alphaviruses, things are less evident (Rosen 1987), but field vertical transmission was put in evidence in *Aedes* species (Ross River and Sindbis viruses in *Ae. camptorhynchus* in Australia (Dhileepan *et al.* 1996); Western equine encephalomyelitis virus in *Ae. dorsalis* in California (Fulhorst *et al.* 1994)). Transovarial transmission of CHIKV in *Ae. albopictus* progeny occurred in laboratory, with an infectious rate of 0.43% of adults in an Italian strain (Bellini *et al.* 2012). Recent outbreaks in tropical and subtropical regions put in evidence vertical transmission in the field for CHIKV in Island of La Reunion (Delatte *et al.* 2008) and Madagascar (Ratsitorahina *et al.* 2008) and for serotypes 2 and 3 of DENV in Brazil (Cecílio *et al.* 2009 ; Martins *et al.* 2012). Consequently this risk of overwintering of transovarial infection in diapausing eggs of *Ae. albopictus* must be determined in temperate area (Guo *et al.* 2007).

2.5. Potential mosquito control methods

Control of any mosquito species is difficult, but the control of *Ae. albopictus* is a challenging one. The current and future potential vector control tools that can be used against the Asian tiger mosquito are presented in a recent review (Baldacchino *et al.* 2015) (figure I.4). In Europe, mosquito control strategies are based on the community source reduction, *i.e.* the removal of potential mosquito habitats by inhabitants (Focks 2003), and on the spray of

adult insecticide (pyrethroids) in specific cases as vectorial transmission suspicion and outbreak. Others control method are not yet largely applicable (copepods and sterile insect technique for example) or “ready to use” for operators (autodissemination of insect growth regulators, cytoplasmic incompatibility, etc.).

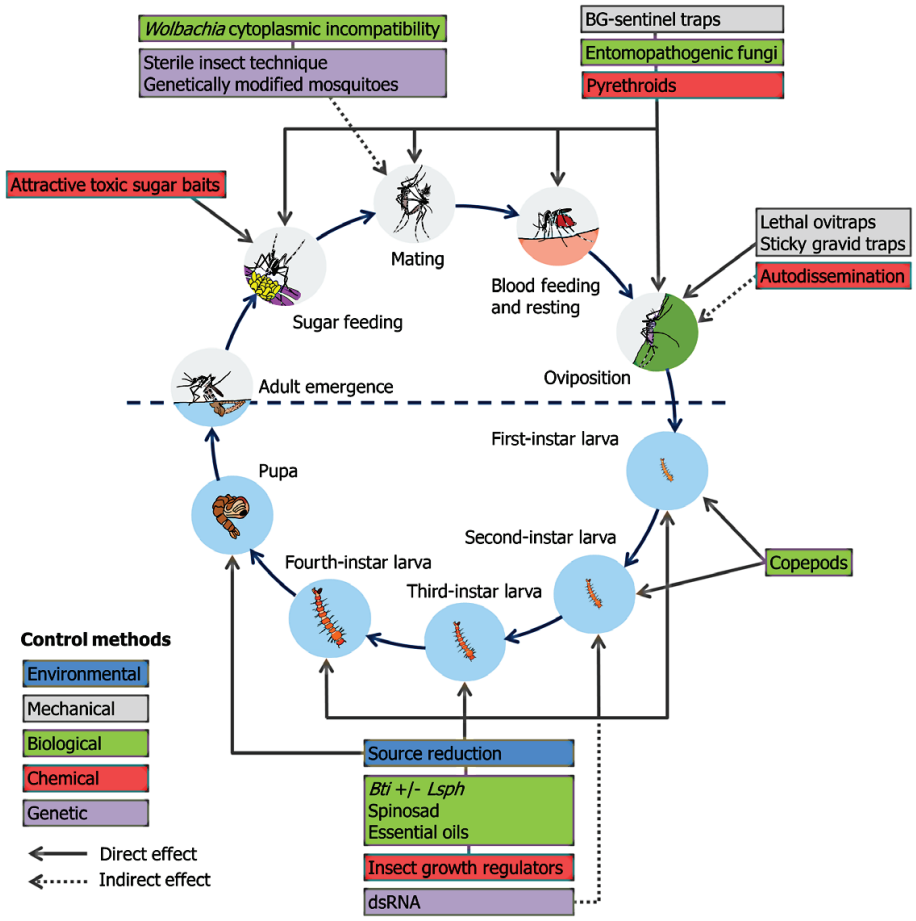


Figure I.4. Control methods available against *Aedes* sp. (Bti: *Bacillus thuringiensis* var. *Israelensis*; Lsph: *Lysinibacillus sphaericus*; dsRNA: double-stranded RNA) (from Baldacchino *et al.* 2015).

III. Diapause

3.1. Definitions of dormancy forms

We referred to the following definitions to avoid any misleading interpretation between the terms *dormancy*, *quiescence* and *diapause*.

Dormancy is "*a generic term covering any state of suppressed development (developmental arrest), which is adaptive (that is ecologically or evolutionarily meaningful and not just artificially induced), and usually accompanied with metabolic suppression*" (Kostal 2006).

There are 2 forms of dormancy: the quiescence and the diapause.

Quiescence is "*an immediate response (without central regulation) to a decline of any limiting environmental factor(s) below the physiological thresholds with immediate resumption of the processes if the factor(s) rise above them*" (Kostal 2006).

Diapause is "*a more profound, endogenously and centrally mediated interruption that routes the developmental program away from direct morphogenesis into an alternative diapause program of succession of physiological events; the start of diapause usually precedes the advent of adverse conditions and the end of diapause need not coincide with the end of adversity*" (Kostal 2006).

3.2. Ecological definition of diapause

Diapause is an adaptation and a complex physiological process which can be simply defined as (Denlinger and Armbruster 2014):

> *"a form of dormancy that is hormonally programmed in advance of its onset and is not immediately terminated in response of favourable conditions"*

Diapause has a genetic basis, but genes involved in diapause are still unknown in most of species. A same species can be able or unable of diapause according its genotype. The activation of diapause is a plastic adaptive response to a prolonged adverse environment (Tauber *et al.* 1986).

It is a spectacular and famed example of phenotypic plasticity in arthropods (West-Eberhard 2003):

> *"Phenotypic plasticity is the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity."*

This plastic response is a major determinant of the ecological-tolerance strategy in seasonal environments (Willmer *et al.* 2000).

3.3. Diapause in insects

The word *diapause* was used for the first time to describe the embryonic diapause in the *Xiphidium* (*Conocephalus*) grasshopper (Wheeler 1893). The use of this word was later broadened to describe developmental arrest, growth and reproduction occurring in all insect stages. The term *diapause syndrome* defined all the physiological and behavioral changes observed in this type of dormancy (Tauber *et al.* 1986). As the definition of diapause evolved over time, we will always refer in this study at the given ones in the review of Kostal (Kostal 2006).

Diapause is a widespread life-history trait in insects, allowing them to colonize areas with severe seasonal conditions (through winter diapause or aestivation). It seems evident that diapause had contributed –and still contributes- to the evolutionary success of insects (Tauber *et al.* 1986, Denlinger 2002). Diapause can be obligatory in univoltine species or facultative. The day length and the temperature are the main stimuli used by sensitive stages in insects to determine the moment to initiate diapause (Tauber *et al.* 1986). Diapause was discovered in every developmental stage: in different embryonic phases, in the larval stage, the pupal stage (very common in Dipterans) and in adults (Denlinger and Armbruster 2014). However, only one and sometimes two stages can potentially initiate diapause in a particular insect species (figure I.5).

Box 1. Bibliometric analysis

We put in evidence the evolution of the scientific works on diapause by a bibliometric study using the *Web of Science* (WoS) produced by Thomson Reuters. Both series *Science Citation Index Expanded* and *Social Sciences Citation Index* of the WoS were interrogated. The scientific community had an increasing interest toward the diapause thematic (figure B1.1).

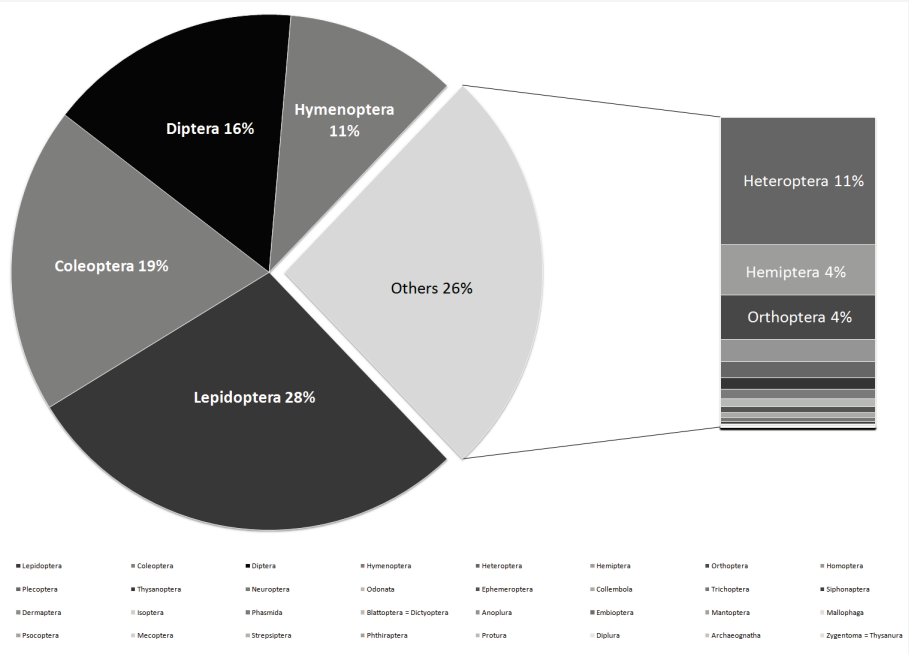


Figure B1.1. Taxonomic repartition among the scientific papers (*n*=1484) on diapause in Insects during the period 1981-2011.

Over the past 4 decades, the global number of scientific papers treating of the diapause (with the word « diapause » in the title, abstract or keywords) was multiplied by 4, reflecting a continuous increase of interest to the diapause process, not only *per se* but also widely integrated in the large picture of the biology of organisms (figure B1.2a). The most studied families are obviously from economic and agronomic interest, with *Bombyx morii* (Lepidoptera) as the first biological model for diapause study. The papers dealing with culicid diapause represent 2% of the global corpus, but double during the last decade (figure B1.2b). It may reflect of the increasing interest for the biology of mosquitoes in temperate areas, and the need to study diapause in other biological models than the limited Drosophila one.

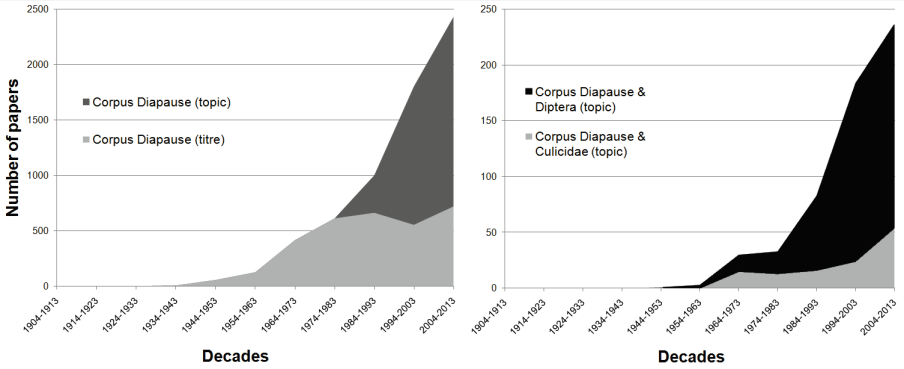


Figure B1.2. Chronological analysis from 1904 to 2013 of the “diapause” corpus and of the sub-corpus “diapause & Diptera” and “diapause & Culicidae”.

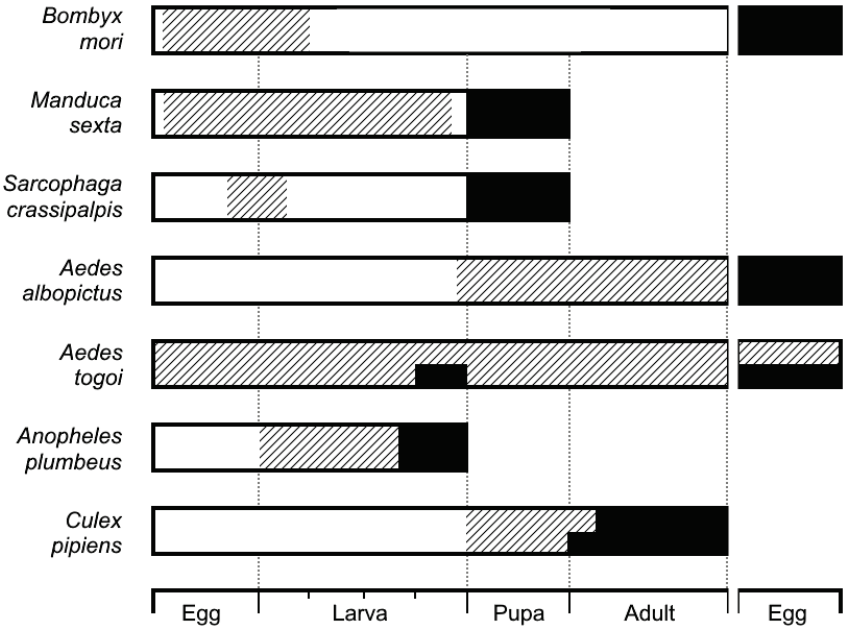


Figure I.5. Examples of the variability of diapause induction and initiation stages in butterflies, flesh fly and mosquitoes. The hatched area represents the photosensitive stages and the black area the stage of diapause initiation (adapted from Denlinger *et al.* 2005).

3.4. Mosquito diapause

Culicid undergo reproductive diapause (only in females at the imaginal stage), larval diapause (at different instars) and egg diapause (at the pharate larva stage) (figure I.5). The Vinogradova's review described these 3 types of diapause in detail and examples. Neither embryonic diapause *sensu stricto* nor pupal diapause has been documented in mosquitoes (Vinogradova 2007).

The evolution of diapause in mosquito was investigated with a dataset of 58 culicid species (Denlinger and Armbruster 2014) (figure I.6). The results support multiple changes in diapause capacity during the evolution. Among mosquitoes able to diapause at egg stage, the genus *Aedes* is the only one specialized in egg diapause; all *Aedes* species which diapause at the larva stage are able of egg diapause too. It is a cue that these both diapause implied probably much closed molecular mechanisms for diapause initiation and maintenance.

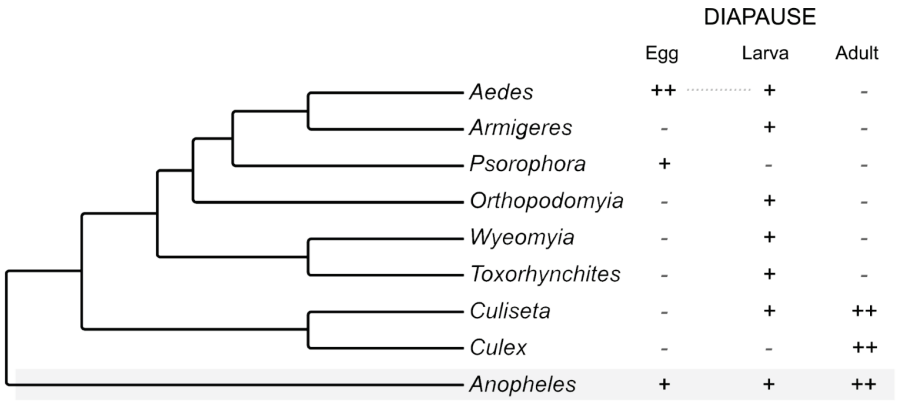


Figure I.6. Evolution of life cycle stage of diapause in mosquitoes (Cladogram adapted from Denlinger and Armbruster 2014). Diapause is observed in the 3 stages of the primitive genus *Anopheles* (light grey strip). The *Aedes* genus is specialized in egg diapause, all species able of larva diapause being able of egg diapause too (as indicated by the grey dashed line).

Numerous examples show that the diapause status of the progeny is determined by the exposure of ovipositing females to short photoperiod (Mousseau and Fox 1998). The annual variation in day length is the most reliable indicator of seasonality in temperate areas. Photoperiodism, defined as the ability to determine day length with precision and to react to changes,

is in most organisms a major adaptation to avoid harsh seasonal environmental conditions (Danilevskii 1965, Tauber *et al.* 1986). As a result it is used to initiate biological events essential to survival, such as reproduction, migration and dormancy (Danilevskii 1965).

Egg diapause

Egg diapause is currently associated with any condition of suspended hatchability in temperate species that overwinter as cold hardy eggs. As described in Lepidoptera, egg diapause can be initiated early in the embryogenesis phase in the late gastrula stage as in the silkworm *Bombyx morii*, or at the end of the embryogenesis in the pharate larva stage, with a fully-developed larva still contained and compacted in the egg, as in *Lymantria dispar* and *Antheraea yamamai* (Denlinger and Armbruster 2014). Ecological mechanisms of egg diapause are quite similar among insect organisms, but strong differences have been observed between their molecular processes (Tauber *et al.* 1986).

The egg diapause in mosquitoes occurred only at the pharate larva stage, none early diapause was observed. The pharate larva is the only clearly defined stage of diapause in the Asian tiger mosquito (Vinogradova 2007).

3.5. Egg diapause in *Aedes albopictus*

Egg diapause is genetically controlled in *Ae. albopictus*; some strains are not able of diapause, tropical strains for example. The diapause ability can be counter-selected in laboratory in short-day rearing conditions in 6 generations (Pumpuni 1989). Egg diapause is facultative in *Ae. albopictus* (Vinogradova 2007).

In this section the phases of the diapause syndrome will be described according to the review of Kostal (Kostal 2006). Each phase will be illustrated with the relevant description of diapause in The Asian tiger mosquito (figure I.7). Majority of the knowledge about diapause metabolism in *Ae. albopictus* was unknown at the beginning of this thesis. The recent work of the Denlinger team in the United States revealed a good overview of the global metabolism of the diapause process (Denlinger and Armbruster 2014).

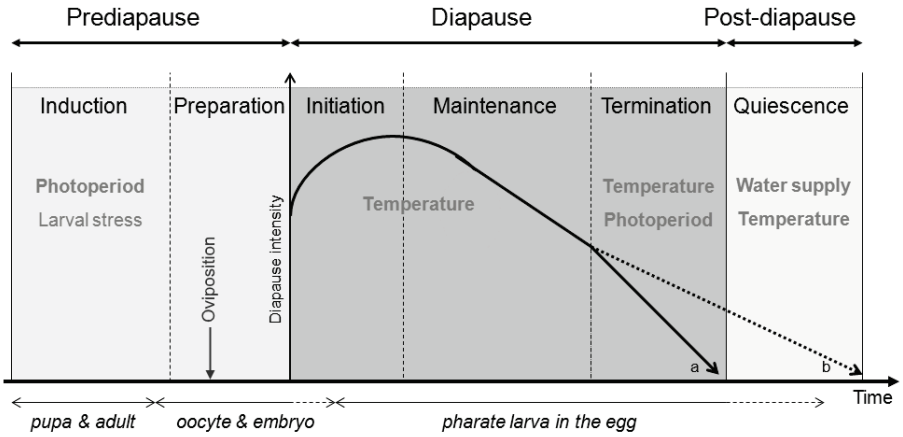


Figure I.7. Schematic description of phases and terms of the egg diapause process in *Aedes albopictus* (adapted from Kostal 2006). Thick line with arrowhead in the lower part of the picture indicates the passage of time starting from photosensitive stages to the hatching or death of the offspring. The 3 major phases of diapause, namely prediapause, diapause and post-diapause, are described in the upper part. The sub-phases of induction, preparation, initiation, maintenance, termination and quiescence (facultative, dependent of favorable environmental conditions and water supply) are indicated by vertical lines.

The first major phase of the diapause process is the prediapause phase, subdivided in two phases, the diapause induction and the diapause preparation.

Diapause maternal induction

Diapause induction phase is defined as (Kostal 2006):

> *The induction phase occurs during genotype specific ontogenetic stage(s) (sensitive period) when cues from the environment are perceived and transduced into switching the ontogenetic pathway from direct development to diapause when the token stimuli reach some critical level (the response may be modified by other environmental factors).*

Diapause is induced by short day length in *Ae. albopictus*, with photoperiod between 12.3 and 14.5 hours in USA and Japan (Pumpuni 1989, Urbanski *et al.* 2012). The pupae and adults are the photo-sensitive stages. The induction of diapause in ovocyte/egg depends of the transmission of a signal from the mother; it is consequently a maternal effect (Mousseau and Fox 1998). The critical photoperiod (CPP), inducing diapause in 50% of eggs, is a very plastic trait submitted to natural selection and resulting in clinal response to latitude and elevation (Urbanski *et al.* 2012). The photoperiodic stimulus triggers hormone release likely from the corpus cardiacum and the corpus allatum (Meuti and Denlinger 2013). The diapause induction also depends of other environmental conditions; high temperature undergone by sensitive stages will decrease the diapause incidence when low food-quality of larval diet will increase the diapause incidence (Pumpuni *et al.* 1992). Contrary to some other *Aedes* species, laid eggs of *Ae. albopictus* are not capable to initiate diapause without the maternal induction (Sinègre 1974, Vinogradova 2007).

Diapause preparation phase

Diapause induction phase is defined as (Kostal 2006):

> *The preparation phase occurs where the phases of diapause induction and initiation are separated by a period of direct development during which the individual is covertly programmed for later expression of diapause. Behavioral and physiological preparations for diapause may take place.*

The preparation phase is easy to determine in maternally-induced diapause: it begins in oocytes and ends at the pharate larval stage. The information about diapause-destiny is stored under an unknown way during the preparation phase. Transcriptional and physiological activities are modified in the oocyte (Urbanski *et al.* 2010b, Poelchau *et al.* 2011) and embryo during the preparation phase. This developmental phase is frequently prolonged in insects, but was not demonstrated in *Ae. albopictus* (Denlinger 2002, Poelchau *et al.* 2013a).

Diapause initiation

Diapause initiation phase is defined as (Kostal 2006):

> *Direct development (morphogenesis) ceases, which is usually followed by regulated metabolic suppression […]. Physiological preparations for the period of adversity may take place and intensity of diapause may increase.*

As mentioned previously, physiological preparation has begun before the developmental arrest in *Ae. albopictus*. The embryo finishes its development and initiates diapause at the pharate larva stage.

Maintenance

Maintenance phase is defined as (Kostal 2006):

> *Endogenous developmental arrest persists while the environmental conditions are favorable for direct development. Specific token stimuli may help to maintain diapause (prevent its termination). Metabolic rate is relatively low and constant. Unknown physiological process(es) lead to more or less gradual decrease of diapause intensity and increase of sensitivity to diapause terminating conditions.*

Molecular mechanisms of diapause maintenance are unknown in *Ae. albopictus* (Denlinger 2002). A differential expression of 2 genes suggested a low level of juvenile hormone (JH) in diapausing pharate larvae (Poelchau *et al.* 2013b). However an over-expression of a gene implied in the transport of JH was also observed. The JH appears to be a regulatory hormone of diapause maintenance in mosquitoes, but its action is not clear. Heat shock proteins (Hsp) as Hsp 23 and Hsp70 are currently upregulated in diapausing insects, as a key component of cold temperature response. Interestingly, there was no diapause-upregulation of Hsp in *Ae. albopictus* (Poelchau *et al.* 2013b) and *Cx. pipiens* (Rinehart *et al.* 2006), suggesting an absence of link between Hsp and diapause syndrome.

Diapause termination and post-diapause

Diapause termination phase is defined as (Kostal 2006):

> *Specific changes in environmental conditions stimulate (accelerate or resume) the decrease of diapause intensity to its minimum level and thus synchronize individuals within a population. By the end of the termination phase, a physiological state is reached in which direct development may overtly resume (if the conditions are permissive) or covert potentiality for direct development is restored but not realized (if the conditions are not permissive).*

At the end of diapause process, there is a transcriptional convergence between diapause towards quiescence process (Poelchau *et al.* 2013b). It implies a modification of the diapause syndrome, and most likely the progressive lost of the diapause in old pharate larvae. However, the lipid metabolism in eggs staid different at the end of the diapause than which observed in quiescent eggs (Poelchau *et al.* 2013b).

Diapause provides advantages for an invasive species during transportation of propagules (protected from environmental conditions and insecticide treatments), naturalization (higher winter survival rate, which can be decisive to preserve the limited number of founders) and spread (Tauber *et al.* 1986).

Global objectif of the study

This Ph.D. thesis is anchored within the disciplines of physiology and ecology. The study will focused on the egg diapause in the life cycle of the mosquito *Ae. albopictus* (figure I.8).

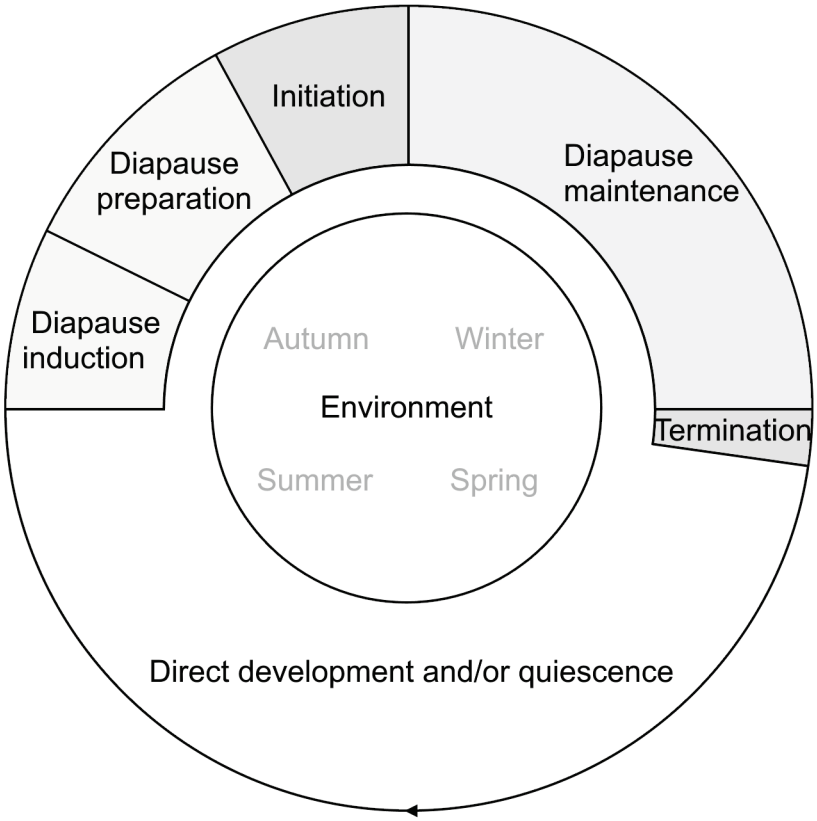


Figure I.8. Schematic representation of the life cycle and the diapause process (shaded parts) of *Aedes albopictus* in temperate area. This figure was modified in the following chapters/papers to represent the methodological approach and the sections studied during this Ph.D thesis.

Diapause is a good example of phenotypic plasticity, which constitutes an adaptive response to new environments faced by this invasive species, and plays a role in the evolutionary process (figure I.9). We first aim to understand the mechanisms and the magnitude of ecological consequences on the diapause process in an adaptive context. The advantage of diapause for the adaptive capacity of the species will be investigated. Because of public health issues linked to the Asian tiger mosquito, applied aspects will be also deal with taking into account the diapause process and the possibility it offers to act on the life cycle of the mosquito.

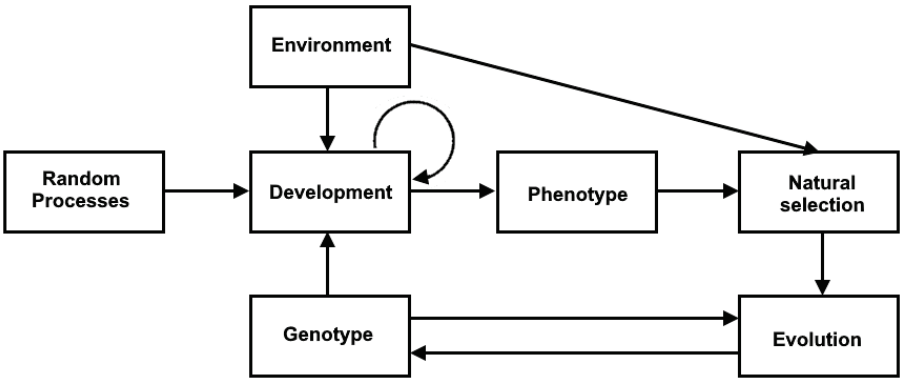


Figure I.9. Schematic description of the evolutionary process. Relationship between genetics, development, environment and evolution (adapted from Scheiner 1993).

In the second chapter of the thesis, we investigated the influence of the prediapause phase on the embryogenesis kinetics (Paper I). The prediapause stage manipulates the embryo physiology and metabolism in order to improve the survival of diapausing eggs during winter. These changes generally increase the embryogenesis timing in insects (Denlinger and Armbruster 2014). We expected that the embryogenesis kinetics of diapausing embryos differed from non-diapausing embryos of a temperate and a tropical strain; the embryogenesis kinetics of non-diapausing embryos of different strains should be similar. As prediapause phase aims to increase the fitness of the diapause-destined pharate larva, phenotypic changes in eggs morphometry are expected to result from the diapause process and not from the change of photoperiodic regime. Measurements of egg length and width were made to verify this hypothesis.

In the third chapter (Paper II), a field survey of a French *Ae. albopictus* population was made during 2 years. The objective was to determine the adequacy of the diapause initiation and termination with the seasonal environmental conditions. The critical photoperiod was determined in laboratory, and diapause initiation and termination were analyzed using a degree-day model. We can hypothesis that a well adapted prediapause process will happen close to winter arrival in order to let a maximum of time to population growth during the favorable season. The diapause termination is temperature-dependant but photoperiod play often a role in other mosquito species (Vinogradova 2007). The degree-day model should be informative on the importance of the temperature in the diapause termination.

If seasonality is the cause of diapause activation, the phenology is the resulting consequence of the diapause syndrome under a temperate environment. In Paper III, we investigated another potential consequence of the diapause process during a chikungunya outbreak. As transovarial infections and the survival of arboviruses in diapausing eggs are both proved existing phenomenon in *Ae. albopictus*, a low rate of viral overwintering is possible in temperate area after an outbreak. A prevalence study was made during the outbreak and after the winter to estimate this risk.

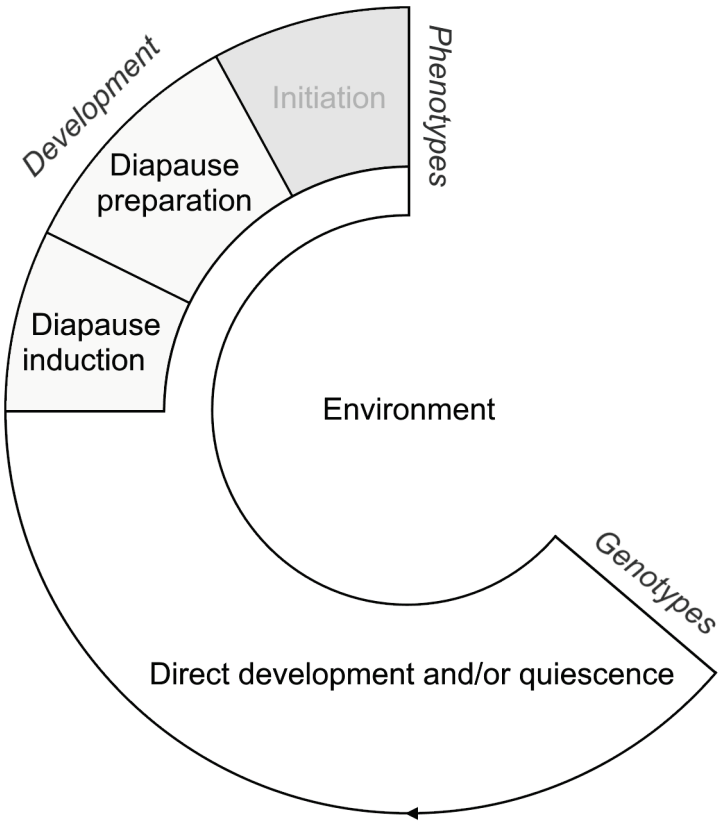
In the next results chapter of the thesis, an integrated approach was realized to determine the adaptive advantage of the diapause process in a temperate area. We based our experimentation on the comparison of a diapausing population and a non-diapausing population of *Ae. albopictus.* Semi-controlled experiments being not appropriate to compare during several years large population of mosquito in a large-scale area, a model was developed to simulate field experimentation. The paper IV describes the development of the generalist deterministic model of the mosquito abundance, implemented with biological parameters of a local strain of *Ae. albopictus.* The diapause process was expected to be one of the most important determinants of the annual mosquito abundance.

In the paper V, the survival and fitness of overwintering populations were determined in a semi-field experiment. A tropical and a temperate strain were used, with or without diapause induction, and laid eggs were placed outside, protected from rainfall to prevent winter egg hatching. As the diapause process improves the egg phenotype (Urbanski *et al.* 2010a, Thomas *et al.* 2012), especially egg desiccation and cold resistance, the

winter survival rate of *Ae. albopictus* strains were supposed to be classified as the following growing order: tropical strain ≤ non-diapausing temperate strain < diapausing temperate strain. The life history traits (larval survival, wing morphometry) of the overwintering progeny were expected to be modulated by the physiological status (diapause) of the egg stage and also be different between the studied strains. The results were implemented in the model (Tran *et al.* 2013), and the dynamics of the theoretical populations were simulated. The extinction of the non-diapausing invasive population was expected if diapause was strictly necessary under Mediterranean climate.

The last paper (paper VI) aims to use our results for applied mosquito control. The diapause shut-down simulated in the paper V was tested under laboratory conditions. A night-interruption with light can partially inhibits the diapause induction in insects (Barker *et al.* 1964). The photoperiodic induction of the diapause process in *Ae. albopictus* should be prevented by an altered photoperiod, and this photo-inhibition must have a deleterious impact on field populations. Laboratory experiments were done to determine the efficacy of the photo-inhibition of diapause in *Ae. albopictus*. The optimal moment and duration of the light source during the scotophase were determined. The maximal efficacy obtained in laboratory was implemented in our deterministic population model and the theoretical efficacy of photo-inhibition was simulated.

Chapter II. Biological effects of photoperiodism and prediapause on embryo development of *Aedes albopictus*



In this paper, the embryogenesis of *Ae. albopictus* was investigated to discriminate the causality (environment or diapause) and effects of plastic responses (reaction norms of development kinetics and egg size) in two strains (different genetic basis) of the Asian tiger mosquito. Indeed, the physiologic and metabolic changes occurring during the diapause preparation generally increase the embryogenesis timing in insects (Denlinger and Armbruster 2014). The determination of the egg diapause status must be done by egg hatching once pharate larvae are fully developed; in consequence, the knowledge of the development timing of embryo is necessary for this Ph.D work. Phenotypic changes in eggs morphometry (size and volume) were also investigated to estimate their use as potential makers of diapause status.

Highlights (paper I)

- Effects of maternal photoperiod and diapause were examined in *Aedes albopictus* eggs.
- The tropical and the temperate strains have distinct kinetics of embryogenesis.
- Diapause increases the embryonic developmental timing in the temperate strain.
- Maternal photoperiod influences the timing of embryogenesis.
- Short day length results in larger egg size in both temperate and tropical strains.

When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae).

Paper I

Guillaume Lacour[1,2], Florian Vernichon[1], Nicolas Cadilhac[1], Sébastien Boyer[3], Christophe Lagneau[1], Thierry Hance[2]

Journal of Insect Physiology 2014; 71, 87–96

Abstract

The diapause of *Aedes albopictus* is maternally induced by photoperiod and initiates at the pharate larvae stage in eggs. This pre-diapause results in enhanced survival eggs during the winter. This study aims to disentangle the effects of photoperiod and diapause on embryonic developmental time and egg size in *Ae. albopictus*. A temperate strain capable to perform diapause and a tropical strain unable of diapause were reared at 21°C with long- (LD) and short-day (SD) lengths. Four distinct traits were studied on embryos and eggs were measured at the end of embryogenesis.

The chronologies of embryo development for both strains were influenced by maternal photoperiod, especially in the temperate strain in which the development of SD eggs took longer than LD eggs. The delay increased gradually in the temperate strain, and reached up to 38 hours at the end of embryogenesis. The kinetics of embryogenesis differed among the temperate and the tropical strains, each one of the 4 studied traits showing differences. For example the serosal cuticle was secreted precociously in the tropical strain. Egg width and volume are influenced by the maternal photoperiod and the strain × photoperiod interaction. For both strains, larger eggs were laid by female reared under SD when compared to LD.

The influence of several maternal effects was demonstrated in this study. The diapause process modifies greatly the length of embryogenesis in the temperate strain, whereas the maternal photoperiod has a direct influence on egg size and embryogenesis regardless of the strain considered. These

[1] EID Méditerranée, Montpellier, France
[2] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium
[3] MIVEGEC, UMR IRD224-CNRS5290-UM, IRD, Montpellier, France

findings provide useful data on chronology of embryonic development for integrative biology studies of egg pre-diapause stages.

Keywords

Embryo development; diapause; day length; phenotypic plasticity.

Background

The Asian tiger mosquito *Aedes albopictus* is an emerging model for the study of the eco-physiology of egg diapause. It is a successful invasive species now present in all continents excepted Antarctica (Bonizzoni *et al.* 2013). It was first introduced in tropical countries around the 18t^{h} century, in water-stock of migrants' ships, and a second wave of invasion is still ongoing in tropical areas (Delatte *et al.* 2011). The colonisation of temperate areas began in the middle of the 20th century, through used tire traffic by ships from Asian subtropical areas (Kuno 2012; Urbanelli *et al.* 2000). As it is an effective vector for many arboviruses (Gratz 2004), this species represents a new public health threat for the US and Europe. Its role as the exclusive vector in epidemics in temperate areas has been proven for chikungunya and dengue fevers (Rezza *et al.* 2007, Vega-Rua *et al.* 2013). There is a remote risk that diapausing eggs will be infected by arboviruses (Guo *et al.* 2007); this could lead to the persistence of arboviruses in mosquito populations in temperate countries.

Diapause is an adaptation and a complex physiological process defined as "a form of dormancy that is hormonally programmed in advance of its onset and is not immediately terminated in response of favourable conditions" (Denlinger and Armbruster 2014). Diapausing individuals are indeed usually more resistant to harsh environmental conditions than non-diapausing ones. Desiccation (Sota and Mogi 1992a, Urbanski *et al.* 2010a) and cold resistance are generally enhanced during diapause (Hanson and Craig 1995a) and post-diapause (Thomas *et al.* 2012) as for *Ae. albopictus* eggs. Diapause also enhances nutritive resources (Hahn and Denlinger 2011), and even irradiation resistance (Brower 1980) in insects. Numerous examples show that the diapause status of the progeny is determined by the exposure of ovipositing females to short photoperiod (Mousseau and Fox 1998). The annual variation in day length is the most reliable indicator of seasonality in temperate areas. The photoperiodism, defined as the ability to determine day length with precision and to react to changes, is in most organisms a

major adaptation to avoid harsh periodical environmental conditions (Danilevskii 1965, Tauber *et al.* 1986). As a result it is used to initiate biological events essential to survival, such as reproduction, migration and dormancy (Danilevskii 1965).

Egg diapause is a useful phenotype to study photo-induced maternal effects. Maternal effect is environmentally modulated transgenerational phenotypic plasticity (Mousseau and Fox 1998). Investigating the pre-diapause process in the egg is of particular interest to elucidate the molecular process of the photo-induced maternal effects, from maternal induction to phenotypic initiation. Egg diapause is currently associated with any condition of suspended hatchability in temperate species that overwinter as cold hardy eggs. As described in Lepidoptera, egg diapause can be initiated early in the embryogenesis phase in the late gastrula stage as in the silkworm *Bombyx morii*, or at the end of the embryogenesis in the pharate larva stage, with a fully-developed larva still contained and compacted in the egg, as in *Lymantria dispar* and *Antheraea yamamai* (Denlinger and Armbruster 2014). The Asian tiger mosquito has only one clearly defined stage of diapause, the pharate larva (Vinogradova 2007). The changes in the eggs occurs during diapause preparation, before the initiation *sensu stricto* (Koštál 2006), resulting in phenotypes with differences in morphology, development time and physiology. The developmental period preceding the stage of diapause initiation is frequently prolonged in insects (Denlinger 2002, Harrat and Petit 2009). This increased duration is linked to changes in metabolism, including protein synthesis and additional lipid storage (Denlinger 2002).

In addition to diapause effects, photoperiod generates direct impacts on mosquito development and life history traits. For example, some larvae of Aedes and Culiseta species cannot reach maturity in the absence of light exposure (Clements 1963), and the development time of *Ae. albopictus* larvae from the US is affected by the rearing photoperiod (Yee *et al.* 2012). Nevertheless it can be difficult to discriminate between effects of the mechanisms of a photoperiod-induced diapause and direct effects of photoperiod on organisms. It is the case for *Ae. mariae*, where diapause-programmed females preferentially seek sheltered holes in rock pools, providing them an appropriate hibernaculum against winter events like storms (Coluzzi *et al.* 1975). We can use tropical and temperate populations of *Ae. albopictus* to study this type of phenomenon in mosquitoes. Tropical strains are unable to perform diapause, contrary to temperate and subtropical strains which perform photo-induced diapause (Pumpuni 1989). This fundamental difference between strains occurs naturally, contrary to other biological models of insects where strains must be artificially selected (Lee *et al.* 1997). It allows for the set-up of a discriminative comparison test

of the consequences of change in daylength on the embryonic development of eggs with or without diapause.

The objective of this paper is to disentangle the effects of photoperiod and diapause on egg size and embryonic developmental time in *Ae. albopictus*. We predict that diapause induction in eggs will generate a prolonged embryo development sometime before the diapausing initiation. To test this prediction, we will investigate the effects of photoperiod and of the diapause syndrome by recording the size of eggs as related to an indicator of mother size (maternal wingspan), and by hourly monitoring the appearance of four features representing successive steps in the embryo development. The simultaneous study of a diapausing temperate strain and a non-diapausing tropical strain under long and short daylengths will allow us to disentangle the effects on development of the daylength experienced by the mother.

Methods

Ethic statement

The animal facility of the “Entente Interdépartementale pour la Démoustication du littoral méditerranéen” has received accreditation from the French Ministry of Agriculture to perform experiments on live guinea pig (permit number B34-172-29) in appliance of the French and European regulations on care and protection of Laboratory Animals.

Mosquitoes

Two strains of *Ae. albopictus* were used in this study. The European temperate strain named SPAM was collected in 2007 in the coastal area of Nice, France (43° 41′ 45″ N, 7° 16′ 17″ E). The tropical strain is native of La Reunion Island, located south-east of Africa near the Madagascar island, and was collected in 2011 in the coastal area of Saint-Denis Providence city (20° 52′ 44″ S, 55° 26′ 53″ E). The F16-F17 and F2-F3 maternal generations were used respectively for the temperate and tropical strains.

General rearing protocol

Mosquitoes of both strains were maintained in a laboratory room under a constant environment of 21.5 ± 0.3°C, 80.1 ± 2.4% relative humidity, a photoperiod of 16 hours of light and 8 hours of darkness. Larvae were

reared in batches of 500 larvae per pan (30.5x20x6 cm) in 2 liters tap water and fed with 3.5 g of milled dog food during larvae development. This standardized protocol was chosen to produce an optimal expression of photoperiodic response, as it has been shown that this response is sensitive to temperature and larval diet (Pumpuni *et al.* 1992). After pupation, 500 pupae were placed per pan and transferred in cages in photoperiodic chambers. They were either submitted to non-diapausing long-days conditions (LD) with a light:dark cycle of 16h:8h, or short-days conditions (SD) inducing diapause in temperate strain with a light:dark cycle of 9h:15h. Photoperiodic chambers consisted of windowless plastic boxes (65x65x40 cm) with a zipper opening in black-cloth placed in the rearing room. Individual chambers were maintained at a constant temperature of 21.5 ± 0.4°C and 79.1 ± 2.3% relative humidity, using a fan-produced air flow and a periodic air dampening system made of a water pot stirred using an aquarium air-pump. Light cycle was computer controlled and generated with a 3 watt bar of 42 white LED per photoperiodic chamber. There was no light transition between photophase and scotophase. Adult mosquitoes were left in the cages in photoperiodic chambers and were supplied with a 10% sucrose solution. The first blood meal was provided on anesthetized guinea pig 10 days after emergence, to make sure enough time was given to strongly induce diapause in SD temperate females (Pumpuni 1989).

Synchronous egg laying

Constraining females to lay eggs synchronously is necessary to know precisely the age of embryos. The protocol employed is adapted from (Rezende *et al.* 2008). One day sugar-deprived females were blood-fed on anesthetized guinea pig. In order to hasten the laying of eggs when transferred into a suitable oviposition surface (nest-box), females were forced into egg retention during the 6 following days. Nest-boxes consisted of cotton filled cups humidified with larval rearing water and covered with a Whatman N°1 paper disk. Cups are closed by a piece of cloth, creating a space of 20/30 mm of height and 75 mm of diameter for about 7 female mosquitoes per cup. Nest-boxes containing mosquitoes were placed in an incubator to begin oviposition at 21°C in darkness. Egg laying was allowed during 30 minutes for eggs destined to the study of serosal cuticle appearance, and during 60 minutes for eggs used to determine timing of segmentation, eyes and egg burster apparition and egg volume, afterwards females were removed from nest-boxes. The middle of the synchronous egg laying period determines the 0 hour after egg laying (HAE).

Humid paper disks with eggs were stored in Petri dishes in incubators at 21°C, and in darkness to avoid any possible reaction due to embryonic light sensitivity. Each replicate in the following experiments used different paper disks, with eggs laid by different females. Eggs were transferred out at different hours after egg laying for egg hatching calculation, egg volume measurements and embryonic observations.

Diapause incidence

This experiment was performed to verify that diapause was initiated only in temperate strain under maternal short days. Three replicates of at least 400 10-days old eggs produced in the first gonotrophic cycle were submitted to the following hatching protocol: eggs were immersed in oxygenated tap water during 30 minutes, for each test group of strain type and maternal photoperiod. A dose of 100 mg of ascorbic acid per litre of water was added to consume dissolved oxygen, in order to suppress the quiescence, a form of dormancy directly triggered and terminated by environmental conditions (Sinègre 1974, Denlinger and Armbruster 2014). The next day, eggs were brought out and let out to dry during 2 hours, and were submitted to the hatching protocol a second time. Hatched eggs were counted. Unhatched eggs were bleached in a bath of Trpiš solution (Trpiš 1970) during 30 minutes. The presence of ocelli and egg burster (a spine on the clypeus of embryo used to break the egg chorion in hatching) was investigated, as indicator of embryogenesis completion (Farnesi *et al.* 2009). Unhatched eggs were considered to be in diapause only when embryos were fully developed and without deformity. Egg hatching rate was calculated with embryonated and hatched eggs:

Hatch % = (hatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)

Wing size measurement

In insects, female size can strongly affect reproductive output, including egg size (Berrigan 1991). Wing length was investigated to confirm that our standardized rearing protocol produced adults of similar size. At least 30 *Ae. albopictus* females were killed by freezing in each test group of strain type and maternal photoperiod. Their right wings were removed and flattened between microscope slide and cover glass. A stereomicroscope Zeiss Stemi SV6 fitted with a CCD camera Sanyo VCC-2972 was used to capture pictures of each wing using the software EllixTM (Version 6.1.5, Microvision Instruments, Evry, France). Wing length was measured from the

notch between the alula and the posterior margin of the wing to the distal tip of the wing excluding the apical fringe.

Egg volume determination

Sixty eggs in each test group were isolated from 6 nest-boxes at the rate of 10 eggs per petri dish nine days after egg laying. Each egg was placed on a damp Whatman paper onto dorsoventral position with a micro teasing needle, in order to show its side of prolate spheroid shape. Maximal width measurement can be subjective in the absence of landmark on the egg chorion. In order to limit the observational error, egg length and width measurements were repeated 3 times per egg and means were used to calculate each egg volume:

Volume = 1/6.π.Length.Width² (Urbanski *et al.* 2012)

Measurements were performed with the system described in the *Wing size measurement* section.

Embryonic development speed determination

The first trait studied in order to determine the developmental time of embryos was the serosal cuticle. The desiccation resistance of Aedes species eggs is acquired with the complete formation of the serosal cuticle, an extracellular matrix that is resistant to chlorine digestion contrary to the dark colored chorion (Rezende *et al.* 2008). Three replicates of 150 eggs per HAE from temperate and tropical strains reared under SD and LD conditions were exposed to chorionic digestion with a 3.6% chlorine solution during 30 minutes. Eggs without their serosal cuticle lose their cellular content; on the contrary eggs with their serosal cuticle remain intact. Eggs were killed by the treatment.

The three other morphological traits used were studied by direct observation of embryo morphology. Three batches of 50 eggs/HAE bleached by Trpiš solution (Trpiš 1970) during 30 minutes were used to observe embryo morphology under Zeiss stereo microscope STEMI SV6. Presence or absence of 3 morphological criteria was noted: embryo segmentation, pigmented ocelli and egg burster (figure II.1). The presence of embryo segmentation was validated when the germ band presented 8 regular abdominal segments on its ventral side and an anterior lobe with cephalic segments. The egg burster, also called hatching spine, is the last morphological trait to appear (Farnesi *et al.* 2009). Morphological

observations were compared to referenced stages of embryogenesis in *Ae. sticticus* (Trpiš 1973), *Ae. aegypti* (Vital *et al.* 2010), *Culex molestus* (reviewed in Christophers 1960), *Cx. pipiens* (reviewed in Clements 1963) and *Ae. caspius* (Sinègre 1974).

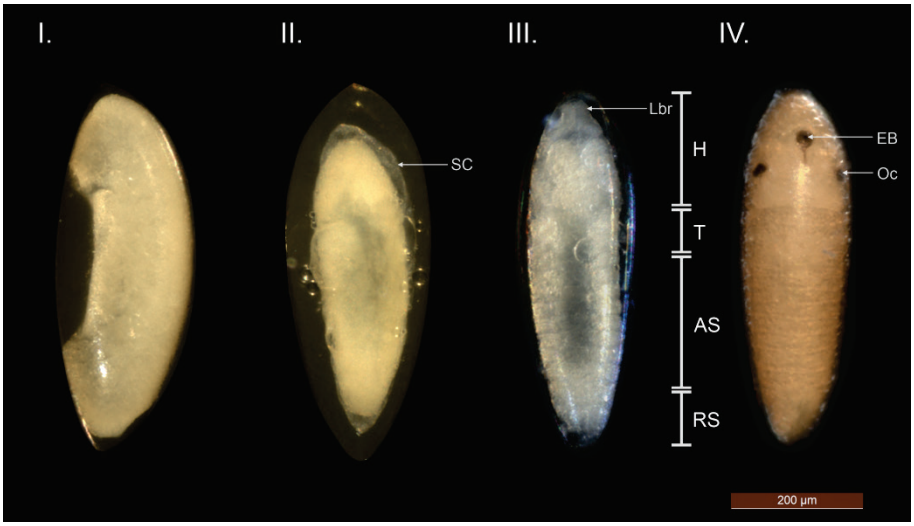


Figure II.1. Embryo morphology of temperate eggs of *Aedes albopictus* developed at 21°C and bleached with Trpis solution. I. Lateral view of early embryogenesis without serosal cuticle 30 hours after egg laying (HAE). II. Lateral view of egg with serosal cuticle (SC) 47 HAE. III. Ventral view of embryo with appearance of the head (H) with labrum (Lbr), thorax (T), eight abdominal segments (AS) and respiratory siphon (RS) and associated structure at 87 HAE. IV. Dorsal view of first-instar-larvae with ocelli (Oc) and egg burster (EB) 160 HAE.

Statistical analysis

Statistical analyses were performed using R 3.0.2 software (R Development Core Team 2013) and a predetermined significance level of 0.01. Female wing size was compared between strains and rearing photoperiod using a two-way analysis of variance (ANOVA) including an interaction term. The effects of maternal photoperiod and strains origin on egg size descriptors (length, width and volume) were evaluated by a multivariate analysis of variance (MANOVA). Wing size was not included in the MANOVA due to a smaller number of replicates which would have unbalanced the data. Whenever a MANOVA was significant on 4 multivariate statistics (Pillai-

Bartlett criterion, Wilk's lambda, Roy's largest root and Hotelling-Lawley trace), an ANOVA was realized for each dependent variable. The Kruskal-Wallis tests were used to confirm the results of the ANOVAs on significant effect of variables.

Embryonic development time was analysed through reaction norms modeling of the frequency of occurrence for desiccation resistance, segmentation, ocelli or egg burster (*Y*), fitted by HAE with an iterative logistic model with a four parameters (*a*, *b*, *c*, *d*) logistic regression:

$$Y = a + \frac{b-a}{1+\exp\left((c-HAE)/d\right)}$$

The parameters *c* and *d* were rendered dependent of the maternal photoperiod to test their influence on the embryogenesis time:

$$Y = a + \frac{b-a}{1+\exp\left(\frac{c0+c1*photoperiod-HAE}{d0+d1*photoperiod}\right)}$$

The parameters *a*, *b*, *c0* and *d0* were estimated per trait and per strain separately.

Results

Diapause incidence

As expected, only the eggs of the temperate strain maternally reared under short days were found to be in diapause, with a hatching ranging from 0.1% to 13.6% (figure II.2). The egg hatching rate of each biological replicate ranged from 92.1% to 99.5%, both in the tropical strain eggs under both photoperiodic treatments and the temperate strain eggs under long day treatment.

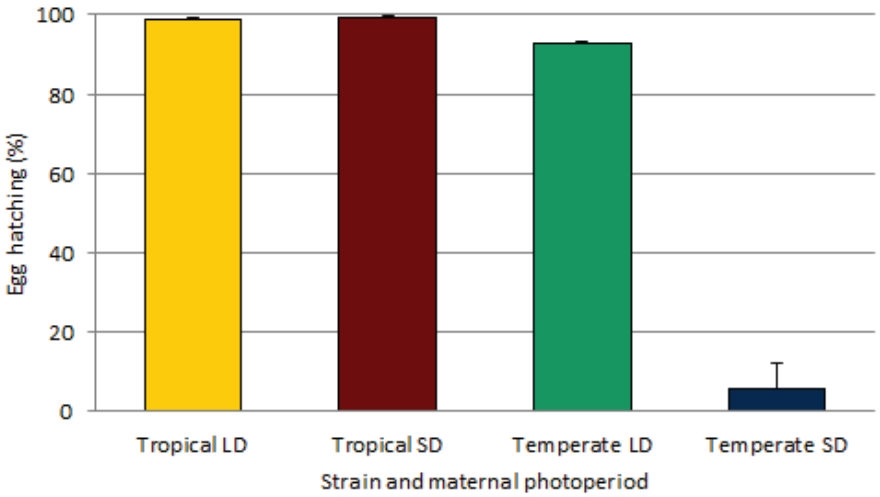


Figure II.2. Percentage (mean ± sd) of egg hatching in *Aedes albopictus* of tropical and temperate strains reared under long-days (LD) and short-days (SD) conditions at 21°C. Only the temperate strain exposed to SD laid eggs in diapause process which do not hatch. Diapause was not induced by LD exposure of the temperate strain, and the tropical strain is not able to perform diapause whatever the rearing photoperiod.

Wing size

The MANOVA results showed a significant effect of the strain variable on female wing size, the latter is therefore not affected by the photoperiodic rearing conditions nor the strain × photoperiod interaction (table II.1, figure II.3D). The Kruskal-Wallis test confirms the significant difference between wing size (means ± sd) of temperate (2503 ± 82 µm) and tropical (2433 ± 76 µm) strains (KW = 27.6, df = 1, $p < 0.01$) and the lack of difference between wing size of SD and LD groups ($p > 0.01$).

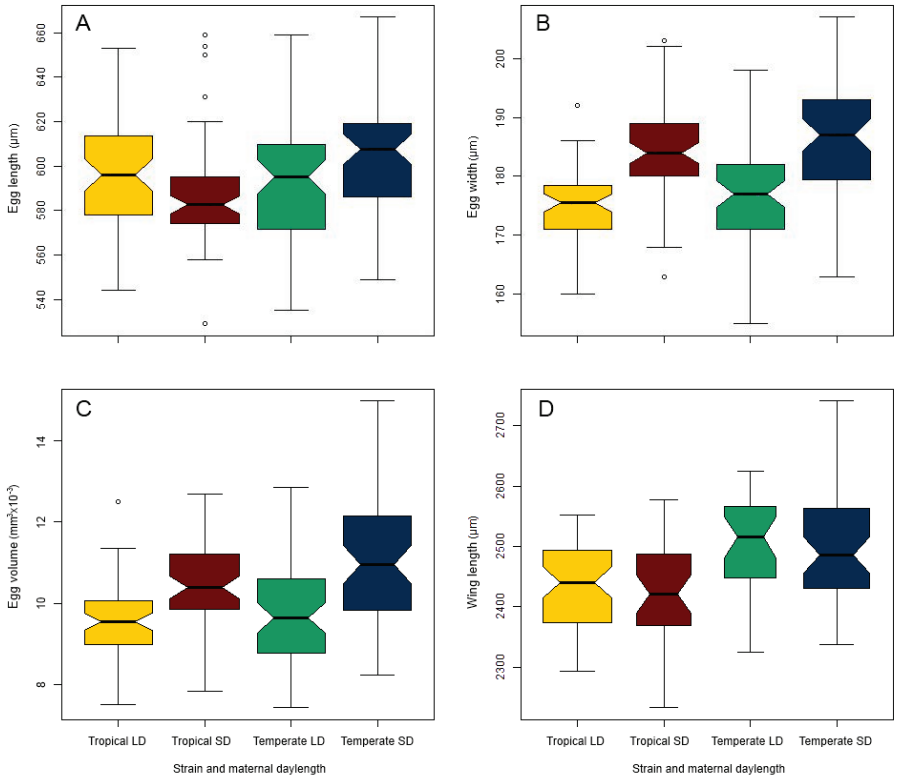


Figure II.3. Boxplots of *Aedes albopictus* egg and wing size measurement. Length (A), width (B) and volume (C) of 9-days old eggs. Female wing size (D) of tropical and temperate strains of *Ae. albopictus* reared under long days LD and short days SD at 21°C. The solid horizontal line represents the median, the box encompasses the lower and upper quartiles, and the boxplot whiskers encompasses the data with values inferior or equal to 1.5 times the interquartile range from the box. Atypical data which values superior to 1.5 times are represented with empty circles. Eggs laid under short days conditions are wider and more voluminous, independently of population origin. Maternal wing size depends of strain origin.

Table II.1. ANOVA extracts the effects of maternal photoperiod, strain and interaction of strain and maternal photoperiod on the size of eggs of *Aedes albopictus*. Univariate ANOVA on egg size was extracted from MANOVA test; ANOVA on wing size were done separately.

Response variable	Explanatory variable	df	SS	F	p[a]
Wing length	Strain	1	247963	35.71	***< 0.01***
	Photoperiod	1	10341	1.49	0.22
	Strain × Photoperiod	1	0	0.00	0.10
Egg length	Strain	1	1747	2.48	0.12
	Photoperiod	1	90	0.13	0.72
	Strain × Photoperiod	3	7857	3.82	0.01
Egg width	Strain	1	145.9	1.69	0.19
	Photoperiod	1	5092.7	77.55	***< 0.01***
	Strain × Photoperiod	3	5254.2	26.72	***< 0.01***
Egg volume	Strain	1	521.62×10^{10}	3.41	0.07
	Photoperiod	1	6783.8×10^{10}	53.48	***< 0.01***
	Strain × Photoperiod	3	7505.8×10^{10}	20.03	***< 0.01***

[a] Significant p-values are in italic and boldface.

Eggs size

The MANOVAs revealed a significant main effect on egg measurements of the rearing maternal photoperiod (Pillai's criterion = 0.25, F3, 238 = 26.87, p <0.01), the strain origin (Pillai = 0.05, F3, 238 = 4.01, p <0.01) and the strain × photoperiod interaction (Pillai = 0.35, F9, 714 = 10.37, p < 0.01). The other multivariate statistics (Wilk's lambda, Roy's largest root and Hotelling-Lawley trace) were all significant. The ANOVAs showed an effect of the maternal photoperiod and the strain × photoperiod interaction on egg width and egg volume, as opposed to egg length that is not influenced by the explanatory variables (table II.1). A strain effect on egg size was only revealed by the MANOVA and not by the ANOVA, underlining that this effect is probably small. The maternal photoperiod is also the factor having the main effect on egg size, even if other factors may still interact. The mean volume of SD eggs is larger than LD eggs, regardless of the temperate or tropical origin of strains (Kruskal-Wallis = 43.5, df = 1, p < 0.01) (figure II.3C). The egg length is not linearly related to egg volume (R^2=0.31) and represents a relatively small contribution in the calculation formula of the egg volume, as opposed to egg width which is positively correlated with the egg volume (R^2= 0.87).

Embryonic development speed

The parameters values used to model reaction norms are presented in appendix (table A.1). The reaction norms for the tropical strain reared under different day lengths remained closed for each studied trait while reaction norms for the temperate strain reared under SD and LD conditions moved away from each other during embryogenesis (figure II.4).

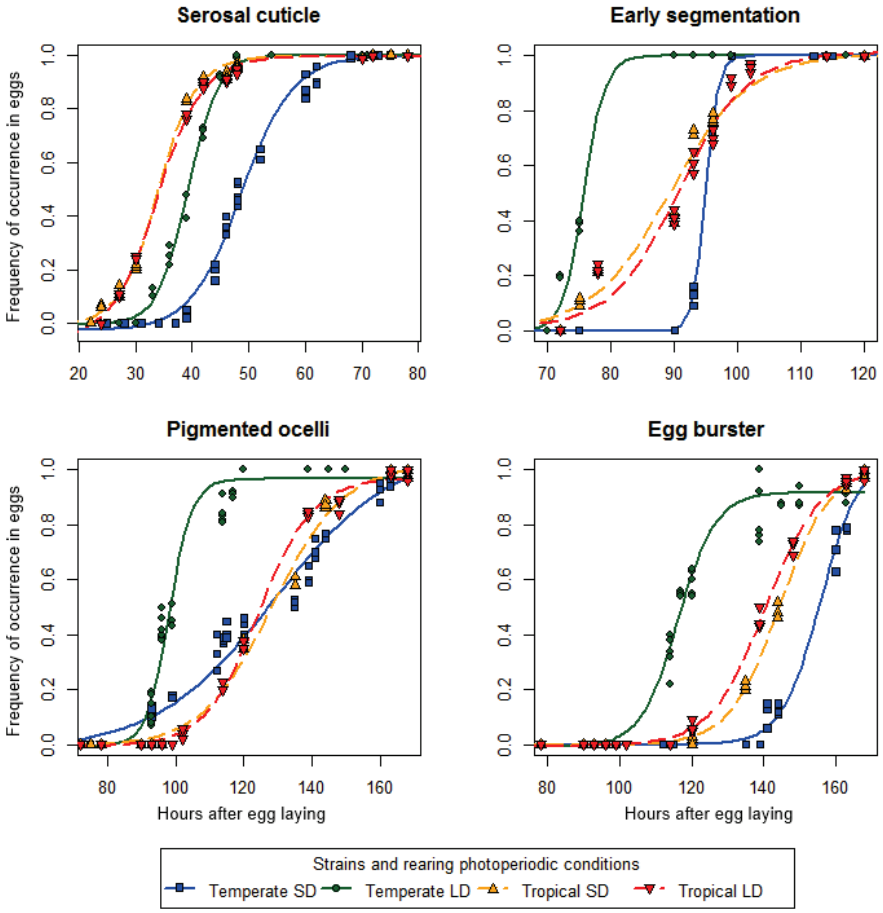


Figure II.4. Photoperiodically-induced egg diapause increases embryogenesis duration in temperate strain of *Aedes albopictus*. Experimental results (plots) and sigmoid nonlinear models (line) of reaction norms for development time of serosal cuticle, early segmentation, pigmented ocelli and egg burster during embryogenesis in tropical and temperate strains of *Ae. albopictus* reared under long days LD (16h daylength) and short days SD (9h daylength) at 21°C.

Table II.2. Developmental duration of *Aedes albopictus* embryos of temperate and tropical strains under different photoperiodic conditions. Development embryonic time is determined by following the formation of 4 morphological indicators: the serosal cuticle providing desiccation resistance, the abdominal segmentation, the ocelli and the egg burster. HAE values were assessed from sigmoid nonlinear models of traits appearance.

Strain	Maternal photo-period	Moment of trait acquisition in 50% of embryos							
		Serosal cuticle		Abdominal segmentation		Ocelli		Egg burster	
		HAE[a]	%[b]	HAE[a]	%[b]	HAE[a]	%[b]	HAE[a]	%[b]
Temperate	LD	39.3	*34*	75.8	*65*	98.4	*84*	117.2	*100*
Temperate	SD	48.9	*31*	94.9	*61*	127.2	*82*	155.3	*100*
Tropical	LD	34.3	*24*	89.3	*64*	125.1	*89*	140.5	*100*
Tropical	SD	34.1	*24*	90.6	*64*	128.2	*89*	144.4	*100*

[a] HAE: hours after egg laying
[b] Percentage of total embryonic development time, relative to egg burster formation.

The difference in the timing of the serosal cuticle, segmentation, ocelli and egg burster appearance is described below as measured when 50% of the analysed embryos had acquired these traits (table II.2). The LD temperate embryos' serosal cuticle appeared five hours later than in the tropical strain ones, and in temperate embryos reared under diapause-inducing conditions it appeared almost 10 hours later. This difference between SD and LD temperate eggs was supported by direct observations: embryos aged from 18 to 47 HAE showed different stages of development between diapause-induced and non-diapause-induced embryos (figure A.2). The abdominal segmentation appears on the third day of embryonic development, first on LD embryos of the temperate strain. In this strain, diapause-programmed embryos are segmented 19 hours after those reared under non-diapause-inducing photoperiod. The segmentation of SD and LD tropical embryos presents a temporal development intermediate to temperate embryos' development. Pigmented ocelli first appeared at 93 HAE in temperate strains and at 102 HAE in the tropical strain. The ocelli development began at the same time for LD and SD temperate strains, but finished 2 days earlier in the LD temperate strain. Egg burster formation in SD temperate strains took 38 hours longer than in LD strains. The maternal photoperiod has a significant effect on the embryogenesis time for all the studied traits in the temperate strain and estimates of c1, representing the distance between turning points, all being positive for SD model (table II.3). There is also an effect of the photoperiod in the tropical strain but not generalized to all the studied traits. In addition, c1 estimates for the tropical SD models are not

structured and have lower values than the temperate SD models, which demonstrate a lack of noticeable tendency in the differentiation of embryogenesis timing for the tropical strain. This corroborates the hypothesis that the diapause syndrome is responsible for the large embryonic developmental delay.

The delay between traits appearance during embryonic development of LD and SD temperate strains increases of approximately 10 hours for each of the analysed trait (table A.3). This increase also seems not to be periodic but continuous during embryogenesis: whatever the strain and the maternal photoperiod considered, abdominal segmentation appeared among 61-65% of total embryogenesis and ocelli were formed among 82-89% of total embryogenesis (table II.2).

Regardless of the morphological feature investigated in the embryo, there are 4 constants: Firstly, temperate and tropical strains have different embryonic kinetics. Secondly, maternal photoperiod modifies the developmental time in both strains, but to a larger extent in the temperate strain. Thirdly, for the temperate strain, females with LD conditions produce eggs with a faster embryonic development that female exposed to diapause-inducing photoperiod. Fourthly, in all test groups the studied traits (except the serosal cuticle) appeared at the same percentage of total development, although the entire embryo development period differs among strains and temperate photoperiods. These results argue in favour of the effect of a progressive diapause preparation process rather than just punctual changes in the embryonic program of the temperate strain.

Table II.3. Effect of maternal photoperiod on development of *Aedes albopictus* embryos of temperate and tropical strains. Results are presented for SD reared mosquitoes. There is one degree of freedom for each parameter. The estimate and t-value for LD reared mosquitoes are the same but multiplied by -1. A statistically significant effect on dependent-parameters c1 or d1 demonstrates an influence of the maternal photoperiod on the embryonic developmental time.

Strain	Parameter	Serosal cuticle			Segmentation			Ocelli			Egg burster		
		Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]
Temperate	c1	9.69 (±0.14)	70.79	***<0.01***	19.14 (±0.10)	184.84	***<0.01***	26.62 (±0.79)	33.82	***<0.01***	36.87 (±0.64)	57.58	***<0.01***
	d1	1.68 (±0.14)	11.90	***<0.01***	-0.62 (±0.09)	-7.12	***<0.01***	11.30 (±0.70)	16.18	***<0.01***	-0.80 (±0.54)	-1.47	0.14
Tropical	c1	-0.51 (±0.17)	-3.02	***<0.01***	-1.16 (±0.49)	-2.35	0.02	2.39 (±0.56)	4.28	***<0.01***	3.64 (±0.28)	12.94	***<0.01***
	d1	-0.31 (±0.11)	-2.96	***<0.01***	0.73 (±0.50)	1.45	0.15	1.46 (±0.45)	3.23	***<0.01***	-0.79 (±0.28)	-2.82	0.01

[a] Significant p-values are in italic and boldface.

Discussion

Based on a detailed morphological analysis, we demonstrated for the first time the modulation of embryonic developmental rate due to diapause preparation in *Ae. albopictus* eggs. The preparation stage of diapause syndrome implies numerous physiological adaptations which necessarily involve an energetic investment. Recent transcriptional works already suggested the existence of a developmental delay of embryos during diapause preparation: a delayed expression of cell-cycle regulators and genes in diapausing SD eggs compared to LD eggs was put in evidence in a US temperate strain of *Ae. albopictus* (Poelchau *et al.* 2013a). However, these delays in physiological processes were not correlated to visible morphological differences in the development (Reynolds *et al.* 2012, Poelchau *et al.* 2013a). Hence, regardless of the origin of the strain, embryogenesis is also slightly sensitive to the maternal photoperiod.

The embryonic time varies between tropical and temperate strains. Both strains have been crossed and gave a viable and fertile offspring, confirming that tropical and temperate strains are of the same species, as it was already attested on other strains (Hanson *et al.* 1993). Globally, at lower temperatures tropical strains of *Ae. albopictus* have an expanded embryonic developmental time compared to temperate strains (Waldock *et al.* 2013). Variability in the studied developmental patterns could be the result of an absence of directional selection or can reflect a selection process to the local environment (Bradshaw *et al.* 2004). As this study's experimental rearing temperature corresponds to the natural coldest developmental conditions on La Reunion Island shores, the tropical strain was reared under a more stressful environment than the temperate strain whose fitness is optimized for this average temperature. However, even among tropical strains of *Ae. aegypti*, developmental rates have been demonstrated to be heterogeneous under the same temperature (Couret and Benedict 2014). Different larval developmental rates between populations have previously been observed in other mosquito species like *Ae.* (*Oc.*) *triseriatus* (Holzapfel and Bradshaw 1981), but not in *Ae. albopictus* (Waldock *et al.* 2013). This underlines the need to compare several temperate and tropical strains in order to confirm our observations.

Aedes species are particularly exposed to desiccation, and its serosal cuticle development is more effective than in other mosquito genera (Vargas *et al.* 2014). Interestingly, only the serosal cuticle's complete secretion took place faster in our tropical strain as compared to our temperate strain. The first hypothesis to explain this kinetic difference is that this protection mechanism against a dried-up environment must be subject to a strong selective

pressure, especially in the tropics where the threat of death by desiccation is extreme (Tauber *et al.* 1986). However *Ae. albopictus* eggs are laid in outdoor water containers likely to dry up, and most eggs in diapause process are laid during the favorable season when average temperature is still high in temperate area. The additional delay in the formation of serosal cuticle generated by diapause preparation incurs a longer period of sensitivity to desiccation in a context of strong evaporation. Thus the induction of diapause syndrome could have hazardous effects on eggs, even if diapause-programmed eggs later offset this risk notably with a higher quantity of surface lipids on the chorion which increases desiccation resistance (Sota and Mogi 1992a, Urbanski *et al.* 2010a). The serosal cuticle is an important structure protecting from desiccation but not the only one (Rezende *et al.* 2008). The second hypothesis is that tropical strains developed other structural and metabolic mechanisms to improve the egg's waterproof quality, and counterbalance a more rapid but potentially weaker or thinner serosal cuticle. This hypothesis is supported by the presence of a higher quantity of surface hydrocarbons on tropical eggs than temperate eggs (Urbanski *et al.* 2010a). Temperate strains would favour the production of a stronger or improved serosal cuticle than tropical strains, which requires a prolonged period of formation.

Previous articles provided information about *Aedes* eggs morphology affected by the duration of light exposure. Eggs of the tropical species *Ae.* (*Oc.*) *epactius* reared under SD were wider than those reared under LD. Electron microscopy studies of eggs of the close temperate species *Ae.* (*Oc.*) *atropalpus* able of diapause revealed different and stronger modifications in size and shape: LD eggs were longer and narrower than SD eggs, with changes in the outer chorion structure (Linley and Craig 1994). However no differentiation of the possible factors, day length and diapause, responsible for these changes was obtained. Our study is thus the first to demonstrate that maternal photoperiod, and not diapause, influences egg volume in an Aedes species capable of diapause.

The structure of mosquito eggs is therefore sensitive to several seasonal factors. Indeed, *Anopheles sacharovi* (Favre) and *An. punctipennis* (Say) produce "winter" eggs almost totally covered by exochorion (Theodor 1925, Fritz and Washino 1992), and "winter" eggs of *An. sacharovi* possess a small float and are larger than "summer" float-less eggs. In these cases, the morphological differentiation originates in response to temperature fluctuations, and not from the diapause syndrome, as diapause occurs at the larval or adult stages in *Anopheles* species (Theodor 1925). The latter are capable of egg quiescence, a process fairly similar to diapause at the

molecular level (Poelchau *et al.* 2013b). However quiescence is by definition an aseasonal state of inactivity (Vinogradova 2007).

The mechanisms involved in egg structure variability in mosquito are not determined and may be multiple. Concerning the photoperiodic causality, we suspect that a circadian rhythm plays a part in the hormonal production and reserve storage, such as was demonstrated in several insect groups, including mosquitoes (Bloch *et al.* 2013). Egg production is regulated by hormones which are photophase dependent, as demonstrated in Hemiptera *Rhodnius prolixus* (Vafopoulou *et al.* 2012). Lipids represent the major energetic source of eggs and are essential for the development of the embryo. Lipid reserve in eggs is provided by the mother (Ziegler and Van Antwerpen 2006). If that storage is dependent of photoperiod, and is more particularly developed during scotophase, long nights will enhance egg volume. Organism size cannot be explained by the simple sum of mechanisms that regulate the size and number of cells in organs (Nijhout 2003), but a positive relationship exists with the energy stock and egg size in some species, like the butterfly *Bicyclus anynana* (Geister *et al.* 2009). A study carried out on a US temperate strain of *Ae. albopictus* found a lipid reserve more important by 30% in diapause-induced pharate larvae (Reynolds *et al.* 2012), linked to an increase in egg volume. Unfortunately the lack of tropical strain as control group in Reynolds experiment led them to conclude that the increase in egg volume was a consequence of diapause expression, whereas the presence of the control group in our experiment allowed us to demonstrate the photoperiodic causality.

Nevertheless, we cannot exclude the possibility that this increase in egg size under short day length could be a relic feature of a lost diapause capacity in tropical populations. Indeed, diapause can be quickly counter selected in laboratory (Pumpuni 1989), or in field populations as described during the species' colonization of Florida (Lounibos *et al.* 2003). On the contrary, even though Brazil was colonized by tropical strains of *Ae. albopictus*, in laboratory some populations still show a small but significant decrease in egg hatchability under short photoperiod (Lounibos *et al.* 2003). In consequence it is possible that tropical populations could be selected to express diapause again. The black cutworm *Agrotis ipsilon* is a good example of persistence of diapausing metabolism: even though larvae and adults of this species are able of diapause elsewhere in the world, Japanese populations migrate from north to south of the island to overwinter. It allows them to avoid diapause initiation, but adults reared under short day length show a delay in the development of their ovarian maturation (Tauber *et al.* 1986).

We make the hypothesis that short day length is interpreted by females as stressful environmental conditions. If bigger eggs are better adapted to survival in harsh environment, then females will anticipate poor development conditions for its offspring by laying eggs of bigger size. For example, a higher amount of yolk in bigger eggs could increase the survival of embryos. On an interspecific scale, larger eggs have an increased developmental duration (Gillooly and Dodson 2000), and in *Aedes* (Stegomyia) species larger eggs are more resistant to desiccation (Sota and Mogi 1992b). It is unclear how these interspecific observations could be verified inside a species. No modulation of desiccation resistance was observed in eggs of a tropical strain of Kuala Lumpur, Malaysia, reared under different photoperiodic conditions (Urbanski *et al.* 2010a).

Photoperiodic rearing conditions don't modify maternal wing length; consequently we dismiss a possible indirect effect of maternal size on eggs volume. Previous works on mosquitoes showed that egg length is neither influenced by mosquito wing size (Shannon and Hadjinicalao 1941, Pumpuni *et al.* 1992) nor by maternal larval nutritional regime (Pumpuni *et al.* 1992). However it must be noted that these studies did not measure egg width, which seems to be the main factor of egg volume variability. Finally, the ecological meaning of photoperiod is questioned in tropical populations. Indeed, annual day length variation is subtle under tropics and less well correlated with environmental events (Tauber *et al.* 1986).

Conclusions

The comparisons of the embryogenesis chronology and egg size demonstrate the existence of photo-induced maternal effects in temperate and tropical populations of *Ae. albopictus*. This study reveals the major influence of maternally induced diapause on the developmental time of the temperate embryos. It is a helpful tool to explore the many facets and implications of diapause metabolism on the organism. It underlines the need to investigate the physiological consequences of diapause preparation in *Ae. albopictus* by genomic and proteomic approach. Recently the genome of the yellow fever mosquito *Ae. aegypti* (Nene *et al.* 2007) was sequenced and will thus become a reference model for developmental studies (Clemons *et al.* 2010). Although unable of diapause, it is a closely related species of the Asian tiger mosquito (Reinert *et al.* 2004) and will provide precious data for comparison. Genomes of an Italian and a Chinese strain of *Ae. albopictus* are currently sequenced and annotations are expected this

year (Bonizzoni *et al.* 2013). These will help to improve our knowledge on the molecular processes of diapause, already initiated on early diapause preparation in oocytes (Urbanski *et al.* 2010b), embryonic diapause preparation (Reynolds *et al.* 2012), diapause initiation and maintenance (Poelchau *et al.* 2013b) and diapause termination.

Understanding the course of diapause could be useful to develop a new strategy for mosquito population control, by inhibiting diapause and foiling winter survival (Tauber *et al.* 1986, Hanson *et al.* 1993). In the light of these elements *Aedes albopictus* emerges as a fantastic biological model for the study of maternal effects and egg diapause.

Acknowledgements

We appreciate the technical assistance of Jean-Sebastien Dehecq (ARS Océan Indien) and Gilbert Le Goff (IRD), and the helpfully statistical advices of Jean-Yves Barnagaud (CIRCE, Aarhus University) and Alain Guillet (SMCS UCL). Many thanks are addressed to Pesser's fellows for laboratory assistance and Nathalie Barras for English revision (EID). We also thank the two anonymous reviewers for relevant comments on earlier version of the manuscript. A preliminary report of these findings was made at the 18th "European Society for Vector Ecology" conference, Montpellier, France, October 2012. This paper is number 320 of the Biodiversity Research Centre.

Author Contributions

Conceived and designed the experiments: GL, FV, TH. Provided the strains: SB, CL. Performed the experiments: GL, FV, NC. Analyzed the data: GL, FV, NC, TH. Wrote the paper: GL, SB, TH.

Supporting Information

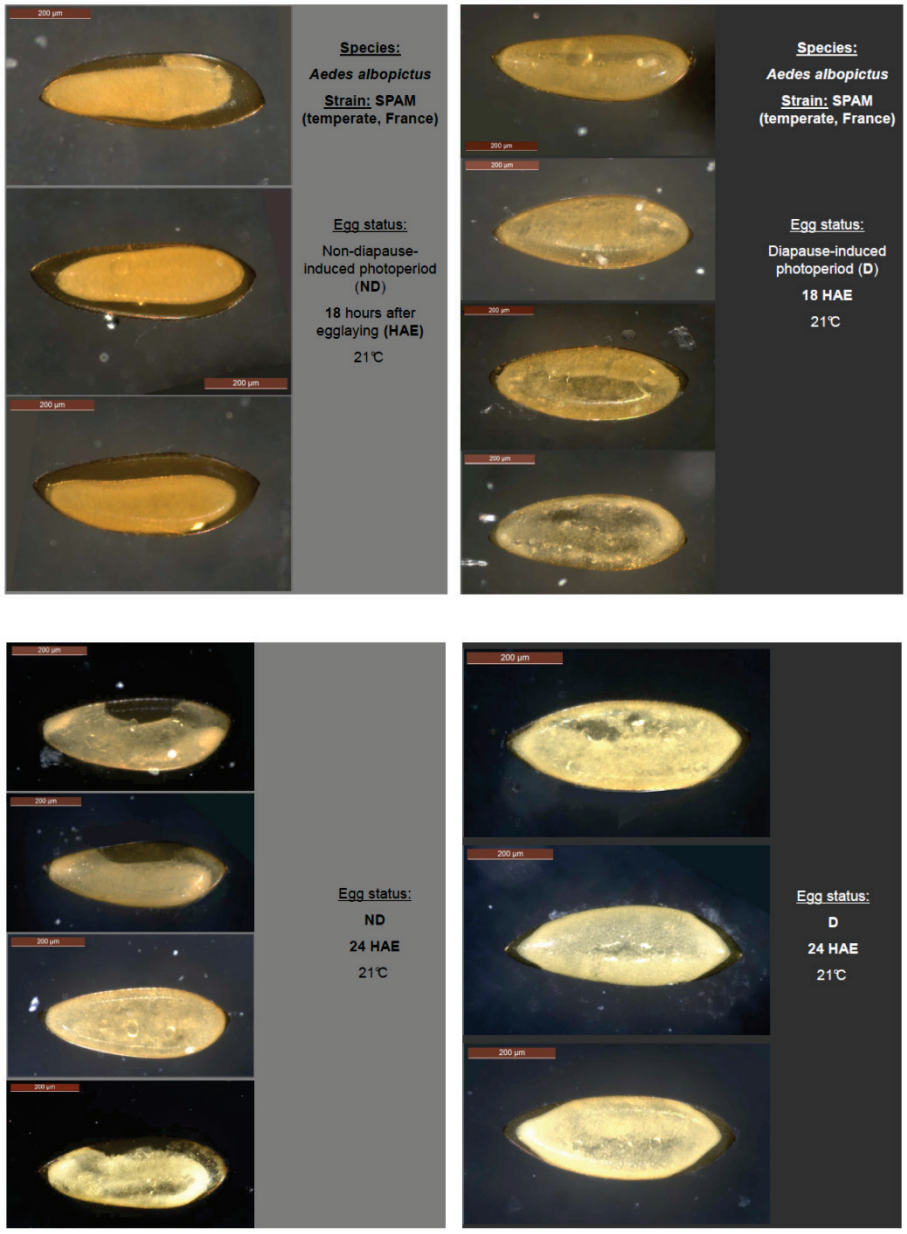
Supporting Information 1

Table A.1 – Parameters values of the reaction norm models for development time of two strains of *Aedes albopictus* reared under different daylengths. Reaction norms were modelled by nonlinear regressions for development time of serosal cuticle, early segmentation, pigmented ocelli and egg burster during embryogenesis in tropical and temperate strains of *Ae. albopictus* reared under long days LD (16h daylength) and short days SD (9h daylength) at 21°C.

Strain & photoperiod	Parameter	Serosal cuticle			Segmentation			Ocelli			Egg burster		
		Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]	Estimate (±S.E)	t	p[a]
Temperate LD	a	0.00 (±0.01)	-0.53	0.59	0.00 (±0.00)	-0.30	0.77	-0.01 (±0.01)	-0.92	0.36	-0.01 (±0.01)	-0.85	0.40
	b	1.00 (±0.00)	563.83	***<0.01***	1.00 (±0.00)	300.89	***<0.01***	0.97 (±0.01)	109.48	***<0.01***	0.92 (±0.01)	80.88	***<0.01***
	c	39.27 (±0.08)	495.39	***<0.01***	75.76 (±0.13)	570.78	***<0.01***	98.14 (±0.31)	318.01	***<0.01***	116.28 (±0.36)	322.82	***<0.01***
	d	2.66 (±0.06)	40.53	***<0.01***	1.64 (±0.12)	13.57	***<0.01***	3.35 (±0.31)	10.84	***<0.01***	5.18 (±0.56)	9.27	***<0.01***
Temperate SD	a	-0.03 (±0.01)	-3.85	***<0.01***	0.00 (±0.00)	-0.68	0.51	-0.02 (±0.01)	-2.65	0.01	0.00 (±0.00)	-0.15	0.88
	b	1.00 (±0.00)	357.42	***<0.01***	1.00 (±0.00)	942.02	***<0.01***	1.08 (±0.03)	38.64	***<0.01***	1.05 (±0.015)	68.29	***<0.01***
	c	48.70 (±0.15)	333.88	***<0.01***	94.90 (±0.02)	5480.44	***<0.01***	128.93 (±1.31)	98.36	***<0.01***	155.90 (±0.39)	395.95	***<0.01***
	d	4.49 (±0.16)	27.22	***<0.01***	1.02 (±0.01)	89.48	***<0.01***	17.45 (±1.02)	17.07	***<0.01***	5.45 (±0.25)	22.15	***<0.01***
Tropical LD	a	-0.06 (±0.01)	-4.79	***<0.01***	0.01 (±0.01)	0.87	0.39	-0.01 (±0.00)	-2.12	0.04	0.00 (±0.00)	-1.31	0.20
	b	1.00 (±0.00)	410.40	***<0.01***	1.01 (±0.01)	98.58	***<0.01***	0.97 (±0.01)	117.79	***<0.01***	1.00 (±0.01)	98.29	***<0.01***
	c	33.83 (±0.19)	174.84	***<0.01***	90.72 (±0.39)	234.92	***<0.01***	124.43 (±0.41)	303.19	***<0.01***	140.51 (±0.29)	477.77	***<0.01***
	d	4.04 (±0.11)	37.90	***<0.01***	5.37 (±0.38)	14.10	***<0.01***	7.99 (±0.31)	26.04	***<0.01***	7.63 (±0.33)	23.06	***<0.01***
Tropical SD	a	-0.01 (±0.01)	-0.79	0.43	0.00 (±0.01)	0.04	0.97	-0.01 (±0.00)	-1.55	0.13	0.00 (±0.00)	-0.64	0.52
	b	0.99 (±0.00)	362.19	***<0.01***	1.00 (±0.01)	92.10	***<0.01***	1.02 (±0.01)	71.13	***<0.01***	1.02 (±0.01)	106.19	***<0.01***
	c	33.98 (±0.17)	197.52	***<0.01***	89.29 (±0.43)	208.07	***<0.01***	128.44 (±0.60)	214.61	***<0.01***	144.53 (±0.28)	507.81	***<0.01***
	d	3.37 (±0.10)	34.10	***<0.01***	6.18 (±0.40)	15.31	***<0.01***	10.40 (±0.54)	19.37	***<0.01***	7.06 (±0.27)	25.89	***<0.01***

[a] Significant p-values are in italic and boldface.

Supporting Information 2



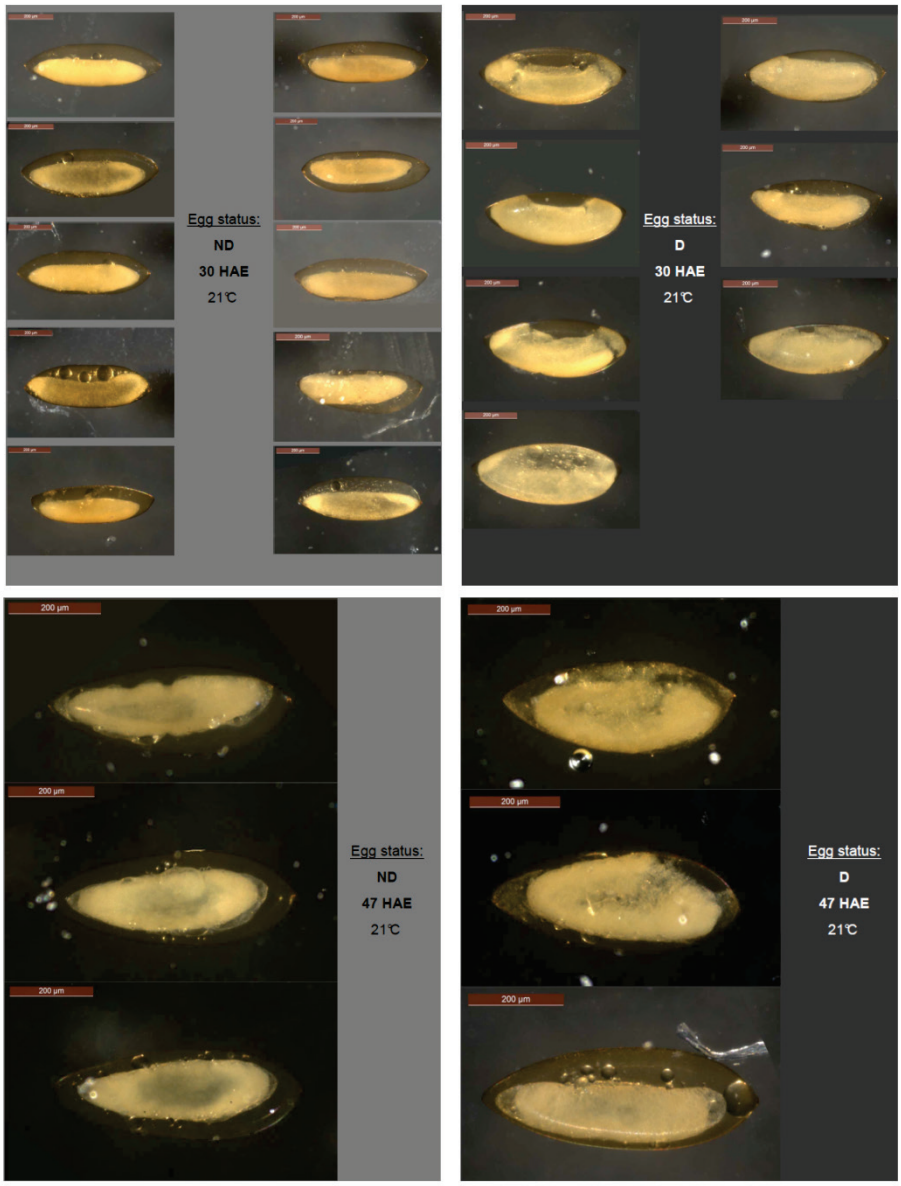


Figure A.2 – Compared morphology of early embryos of temperate SPAM strain of *Aedes albopictus*. Eggs aged 18, 24, 30 and 47 hours after embryogenesis. Pictures were taken immediately after Trpis bleaching with stereomicroscope (LEICA) coupled with multifocal system LAS V 3.7 (magnification x115). 18 HAE ND and D: blastoderm stage. 24 HAE ND: beginning of the germ band elongation. 30 HAE ND: middle of the germ band elongation. 30 HAE D: beginning of the germ band elongation. 47 HAE ND: beginning of the germ band retraction. 47 HAE D: end of the germ band elongation.

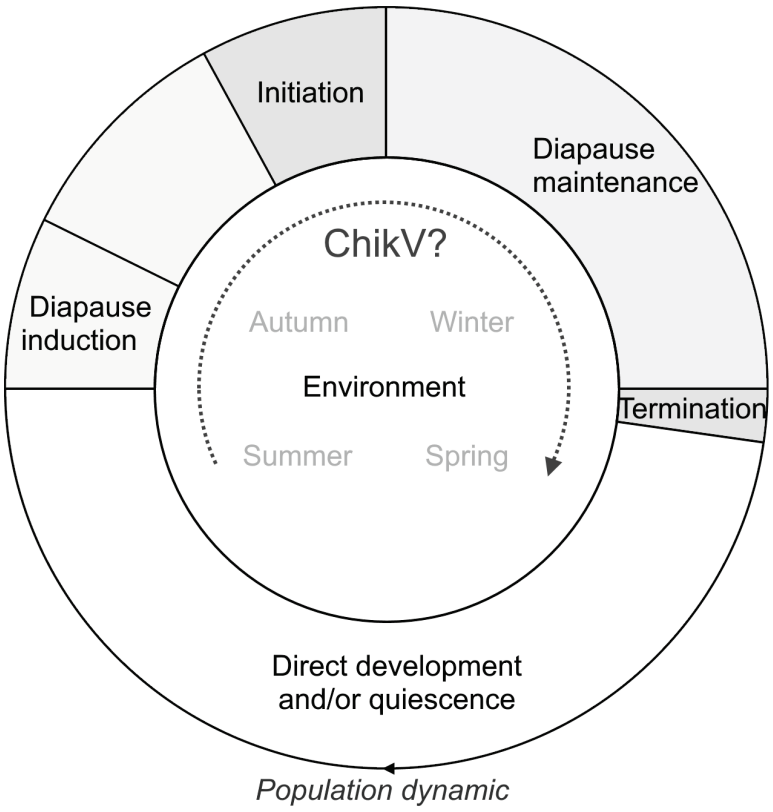
Supporting Information 3

Table A.3 – Cross comparison of development time of a trait in 50% of embryos laid by females of a temperate and a tropical strain of *Aedes albopictus* reared under short-days (SD) and long-days (LD) conditions. The 4 morphological indicators studied are the serosal cuticle (SC), the abdominal segmentation (AS), ocelli (Oc) and egg burster (EB). Time intervals were calculated from time values assessed from theoretical time sigmoid nonlinear models of traits appearance.

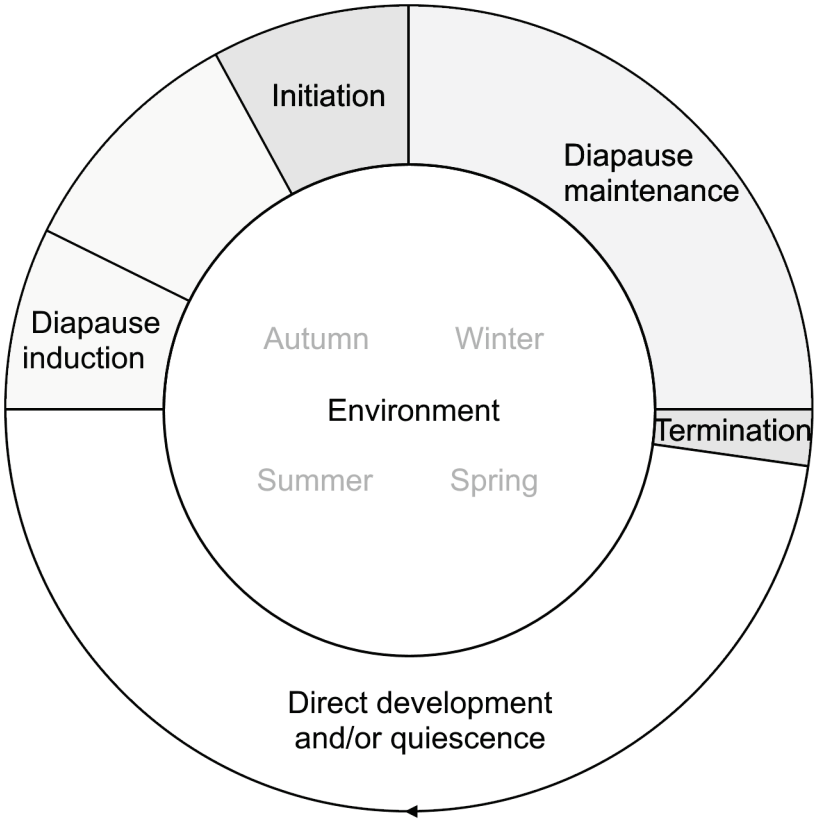
	Strain of comparison		
	Temperate SD	Tropical LD	Tropical SD
Reference strain	Hour (trait)[a]	Hour (trait)[a]	Hour (trait)[a]
Temperate LD	+9.6 (SC) +19.1 (AS) +28.8 (Oc) +38.1 (EB)	-5.0 (SC) +13.5 (AS) +26.7 (Oc) +23.3 (EB)	-5.2 (SC) +14.8 (AS) +29.8 (Oc) +27.2 (EB)
Temperate SD		-14.6 (SC) -5.6 (AS) -2.1 (Oc) -14.8 (EB)	-14.8 (SC) -4.3 (AS) +1.0 (Oc) -10.9 (EB)
Tropical LD			-0.2 (SC) +1.3 (AS) +3.1 (Oc) +3.9 (EB)

[a] Hour (trait) = time of appearance of trait in 50% of embryos of the strain of comparison - hour of appearance of trait in 50% of embryos of the reference strain.

Chapter III. Diapause and the mosquito phenology



The results obtained in the chapter II were used in the following study of the diapause process in the field. In absence of morphological markor of diapause in eggs (egg volume being dependent of photoperiod), the determination of the diapause status must be made by egg hatching and bleaching. The embryogenesis kinetics of non-diapausing eggs or diapause-induced eggs of *Ae. albopictu*s were used in a degree-day model to analyze the synchronization of the diapause initiation in the field.



Highlights (paper II)

- The critical photoperiod (CPP) of French Mediterranean population of *Aedes albopictus* is equal to 13.5 hours of light.
- Field diapause incidence increases during September until a level of 95%, but declined from mid-October.
- Half of eggs are in prediapause stage 17 days after the occurrence of CPP in the field, corresponding to the time of diapause preparation.
- Dormance termination occurs in the first half of March.
- The overwintering generation passes 20-26 weeks in egg stage and about 7 weeks in larval stage, a long time which is an opportunity for vector control.

Seasonal Synchronization of Diapause Phases in *Aedes albopictus* (Diptera: Culicidae)

Paper II

Guillaume Lacour[4,5], Lionel Chanaud[4], Grégory L'Ambert[4], Thierry Hance[5]

PLoS ONE 2015 ; 10(12), e0145311.

Abstract

In temperate areas, population dynamics of the invasive Asian tiger mosquito *Aedes albopictus* is strongly affected by winter. The work we present here analyzes the adaptive synchronization of the diapause process in the wintry generation of *Ae. albopictus*, where the egg stage is exposed to adverse winter conditions. The seasonal pattern of egg laying activity of a French Mediterranean population of the Asian tiger mosquito was monitored weekly for 2 years with ovitraps. The field diapause incidence and the critical photoperiod (CPP, i.e. the maternal day length inducing diapause in 50% of the eggs), were determined by hatching experiments on the collected eggs. The period of diapause termination was estimated by a field survey of the first hatchings for both years. The CPP is equal to 13.5 hours of light and occurs in the field on the 25th of August. Thus, it is on September 11th, 17 days after the CPP, that 50% of the eggs are in a prediapause stage in the field. The egg diapause rate increases rapidly during September, whereas the mean number of eggs laid decreases sharply after mid-September. Surprisingly, after having reached a peak of 95% at the end of September, from mid-October the diapause incidence declined and stayed below 50%. Indeed, both years the diapause initiates before the rapid decrease of the environmental temperature. This leaves a sufficient period of time to the complete development of one generation of *Ae. albopictus* with effective induction of diapause in the laid eggs. The very first larvae hatched were sampled both years in the first half of March. With 20 to 26 weeks in the egg stage and about 7 weeks in the larval stages, the first annual generation spends a long time in immature stages. On a practical point of view, this long development time represents a wide window for eggs and larvae control in early spring.

[4] EID Méditerranée, Montpellier, France.
[5] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium

Keywords

Winter; diapause; phenology; egg hatching rate; seasonal adaptation; *Aedes albopictus*.

Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse 1894) is an invasive species, extending its distribution range faster than any mosquito species during the last 30 years (Bonizzoni *et al.* 2013). Native to South-East Asia, the first efficient establishment of *Ae. albopictus* in a temperate country outside its native area was recorded in Albania in 1979 (Adhami and Reiter 1998). In 1985 it started to invade the United States of America from Japan (Kuno 2012) and in 1990 Western Europe, especially Mediterranean areas, from the United States (Dalla Pozza *et al.* 1994). In both cases the species was imported as dormant eggs through used tires trade (Kuno 2012, Mitchell 1995, Urbanelli *et al.* 2000). The vector competence of the Asian tiger mosquito was demonstrated for 27 arboviruses, but its role in field outbreaks was only proved for dengue, chikungunya and Zika viruses (Paupy *et al.* 2009, Grard *et al.* 2014). The risk of emergence or re-emergence of these diseases is relevant with regards to the European outbreak history. The most explosive and virulent dengue outbreak was recorded in 1927-28 in Greece, and was transmitted by another urban and anthropophilic vector, *Ae. aegypti* (Halstead 2008). It is a topic of high concern with autochthonous cases of dengue fever reported in France repeatedly since 2010 (La Ruche *et al.* 2010, Marchand *et al.* 2013, Giron *et al.* 2015) and in Croatia in 2010 (Gjenero-Margan *et al.* 2011). Similarly, *Ae. albopictus* is responsible for a chikungunya outbreak in Italy in 2007 (Rezza *et al.* 2007) as for indigenous transmissions in France in 2010 (Grandadam *et al.* 2011) and in 2014 (Delisle *et al.* 2015). However detailed field data on its phenology are still lacking, and particularly on the incidence of winter dormancy, to better understand its seasonal dynamics. These data are needed to assess the risk of mosquito-borne diseases transmission and to develop adequate vector control strategies in temperate areas.

The Asian tiger mosquito shows a remarkable ecological and physiological plasticity (Paupy *et al.* 2009). The environmental parameters of importance to define this species suitable habitat have been identified (Kobayashi *et al.* 2002, Caminade *et al.* 2012, Mogi *et al.* 2012) as well as the epidemiological hazard (Brady *et al.* 2014). In temperate areas, winter temperature is the

major factor limiting the persistence of *Ae. albopictus* (Thomas *et al.* 2012, Waldock *et al.* 2013). Temperate populations are able to withstand winter at the stage of egg diapause (Bonizzoni *et al.* 2013). Diapause is a process of arrested development of the organism occurring before environmental conditions become harsh, permitting the optimal temporal adaptation of the species to its environment (Denlinger and Armbruster 2014). The seasonal dynamics of the diapause process is of prime importance to decipher its phenology. The terms and definitions of the diapause phases used here refer to the review of Koštál (Koštál 2006). The facultative egg diapause is induced by maternal photoperiod (Pumpuni 1989). Females are sensitive to short photoperiod at pupal and adult stages. They then produce a signal to trigger diapause preparation in their oocytes. Diapause initiation is effective once the embryo is fully developed (Lacour *et al.* 2014). The prediapause phase includes all events from diapause induction to diapause initiation. The diapause phase ends by the diapause termination, as the gradual reactivation of the eggs after winter. In many insects, the major environmental factors in diapause termination are low temperatures and an extended photoperiod (Vinogradova 2007). Eggs will stay in a post-diapause quiescence after the diapause termination up until they experience a springtime hatching stimulus. As winter temperatures in Southern France are a strong constraint for the survival of this species, the invasive populations probably underwent a strong selection pressure. The adaptation of the photoperiodic induction of diapause in a particular area is known to happen quickly in *Ae. albopictus* (Urbanski *et al.* 2012). Thus we expect that *Ae. albopictus* populations from the French Riviera are able to respond to the local photoperiodic conditions through a maternally induced diapause allowing them to avoid temporal harsh conditions.

The work presented here attempts to analyze the complete diapause process of the wintry generation of *Ae. albopictus*, from events occurring during their maternal oogenesis to the oviposition of their offspring. For this purpose, we isolated and quantified the effects of photoperiod on diapause incidence in a natural population. (1) Using individuals sampled in the field, we determined the critical photoperiod (CPP) of the population, i.e. the day/night ratio that the laying females must be exposed in order to induce diapause in 50% of the eggs laid. The CPP was compared to the field diapause incidence. (2) The period of dormancy termination was then estimated by a field monitoring of the first hatchings on both years. (3) Lastly, using a day degree model, the egg laying activity data of 2 consecutive sliding years were analyzed according to the phenology of diapause and climatic conditions.

Materials and Methods

Ethic statement

The animal facility of the ‘‘Entente Interdépartementale pour la Démoustication du littoral méditerranéen’’ has received accreditation from the French Ministry of Agriculture to perform experiments on live guinea pig (permit number B34-172-29) in appliance of the French and European regulations on care and protection of Laboratory Animals. Field survey was realized with permission from the Direction Générale de la Santé (DGS) of the French ministry in charge of health.

Experimental field area

The diapause incidence survey was performed in the Alpes-Maritimes region, France, where *Ae. albopictus* is established since 2004 (Delaunay *et al.* 2007) (S1 Table 1). It is the only species of the subgenus Stegomyia present in France since the 1950’s eradication of *Ae. aegypti* (Fontenille *et al.* 2007). An ovitrap network was installed at the summer solstice 2010 in Cagnes-sur-Mer (43°66'N, 7°16'E), a city colonized by the species since 2006 (figure III.1). A residential area mainly composed of individual houses with gardens was chosen for the monitoring. During our experimental period, no insecticide treatment was realized in the vicinity of the ovitraps, even though this experiment took place within the framework of the national anti-dissemination plan for chikungunya and dengue viruses (Ministère de la santé et des sports 2010). Sites in the neighboring area were chosen to survey the spring larval development. Eggs collected on ovitraps up until July 26th 2010, a period of the year not expected to induce diapause, were reared as progenitors (F0 generation) of laboratory mosquitoes.

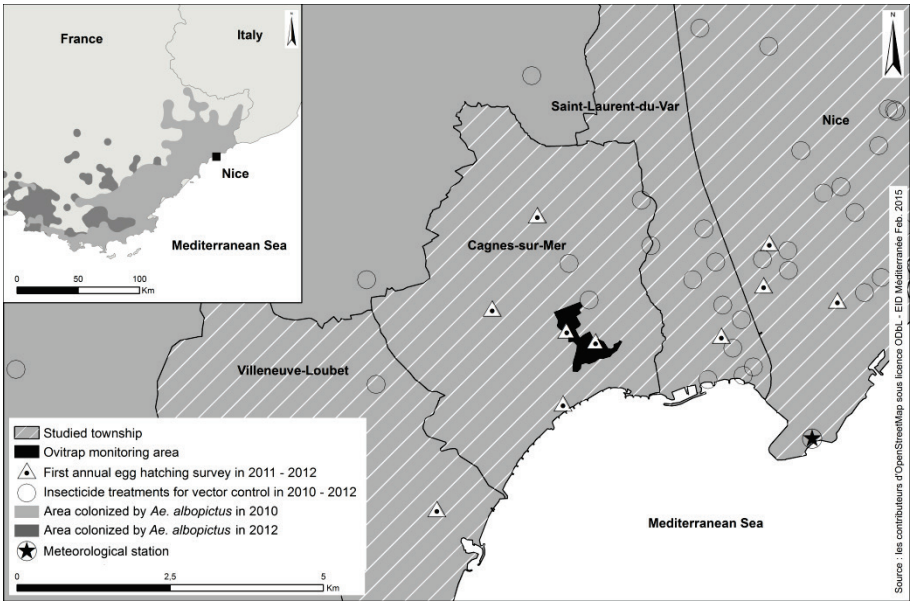


Figure III.1. Experimental area for seasonal egg laying dynamics and first annual egg hatching monitoring of *Aedes albopictus* in Mediterranean France. The area monitored by ovitraps from summer solstice 2010 to summer solstice 2012 was computed as the surface of the smallest polygon including all traps with a buffer distance of 50 m (black area). This area was safe from outdoor insecticide spraying for vector control (circles). Ten infested places localized around the ovitraps network were inspected daily in March 2011 and 2012 to detect the first larvae of the year (triangles).

Diapause incidence

Experimental determination of diapause incidence and critical photoperiod

The purpose of this experiment was to determine the photoperiod response of a sampled population of *Ae. albopictus*. Specifically, the objective was to calculate the critical photoperiod (CPP). Since day length is changing daily in the field, the CPP could hardly be deduced from observations and is more surely determined under fixed day length in the laboratory. Two generations of mosquitoes were reared at 21°C, 80% relative humidity and a light:darkness (L:D) 16:8 photoperiod (figure III.2). Eggs of F2 generation were stimulated to hatch under standardized conditions, and larvae were reared in batches of 250 larvae per pan of 1 liter tap water and fed with 1.75 g of milled dog food. Eleven days after egg hatching, pans containing fourth instar larvae with first pupae were transferred in cages in photoperiodic

chambers. Three batches of larvae were used per photoperiodic conditions, as follows: 13:11, 13.25:10.75, 13.5:10.5, 13.75:10.25 and 14:10 L:D hours. Photoperiodic chambers consisted of windowless plastic boxes (65 x 65 x 40 cm) with a black-tissue zipper opening. Individual chambers were maintained at a constant temperature of 21.5°C and 80% relative humidity, using a fan-produced air flow and a periodic air dampening system made of a water pot stirred using an aquarium air-pump. Light cycle was computer controlled and generated with a 3 watts bar of 42 white LED per photoperiodic chamber. There was no light transition between photophase and scotophase. Adult mosquitoes were supplied with a 10% sucrose solution. Females were blood-fed on an anesthetized Guinea pig 10 days after emergence. Five days after blood feeding, each cage was provided with an oviposition cup containing water and a strip of paper. The paper was removed 5 days later and eggs were conserved in petri dishes in darkness during 10 days to allow complete embryo development (Lacour *et al.* 2014).

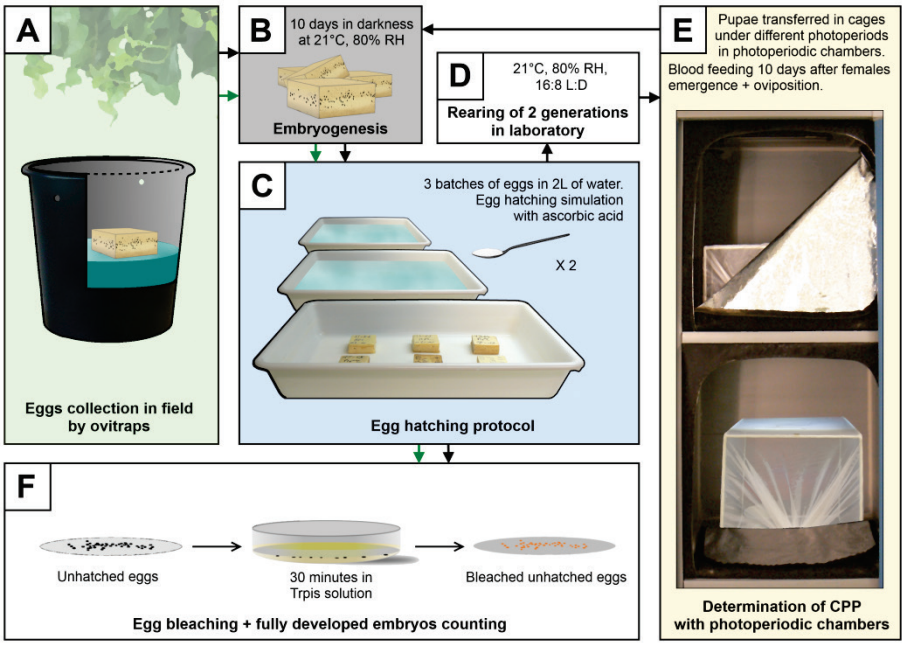


Figure III.2. Protocol for the determination of egg diapause incidence in *Aedes albopictus*. The critical photoperiod (CPP) of the population is determined by an experiment including all steps in this order: A, B, C, D, E, B, C and F (black arrows). The survey of diapause incidence in the field follows steps A, B, C and F (green arrows).

In order to determine the egg diapause rate, the resulting batches of eggs were immersed in containers filled with 2 liters of water (figure III.2). A decrease in dissolved oxygen concentration was generated with the addition of 100 mg of ascorbic acid per liter of water 30 minutes after flooding (Sinègre 1974). This powerful hatching stimulus suppressed egg quiescence (Vinogradova 2007). This protocol was repeated one day later, after air drying the eggs during 2 hours, to ensure the efficiency of the hatching stimulation. After two consecutive hatching stimulations, hatched eggs were counted rather than hatched larvae, the latter being prone to mortality and more difficult to count (Fig A.II.1). To determine the viable state of unhatched eggs, they were first bleached in Trpiš solution (Trpiš 1970) to allow embryo observation. Eggs were considered viable when the embryo presented pigmented ocelli, an egg burster and were without abnormal pigmentation or malformation (Lacour *et al.* 2014). Diapause rate was calculated with hatched and unhatched embryonated egg numbers:

Diapause incidence % = (embryonated unhatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)

Field survey of diapause incidence

Once collected in the ovitraps (see below), eggs were stored for at least of 10 days in a humid atmosphere under complete darkness at 21°C. In 2010, eggs collected in the same week were studied as a uniform batch. For increased accuracy, 2011's eggs were weekly separated in three batches of 100 eggs minimum in order to obtain the standard deviation values. All eggs were studied together when the weekly number of eggs became less than 300. The egg hatching and counting protocol described previously was used to determine the field diapause incidence.

Dormancy termination

The diapause termination was also investigated in the field. Ovitraps being inappropriate to determine the beginning of the period of activity for the first generation of immature stages of the year, in 2011 and 2012 daily active surveys were conducted around the study area to find the first larvae hatchings (figure III.1). Ten places documented as infested by *Ae. albopictus* and localized in a five kilometer radius around the ovitraps network were inspected daily from March 1st. These places displayed unmovable breeding sites of varied nature (concrete blocks holes, stone containers, tree holes, etc.) and were maintained with the addition of water during dry conditions.

Monitoring of mosquito population dynamics

The population dynamics was monitored weekly using a network of 15 ovitraps set up on June 21st 2010 (Tran *et al.* 2013). Ovitraps are artificial containers made up of 3 L black plastic buckets (Dillewijn Zwapak©, Aalsmeer, The Netherlands) filled with 2 L of tap water changed weekly. In each container a floating polystyrene square (5 x 5 cm) is added to provide a support for oviposition. Ovitraps were hidden under vegetation and egg batches were collected weekly and brought back to laboratory for counting. The collection was stopped in November after two consecutive negative reports, marking the end of the active season of the Asian tiger mosquito. In 2011 and 2012 the network was reinstalled from the end of March and was expanded to 18 ovitraps. The surface of the trapping area was computed as the surface of the smallest polygon including all ovitraps with a buffer distance of 50 m, and covered 30.5 hectares in 2010 and 41.6 ha the following years. Both hatched and unhatched eggs of *Ae. albopictus* collected in ovitraps were counted in laboratory under a stereomicroscope Zeiss Stemi SV6.

Photo-thermal responses in population dynamics

The seasonal dynamics were studied according to the meteorological influence. The daily minimum (T_{min}), maximum (T_{max}) and average (T_{mean}) air temperatures and daily precipitation were obtained from the Nice Côte d'Azur airport meteorological station, distant of 3 km from the study area (figure III.1). The daylight duration was provided by the “Institut de mécanique céleste et de calcul des éphémérides”. Daily data were pooled to calculate the weekly mean temperatures, total weekly precipitations and weekly mean daylight durations.

The development of poikilothermic organisms such as mosquitoes, being dependent of environmental temperature, the span of the mosquito life cycle should be analyzed according to the temperature range allowing its development. The time units (days) were reported to the number of accumulated daily degree (ADD), calculated as follows:

$$ADD = \sum(((T_{max} + T_{min}) / 2) - T_{base})$$

with (T_{base}) the critical temperature defined as the thermal threshold below which the development cannot occur, corresponding to 11°C (Kobayashi *et al.* 2002, Waldock *et al.* 2013).

The numbers of heat units required for different development times were calculated using demographic parameters of two strains. There are no differences in development rates between tropical and temperate strains, except in the embryonic stage (Waldock *et al.* 2013). Thus, we used development data from a tropical strain (Delatte *et al.* 2009), completed with the embryonation rates – with or without diapause induction – of a local temperate strain (Lacour *et al.* 2014) to calculate our day degree model.

Statistical analyses

The diapause incidence percentages from laboratory and field eggs batches were modeled through sigmoid response curves fitted by day length using an iterative logistic model with a four parameters logistic regression. It allowed calculating the CPP for this population. The reaction norms were compared to determine the delay between maternal diapause induction and diapause initiation in offspring.

The threshold temperatures of the beginning of the egg laying activity and of the first seasonal egg hatching were determined by linear regressions of day length and the average minimum temperature of the week preceding egg laying activity.

Statistical analyses were performed using R 3.0.2 software (R Development Core Team 2013).

Results

Diapause incidence

Experimental diapause incidence

As expected, under fixed temperature and photoperiod in the laboratory, the complete shift in the number of eggs entering diapause incidence occurs between a day length of 13.0 and 14.0 hours. The CPP of this population is equal to 13.5 hours of light according to the sigmoid curve model of diapause induction (figure III.3).

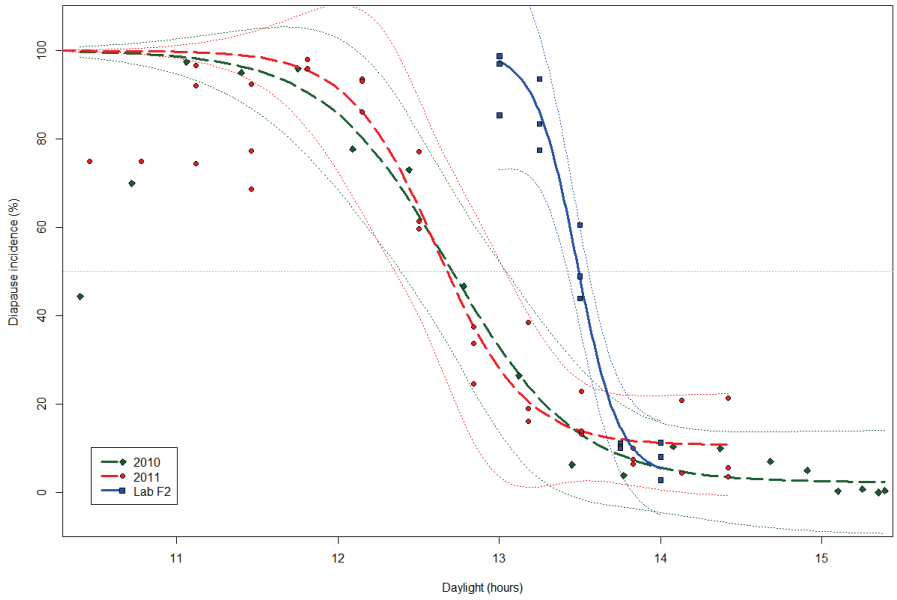


Figure III.3. Photoperiodic analyses of egg diapause induction and initiation in *Aedes albopictus*. X axis represents the mean daylight duration during the egg laying week in the field in 2010 (green diamonds, n=10) and 2011 (red dots, n=36). Photoperiod inducing diapause was studied at fixed day length in laboratory (blue squares, n=15). Sigmoid curves modeled for egg diapause incidences are in bold solid line (laboratory) or solid dashed lines (field). Smooth dotted lines represent the 95% confidence intervals. The sigmoid response curves ignore the field data points of decreasing diapause incidence for day length ≤ 11.5 hours, as these outliers are probably the result of a selection trend. The horizontal dot line (light grey) marks 50% of diapause incidence. The difference between blue curve (laboratory data) and green and red curves (field data) highlights the delay between maternal diapause induction and diapause preparation in offspring.

Diapause incidence in the field

The diapause incidence was compared to the average day length (figure III.3) of the week of field monitoring. The diapause rate showed a similar pattern between 2010 and 2011. It increases rapidly in the field during September (figures III.4A and III.4B). Half of the eggs laid in the second week of September (week 36) were programmed to enter into diapause. The moment when 50% of the eggs initiate diapause corresponds to a day length of 12.7 hours (12h41), and occurred on September 11th. After the 39th week, more than 95% of eggs laid were in prediapause stage, the diapause incidence stayed high for the first half of October. It decreased significantly from mid-October while the number of laid eggs decreased sharply.

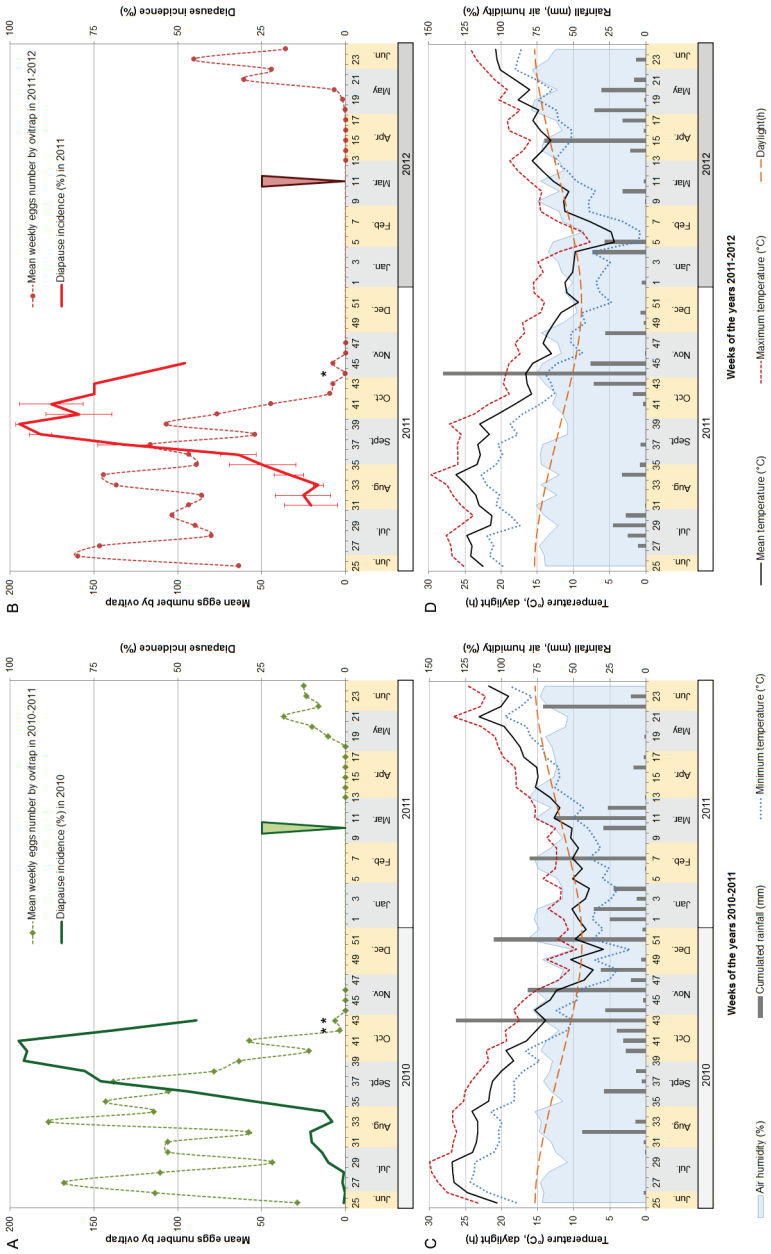


Figure III.4. Seasonal profiles of oviposition activity and diapause timing of *Aedes albopictus* population (A,B) from summer solstice 2010 to summer solstice 2012 under the Mediterranean climatic conditions (C,D) of the city of Cagnes-sur-Mer, France. Asterisk represents positive samples with less than 100 eggs collected. Arrows show the week of sampling of the first larvae of the year.

Comparison of experimental and field diapause incidence

The experimentally determined CPP is of 13.5 hours; in the field this day length corresponds to the 25th of August. The CPP is higher by 0.8 hour than the day length monitored in the field when embryonic diapause would be programmed in 50% of the eggs. This difference represented a time span of 17 days after the date of the CPP. It suggests the influence of environmental factors as well as a physiological delay during maternal diapause induction. The transition from a non-diapausing day length to a day length inducing the maximal diapause incidence should last theoretically 20 days (with the laboratory determined day lengths), but it lasted twice that time in the field.

Dormancy termination

The very first larvae were sampled both years in the first half of March (the 8th in 2011 and the 13th in 2012) and in the same breeding site (holes in concrete stud). A period of 25 (in 2010) to 26 weeks (in 2011) was needed between the time when 50% of eggs entered in diapause and the observation of the first viable hatched larva the following year. This period was similar from year to year despite different winter conditions (see below).

Seasonal pattern of oviposition

A total of 53790 eggs of *Ae. albopictus* were collected during the field samplings (21126 eggs during the period between the summer solstices of 2010 and 2011, and 32664 during the period between the summer solstices of 2011 and 2012). A mean density of 68.4 ± 49.0 eggs per ovitrap per week was collected from weeks 19 to 45 in 2011. All the ovitraps were found positive during 14 weeks in 2010 and 12 weeks in 2011, between June 27th and November 2nd. The egg laying activity highly increased after the summer solstices of both years and remained globally high despite variations until the second half of September (figures III.4A and III.4B). After that, the mean number of eggs per ovitrap sharply decreased. No egg laying activity was detected after November 2nd in 2010 and November 14th in 2011 (data already published in (Tran *et al.* 2013)).

The first eggs laid by the first annual generation of adults were detected in the first half of May for both years. Taking into account the date of first egg hatchings, it corresponds to a development length of 9 weeks in 2011 and of 7 weeks in 2012.

Photo-thermal responses in population dynamics

The elapsed time at different phases of the diapause process was converted in ADD above 11°C, the estimate thermal units available for mosquito development (table III.1). We considered here the 11th day of September as the generic day of diapause initiation, with 50% of the eggs laid in diapause. Diapause was induced in 50% of eggs 15 days before the date of diapause initiation (figures III.3, III.4A and III.4B). In the field the fortnight elapsed between these estimated dates correspond to 205 to 225 ADD. It is more than the heat units necessary in *Ae. albopictus* for the development from pupae to first egg laying, or from pupae to the end of the second gonotrophic cycle. The photothermic analysis of the prediapause stage suggests that diapause was not completely induced in field eggs of the first gonotrophic cycle. This is corroborated by the laboratory experiment, where females exposed to short photoperiod during at least 11 days show a switch to complete induction of diapause (S3 Fig 1).

Table III.1. Comparison of theoretical and field observed development time of *Aedes albopictus* stages using a day-degree model. The thermal threshold is equal to 11°C.

Development time	Theoretical ADD	Observed ADD (year)
From egg hatching to first oviposition	245[a]	
From pupation to first gonotrophic cycle end	142[b]	
From pupation to second gonotrophic cycle end	198[b]	
Mean generation (without diapause)	295[c]	
Mean generation (with diapause)	310[c]	
From CPP to diapause initiation		205 (2010)[b]
		225 (2011)[b]
From CPP to the end of the oviposition activity		558 (2010)[c]
		729 (2011)[c]
From diapause initiation to the first spring hatching		424.85 (2010-2011)[d]
		615.5 (2011-2012)[d]
From the first spring hatching to the first oviposition		270 (2011)[a]
		201 (2012)[a]

[a,b,c]: data compared against each other's.

For both years, from the date when field day length was equal to the CPP to the end of the monitored oviposition activity, there were actually more heat units available than required to complete one generation of *Ae. albopictus* with effective induction of diapause in the laid eggs. The sum of heat units

available even allowed the complete development of 2 generations in 2011 (605 ADD necessary).

The 2010-2011 winter was rainier than the 2011-2012 winter (324.1 mm versus 85.3 mm of cumulated rainfalls) (figures 4C and 4D). The average mean temperature was similar between both winters (9.66 ± 1.78°C for the 2010-2011 winter versus 9.60 ± 2.85°C for the 2011-2012 winter), however the last winter was more contrasted: it started hot and dry and followed by two cold weeks with 8 days of negative minimal temperature. Consequently, from the generic day of diapause initiation to the date of observation of the first larva hatching, there was 424.85 ADD during the winter 2010-2011 and 615.5 ADD during the winter 2011-2012. This represents an increase of 45% ADD.

The two days following the observation of the first larva in 2011 were the last days of spring where the mean temperature was less than 10°C (S4 Fig 1) with a minimum temperature less than 5°C. In 2012 such low temperatures ended a week before the observation of the first larva. Only 2 days of chilling with Tmean ≤ 5°C were encountered in both winters. These winters differed little in their average temperature: the 2010-2011 winter was 1.3°C colder than the 2011-2012 winter, with respectively 73 days versus 38 days under an average temperature of 10°C. Only the 2011-2012 winter encountered a freezing period with 9 days of Tmin ≤ 0°C. However when looking at the cumulated thermal degree units available for *Ae. albopictus*, both winters differed a lot. Indeed, the 2011-2012 winter had 45% more ADD than the winter 2010-2011. As the first annual larvae appeared quite simultaneously both years despite different winter meteorological conditions, other factors may be involved. The day length corresponding to the first egg hatching was equal or superior to 11.5 hours.

The average minimum temperature of the week preceding egg laying activity is estimated with an intercept of the linear regression at 12.5°C. From egg hatching to the first oviposition, the development time measured in 2011 in the field corresponded closely to the heat units necessary (245 ADD). However in 2012, only 201 ADD were observed between the monitored hatching and egg laying; a correspondence in ADD measured is observed when considering the time where the number of eggs laid reached 5% of the annual weekly mean number of eggs laid.

Discussion

Diapause induction

The CPP is under polygenic control, evolving with latitude (Urbanski *et al.* 2012), and is a very plastic life history trait sensitive to high temperature and larval nutritive state (Pumpuni *et al.* 1992). The observed difference in the field of 0.8 hour between the CPP and the diapause preparation into 50% of the eggs is important and could be partly explained by environmental conditions. Indeed, individuals of the natural population are exposed to fluctuating temperatures, which can slightly interact with the photoperiodic signal. In the northern tamarisk beetle *Diorhabda carinulata*, high daily thermal amplitude was demonstrated to reduce the CPP by 0.15 hour for laboratory controlled amplitude of 10°C (Bean *et al.* 2012). This kind of effect could exist in *Ae. albopictus*, however the median daily thermal amplitude did not reach 6.7°C during the month preceding and following the day of CPP. Furthermore, the effects of thermal amplitude in the field should be counterbalanced by the broad variability of larval nutrition; indeed, adults starved at larval stages showed a CPP lengthened up to 0.25 hour (Pumpuni *et al.* 1992). The different temperature and food conditions experienced by individuals do not entirely explain the difference in photoperiodic responses between the field and the laboratory populations. The photothermic analysis of the prediapause stage suggested diapause was not completely induced in eggs of the first gonotrophic cycle in the field. Indeed, there was enough ADD during this time for sensitive stages to develop from the pupae to the end of the second gonotrophic cycle (table III.1). This supposition is corroborated by laboratory experiment at 21°C on this strain, showing a switch to complete induction of diapause in eggs of females exposed to short photoperiod during at least 11 days (S3 Fig 1). A similar observation was made on North America strains (Pumpuni 1989). Thus diapause induction should only be considered fully effective for eggs from the second gonotrophic cycle. As a physiological process, diapause induction could occur slightly faster in the field where mean temperatures are higher by 1 to 3°C (figures III.4C and III.4D).

Egg diapause must be induced at the appropriate time to obtain an initiation accurately synchronized with seasonal conditions. In the field, the maximum diapause incidence occurred a month before the cooling of the minimal temperature under 15°C (figure III.4). After diapause induction it is essential that the environment remains sufficiently warm to allow for the completion of at least one full generation of *Ae. albopictus* progenitors and of a diapausing offspring (Tauber *et al.* 1986). The sum of heat units recorded both years in

our field study allowed the complete development of one, and sometimes two generations of progenitors. Does it mean the CPP of this Asian tiger mosquito population is not perfectly adapted to the local Mediterranean environment? Even though it is not possible to assert this with certainty, the data are consistent with a “bang-bang” strategy (McNamara 1994): during the favorable period mosquitoes reproduce and do not enter diapause, up to a specific date, illustrated as the CPP, when all the organisms will have to initiate diapause. The apparent precocity of prediapause stage could then be explained by the overlapping generations of *Ae. albopictus* and the stochasticity of rainfalls occurrence or intensity during Mediterranean summers.

Most of the population enters diapause at the end of September, however a decrease of the diapause incidence is observed in eggs laid after that date on both years. This trend is not directly induced by the photoperiod, as the diapause incidence of our local temperate populations in the laboratory remains higher than 90% for rearing day lengths of 13.0 as well as 9.0 hours (Lacour *et al.* 2014). As diapausing eggs are not sensitive to environmental stimuli, only non-diapausing eggs can hatch to produce a new generation of breeders at the end of the season. Thus the overall decrease in the number of active *Ae. albopictus* at end of the season – decrease caused by the initiation of diapause – leads to a selection of phenotypes that express less or no diapause in the remaining active population. Under favorable climatic conditions this selection phenomenon allows for a rapid decline of diapause in the population, such as documented in previous studies: In Rome, Italy, the minimum egg hatching rate obtained after a field sampling of *Ae. albopictus* eggs realized in the fall of year 2000 was 17% - as compared to less than 5% in our study – meaning that a significant part of the *Ae. albopictus* population had not entered diapause (Toma *et al.* 2003). In southernest's cities of Spain, a small part of the Asian tiger mosquito population is probably unable of winter diapause (Collantes *et al.* 2014, Bueno-Mari *et al.* 2015). This phenomenon is even more evident in North America where a progressive selection of the non-diapausing phenotypes could explain that in 2011 more than 60% of eggs laid by the strain of Miami are not in diapause (Lounibos *et al.* 2003, Lounibos *et al.* 2011). The non-diapausing individuals must be advantaged in areas with warm winters, having the exclusive benefit of a longer period of time for reproduction and proliferation.

In metropolitan France, non-diapausing phenotypes are probably eliminated afterwards by winter conditions. Indeed, in our study the complete end of the egg laying period occurred at the same time as the most important rainfalls of the year (figure III.4). These exceptional rainfall events occurring on week

44 may have precipitated the end of the active season of the Asian tiger mosquito by causing an important mortality of the immature stages (Dieng *et al.* 2012) and remaining adults. However, a continuous residual egg laying activity was observed during the winter in Rome (Romi *et al.* 2006) and in southern Spain (Collantes *et al.* 2014, Bueno-Mari *et al.* 2015), but none was reported in other southern cities, such as Athens, Greece (Giatropoulos *et al.* 2012) or Tirana, Albania (Velo *et al.* 2012) where the oldest European populations of the Asian tiger mosquito are found. In Rome (41°53′N), despite repeated flooding during winter the eggs remained unhatched, thus confirming their diapause status and leading to the strong assumption that they had been laid by long-lived *Ae. albopictus* females of the last seasonal generation (Romi *et al.* 2006). The anthropogenic modification of the environment is likely to be involved in the winter survival of mosquitoes (Andreadis *et al.* 2010). However in France, based on a 6 years long population sampling - winter monitoring included -, the persistence of adult *Ae. albopictus* populations during winter seems unlikely to happen soon (Tran *et al.* 2013).

Dormancy termination

The beginning of diapause termination in the field was estimated in our study by a monitoring of egg hatching, although this method cannot discriminate the termination per se of the post-diapause quiescence. This quiescence follows diapause termination until environmental conditions become favorable. The monitored breeding sites were flooded and maintained with water by the investigator, therefore only eventual low temperatures would have sustained quiescence and have substantially delayed egg hatching. Based on our study, and according to previous field observations (Toma *et al.* 2003) and models (Caminade *et al.* 2012), the values for the hatching onset parameters correspond to a spring temperature above 10.5°C and a spring photoperiod above 11.25 hours. The termination of diapause leads to a gradual reactivation of eggs in the field. The factors involved in this process where studied in laboratory and differed with regards to the mosquito species (Vinogradova 2007). Mainly, a long-term exposition at chilling but not freezing temperature triggers egg reactivation. Photoperiodism is also effective, long day treatment having a strong reactivation effect in eggs of *Ae. caspius* and *Ae. triseriatus*. The adaptive advantage of photothermic interaction is obvious in the field, photoperiodism preventing early and inappropriate hatchings under changing temperatures during winter. Our data are consistent – but not conclusive – with a reactivation dependent of a photothermic interaction. The estimation of the average minimum temperature of the week preceding egg laying activity

found in this study is inferior by 0.5°C from the 13°C found in northern Italy on adult female emergence (Roiz *et al.* 2010). As some precocious egg laying and adults were observed in 2012, earlier undetected hatchings could explain this discrepancy between monitored and development time. However, there occurrence resulted more likely from an accelerated larval development in breeding sites warmer than field conditions, such as rain barrels exposed to the sun.

The long generation

Maternal induced egg diapause plays a central role for the wintry long generation, with phases studied here in a field population: from the induction through to the diapause initiation, and following up to diapause termination (figure III.5). The first annual generation spends a long time in immature stages, with 20 to 26 weeks at the egg stage and about 7 weeks at the larval stages. This long development time represents for mosquito control operators a great opportunity to thwart the species population dynamics by targeting the eggs and larvae of a unique generation. The mechanical suppression of breeding containers where eggs have accumulated as a result of female seasonal oviposition choice during the fall (Fonseca *et al.* 2015) would be an especially efficient procedure for that purpose. As mosquito control operators cannot contact every inhabitants living in the suburban areas where the major biting discomfort is experienced, the main issue to overcome, particularly in a period with no adult nuisance, will deal with communication aiming to modify community habits (Claeys and Mieulet 2013).

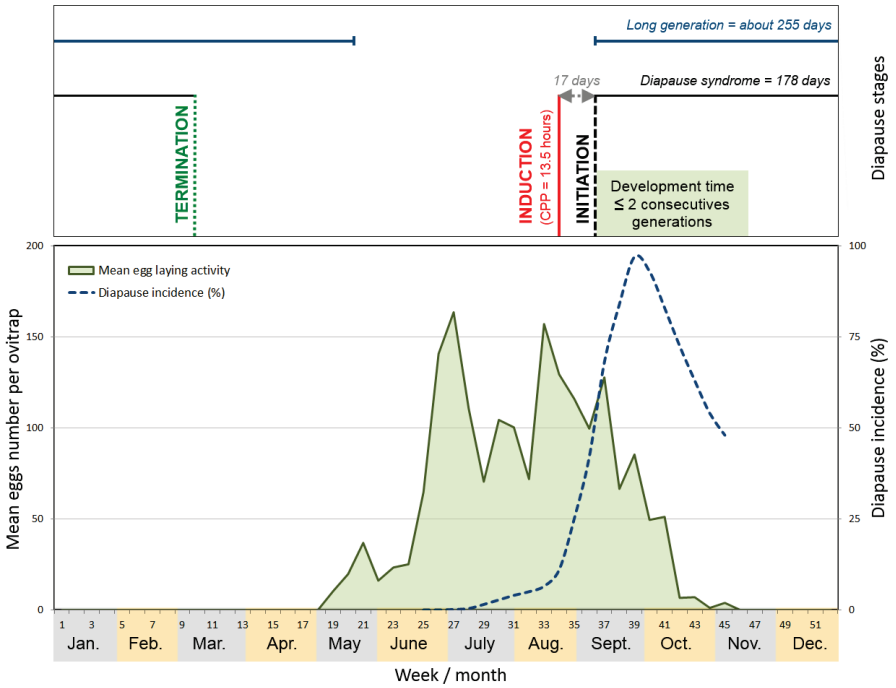


Figure III.5. Schematic depiction of the seasonal activity and diapause process of *Aedes albopictus* population in the Mediterranean city of Cagnes-sur-Mer, France. Horizontal lines represent the mean durations of diapause syndrome and of the long generation of *Ae. albopictus*. Vertical lines delineate the moment when diapause is induced (in red) or initiated (in dark) in 50% of eggs, and the start of diapause termination (in green).

When compared to models based on Medlock *et al.* (Medlock *et al.* 2006), the time span of mosquito activity measured in our study is already longer than projections for the current period (ECDC 2013) and even equal to projections for 2030-2050 (Caminade *et al.* 2012). In these models, the Mediterranean area is expected to be very vulnerable to climatic change, with a strong overall warming – including a temperature increase of 2-4°C during fall and winter up to the years 2071-2100 – and a reduction in precipitation (Giorgi and Lionello 2008). These models slightly underestimate the extent of the growing season in urban areas, where temperature is generally higher than in natural environments. Consequently, an upward revision of the estimated period of activity of *Ae. albopictus* should be considered for cities. The distribution area, the extent of the growing season and developmental rates are all expected to increase due to global warming. It would notably make possible the production of one or two additional yearly

generations (Musolin and Saulich 2012). The major biological response to climate change in mosquitoes is expected to be an adaptation of the diapause induction and termination to adjust to shorter winters (Denlinger and Armbruster 2014).

Conclusions

The phenological population dynamics of *Ae. albopictus* was studied in the light of the major role played by the evolutionary process of diapause. The photoperiodic data of diapause induction, initiation and termination of the population of Asian tiger mosquito will serve as reference for future work on the evolution over time and dissemination of this invasive species, with regards to local adaptation and global warming. This study provides a basis of field and phenological data that will be useful to apprehend the future expansion of *Ae. albopictus* population toward Northern Europe, and to develop temporal abundance models in order to simulate and test innovative strategies for vector control operators.

Author Contributions

Conceived and designed the experiments: GL TH. Performed the experiment: GL LC. Analyzed the data: GL GLA TH. Wrote the paper: GL LC GLA TH.

Supporting Information

S1 Table. Geographic coordinates of *Aedes albopictus* monitoring devices.

S2 Figure. Comparison of counted hatched egg and larvae methods.

S3 File. Time requirement for maternal induction of diapause.

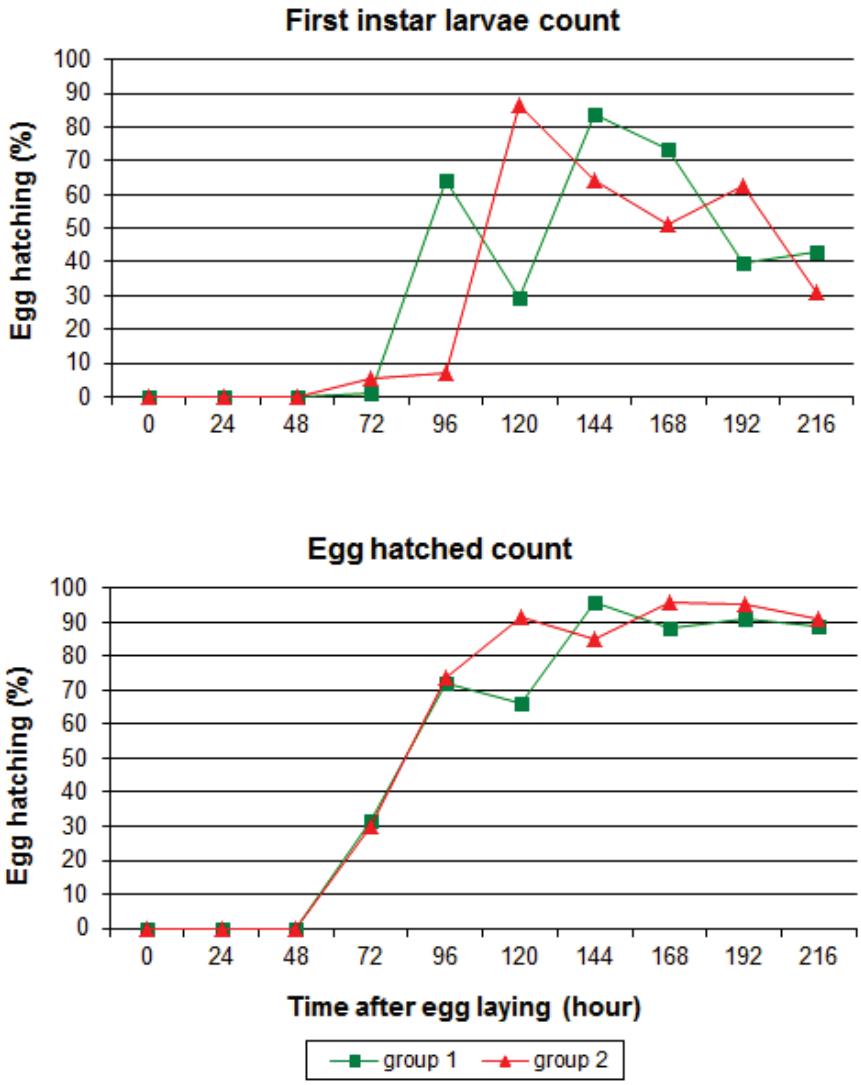
S4 Figure. Temperature conditions during the first annual egg hatchings.

Supplementary information 1: coordinates of monitoring devices.

S1 Table. Geographic coordinates (decimal degrees, system WGS84) of ovitraps and breeding sites used for the monitoring of population dynamics of *Aedes albopictus* from 2010 to 2012 around Cagnes-sur-Mer, France.

Type	Code	Latitude	Longitude
Breeding site	**A**	43.67742194	7.20158278
Breeding site	**B**	43.66843000	7.21472083
Breeding site	**C**	43.64094389	7.13159000
Breeding site	**D**	43.66993306	7.14489583
Breeding site	**E**	43.67124806	7.19998972
Breeding site	**F**	43.66417889	7.19090583
Breeding site	**G**	43.68328889	7.15502389
Breeding site	**H**	43.66431000	7.16542694
Breeding site	**I**	43.65548389	7.15819083
Breeding site	**J**	43.66614889	7.15972278
Ovitrap	**1**	43.66408056	7.17061944
Ovitrap	**2**	43.66246111	7.16865556
Ovitrap	**3**	43.66274722	7.16825278
Ovitrap	**4**	43.66428056	7.16549722
Ovitrap	**5**	43.66444167	7.16582222
Ovitrap	**6**	43.66542778	7.16074722
Ovitrap	**7**	43.66565556	7.16053056
Ovitrap	**8**	43.66048333	7.16169444
Ovitrap	**9**	43.66066667	7.16134444
Ovitrap	**10**	43.66696389	7.15885833
Ovitrap	**11**	43.66681111	7.15902778
Ovitrap	**12**	43.66843333	7.16146389
Ovitrap	**13**	43.66598889	7.16161667
Ovitrap	**14**	43.66678333	7.16015833
Ovitrap	**15**	43.66896389	7.15858611
Ovitrap	**16**	43.66992778	7.16303611
Ovitrap	**17**	43.66914444	7.16206944
Ovitrap	**18**	43.66316667	7.16789167

Supplementary information 2: comparison of counted hatched egg and larvae methods.



S2 Fig 1. Egg hatched count is a more reliable method than larval count, the latter being sensitive to mortality of first instar larvae. Independent batches of *Aedes albopictus* eggs (n>300) were stimulated during 3 hours with ascorbic acid to hatch. Eggs were removed, dried, and then live first instar larvae (A) and hatched eggs (B) were counted.

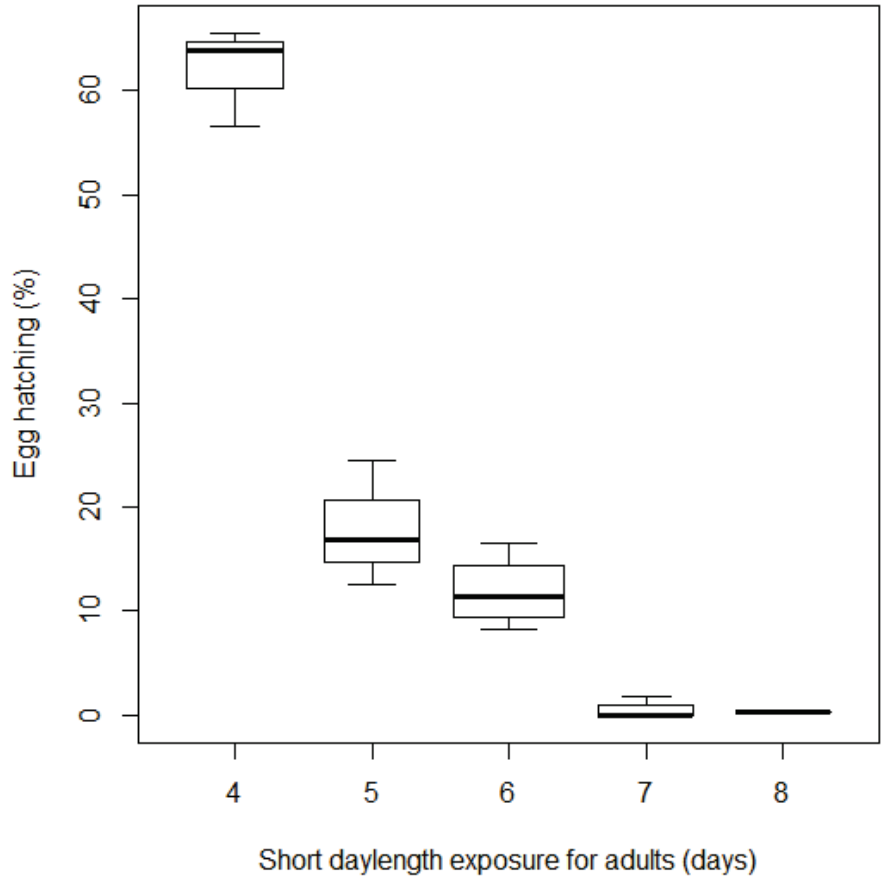
Supplementary information 3: Time requirement for maternal induction of diapause

In order to determine the duration under SD the female must be exposed to, to induce diapause in her eggs, long-days reared larvae of SPAM strain were transferred under short-day exposure at the moment of pupation. Thirty newly emerged females were put in cage with 30 males under SD with 10% sucrose solution. Cages were transferred under LD exposure between 5 and 8 days after female emergence and provided with a blood meal on anesthetized guinea pig and an oviposition site.

Batches of eggs from oviposition sites were collected 7 days after the blood meal and maintained in incubator in darkness at 21°C and 70% RH. Three replicates of at least 400 10-days old eggs were immerged in over-oxygenate tap water, and with an addition of 100 mg of ascorbic acid per liter of water which is a strong hatching stimulus. This hatching protocol is repeated next day to make sure of having real hatching rate. Unhatched eggs are considered in diapause if embryos are fully developed without deformity. The presence of ocelli and egg burster (a spine on the clypeus of embryo by which it breaks the egg chorion in hatching) on embryos is inquired into eggs decolorized with Trpiš solution (Trpiš 1970).

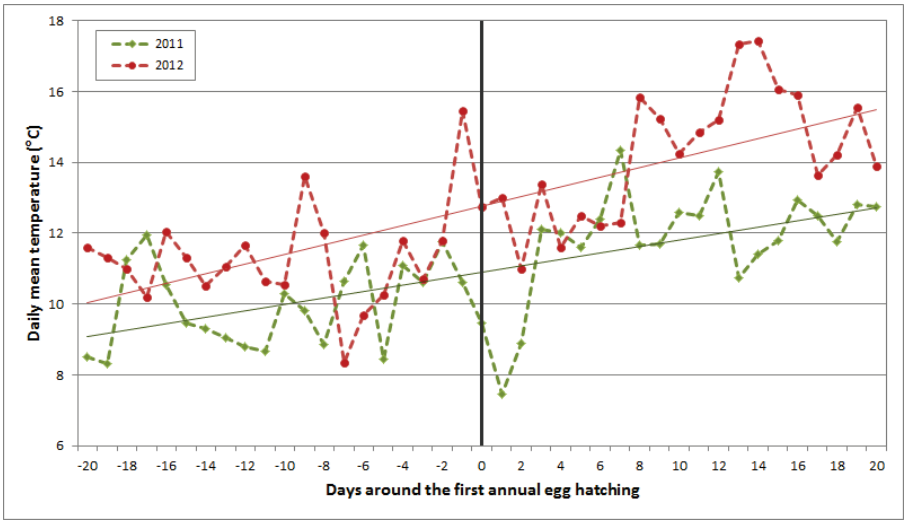
The duration of SD exposure needed for a female to induce diapause in her eggs is deducted by the egg hatching rate, calculated with embryonated and hatched eggs:

Hatch % = (hatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)



S3 Fig 1. Percentage of egg hatching in function of the number of short days underwent by the adult mother of a temperate strain of *Aedes albopictus* at 21°C. The SD exposure began at pupae stage. Horizontal axe indicate the day of switch from diapausing short days (9L:15D) to long days (16L:8D) imaginal exposure. An exposure of adult females during 7 days under a short day photoperiod from the end of the pupal stage to the first blood meal is required to induce diapause in all her progeny. Taking account a mean duration of 4 days under pupal stage, 11 days under short photoperiod during photosensitive stages induce more than 90% of diapause. Interestingly, same induction duration was found for the North-American INDY strain of *Ae. albopictus* (Pumpuni 1989), suggesting that the induction duration is similar for temperate strains.

Supplementary information 4: Temperature conditions during the first annual egg hatchings



S4 Fig 1. Daily temperature during the period of diapause termination in spring 2011 and 2012 in Nice, France. Value (dotted line) and linear regression (solid line) of daily mean temperature during the 3 weeks preceding and following the first annual hatching of 2011 (light diamond) and 2012 (dark plots).

The seasonal dynamic of *Ae. albopictus* have a major influence on viral circulation in temperate area. Arboviruses are indeed imported by travellers coming from an epidemic or endemic country (Tomasello and Schlagenhauf 2013). In mainland France, all outbreaks occurred during the period of diapause induction and initiation (figure III.6).

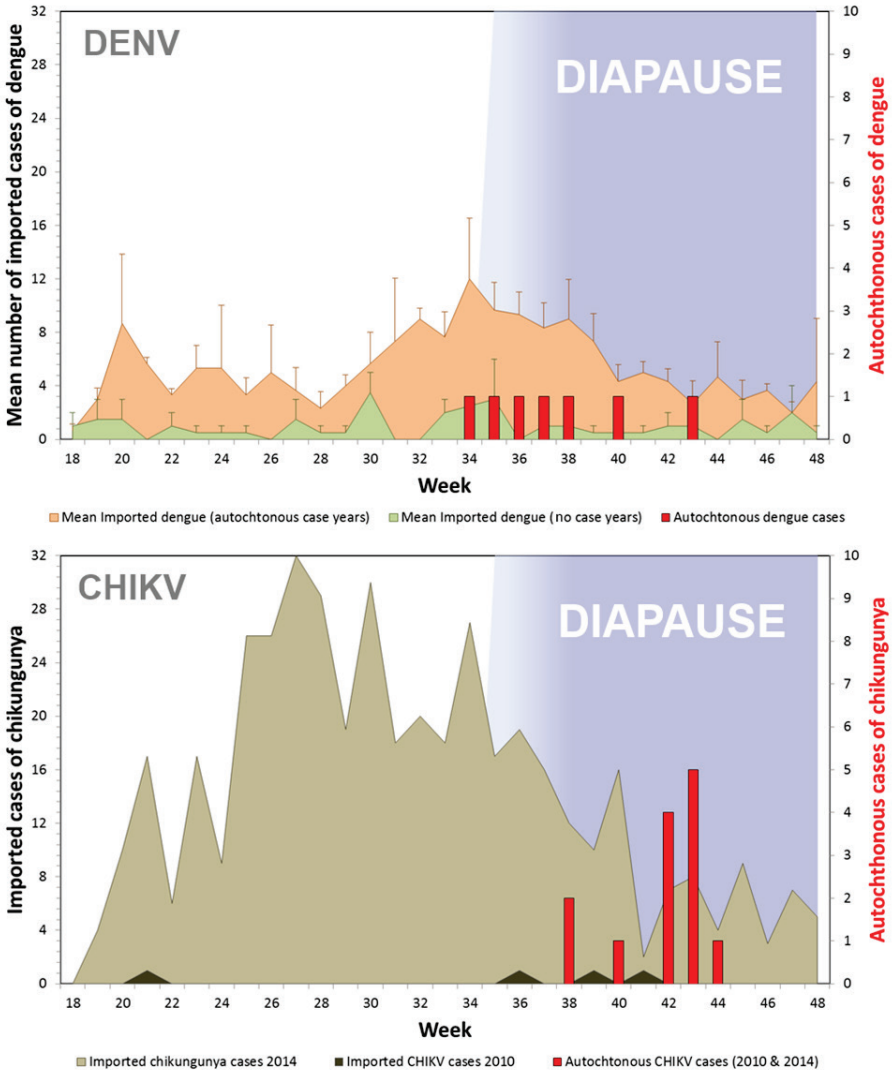
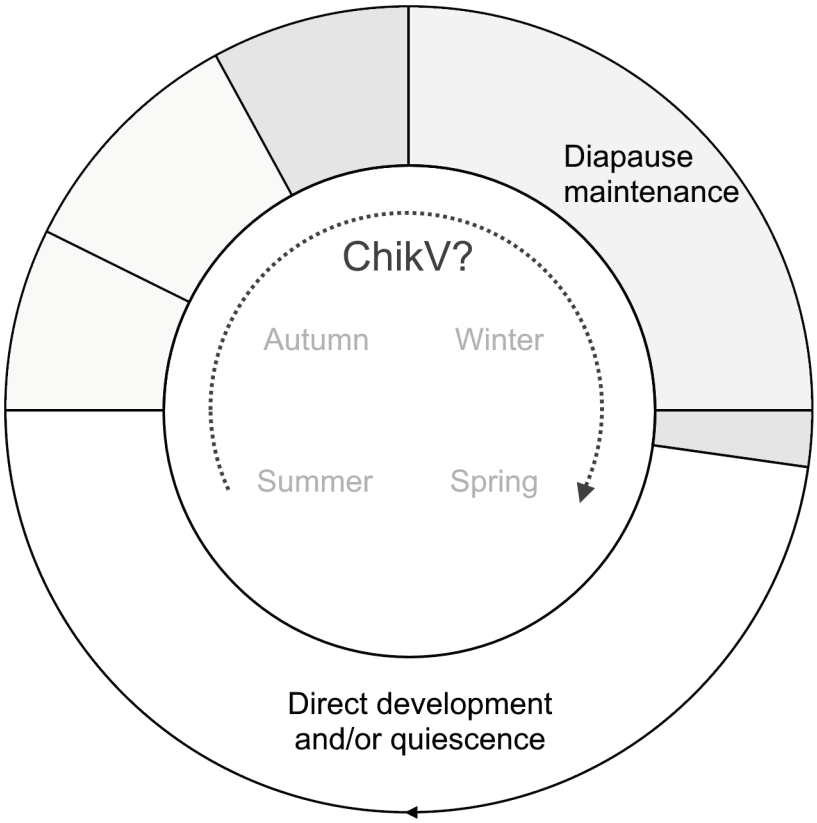


Figure III.6. Imported and autochthonous cases registered from 2010 to 2014 of (A) dengue fever (DENV) and (B) chikungunya (CHIKV) in Metropolitan French areas colonized by *Aedes albopictus* (data: INVS), during the period of activity of the Asian tiger mosquito from May to November (blue area represent the period of diapause initiation in the field).

As recommanded (ECDC 2012), we performed a surveillance of vertical transmission and overwintering of arboviruses during the chikungunya outbreak in Montpellier in 2014 (Delisle *et al.* 2015). Indeed, diapause may permit the persistence of chikungunya in overwintering eggs from year to year. The vector density was also investiguated until the following year in different districts to estimate the effect of antivectorial treatments (insecticides and source reduction) and winter on both vector population and sociological aspects.



Highlights (paper III)

- The vector abundance and the prevalence of *Aedes albopictus* for chikungunya virus (CHIKV) was investigated during the outbreak occurred in Montpellier in autumn 2014.
- There was a low prevalence of CHIKV in the infested district in autumn (0.18-1.27%).
- No evidence of CHIKV overwintering through transovarial infection in the spring generation in spring 2015.
- Few containers and negligence habits are responsible of most of the vector abundance.
- High levels of Breteau index (≥ 100) and House index (≥ 38) characterizes former outbreaks in temperate area.
- The current mosquito control strategy based on source reduction by community did not reach the prophylactic level.

Entomological Investigation on the Chikungunya Outbreak in Montpellier, 2014: Vector Abundances, Viral Detection and Viral Overwintering

Paper III

Guillaume Lacour[6,7], Jean-Baptiste Ferré[6], Charles Jeannin[6], Grégory L'Ambert[6], Eric Le Monnier[6], Isabelle Leparc-Goffart[8], David Roiz[9], Charles Tizon[6], Florian Vernichon[6], Christophe Lagneau[6], Thierry Hance[7].

Submitted

Abstract

The establishment of the invasive vector *Aedes albopictus* in temperate area represents a new health risk issue, which resulted in several chikungunya (CHIKV) and dengue fever outbreaks in Europe since 2007, the most often in mainland France. The entomological component of the CHIKV outbreak that occurred in Montpellier in 2014 is described. The prevalence of *Ae. albopictus* was investigated during the outbreak and the following spring in adults. One pool of 7 mosquitoes was found infected in autumn (0.18-1.27%), but none of the adults emerged from overwintering eggs was infected in the spring 2015. Only 12% of the productive containers (barrels, water tanks and plant pot) produced half of the total estimated mosquito abundance. The presence of water in productive containers resulted mainly of negligence habits. One year after the outbreak, the Stegomyia indices and refusal of entrance in the CHIKV infested district have recovered with comparable levels than other districts. The efficiency of vector control treatments is confirmed but the strategy of community-based source reduction did not reach the expected prophylactic level.

Keywords

Aedes albopictus; mosquito-borne disease; temperate area.

[6] EID Méditerranée, Montpellier, France.
[7] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium.
[8] Centre national de référence des arbovirus, IRBA, Marseille, France.
[9] MIVEGEC, UMR IRD224-CNRS5290-UM, IRD, Montpellier, France.

Background

Most of European countries are increasingly concerned by the major invasive species *Aedes albopictus,* the Asian tiger mosquito. Firstly introduced in Albania in the 1970s, it really spread out in Europe since its introduction in Italy in 1990. It has now colonized almost all the Mediterranean coast line of Europe and rises to northern latitudes (Medlock *et al.* 2015). This anthropophilic species is a competent vector for at least 25 arboviruses, which involves a real risk of emergence or re-emergence of tropical diseases in Europe (Paupy *et al.* 2009). It is relevant with regards to history as the Asian tiger mosquito was the main vector in large field outbreaks of dengue fever virus (DENV) in Japanese cities of Nagasaki in 1942-44 (Oki and Yamamoto 2013) and Tokyo in 2014 (Kutsuna *et al.* 2015) and chikungunya virus (CHIKV) in Italy in 2007 (Rezza *et al.* 2007). The current outbreak of Zika virus in the Caribbean area exposes the European area to viral introduction and indigenous transmission, *Ae. albopictus* being vector and responsible of an outbreak in Gabon (Chouin-Carneiro *et al.* 2016, Grard *et al.* 2014).

Mainland France is the European country the most regularly concerned by indigenous arboviruses transmission with 8 local foci reports and 27 autochthonous cases from 2010 to 2015. Mainland France is indeed the first destination of airline travelers coming from areas affected by arboviruses (Semenza *et al.* 2014), but is also the country with the most complete surveillance of imported cases. Indigenous DENV cases were reported in Nice in 2010 (La Ruche *et al.* 2010), in Aix-en-Provence in 2013 (Marchand *et al.* 2013), in Aubagne and Toulon in 2014 (Giron *et al.* 2015) and in Nimes in 2015 (INVS 2015). Two local foci of CHIKV in Fréjus in 2010 (Grandadam *et al.* 2011) and in Montpellier in 2014 (Delisle *et al.* 2015) were identified. These both CHIKV infectious episodes were caused by the viral strain from East-Central-South-Africa genotype, particularly adapted to local *Ae. albopictus* populations and temperate conditions (Zouache *et al.* 2014). In all the identified cases, the virus was imported by a traveler returning from countries with endemic viral circulation. The autochthonous transmission of arboviruses can occurred as early as 3 years after mosquito detection in French area, as happened for half of the French indigenous episodes (Fréjus 2010, Aix-en-Provence 2013, Aubagne 2014, and Montpellier 2014).

In mainland France, a response plan is activated in departments colonized by *Ae. albopictus* to prevent viral transmission of CHIKV and DENV. Each outbreak represents an opportunity to evaluate the efficacy of the sanitary and vector surveillance system and to assess possible improvements of the plan. From September to the end of October 2014, a CHIKV outbreak

occurred in the *Mas de Tesse* district of Montpellier, France, and caused at least 11 autochthonous cases. The epidemiological and operational aspects of this outbreak are described in Delisle *et al.* (2015). In this study, the vectorial issue of this outbreak is investigated, which is of prime importance for vector control structures. The vector density and the effectiveness of treatments and source reduction during the outbreak were assessed. Different sessions of Stegomyia indices were realized to compare the evolution of mosquito abundance in the *Mas de Tesse* district with other neighboring districts. The prevalence of CHIKV in the vector population was investigated during the outbreak and the adults emerging from the overwintering offspring were also analyzed to confirm the absence of vertical transmission.

Methods

Area

The study focused on the city of Montpellier and its outskirts (France). The CHIKV outbreak occurred in the *Mas de Tesse* district (43°36'52"N 3°50'46"E), a square of side 250m (Delisle *et al.* 2015). The other zones concerned by larval investigations from 2014 to 2015 were the *Saint-Martin* district (43°35'58"N 3°52'56"E) and the *Croix-d'Argent* district (43°35'14"N 3°52'03"E) in Montpellier, and the *Goélands* district (43°33'58"N 4°05'36"E) in the neighboring town of La Grande-Motte. All the studied districts referred in this paper are in urban area and present the same discontinuous urban fabric, including individual houses with gardens and some small buildings, and a similar level of infestation by *Ae. albopictus*.

Estimation of vectorial abundance during the outbreak

After the report of a confirmed autochthonous infected transmission, eight adult mosquito traps (BG Sentinel^{TM}) were installed over an area of 3 ha, in gardens around the primary case, the index cases and all the other autochthonous cases. Traps were operational from 15th to 28th October 2014, covering the beginning of adulticide treatments done in this area the 19th, 23th and 30th October, with Ultra Low Volume spraying by pick-up and pedestrian thermal fogging of deltamethrin (Cerathrin and Aqua-KOthrine). Trapped mosquitoes were killed in freezer, then identified and sorted on a frozen table according to species and sex. Females of *Ae. albopictus* were pooled by trapping date and stored at -80°C for further viral detection. The

number of *Ae. albopictus* adults was compared with data on density of Asian tiger mosquito in 24 BGs positioned in 8 locations in Montpellier in 2014 (Roiz *et al.* 2015). The spatio-temporal abundance data were statistically analyzed with the Wilcoxon rank sum test to determine the treatment efficiency.

Entomological indices

The investigations took place in the district of the *Mas de Tesse* in Montpellier city, including the imported case and all the autochthonous cases declared in September 2014. Three door-to-door campaigns were done in autumn 2014, spring 2015 and autumn 2015. Two entomological indices were calculated: the Breteau index (BI) (number of breeding site containing immature stages of *Ae. albopictus* per 100 houses) and the House index (HI) (number of houses with positive container per 100 houses) (Focks 2003). The productivity of breeding sites was estimated by the Abacus 5 method (Carron *et al.* 2003). For practical reasons and sociological considerations, we calculated a Reluctance index (RI) (number of houses with residents opposed to entomologic investigations per 100 houses).

The first door-to-door campaign was done the 22-23th October by teams composed of a mosquito control agent and a physician. All the water-containing receptacles around the houses were investigated. The small breeding sites were suppressed, whereas the breeding sites considered "not suppressible" were treated with larval insecticide *Bacillus thuringiensis* var. *israelensis* (Vectobac DT®). The second door-to-door survey occurred the 9-13-17th April 2015, before the emergence of the first generation of *Ae. albopictus*, when all the population is at larval-pupal stages (Lacour *et al.* 2015). Gardens were thoroughly prospected by teams of mosquito control agents. Larvae and their growing media were transferred in plastic cups for rearing in laboratory. The third and last entomological survey in the *Mas de Tesse* district was done in autumn on the 16-18-22th September 2015, one month earlier than in 2014, to correlate with the meteorological events that occurred before the 2014 chikungunya outbreak (about 24 days after heavy rainfall, with respectively 300 and 169 mm in 2014 and 2015).

The Stegomyia indices were compared with another surveys realized in the districts of *Croix-d'Argent*, *Saint-Martin* and *Goélands*. The objective is to discriminate the seasonal environmental influence from the effects of mosquito control interventions.

Research of chikungunya virus in mosquitoes

Aedes albopictus females trapped during autumn 2014 were pooled by trapping date and stored at -80°C for viral detection. *Aedes albopictus* larvae and pupae collected in spring 2015 were reared in laboratory under 21°C, 16:8 photoperiod in plastic containers (Nalgène®), with approximately 450 mL of their natural growing media, including organic material and water. Samples were daily inspected, dead individuals removed and counted. Adults were separated in cage according to the emergence day, and fed with a 10% sucrose solution. The 5 or 6 days old adults were separated by sex and emergence date, and frozen at -80°C. The French Reference Center for Arboviruses realized the viral investigation on adult mosquitoes captured in autumn 2014 and adult emerged in spring 2015. Mosquito pools were tested by processing with CHIKV reverse transcription–PCRs.

Results

No evidence of chikungunya virus overwintering

One pool of 7 female mosquitoes trapped during the night of the 20th October 2014 was positive to CHIKV. Based on 553 females analyzed, the minimum infectious rate of *Ae. albopictus* population is equal to 0.0018.

The next spring, at least 572 larvae and pupae were collected, representing a sample of 64% of the number of households in the district. During the rearing of field larvae in the laboratory, a mortality rate of at least 0.11 was observed. A total of 499 5-days old imagoes (232 females and 277 males of *Ae. albopictus*) were frozen for CHIKV analysis. All the samples tested were negative to CHIKV.

Estimation of vectorial abundance during the outbreak

There was a high density of Asian tiger mosquitoes at mid-October (figure III.7), corresponding to the maximal peak of the year 2014 (Roiz *et al.* 2015). For example, a BG-trap captured 346 mosquitoes during the 4 days before the adulticide treatment. There was no significant difference of mosquito density between the Mas de Tesse district and Montpellier city during the 15-17th October 2014 (Wilcoxon test; Females: p=1; Males: p=0.12). A metal barrel, treated by mosquito control operators, had been identified during the larval survey as a very productive breeding container in the garden of the

primary case (abacus V). The abundance of trapped mosquito increased dramatically at the surroundings of the barrel (figure III.8); a good linear correlation was found on the daily number of females (r^2=0.88) and males (r^2=0.87) mosquito trapped in function of the distance from the barrel (from 0 to 35 meters). There was a significant difference between the abundance of female (Wilcoxon test, p=0.0036) and male (Wilcoxon test, p=0.0014) mosquito trapped from 0 to 40 m or trapped further than 40 m.

Trap results showed a high effectiveness of the first treatment on the vector density: the strong decrease in mosquito abundances after the vector control treatment was not observed in other districts of Montpellier but in the outbreak area where adulticide spraying occurred (Wilcoxon test; Females: p=0.0003; Males: p=0.0033). It excluded an influence of meteorological or seasonal parameters such as diapause in the decrease of population recorded. The effectiveness of the first treatment was not absolute, as at least one mosquito trapped during the night following the first treatment was positive to CHIKV. The second treatment, done 5 days later, did not provide any supplementary reduction of the vector density. Although trapping were not carried out anymore, we are pretty confident that the density of vector did not increase later. Indeed, the mosquito density strongly decreased from the last week of October everywhere in the town; there was no difference of abundance between the treated district and Montpellier city during the 27-29th October (Wilcoxon test; Females: p=0.26; Males: p=0.68). It is the consequence of the complete diapause initiation in eggs, fully effective since the end of September (Lacour *et al.* 2015), and the extreme rainfall event of the 29th September 2014 which must have trigger the hatching of all the bank of non-diapausing eggs in containers.

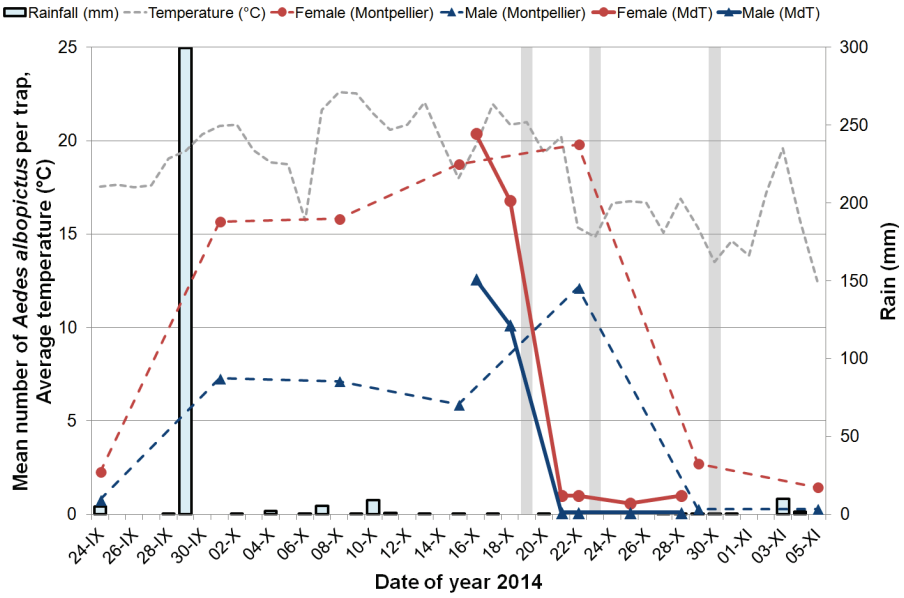


Figure III.7. Comparison of the mean numbers of adults *of Aedes albopictus* females (red dots) and males (blue triangles) per trap per day in Montpellier during the chikungunya outbreak, 2014. The mosquito densities in the district infected by CHIKV (continuous lines) and 8 others districts from Montpellier (dashed lines) are referred with daily average temperature (grey dotted line) and rainfall (bars). The 3 grey bars indicate vector control treatments. Mosquito female abundance in Montpellier is adapted from Roiz *et al.* 2015. Mosquito densities are significantly different between the Montpellier city and the *Mas de Tesse* district during the 20-25th October, after the first insecticide treatment (Wilcoxon-Mann Whitney test, $p<0.005$).

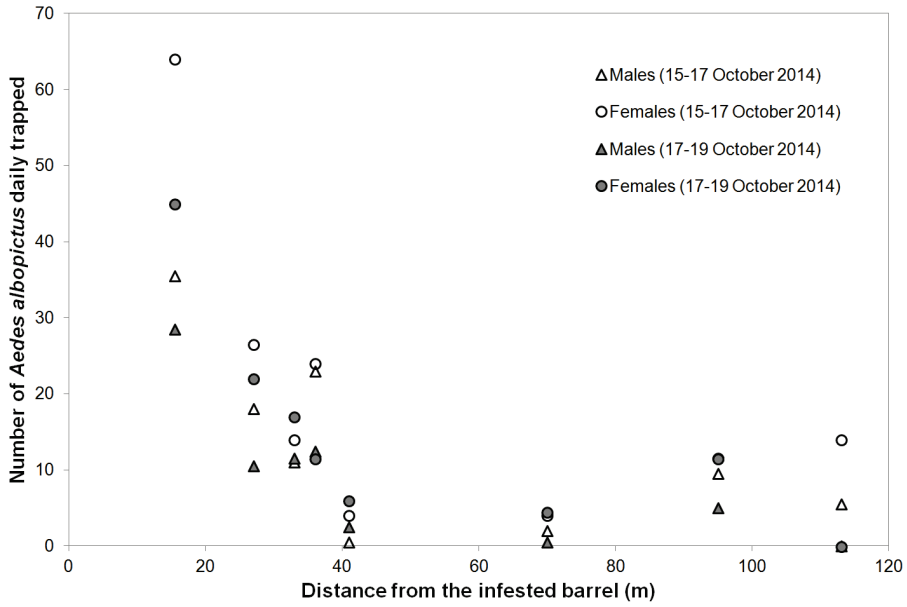


Figure III.8. Abundance of *Aedes albopictus* adults according to the distance of the major breeding site identified (a metal barrel) in the primary case premise in Montpellier during the CHIKV outbreak, October 2014. The data represent the daily number of females (dots) and males (triangles) of *Ae. albopictus* trapped by BG-sentinel trap before adulticide treatments.

Evolution of entomological indices

Compared to autumn 2015, the level of infestation was very high in autumn 2014 in the *Mas de Tesse* district concerned by the CHIKV outbreak, especially the BI reaching 204 (figure III.9). The BI and HI obviously decreased strongly in spring 2015. The winter naturally induced a diminution of the number of colonized containers in all districts. The *Mas de Tesse* district's HI presents almost half the value of the HI in the previous year in other districts; The wintry conditions differed slightly, but the HI diminution likely resulted from treatment control and source reduction operations of the past autumn. One year after the beginning of the outbreak, the indices have recovered with a comparable infestation level in the *Mas de Tesse* and *Goélands* districts. During the year of investigations in the *Mas de Tesse* district, only few unused barrels and water tanks (12.5% of total positive containers) produced most of the larvae (abacuses 4 and 5), corresponding to 51.5% of the total estimated larval abundance.

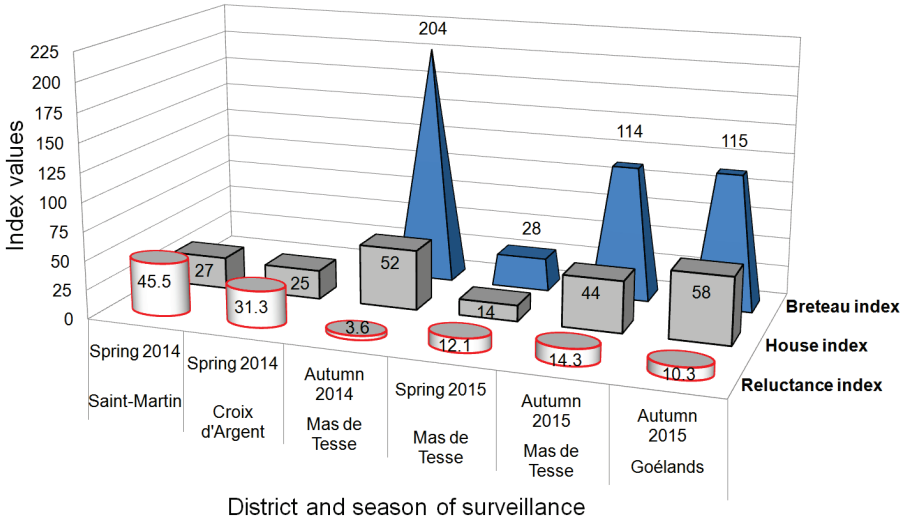


Figure III.9. Entomological indices for *Aedes albopictus* in different districts of Montpellier from the spring 2014 to the autumn 2015. The *Mas de Tesse* district was concerned by a chikungunya outbreak during the autumn 2014. Three indices are represented: the Breteau index (number of breeding site containing immature stages of *Ae. albopictus* per 100 houses), the House index (number of houses with positive container per 100 houses), and the Reluctance index (number of houses with residents opposed to entomologic investigations per 100 houses). Breteau index was not available for spring 2014 surveys.

The RI of autumn 2014 revealed that few inhabitants of the *Mas de Tesse* district were opposed to investigations; they welcomed the agents in a context of current perception of the sanitary risk, strengthened by the media coverage. There was three times less entrance refusal during the vectorial episode than one year later (3.6 then 14.3). In the next spring (2015), the RI was still low, divided with a factor of 2.5 to 3.8 compared to the RIs recorded during the 2014 campaign. The remembrance of the sanitary crisis was still strong despite the 5 months of winter and the absence of a perceptible nuisance in spring. As for entomologic indices, the RI did not differed in autumn 2015 from the control district of *Goélands*, in period of pest annoyance. However, a strong diminution in active breeding sites supplied by voluntary watering was observed (figure III.10). Consequently there is no evidence of a virtuous effect of the CHIKV sanitary episode on the mosquito abundance or the inhabitants' execution of community-based prevention after the spring 2015.

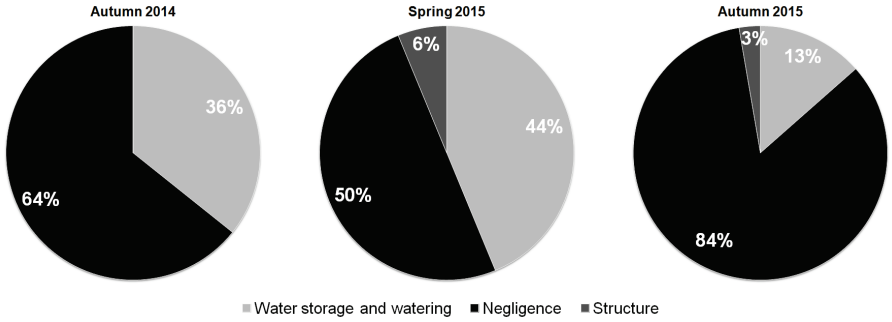


Figure III.10. Origin of water in positive breeding sites in the district of 2014 Chikungunya outbreak in Montpellier, France. Houses visited in autumn 2014 (n=27), spring 2015 (n=58) and autumn 2015 (n=36). "Water storage and watering" concerned voluntary water supplies. "Structure" concerned not removable constructions when "Negligence" is defined by removable containers, both with unintentional water supply.

Discussion

The present study described the vectorial issue of the first significant outbreak of CHIKV in mainland France. History shows that high level of mosquito abundance is a recurrent factor of arboviral outbreaks in temperate area. An unusually high density of *Ae. albopictus* was presumably responsible of the large DENV outbreak in 1942 in Nagasaki, Japan. The culicid infestation was consecutive to the storage of water in tanks, an usual habit in the city due to the traditional Japanese lifestyle and the fire risk consecutive to American bombing (Oki and Yamamoto 2013). In 2004 in Ningbo, China, the HI and BI reached respectively 91 and 261 during a DENV-1 outbreak, as a typhoon previously flooded the area (Xue *et al.* 2007). Unfortunately, European data are lacking and no Stegomyia index was published from the largest European CHIKV outbreak in 2007 in Italy. The vector control operations are logically the priority rather than scientific data collection during such outbreaks. In a robust study carried out the next year in Italy, some quite moderate BI indices were observed (around 50-64), the value increasing in vegetated area badly maintained and/or shaded (Carrieri *et al.* 2011b). In fact, the primary case and the majority of the autochthonous cases (at least 8) in Montpellier were viremic before the extreme rainfall event and the vector control operations (Delisle *et al.* 2015). Due to a delay in identification of autochthonous cases, the entomological indices observed were probably superior to the local situation during the

majority of indigenous transmission. Larval indices observed at the beginning of local transmission of other foci were indeed inferior, with BI and HI respectively equal to 106 and 40 for CHIKV focus in Fréjus in 2010 (77 houses visited) (EID Méditerranée 2010) and 103 and 42 for DENV focus in Nîmes in 2015 (91 houses visited) (INVS 2015). Paradoxically, some high levels of infestation are often observed in the French Mediterranean area without heavy rainfall. Entomological surveys made in two cities of the French Riviera in September 2010 while 2 autochthonous cases of DENV occurred already show average BI and HI values of respectively 163 ± 9 and 58 ± 9 before the seasonal rain (unpublished data).

For arboviruses as DENV and the vector *Ae. aegypti*, the risk of outbreak in tropical areas exists when HI and BI are superior to 5 and the risk is important when BI is > 50 (Focks 2003). The transposition of these thresholds to *Ae. albopictus* is not unwise, even if their pertinence is difficult to assess in temperate area, where numerous opposing factors are involved, like climatic conditions and herd immunity. In mainland France, the threshold of important risk is largely exceeded from August to September, which is the period where all autochthonous infection begun. However, the annual dynamic of *Ae. albopictus* presents high density peaks since June (Lacour *et al.* 2015). It means contamination risk is possible earlier in the season, which could lead to a large scale outbreak, exactly as the Italian CHIKV outbreak that started in the end of June 2007 (Rezza *et al.* 2007). The fact is that data from indices are still lacking in Europe; the collect of entomological information during autochthonous transmission should be assessed as often as possible. It would be helpful to determine the critical threshold for prophylactic and epidemic levels in temperate area, and to improve mosquito control intervention and communication. Our results support that source reduction instructions should give priority to the suppression of the most productive containers (barrels, water tanks and plant pots) (Focks 2003).

The wintry period is the major asset in temperate climate to end mosquito-borne disease circulation. In this CHIKV outbreak, it appears that the diapause process would have naturally eliminated the imago vectors thinly one week later than mosquito control operations did. It does not make optional the resort of heavy vector control measures as done in French. Indeed, the last autochthonous transmission occurred in days preceding the first antivectorial treatment (Delisle *et al.* 2015), in a high vector density context. As old females are the most infectious (Hardy *et al.* 1983), authors are likely to think that without appropriate control measures more autochthonous case would have occurred.

Winter will cause the definitive arrest of transmission of arboviruses like DENV and CHIKV as long as they will not be successfully vertically transmitted to the diapausing progeny. All field studies done in temperate area failed to found a contaminated offspring of *Ae. albopictus* in the long overwintering generation: No CHIKV was found in larvae after the 2006 Italian outbreak (Bellini *et al.* 2012), neither dengue in adults emerged from larvae sampled on May 2005 after the dengue outbreak in Cixi, China (Xue *et al.* 2007). Laboratory studies confirmed the extremely low probability (<0.5%) of transovarial transmission of CHIKV in *Ae. albopictus* to the adult stage (Bellini *et al.* 2012). The infectious rate of field adult mosquitoes during the Montpellier outbreak was low compared to tropical CHIKV outbreaks. In La Reunion island, the adult infectious rate was multiplied by ten (0.017, 24 pools on 1413 adults) but an effective vertical transmission occurred at a very low rate of 0.0003 (2 positive pools on 6853 mosquitoes). The probability to found a transovarial contamination with a lower infectious rate of the vectors was weak. However, there are too few recent outbreaks in temperate area to give final judgment on this possibility (Fontenille *et al.* 2007). Otherwise, epidemiological consequences would be difficult to estimate, ranging up to a dreaded risk of viral endemicity. Fortunately, because of its small size, the first annual generation of *Ae. albopictus* is not sufficient to trigger a rapid outbreak (Carrieri *et al.* 2012). Other implications of a spring-resurgent autochthonous transmission should be more dramatic, as the public image of the health monitoring system and mosquito control operators, and may be a turn-off for the seasonal touristic activity of regions.

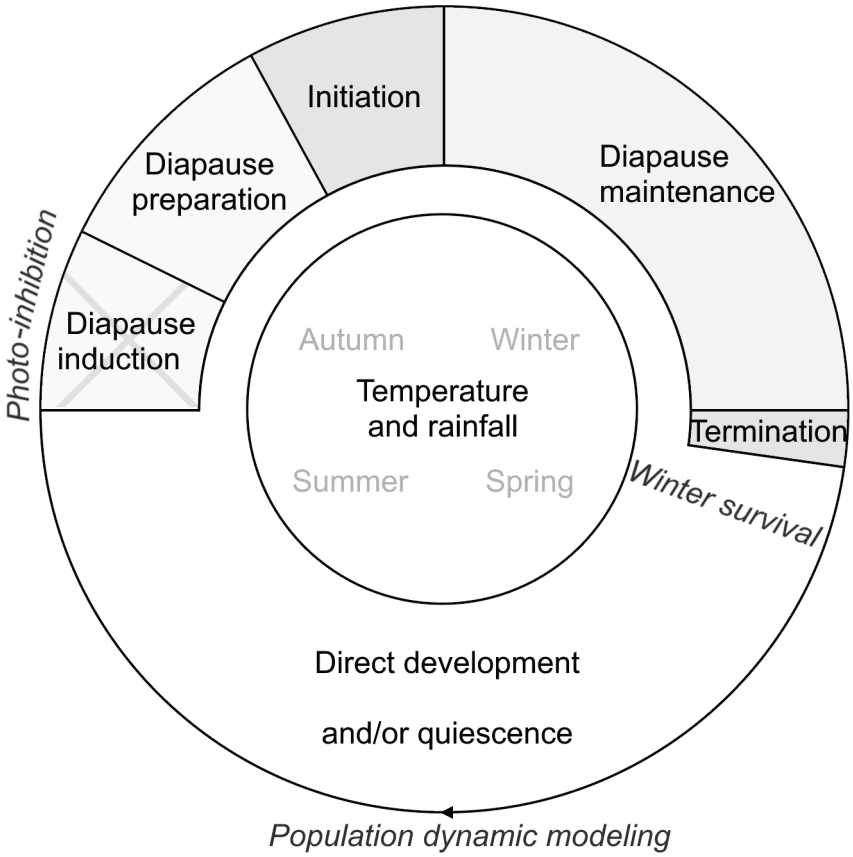
Acknowledgments

The authors are grateful to the agents of EID Méditerranée (Vincent Brun, Sebastien Estaran, Maëliss Gion, Adeline Toro, Fréjorgues agency and others) for helping to provide entomological data and mosquito samples, Christine Prat for viral analyses and Yvon Perrin for proofreading. This work was supported by EID Méditerranée and the French ministry in charge of health, Direction Générale de la Santé (DGS). This paper is number BRC 364 of the Biodiversity Research Centre. The contents of this publication are the sole responsibility of the authors and do not necessarily reflect the views of the institutions.

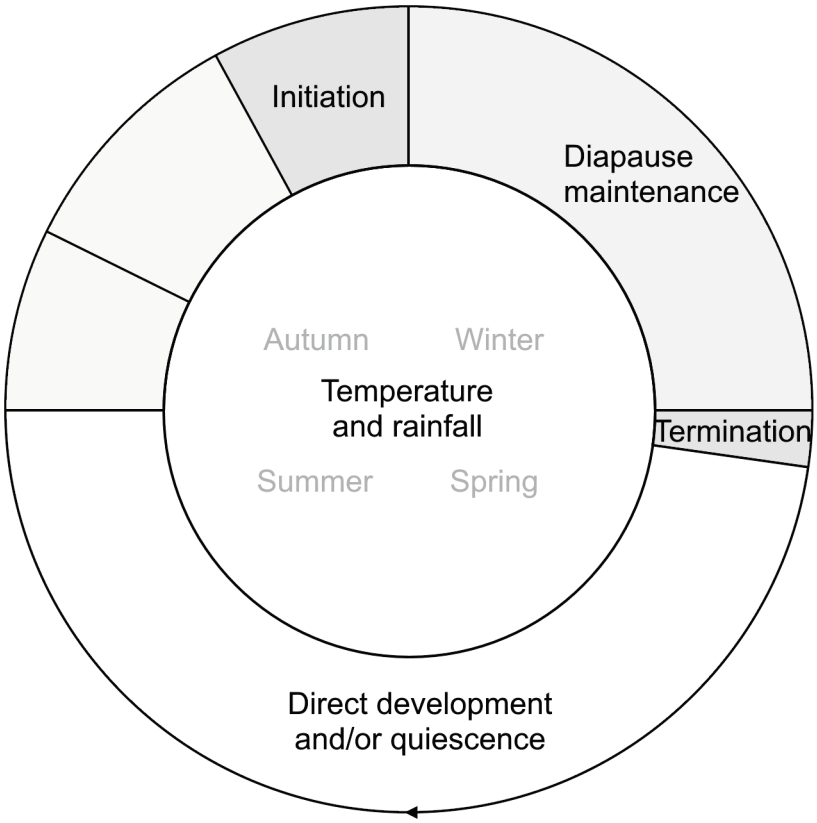
Author Contributions

Conceived and designed the experiments: GL, GLA, ILG, CL, TH. Performed the entomological investigations and experiments: GL, JBF, CJ, GLA, ELM, ILG, DR, CT, FV. Analyzed the data: GL, JBF, DR, ILG, CT. Wrote the paper: GL JBF GLA.

Chapter IV. A model-based approach of the adaptive value of diapause under warm temperate environment



In previous chapters, the causality and effects (at physiological and population levels) of diapause were described. The objective of this chapter is to put in evidence the adaptive value of the diapause in temperate populations of *Ae. albopictus*. We used a comparative approach between physiotypes able or not of diapause to put in evidence the advantages provided by this overwintering process. However, no temperate strains of *Ae. albopictus* exist with both a diapausing and a fixed non-diapausing physiotypes (with the assurance to have the same genotype). Consequently, the use of simulated populations appeared to be necessary in our study. The first step of the work was to develop a deterministic model of population dynamics for *Ae. albopictus*. The model is presented in the following paper.



Population dynamic modeling

Highlights (paper IV)

- A generalist deterministic model of mosquito population dynamics was adapted to *Aedes albopictus*
- The model was developed for a local scale, based on the biology of French local populations and validated with field local ovitrap surveys.
- The model's main control points were related to adult's mortality rates, the carrying capacity in pupae of the environment, and the beginning of the unfavourable period.

A Rainfall- and Temperature-Driven Abundance Model for *Aedes albopictus* Populations.

Paper IV

Tran A[10,11]*, L'Ambert G[12]*, Lacour G[12,13]*, Benoît R[12], Demarchi M[14], Cros M[12], Cailly P[10,15,16], Aubry-Kientz M[15,16,17], Balenghien T[17], Ezanno P[15,16].
(* for equal contributions of authors)

International Journal of Environmental Research and Public Health. 2013; 10(5), 1698–719.

Abstract

The mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) is an invasive species which has colonized Southern Europe in the last two decades. As it is a competent vector for several arboviruses, its spread is of increasing public health concern, and there is a need for appropriate monitoring tools. In this paper, we have developed a modelling approach to predict mosquito abundance over time, and identify the main determinants of mosquito population dynamics. The model is temperature- and rainfall-driven, takes into account egg diapause during unfavourable periods, and was used to model the population dynamics of *Ae. albopictus* in the French Riviera since 2008. Entomological collections of egg stage from six locations in Nice conurbation were used for model validation. We performed a sensitivity analysis to identify the key parameters of the mosquito population dynamics. Results showed that the model correctly predicted entomological field data (Pearson *r* correlation coefficient values range from 0.73 to 0.93). The model's main control points were related to adult's mortality rates, the carrying capacity in pupae of the environment, and the beginning of the unfavourable period. The proposed model can be efficiently used as a tool to

[10] CIRAD, UPR AGIRs, Montpellier, France.
[11] CIRAD, UMR TETIS, Montpellier, France.
[12] EID Méditerranée, Montpellier, France.
[13] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium.
[14] SIRS, Montpellier, France.
[15] INRA, UMR1300 Biologie, épidémiologie et analyse de risques en santé animale, Nantes, France.
[16] ONIRIS, LUNAM Université Nantes Angers Le Mans, Nantes, France.
[17] CIRAD, UMR CMAEE, Montpellier, France

predict *Ae. albopictus* population dynamics, and to assess the efficiency of different control strategies.

Keywords

Aedes albopictus; arbovirus; population dynamics; modelling; sensitivity analysis.

Introduction

The Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) is a competent vector for several arboviruses, such as dengue and chikungunya viruses (Mitchell 1995). Originally indigenous to South-East Asia, the species has spread during the last decades to Africa, the Middle East, Europe and the Americas (Gratz 2004). Since 1990, the Asian tiger mosquito has rapidly colonized Southern Europe (ECDC VBORNET). It is the only invasive mosquito species currently installed in continental France, present since 2004 on the Côte d'Azur region (Delaunay *et al.* 2007, ECDC 2012, Medlock *et al.* 2012). Its continuing spread is of increasing public health concern, strengthened by a recent chikungunya outbreak in Italy in 2007 (Rezza *et al.* 2007), and the first occurrence of autochthonous dengue and chikungunya cases in southern France in 2010 (La Ruche *et al.* 2010, Grandadam *et al.* 2011).

Due to the urgent need for intensive monitoring and risk-based surveillance of *Ae. albopictus* populations, modelling approaches have been used to map the areas of its potential distribution (Neteler *et al.* 2011, ECDC 2009). Moreover, mechanistic modelling approaches have been successfully used to predict the temporal dynamics of *Ae. aegypti* using either stochastic (Otero *et al.* 2006) or deterministic models (Focks *et al.* 1993), or discuss the impact of mosquito population dynamics on chikungunya virus transmission to the human population on La Réunion island, France (Dumont and Chiroleu 2010, Moulay *et al.* 2011). Such models are useful to identify the control points of the population dynamics, and test some control strategies (Dumont and Chiroleu 2010). In Europe, Poletti *et al.* recently modelled the temporal dynamics of *Ae. albopictus* in Italy and discussed the transmission potential of chikungunya virus (Poletti *et al.* 2011).

In this paper, we present a weather-driven abundance model depicting the annual and inter-annual variations of *Ae. albopictus* populations taking into account diapause processes. We used the generic framework proposed recently by Cailly *et al.* (Cailly *et al.* 2012) for modelling mosquito populations, and we defined parameters and functions to adapt this generic model to the Asian tiger mosquito. The model was used to simulate *Ae. albopictus* populations in the Côte d'Azur region, using daily rainfall and temperature data. Entomological collections of egg stage from different municipalities were used for model validation, and a sensitivity analysis was performed to identify the key parameters driving the mosquito population dynamics.

Material and Methods

Study Area

The study area includes the municipality of Nice and neighbouring municipalities from the French Riviera region where entomological longitudinal surveys on *Ae. albopictus* populations are performed since 2008 (figure IV.1). In this region located in South Eastern France, the climate is typically Mediterranean, with warm, very dry summers and mild, wet winters. Total annual rainfall is around 750 mm, and temperatures usually vary between 0 °C in winter, and 34 °C in summer. *Aedes albopictus* populations are mainly installed in urban areas, as in Nice, a densely populated city with a population over 340,000 inhabitants, and in the residential areas of neighboring municipalities.

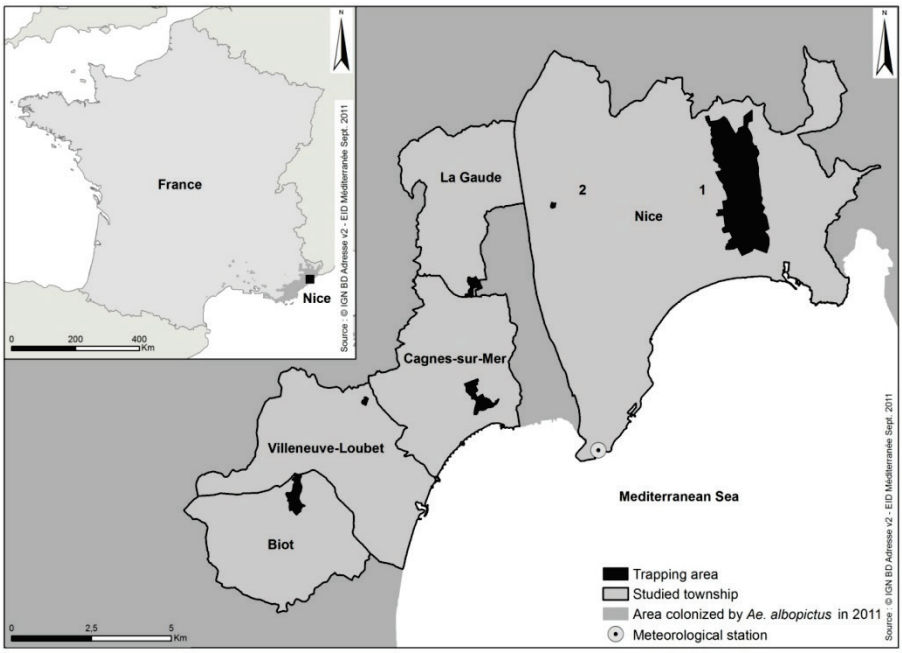


Figure IV.1. Study area including the municipalities of Nice, La Gaude, Cagnes-sur-Mer, Villeneuve-Loubet, and Biot. Source: © IGN BD Adresse v2-EID Méditerranée, September 2011.

Entomological Data

Mosquito sampling was performed in different locations of Nice conurbation, using ovitraps' networks placed mostly in sites shaded by vegetation. The surveillance of eggs was chosen for detecting invasive mosquito species such as *Ae. albopictus* in South-eastern France. It allows targeted and rapid sampling efforts and an optimal cost-benefit ratio (ECDC 2013, Carrieri *et al.* 2011a). Ovitraps are artificial egg-laying containers made up with 3 L black plastic buckets (Dillewijn Zwapak©, Aalsmeer, The Netherlands) filled with 2 L of tap water and the biolarvicide *Bacillus thuringiensis israelensis* (Bti) to prevent the production of mosquitoes in the trap (Carrieri *et al.* 2009), in which a floating polystyrene square (5 x 5 cm) was added to provide a support for oviposition. The sampling was conducted in six different locations of Nice conurbation, mainly in discontinuous urban fabric including individual houses with gardens: Nice (1: Nice downtown, 2: residential area on the outskirts of Nice), Cagnes-sur-Mer, La Gaude, Biot, and Villeneuve-Loubet. Networks of between 15 and 50 ovitraps were collected biweekly, and weekly in Cagnes-sur-Mer (table IV.1). We considered the fortnightly frequency as an acceptable compromise between representative data (average period of time separating two generations during summer, considering around three days for embryogenesis, seven days of larval development and five days for mating, blood engorgement and oviposition) and reasonable trapping effort in the five areas (Roiz *et al.* 2010, Abramides *et al.* 2011). Surveys were stopped at the end of each year after two consecutive negative samples. Trapping areas were considered free of insecticide treatments realized during the plan of anti-dissemination of chikungunya virus from 2008 to 2011, except three ovitraps located in Nice-1 within a distance of 200 m from the insecticide spraying. Thus, results from these three ovitraps after the treatments were removed from the analysis. Hatched and unhatched eggs of *Ae. albopictus* were counted in laboratory under stereomicroscope. Eggs of *Ae.* (*Finlaya*) *geniculatus*, the only other mosquito species to lay eggs into ovitraps in south-eastern France, were morphologically discriminated from *Ae. albopictus* eggs using a stereomicroscope (Matsuo *et al.* 1972, Encinas Grandes 1982).

Table IV.1. Entomological collections for the surveillance of *Aedes albopictus*, Côte d'Azur area, France, 2008–2011.

Campaign		Trapping season		Ovitrap network			Result
Location	Year	Beginning	End	Nb traps	Surface of the trapping area (ha)*	Sampling frequency	Annual max. of the mean number of collected eggs per ovitrap per capture session
Nice 1	2008	25Mar	8Dec	30	328.7	biweekly	170
Nice 1	2009	16Apr	9Dec	50	517.7	biweekly	233
Nice 1	2010	15Apr	2Dec	50	517.7	biweekly	462
Nice 1	2011	21Apr	14Dec	50	517.7	biweekly	311
Cagnes-sur-Mer	2010	21Jun	15Nov	15	30.5	weekly	177
Cagnes-sur-Mer	2011	28Mar	28Nov	18	41.6	weekly	169
La Gaude	2010	16Jul	8Oct	22	17.9	biweekly	566
Biot	2010	16Jul	8Oct	25	40.3	biweekly	830
Villeneuve-Loubet	2011	6May	30Nov	15	3.8	biweekly	1,108
Nice 2	2011	11May	18Nov	15	2.7	biweekly	654

* computed as the surface of the smallest polygon including all ovitraps with a buffer distance of 50 m.

Environmental Data

Daily rainfall and temperature data from 2008 to 2011 recorded in Nice Airport were obtained from the national meteorological service "Météo France". Indeed, we assumed that the population dynamics of *Ae. albopictus* is mainly driven by these two factors: (*i*) temperatures have a strong impact on the survival of *Ae. albopictus* populations, and on the development of aquatic stages (Roiz *et al.* 2010, Delatte *et al.* 2009, Lacour *et al.* 2010); (*ii*) precipitations favor the availability of breeding sites, i.e., any small recipient filled with water where *Ae. albopictus* females lay their eggs. Moreover, we considered that the egg hatching is triggered by rainfall events but also by human water supply (e.g., garden watering in summer) (Roiz *et al.* 2010). Indeed, larval surveys carried out since 2008 showed the importance of small and medium containers sampled in gardens in the productivity *Ae. albopictus*' populations (EID-Méditerranée 2011).

Model Description

Aedes albopictus *Life Cycle*

As for all mosquito species, the life cycle of *Ae. albopictus* includes three water-dependent stages (egg, larva, and pupa), and one aerial stage (adult). The lifespan in each stage depends on several factors, such as temperature, or water availability. After emergence and insemination, female adults successively: (*i*) seek a human host to take a blood meal; (*ii*) rest in a sheltered place during the few days needed for the eggs to mature; and (*iii*) search for sites to lay their eggs. The Asian tiger mosquito breeds in artificial containers of any type (metal, glass, stone, plastic, rubber, etc.), or in small natural water bodies such as tree holes or rock pools (Hawley 1988). Eggs hatch after a desiccation period (few days to several months) when they are submerged in water by rainfall or artificial flooding. The larvae then mature through four stages before entering pupation. Adult mosquito emerges from the pupa at the surface of water. In temperate climates, *Ae. albopictus* survive the unfavorable period (winter) as eggs in dormancy (diapause) that will hatch during the next favorable season (spring).

Modelling Aedes albopictus Population Dynamics

The generic model of mosquito population dynamics developed by Cailly *et al.* (Cailly *et al.* 2012) represents all of the steps of the mosquito life cycle

(figure IV.2). It considers ten different stages: three aquatic stages (*E*, eggs; *L*, larvae; *P*, pupae), one emerging adult stage (A_{em}), three nulliparous stages (A_{1h}, A_{1g}, A_{1o}), and three parous stages (A_{2h}, A_{2g}, A_{2o}). In the adult stage, females only are represented. Parous females are females that have oviposited at least once, whereas nulliparous females have never laid eggs. Adults are subdivided regarding their behaviour during the gonotrophic cycle (*h*, host-seeking; *g*, transition from engorged to gravid; *o*, oviposition site seeking). Once parous, females repeat their gonotrophic cycle until death. The events driving the transitions between stages are: egg mortality or hatching, larva mortality, pupation (moult of larvae to pupae), pupa mortality, adult emergence, mortality, engorgement, egg maturing, and oviposition. The model takes into account density-dependent mortality of the larval stage (Clements 2000), and pupa density-dependent success of adult emergence. Density-dependent mortality was assumed at the larval stage as it is has been often observed (Clements 2000, Lord 1998). Pupa density-dependent success of adult emergence was assumed as emergence success was found negatively correlated to pupa density (Jetten and Takken 1994).

The model is based on a system of ordinary differential equations (ODE). For *Aedes* populations in temperate climate, the eggs stop hatching at the beginning of the unfavorable period, during which diapause occurs. All other stages will continue their development or transition to the next stage. Thus, the ODE system is:

$$\begin{cases} \dot{E} = \gamma_{Ao}(\beta_1 A_{1o} + \beta_2 A_{2o}) - (\mu_E + z^* f_E)E \\ \dot{L} = z^* f_E E - [m_L(1 + L/k_L) + f_L]L \\ \dot{P} = f_L L - [m_P + f_P]P \\ \dot{A}_{em} = f_P P \sigma \exp[-\mu_{em}(1 + P/k_P)] - [m_A + \gamma_{Aem}]A_{em} \\ \dot{A}_{1h} = \gamma_{Aem} A_{em} - (m_A + \mu_r + \gamma_{Ah})A_{1h} \\ \dot{A}_{1g} = \gamma_{Ah} A_{1h} - (m_A + f_{Ag})A_{1g} \\ \dot{A}_{1o} = f_{Ag} A_{1g} - (m_A + \mu_r + \gamma_{Ao})A_{1o} \\ \dot{A}_{2h} = \gamma_{Ao}(A_{1o} + A_{2o}) - (m_A + \mu_r + \gamma_{Ah})A_{2h} \\ \dot{A}_{2g} = \gamma_{Ah} A_{2h} - (m_A + f_{Ag})A_{2g} \\ \dot{A}_{2o} = f_{Ag} A_{2g} - (m_A + \mu_r + \gamma_{Ao})A_{2o} \end{cases} \quad , with\ z = \begin{cases} 0 & during\ diapause \\ 1 & otherwise \end{cases} \qquad (1)$$

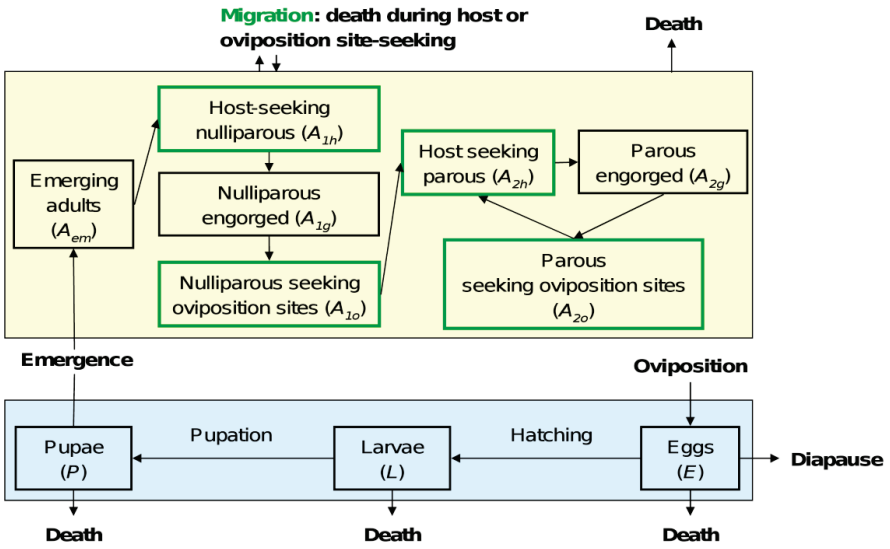


Figure IV.2. Model diagram of *Aedes albopictus* population dynamics in temperate climate. Aquatic stages are drawn in blue, adult females in yellow. The green compartments indicate the adult females which move to seek for a host or an oviposition site. Adapted from Cailly *et al.* (2012). Reprinted from Ecological Modelling, 227/24 February 2012, Cailly, P.; Tran, A.; Balenghien, T.; L'Ambert, G.; Toty, C.; Ezanno, P., A climate-driven abundance model to assess mosquito control strategies, Pages 7–17, Copyright (2012), with permission from Elsevier.

Model parameters are in Greek letters. They are constant. For stage X, γ_X is the transition rate to the next stage, μ_X the mortality rate, and β_X the egg laying rate. σ is the sex-ratio at the emergence. μ_r is an additional adult mortality rate related to the seeking behavior, applied only on adult stages involving risky movements (host or oviposition site seeking).

Model functions are in Latin letters. They depend on parameters and weather-driven functions (i.e., functions of temperature, humidity or precipitation varying over time). For stage X, f_X is the transition function to the next stage, m_X the mortality function, and k_X the environment's carrying capacity which limits the population growth due to density-dependent processes.

Parameters and Functions of the Model

We defined specific parameter values, forcing functions, transition functions between stages of the life cycle, and mortality functions to adapt the model to *Ae. albopictus* in the region of Nice. As our model neglects mosquito dispersal (arrival or departure of individuals), the dimensions of the surface used for simulation have to be larger than the active flight distance of mosquitoes: *Ae. albopictus* having a distance of dispersal of about 100 m (Marini *et al.* 2010), our model is used for simulating the mosquito population dynamics over a squared surface greater than 4 ha.

Because of the large phenotypic variability of the Asian tiger mosquito (Hawley 1988), parameter values were based on expert knowledge of local *Ae. albopictus* population biology (Lacour *et al.* 2010, EID-Méditerranée 2011, Lacour *et al.* 2012), supported and completed by scientific literature (table IV.2).

Table IV.2. Parameter values of the model of mosquito population dynamics adapted to *Aedes albopictus* in Mediterranean temperate climate.

Parameter	Definition	Value	Reference
β_1	Number of eggs laid by ovipositing nulliparous females (per female)	95	Lacour *et al.* 2010
β_2	Number of eggs laid by ovipositing parous females (per female)	75	Lacour *et al.* 2010
κ_L	Standard environment carrying capacity for larvae (larvae ha^{-1})	250,000	To our best knowledge
κ_P	Standard environment carrying capacity for pupae (pupae ha^{-1})	250,000	To our best knowledge
σ	Sex-ratio at the emergence	0.5	Delatte *et al.* 2009
μ_E	Egg mortality rate (day^{-1})	0.05	(Lacour, unpublished)
μ_L	Minimum larva mortality rate (day^{-1})	0.08	(Lacour, unpublished)
μ_P	Minimum pupa mortality rate (day^{-1})	0.03	(Lacour, unpublished)
μ_{em}	Mortality rate during adult emergence (day^{-1})	0.1	(Lacour, unpublished)
μ_A	Minimum adult mortality rate (day^{-1})	0.02	Lacour *et al.* 2010
μ_r	Adult mortality rate related to seeking behavior (day^{-1})	0.08	To our best knowledge
T_E	Minimal temperature needed for egg development (°C)	10.4	Delatte *et al.* 2009
TDD_E	Total number of degree-day necessary for egg development (°C)	110	(Lacour, unpublished)
γ_{Aem}	Development rate of emerging adults (day^{-1})	0.4	To our best knowledge
γ_{Ah}	Transition rate from host-seeking to engorged adults (day^{-1})	0.2	To our best knowledge
γ_{Ao}	Transition rate from oviposition site-seeking to host-seeking adults (day^{-1})	0.2	To our best knowledge
T_{Ag}	Minimal temperature needed for egg maturation (°C)	10	Delatte *et al.* 2009
TDD_{Ag}	Total number of degree-days necessary for egg maturation (°C)	77	Delatte *et al.* 2009
t_{start}	Start of the favorable season	10 Mar	Lacour *et al.* 2012
t_{end}	End of the favorable season	30 Sept	Lacour *et al.* 2012

The end of the favorable period is defined as the moment when 90% of eggs laid enter into diapause. Diapause is genetically programmed in *Ae. albopictus* populations. It is induced by a short photoperiod (Hawley 1988). In Nice area, egg diapause initiation occurs gradually during September, so diapause processes occur in the model from 30 September to 10 March the following year (Lacour *et al.* 2012). During this period, only eggs survive until the start of the next favorable season when they hatch if

they are immerged in water. The standard environment carrying capacities for larvae (κ_L) and pupae (κ_P) were estimated as follows: the maximal larval and pupal densities observed in laboratory (10 individuals per cm² of water surface (Tsuda *et al.* 1991)) was multiplied by the surface of a typical *Ae. albopictus* breeding site (~50 cm²), the number of breeding sites per household (~20), and the number of households per hectare (~25), all of these values being estimated from field observations (EID-Méditerranée 2011).

The two forcing function variables are temperature (T) and precipitation (P), both varying over time. Daily mean temperature and precipitation were used. Precipitation is known to trigger egg hatching of *Aedes* species breeding in out-door oviposition sites (Soti *et al.* 2012). However, in human-made environments artificial flooding (e.g., watering of gardens) is likely to be the main driver of *Ae. albopictus* egg hatching in summer, when rainfall events are scarce. Thus, we considered that the development of *Ae. albopictus* eggs is driven by: (*i*) the occurrence of rainfall events in spring; and (*ii*) temperature, using the concept of degree-day, the quantity of accumulated heat necessary for development from one stage to another. In spring, hatching occurs only with rainfall events: the transition function from egg to larva $f_E(t)$ will be null if no rainfall event occurs at time t ($P(t) = 0$). Otherwise, it is driven by temperature, using the degree-day relation, also used to express the development rate of engorged adults becoming gravid at time t:

$$f_X(t) = \begin{cases} \dfrac{T(t) - T_X}{TDD_X} & if \quad T(t) > T_X \\ 0 & otherwise \end{cases}, X \; in \left\{ E; A_g \right\} \qquad (2)$$

Values of T_X and TDD_X are given in Table IV.2.

The development of other aquatic stages (larvae and pupae) is positively correlated to temperature within an optimum range (Delatte *et al.* 2009). Non-linear relations were used to express the relationships between temperature (in °C) and development rate of larvae and pupae according to observations under laboratory controlled conditions (Delatte *et al.* 2009). These functions are given in Table IV.3. It should be noted that atmospheric temperature was used as a proxy of water temperature. This approximation is justified by the small size of the urban breeding sites of *Ae. albopictus* in Nice region.

Table IV.3. Functions of the model of mosquito population dynamics adapted to *Aedes albopictus* in Mediterranean temperate climate.

Function	Definition	Expression
f_E	Transition function from egg to larva	Equation(2)
f_L	Transition function from larva to pupa	$f_L(t)=-0.0007.T^2(t)+0.0392.T(t) - 0.3911$
f_P	Transition function from pupa to emerging adult	$f_P(t)= 0.0008.T^2(t) - 0.0051.T(t)+0.0319$
f_{Ag}	Transition function from engorged adult to oviposition site—seeking adult	Equation(2)
m_L	Larva mortality (day^{-1})	$m_L(t) = \exp(-T(t)/2) + \mu_L$
m_P	Pupa mortality rate (day^{-1})	$m_P(t) = \exp(-T(t)/2) + \mu_P$
m_A	Adult mortality rate (day^{-1})	$m_A(t)=\max(\mu A;0.04417+0.00217.T(t))$
k_L	Environment carrying capacity of larvae (ha^{-1})	Equation(3)
k_P	Environment carrying capacity of pupae (ha^{-1})	Equation(3)

Temperature also impacts the mortality rates of larvae, pupae and adults. Expressions were derived from Shaman *et al.* (Shaman *et al.* 2006), considering one different function for each stage, and adapted to the observations of *Ae. albopictus* (Delatte *et al.* 2009) (table IV.3).

Finally, we considered that the precipitations impact the environment's carrying capacity (k_X) of aquatic stages (larvae and pupae), increasing the number of breeding sites available for *Ae. albopictus* which is essentially an outdoor breeder (Chan *et al.* 1971, Ho *et al.* 1971, Lourenco-de-Oliveira *et al.* 2004):

$$k_X(t) = \kappa_X * (\mathrm{P}_{\mathrm{norm}}(\mathrm{t}) + 1), X\ in\ \{L; P\} \qquad (3)$$

Values of κ_X, the standard environment's carrying capacity of aquatic stages, are given in Table 2. $P_{norm}(t)$ is defined as the rainfall amount summed over a two weeks period, and normalized in order to vary between 0 and 1.

Model Outputs

The model predicts the abundance of mosquitoes per stage (E, L, P, A_{em}, A_{1h}, A_{1g}, A_{1o}, A_{2h}, A_{2g}, A_{2o}) over time. In addition, the dynamic information

computed by the model was aggregated using average output values following Cailly *et al.* (2012): the adult peak (maximum number of adults observed in a year), the attack rate (average of the daily number of host-seeking adults ($A_h = A_{1h} + A_{2h}$) during the 21 days around the peak dates), and the parity rate (ratio of the total number of parous females to the total number of females). These aggregated outputs were chosen because of their epidemiological importance. They will be used to perform the sensitivity analysis of the model (Section 2.6). Finally, to allow the comparison between the entomological collections from the ovitraps (as described in Section 2.2) and the predictions of the model, the abundance of eggs laid at time t by *Ae. albopictus* females, $E_l(t)$, was computed as:

$$E_l(t) = \gamma_{Ao}\left(\beta_1 A_{1o}(t) + \beta_2 A_{2o}(t)\right) \qquad (4)$$

Initial Conditions and Simulations

The differential equations were discretized using the explicit Euler method that we implemented in Scilab 5.1. Simulations were run over five years (2008–2011, and one first year with average values of precipitation and temperature, not retained for output computation). Initially, the population consisted of 10^6 eggs (stage E), t_0 corresponding to 1 January.

Validation

Because the collected eggs in ovitraps are removed after sampling, we compared the observed average number of eggs per trap (relative to the maximum value of the observed average number of eggs per trap) in each site (Nice-1 and -2, Cagnes-sur-Mer, La Gaude, Biot, and Villeneuve-Loubet) with the simulated abundances of eggs newly laid (E_l) (relative to the maximum value of simulated eggs abundance over the 2008–2011 period). The degree of association between observed and simulated number of eggs at the time of ovitrap collection was assessed for each collection site by calculating the Bravais–Pearson correlation coefficient.

Sensitivity Analysis

We carried out a global sensitivity analysis, varying simultaneously all of the model's parameters described in table IV.2 (20 parameters) using a fractional factorial design (Saltelli *et al.* 2000). Such a design enabled us to estimate the sensitivity indices for the principal effects and the first-order interactions between parameters (3 levels per factor: the nominal value

±10%, generating 2,186 scenarios). Based on the model realizations implemented in this design, the contributions of the variation factors to the output variability were evaluated using a linear regression approach (Saltelli *et al.* 2000). For each aggregated output (as described in Section *Model Outputs*), a linear regression model was fitted with all the principal effects of the factors and their first-order interactions. A minimum variance criterion was defined: factors or interactions accounting for more than 1% of the output variance were retained in the model. The contribution of factor or interaction *i* to the variation in output *y* was the ratio of the sum of squares related to *i* on the total sum of squares of the model for output *y*. The sum of the contributions for output *y* equaled the coefficient of determination of the regression model r^2.

Results

Aedes albopictus *Population Dynamics in Urban Areas of South Eastern France*

The dynamics of *Ae. albopictus* populations in the Côte d'Azur area present a strong seasonal variability with a 6-month period of adult activity and a 6-month period of egg diapause. The first eggs of the year are laid at the beginning of May. Mosquito density is maximal early July, late August and early September, depending on years and cities. Oviposition activity decreases between mid-September and mid-October, with a residual egg laying activity remaining until November or December. Sampling results did not show evidence of continuous oviposition activity during winter, unlike the situation reported in Rome, Italy (Romi *et al.* 2006).

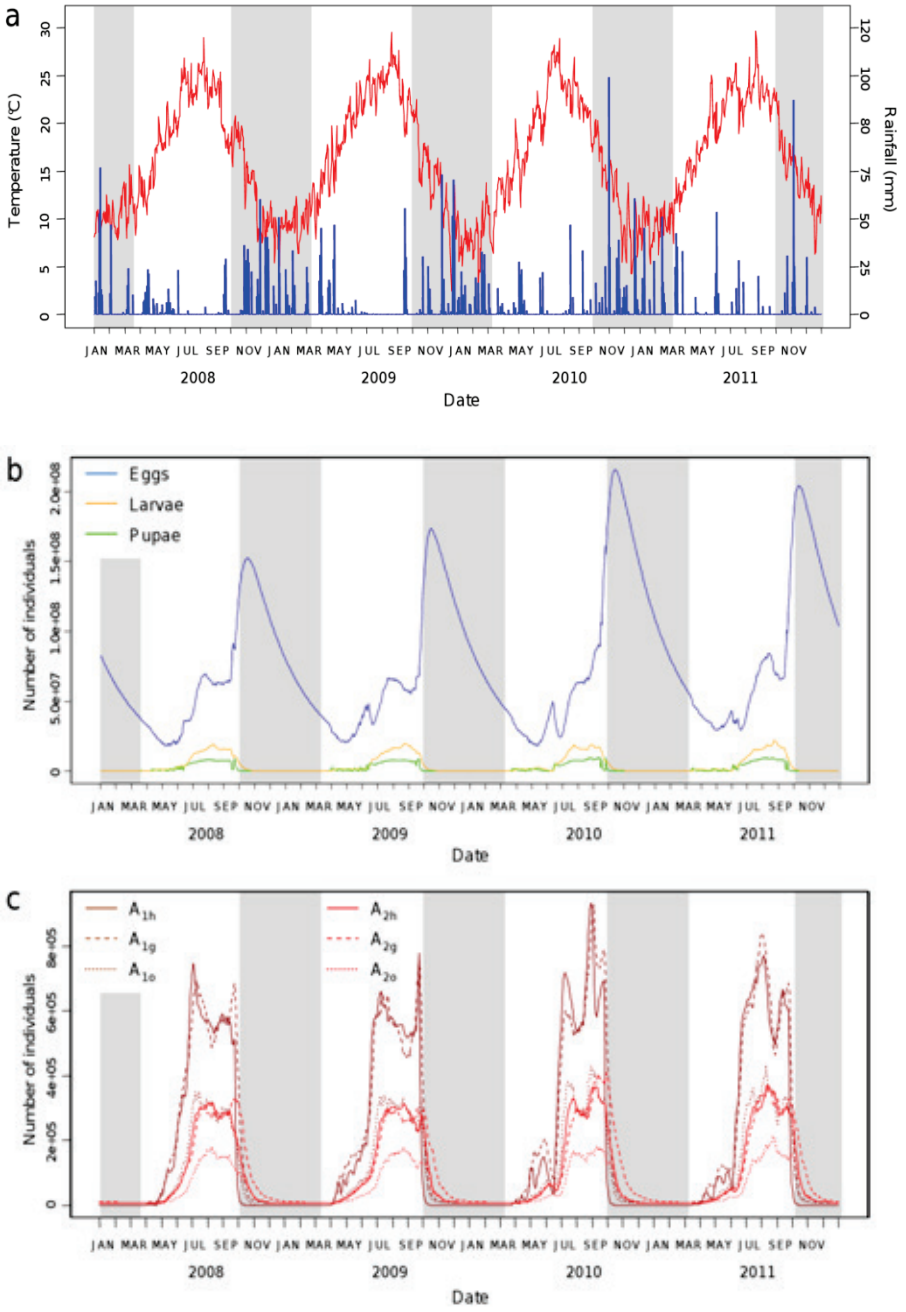


Figure IV.3. *Aedes albopictus* population dynamics simulated over four years according to temperatures and rainfall, Nice region, 2008–2011. (a) Daily mean temperature (red) and rainfall (blue). (b) Simulated number of individuals in the aquatic stages (eggs, larvae and pupae). (c) Simulated number of individuals in the aerial stages (A_{1h}: nulliparous host-seeking; A_{1g}: nulliparous engorged; A_{1o}: nulliparous seeking oviposition site; A_{2h}: parous host-seeking; A_{2g}: parous engorged;

A_{2o}: parous seeking oviposition site). The alternation of favorable and unfavorable periods is represented in white and grey, respectively.

Model Validation

Simulated mosquito abundances were highly consistent with field data collected in Nice-1, 2008–2011 (figure IV.4(a)), with a cross-correlation value $r = 0.79$. For the four years under consideration the model reproduces well the abundance peak of catches occurring in late August. When considering 2010 and 2011, for both years the model simulates well the population growth in May and its decline in autumn. Yet, the model overestimates the abundances of eggs at the start of the favorable period (April–July) in 2008 and 2009, and all over the year in 2008. In 2010 and 2011, egg abundances were also slightly overestimated in April and May. The model also correctly simulated the abundance of *Ae. albopictus* in the five other sites (figure IV.4(b)), with correlation coefficients of 0.73 for Biot (2010), 0.77 for Cagnes-sur-Mer (2010–2011), 0.87 for La Gaude (2010), 0.92 for Nice-2 (2011), and 0.93 for Villeneuve-Loubet (2011). Altogether, these results demonstrate that our model nicely predicts the dynamics of *Ae. albopictus* in various environments of the urban areas of Nice area and for different years.

Based on observed temperatures and precipitations from 2008 to 2011, the model showed *Ae. albopictus* adult mosquitoes to be present in Nice from May to November with a maximum population in late August–early September (figure IV.3). The number of eggs reaches a maximum at the beginning of the unfavorable period, when eggs stop hatching while adult females continue oviposition activity. The egg reserve decreases during winter time, allowing the survival of the population. Differences between years were due to differences in weather variables, the model being otherwise deterministic.

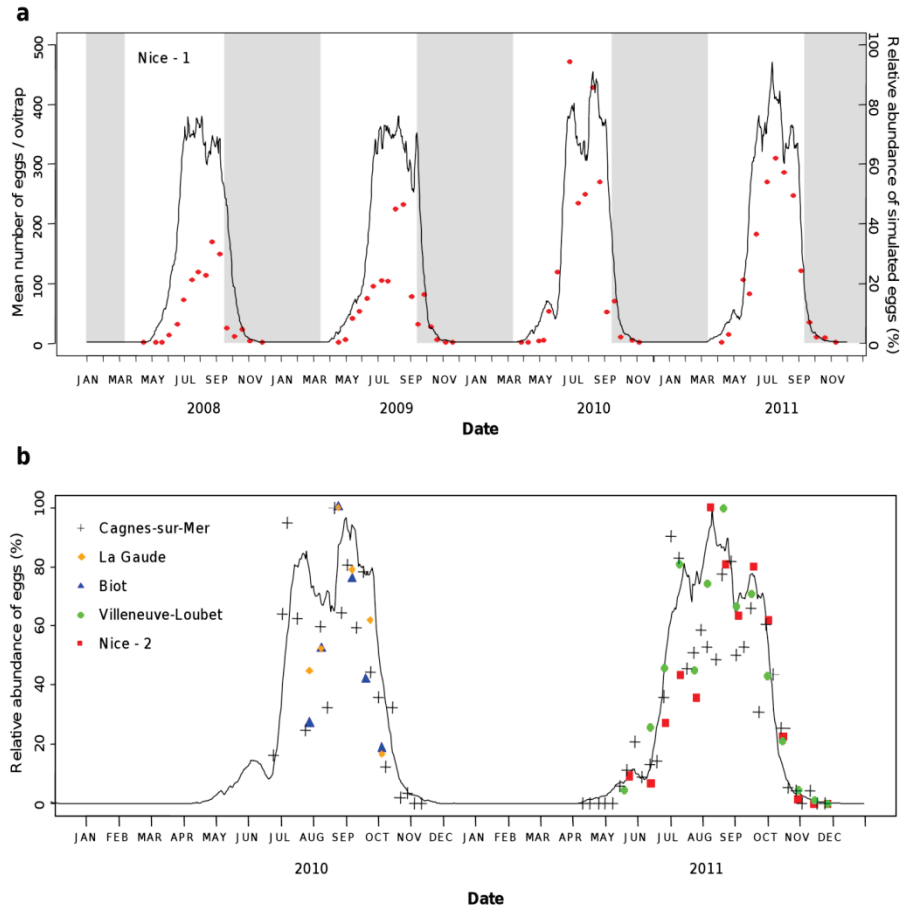


Figure IV.4. Model validation. (a) The simulated dynamics of eggs laid at time t $E_i(t)$ (black line) based on observed temperatures and precipitations from 2008 to 2011 was compared to the mean number of eggs collected per ovitrap in Nice-city area (red dots) using relative abundances. The alternation of favorable and unfavorable periods is represented in white and grey, respectively. (b) Simulated and observed eggs abundances, Nice area, 2010–2011. Symbols represent the observed mosquito abundance data in the different sites, and the black line is the simulated mosquito abundance.

Key Model Parameters

The variations in the peak in adult abundance, in the attack rate and in the parity rate were mainly explained by six of the 20 parameters: the mortality rate at emergence (μ_{em}), the carrying capacity in pupae of the environment (κ_P), the end of the favourable period (t_{end}), the sex-ratio (σ), the transition rate from host-seeking to engorged adults (γ_{Ah}), and to a much lesser extent the transition rate from oviposition site-seeking to host-seeking adults (γ_{Ao}) (figure IV.5).

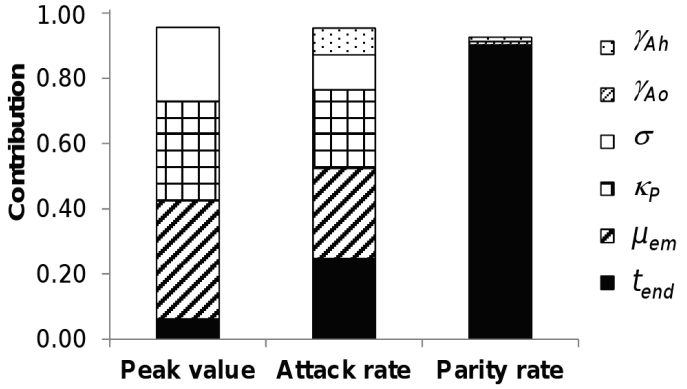


Figure IV.5. Contribution of model parameters to model output variance. Only parameters contributing to more than 1% of output variance were retained here. No interaction was retained.

Therefore, the better these parameters are known, the more precise the model will be in predicting these outputs. Further knowledge thus is needed concerning especially the mortality rate at emergence and the carrying capacity in pupae, which are quite uncertain parameters. A lower mortality at emergence, a higher carrying capacity in pupae, a longer favorable period, and a higher sex-ratio increased the peak abundance in adults and the attack rate (figure IV.6). A shorter development of host-seeking adults decreased the attack rate. As expected, a longer favorable period also favored a higher parity rate.

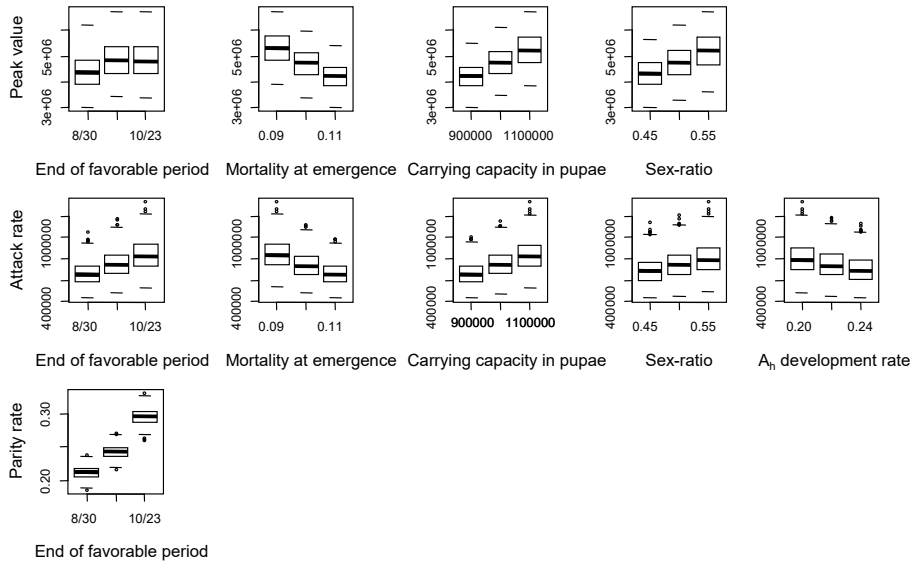


Figure IV.6. Variations of model outputs (in lines) with parameters contributing to more than 10% of their variance (in columns): 3 levels were tested per factor (nominal value ±10%). For each considered parameter and model output, a box-and-whisker diagram graphically depicts the maximum, minimum, median, lower and upper quartiles values of the model output obtained from the simulations with three different levels of the parameter tested.

Discussion

As far as we know, our model is the first mechanistic model of the dynamics of populations of *Ae. albopictus* in a temperate climate country taking into account diapause processes. Driven by two weather variables—temperature and rainfall—it describes the whole mosquito life cycle and takes into account egg diapause. Altogether, the simulations of the model were highly consistent with the number of eggs collected in ovitraps from six different sites of the Nice area over the 2008–2011 period. The model probably overestimates the populations of *Ae. albopictus* in 2008 and 2009 because at that time this invasive species was not yet fully established in Nice area (Delaunay *et al.* 2007).

These results clearly show that the underlying assumptions on the main drivers of Asian tiger mosquito dynamics in this region (*i.e.*, the impacts of temperature, rainfall, and artificial flooding) are correct, and that the model could be used to predict the dynamics of *Ae. albopictus* populations of following years. Yet, additional entomological data on adult populations and

data originating from different cities of Southern France where the Asian tiger mosquito is now installed would strengthen confidence in the model predictions. The model could be applied on other Mediterranean areas where the Asian tiger mosquito is already installed and surveyed, such as Rome (Toma *et al.* 2003), Athens (Giatropoulos *et al.* 2012), Barcelona (Aranda *et al.* 2006), and Tirana (Adhami and Reiter 1998). Moreover, it would be instructive to test the model in other temperate climates (United States of America or Japan) where European invasive populations of *Ae. albopictus* come from (Urbanelli *et al.* 2000).

It should be noted that our model was easily adapted from a generic, mechanistic climate-driven model of mosquito populations developed by Cailly *et al.* (2012). Our results confirm the ability of Cailly's model—applied on *Anopheles* species in (Cailly *et al.* 2012)—to predict the dynamics of different species of mosquito populations, in different geographical areas and over several years. This model could be efficient and useful if used on other exotic mosquito invasive species established in European temperate areas: the Asian bush mosquito *Ae.* (*Finlaya*) *japonicus* (present since 2007 in northern Switzerland, 2008 in southern Germany and 2002 in Belgium), *Ae.* (*Finlaya*) *koreicus* (identified in 2008 in Belgium and 2011 in Italy), the yellow fever mosquito *Ae.* (*Stegomyia*) *aegypti* (established in Georgia, Abkhazia and Russia), and to a lesser extent *Ae*. (*Ochlerotatus*) *atropalpus* (ECDC VBORNET). Indeed, these species have a similar biology with the Asian tiger mosquito: generally introduced by used tire trade, they breed in artificial containers and survive to cold winter temperature; they are human-biters in urban environments, increasing sanitary risk in regard as their proven or potential vector status (Medlock *et al.* 2012).

Using a mechanistic approach, we can study the impact of temperatures and rainfall on the dynamics of *Ae. albopictus*, and our results are consistent with effects demonstrated in correlation studies (Roiz *et al.* 2010). Temperature is recognized to have a stronger influence on *Ae. albopictus* abundance than precipitation (Alto and Juliano 2001), and it is also the main driver of our model. Indeed, most of the mortality and transition rates are temperature-dependent functions. Temperature drives the mortality and transition rates functions in two different ways: higher temperatures favour higher transition rates between stages, although mortality rates decrease with temperature. Yet, according to our simulations in the Nice area, the impact of temperatures is rather favourable to *Ae. albopictus* populations: the peak of abundance occurs with the highest temperatures observed in summer (figure IV.3).

On the other hand, previous observational studies stress the lack of clear relationship between precipitations and *Ae. albopictus* abundances, these studies showing either a positive effect of rainfall (Lourenco-de-Oliveira *et al.* 2004), a negative effect (Roiz *et al.* 2010) or no effect at all (Toma *et al.* 2003). Because *Ae. albopictus* females breed mainly in small artificial containers, Roiz *et al.* (2010) suggest that the seasonal pattern of *Ae. albopictus* population dynamics might be more influenced by variations in human water supply than changes in precipitations. In this study, we followed this assumption to define egg hatching. In our model, this function is driven by rainfall in spring, and temperature-driven later on (Equation (2)), assuming that regular artificial flooding events trigger the egg hatching during the dry months of summer time. Our results stress the relevance and importance of this assumption in the urban environment of the Nice region. Indeed, simulations with an egg hatching function driven by rainfall the whole year (hatching occurring only with rainfall events) show that *Ae. albopictus* populations would be quasi absent during the summer months of dry years such as 2009, and that low rainfalls may lead to an extinction of the *Ae. albopictus* population (figure IV.7).

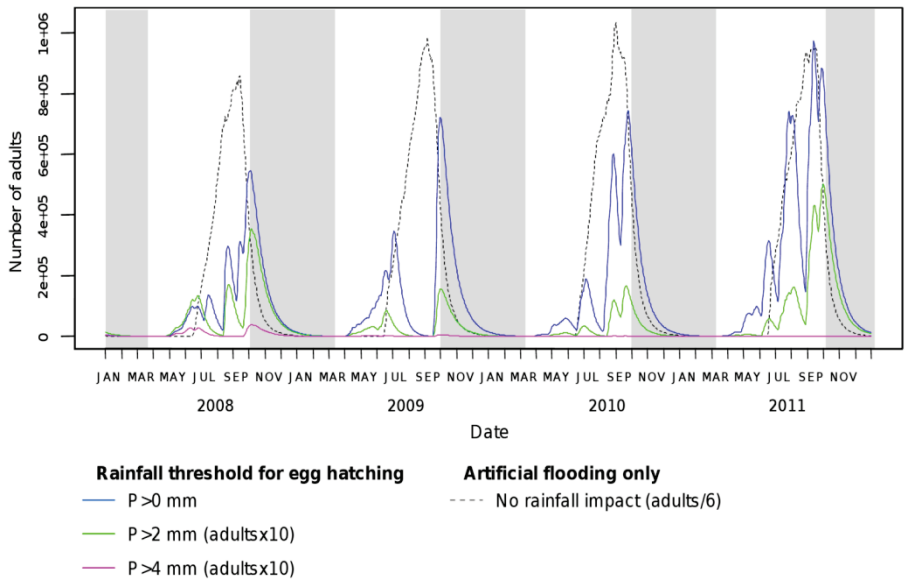


Figure IV.7. *Aedes albopictus* adult population dynamics with an egg hatching function driven either by rainfall or artificial flooding. Simulations were computed for different thresholds applied to daily rainfall for triggering egg hatching (P > 0 mm, P > 2 mm, and P > 4 mm).

The sensitivity analysis identified six key parameters for the population dynamics model of *Ae. albopictus*. An improved knowledge of those parameters through laboratory or field studies will increase the precision of the model predictions. The sex-ratio of *Ae. albopictus* populations has been well studied (Delatte *et al.* 2009). The mortality rate at emergence measured in the field will have to be compared with laboratory data. The transition rates could be better estimated from laboratory studies under controlled temperature conditions.

The sensitivity analysis demonstrates the importance for an invasive mosquito population in a temperate climate of the duration of the favorable period. To survive mosquito phenology must be adapted to their local environment, including a timely, appropriate initiation of the diapause process. *Aedes albopictus* diapause is a crucial adaptation, strengthening the survival capacity of the eggs to winter (Hawley *et al.* 1989). The diapause process is induced by a critical day length which is species- and location-dependent. This photoperiodism response is the main factor of adaptation of species to seasonal change. As compared to other studies on life history traits evolution in other animal species, the evolution in photoperiodism by *Ae. albopictus* is the fastest recorded (Urbanski *et al.* 2010b). Field studies on *Ae. albopictus* populations in Southern France showed that eggs enter diapause progressively between late August and mid-October (Lacour *et al.* 2012). The importance of the end of the favorable period, as emphasized by our results, suggests that the model could be modified to take into account this gradual entry in dormancy of *Ae. albopictus* populations. In the same way, the progressive hatching of diapausing eggs in spring (Toma and Miyagi 1990) could be modeled. Furthermore, a different egg survival rate during both the favorable and unfavorable periods could be integrated in the model, to better simulate the incidences of diapause and weather harshness. Indeed, the modeled winter's egg survivorship is lower than estimations in semi-controlled field studies (Hanson 1995, Hanson and Craig 1995a).

The environment's carrying capacity in larvae and pupae could be better estimated from field studies. These values depend on the number of available breeding sites in the field and on larval and pupal densities, which can reach up to 10 individuals per cm^2 in laboratory (Tsuda *et al.* 1991). *Aedes albopictus* immature stages are mainly found in anthropogenic breeding sites, however there is an extreme spatial heterogeneity of breeding sites composition and abundance in the field. Field studies measuring different entomological indices (House, Breteau and Container

indices (Anon 1972, Breteau 1954)) suggest that the most suitable environment for the development of *Ae. albopictus* is a dense residential area combining individual houses and shaded gardens (Carrieri *et al.* 2011b). Therefore, information on the relationship between the type of urban land cover (residential areas with gardens, high density development areas, etc.) and the number of available breeding sites, complete with field measures of larva and pupa abundances, would help adapt the model according to land cover, with environment carrying capacities varying in time (with the rainfall) and in space. This would allow the development of a space-time population model, taking into account urban environment heterogeneity.

The model's parameters identified as the most influential could be the potential control points of the biological system. Hence, vector-control strategies achieving the modification of these parameters can be expected to influence notably the biting rate and therefore the associated risk of pathogen transmission to humans and animals.

Chemical or biological treatments of larval instars, as well as the physical destruction of breeding containers will increase the mortality rate at emergence and decrease the environment's carrying capacity in pupae. Obviously, adult mosquito control treatments will increase the mortality rate of adults. The use of repellents may raise the length of the host-seeking phase and, consequently, the related lethality.

Modifying the end date of the favorable period of activity for mosquito is a priori inconceivable. However, it is possible to mislead photosensitive stages by using night-interrupted light, thus avoiding diapause initiation and artificially decreasing winter survival of the populations. Previous experiments on mosquitoes and other insects like butterflies brought to light the photoinhibition of diapause as a way of control (Barker *et al.* 1964, Beach and Craig 1979), but data on the efficiency and the applicability of such a method in a context of urban vector control is lacking.

To the best of our knowledge, there is no method to change the sex-ratio at the emergence. Yet, the impact of sterile insect technique (SIT), consisting in releasing sterile males which will compete with wild males for mating with females, could be modeled as a first approximation by diminishing the sex-ratio at the emergence. Indeed, females which have mated with a sterile male will have no offspring, and could be removed from the modeled adult population.

The primary application of our model is its use to elaborate and test effective control strategies against the Asian tiger mosquito. Indeed, there is currently no clearly-defined and efficient vector control strategy. All existing tools present problems for routine large scale applications (Medlock *et al.* 2012). Breeding sites can be physically removed to prevent the proliferation of *Ae. albopictus* populations. Yet, to be efficient such actions require repeated interventions and thus (*i*) the involvement of an important part of the vector control agencies' workforce and (*ii*) a strong mobilisation of the population through an appropriate communication plan. SIT remains very expensive and better adapted to isolated areas like islands. The use of chemicals (*i*) has adverse effects on the environment and health and (*ii*) can led to the development of resistance in mosquito populations (Labbe *et al.* 2007, Marcombe *et al.* 2011). In this context, only an integrated management combining these methods can carry out to an efficient and durable vector control strategy (Abramides *et al.* 2011). However, parameters of these optimal strategies (time of application, duration, number and frequency of the treatments, etc.) are complex to determine. Thus, modeling approaches can be helpful to test and compare mosquito population reduction methods, before long and expensive operational field trials. These studies should address the compared efficiency of control tools according to different conditions of use. Special attention should be paid to the optimal time of application: indeed, if insecticides treatments are usually started and repeated during the second semester of the year, when mosquito abundance is high in the field (Abramides *et al.* 2011), modeling studies suggest that applying treatments earlier—in spring—may be more efficient (Cailly *et al.* 2012). Modeling approaches should also address other conditions of use such as the number of traps, treatments frequencies, the minimal percentage of the targeted mosquito population affected by the treatment, or the level of maintenance of community implication required to reduce mosquito population.

Another perspective of the use of our model concerns the assimilation of the predicted abundance of host-seeking mosquitoes into a predictive model of host-vector contacts taking into account the human population density and exposure (Poncon *et al.* 2008). Such an approach will provide maps of the entomological risk induced by *Ae. albopictus*, taking into account the seasonal variations of host and vector distributions. The *Ae. albopictus* dynamics model could also be linked to an epidemiological model of transmission, such as compartmental models (Dumont and Chiroleu 2010, Chen and Hsieh 2012) or agent-based models (Linard *et al.* 2009), to study in more details the possible spread of dengue or chikungunya viruses.

Conclusions

The population dynamics of *Ae. albopictus* in a temperate climate environment have been modelled for the first time, using a mechanistic approach. The model, driven by temperature and rainfall, correctly predicted entomological field data of egg stage over a four year period. It can be used efficiently as a tool to predict *Ae. albopictus* population dynamics, and to assess the efficiency of different control strategies.

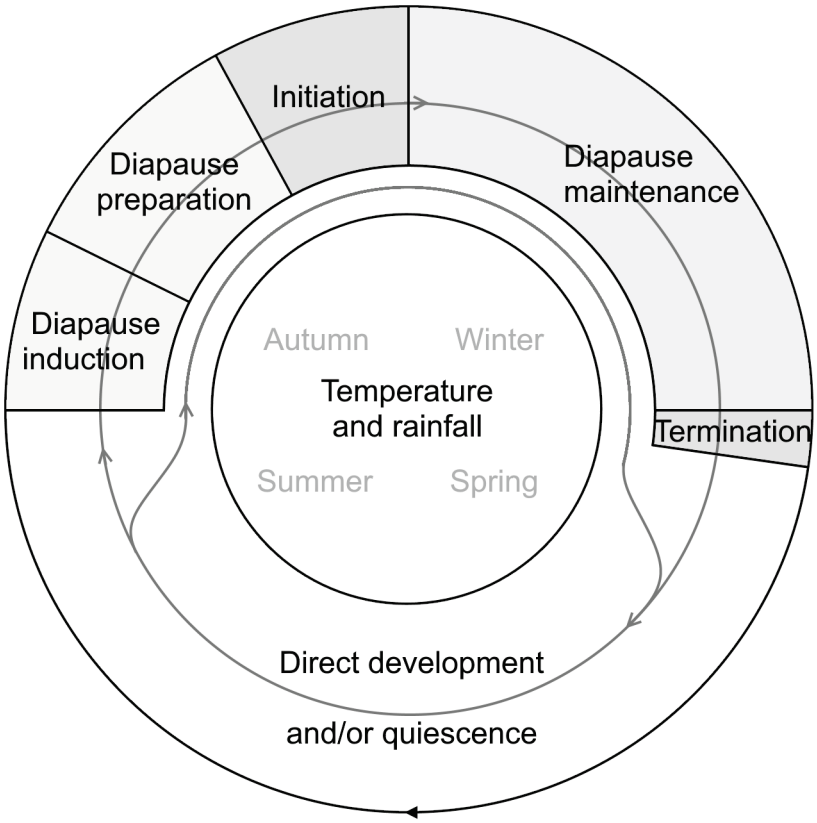
Acknowledgments

This study was conducted within the framework of the European Life+ project ("Integrated Mosquito Control Management", LIFE08/ENV/F/000488). The authors thank Didier Fontenille (Institut de Recherche pour le Développement—IRD), Benjamin Roche (IRD), Lucas Léger (IRD), Yvon Perrin (Centre national d'Expertise sur les Vecteurs—CNEV), and Jean-Michel Roques (AMENYS), for fruitful discussions on the modelling of *Ae. albopictus* populations, and Nathalie Barras (EID) for manuscript revision. Thanks are due to the four anonymous reviewers for valuable comments and advices for improving the text.

Author Contributions

Conceived and designed the model: AT, GLA, GL, MD, MC, PC, MAK, TB, PE. Provided laboratory and field data: GL, RB. Analyzed the data and validated the model: AT, GLA, GL, MC, MD. Wrote the paper: AT, GLA, GL, MD.

In the next paper, we developped an original approach –combining semi-field experiment and modeling with the model previously developped (paper IV)- to demonstrate the adaptive value of *Ae. albopictus* in Mediterranean area on the long term, rather than on one or two generations as previously tested in scientific litterature. The survival data of the overwintering generation of a diapausing and a non-diapausing groups of *Ae. albopitus* were implemented in the model, and the simulated dynamics of a temperate population able or not to diapause were compared.



Highlights (paper V)

- The diapause process did not improve or reduce the survival or wing shape in the overwintering temperate individuals in Mediterranean area.
- Diapause main role is to avoid precocious egg hatching and subsequent larva mortality under fluctuating winter conditions.
- The diapause modeling of the deterministic model of *Aedes albopictus* population dynamics was improved.
- The theoretical diapause shutdown did not lead the population to the extinction but its seasonal population dynamics and abundance was dramatically impacted.

Why Bothered About Winter? The Adaptive Advantage of Diapause for *Aedes albopictus* in Mediterranean Area.

Paper V

Guillaume Lacour[18,19], Annelise Tran[20,21], Jorian Prudhomme[22], Christophe Lagneau[18], Thierry Hance[19]

In preparation

Abstract

The Asian tiger mosquito is a widely spread invasive vector species native in tropical South-East Asia. For the last 40 years, the species has spread to temperate regions of the northern hemisphere due to its egg diapause ability. The aim of this study is to quantify the adaptive advantage of diapause in the context of the colonization of the Mediterranean basin, at the geographic margin of diapause necessity. Firstly, the ability of *Aedes albopictus* to overwinter was investigated. Diapause-induced temperate eggs, non-diapause-induced temperate eggs, and tropical eggs unable of diapause were exposed to winter outdoor environment -sheltered from rainfall- from November 2012 to mid-March 2013. Next, eggs were stimulated to hatch every fortnight in the laboratory, larval survival and wing shapes were analyzed. In the temperate strain egg survival rate was not significantly improved by diapause, but was higher than in the tropical strain. The activation of diapause did not change larval survival and wing size or shape in the temperate strain. The essential function of the egg diapause is to avoid early hatching during mild periods of winter. Secondly, these data were integrated to a rainfall- and temperature-driven abundance model. The generated simulations were validated with field data and were used as the reference model for *Ae. albopictus* dynamics. A knocking-out simulation of the diapause ability was compared to the reference model. The diapause inability resulted in the continued hatching of mosquito eggs up to December instead of October, with host seeking females active up to the end of the year. The mosquito population did not become extinct through time but persisted at a very low density, and with a 3-months delay of the beginning of the population density increase. We conclude that diapause tremendously

[18] EID Méditerranée, Montpellier, France.
[19] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium.
[20] CIRAD, UPR AGIRs, Sainte-Clotilde, Reunion Island.
[21] CIRAD, UMR TETIS, Montpellier, France.
[22] IRD, MIVEGEC, UMR IRD224-CNRS5290-UM, Montpellier, France

increases the survival ability of *Ae. albopictus* in the French Mediterranean area. These results are discussed in terms of control strategies and history of invasive mosquito species' establishment in Europe.

Keywords

Aedes albopictus; egg diapause; winter survival; population dynamics

Background

Winter conditions are a strong limiting factor for physiological processes and for the distribution of ectotherm organisms under temperate regions (Tauber *et al.* 1986). It is also the case in blood-sucking insects for which host availability is not the major constraint, whereas suitable developing environment is needed at larval stage (Denlinger and Armbruster 2014). Seasonal dormancy allows mosquitoes to survive during a long period under adverse conditions. This phenological adaptation allows maintaining viable populations under winter diapause in temperate climate or aestivation during dry and hot season. Winter represents a major obstacle for invasive mosquito species which are accidentally introduced in new northern areas by international trade and mass travel. Many examples of failed establishment of *Aedes* species have been reported over the last decade (Medlock *et al.* 2015). The invasive species *Aedes albopictus*, known as the tiger mosquito, is a good example of an invasive species which expanded to Northern temperate area through intercontinental trade of used tires and plants containing eggs. The introduction of the Asian tiger mosquito was reported in continental USA in 1985 in Texas, and until now it has colonized at least 32 states. Although the first report of *Ae. albopictus* in Europe was in Albania in 1979, the major wave of infestation started from its introduction in Italy since 1990 (Medlock *et al.* 2015). Its continental spread is mainly assured by the passive transport of propagules by road traffic (Roche *et al.* 2015). It has quickly become established in the Mediterranean regions and is expanding its spread toward northern regions. Its northern limit distribution range is currently in France, with a confirmed established population in the city of Strasbourg and some newly founded populations in the outskirts of Paris (EID Méditerranée 2015).

The establishment of self-sustaining populations and the spread of *Ae. albopictus* in temperate area are made possible by its winter egg diapause (Denlinger and Armbruster 2014). Egg diapause is maternally-induced in this species based on the photoperiod and temperature conditions underwent by the females in autumn (Mori *et al.* 1981, Pumpuni 1989). The diapause process improves the egg resistance to desiccation (Sota and Mogi 1992a, Urbanski *et al.* 2010a) and to cold and freezing (Thomas *et al.* 2012). The diapause advantage for egg survival was demonstrated by many field studies in coldest regions of the North hemisphere (Nawrocki and Hawley 1987, Hawley *et al.* 1989, Hanson and Craig 1995a, Kobayashi *et al.* 2002, Mogi *et al.* 2012, Rochlin *et al.* 2013). However, the Asian tiger mosquito must not only be able of diapause but also well synchronized with local seasonal conditions to overwinter, otherwise they failed to establish (Andreadis 2009, Deblauwe *et al.* 2015). The monitoring of *Ae. albopictus* populations in Japan and United States during 20 years shows the extremely rapid evolutionary response of the critical photoperiod, inducing diapause in 50% of eggs laid, to field selection (Urbanski *et al.* 2012). In Southern latitude of North America, the diapause incidence decreases due to a warmer environment (Urbanski *et al.* 2012). In southern Europe, ovitrap field samplings provide clues of the occurrence of this evolution of the diapause incidence. The maximum weekly diapause incidence recorded reached more than 95% in French Riviera compared to 83% in Roma, Italy, 200 km further South (Lacour *et al.* 2015, Toma *et al.* 2003). We have compiled data on the time of the egg laying period in Europe; it shows also a negative correlation with the latitude in Northern area, within a range from Israel to North Italy (figure IV.8). Except for Rome (Romi *et al.* 2006), there is not a continuous period of oviposition monitored during the 52 weeks of a year above a latitude of 40°N. In southern towns, fewer data are available but partial ovitrap survey in Consenza, Italy (39°18′N) and observations in Cartagena, Spain (37°36’N), suggested a continued ELP among years in function of winter meteorological conditions (Bonacci *et al.* 2015; Collantes *et al.* 2015). The egg diapause is effective in *Ae. albopictus* populations of the Mediterranean basin and present a typical latitudinal cline as the diapause incidence and the critical photoperiod in United States and Japan (Urbanski *et al.* 2012).

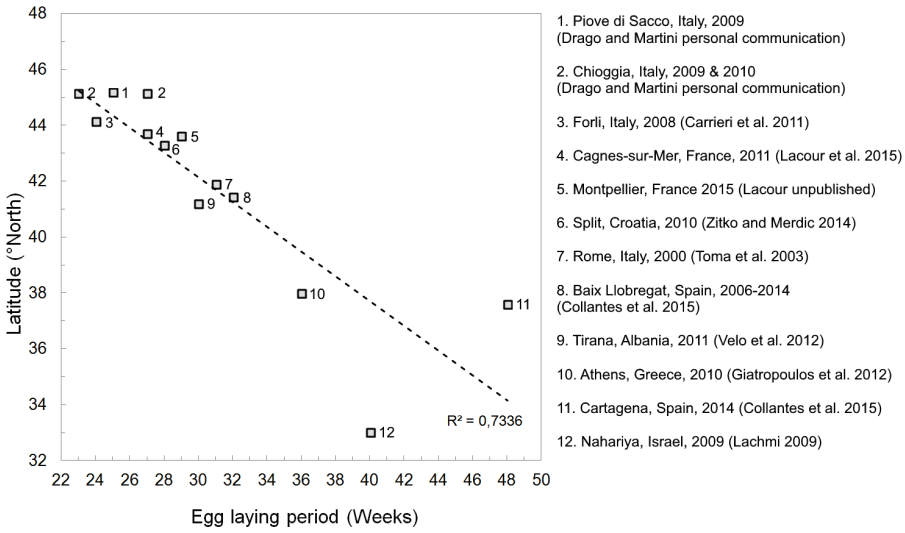


Figure IV.8. Length of egg laying period (ELP) of *Aedes albopictus* in Europe and Mediterranean basin. Only weeks with mean number of eggs/ovitrap > 1.0 were used to calculate the length of egg laying period, based on weekly monitored ovitraps data from different studies. The period of reproductive activity is discontinuous in the Mediterranean area due to winter. The ELP show a latitudinal cline (linear correlation r^2=0.734)

The diapause syndrome is a life-history trait currently found in all *Ae. albopictus* Mediterranean populations. The necessity of diapause can be questioned, as the 10°C coldest-month isotherm which separate continuously breeding populations and obligatory dormant populations of *Ae. albopictus* passes through Mediterranean basin (Mitchell 1995). Indeed, history shows that the closed tropical species *Ae. aegypti*, unable of diapause, was well established in this area. Until 1960's years, the Yellow fever mosquito was widely distributed around the Mediterranean area, including French ports, and even abundant in Italy, Spain and Corsica Island (Holstein 1967). The disappearance of this species was not linked to climatic change, and the species is currently re-establishing in Southern and Eastern areas of the Mediterranean area (Medlock *et al.* 2015). The presence of a non-diapausing species proves that diapause is not crucial for the invasive process in an area characterized by winter temperatures from cool to mild. The question is: what is the adaptive advantage bringing by the diapause to the colonization of the Mediterranean basin by *Ae. albopictus*?

The objective of this study is to demonstrate and to quantify the ecological advantage of diapause on mosquito population, in warm temperate climate, by coupling experimental and modeling approaches. In a first step, a semi-controlled field experiment was realized during winter, to compare the effects of diapause on life-history traits of a tropical and a local temperate *Ae. albopictus* in which diapause was induced or not. The direct effects of diapause were analyzed with egg survival and temporal egg hatching patterns. The potential delayed influence of egg diapause was investigated on phenotype markers of larvae and emerging adults, respectively the survival of 3-day-old larvae and the differences between wing size and shapes. In a second step, these data were integrated in a mechanistic population dynamics model. After validation of the model, simulations were realized to compare the population dynamics between diapausing and theoretical non-diapausing populations. The simulated population abundance changes were analyzed as a macroscopic revelation of the physiological influence of the diapause process. By this modeling approach, we aim to analyze the adaptive advantage of winter diapause for invasive mosquito in Mediterranean area.

Methods

Ethic statement

We used the animal facility of the “EID Méditerranée” (Montpellier, France), which has received accreditation from the French Ministry of Agriculture to perform experiments on live guinea pig (permit number C34-172-29) in appliance of the French and European regulations on care and protection of Laboratory Animals.

Mosquitoes

Two strains of *Ae. albopictus* were used in this study: a temperate one able to diapause and a tropical one unable to diapause (Lacour *et al.* 2014). The temperate strain, named SPAM, was collected in 2007 in the coastal area of Nice, France (43°41′ N, 7°16′ E). The tropical strain is native to La Reunion Island, located South-East of Africa near the Madagascar island, and was collected in 2011 in the coastal area of Saint-Denis city (20°52′ S 55°26′ E). The F18-19 and F4 maternal generations were used respectively for the

temperate and tropical strains. Mosquitoes of both strains were reared according to a standardized protocol under an environment of 21.5°C, 80% relative humidity, and a photoperiod of 16 hours of light and 8 hours of darkness.

Egg preparation for overwintering

Larvae of each strain were reared in batches of 500 larvae per pan (30.5x20x6cm) in 2 L tap water and fed with 3.5 g of milled dog food during larvae development. After pupation, 500 pupae were placed per pan and transferred in cages in photoperiodic chambers. Pupae from tropical and temperate strains were submitted to non-diapausing long-days conditions (LD) with a light:dark cycle of 16h:8h, and pupae from the temperate strain were reared under diapause-inducing short-days conditions (SD) with a light:dark cycle of 9h:15h. Adult mosquitoes were supplied with access to 10% sucrose solution. The first blood meal was provided on anesthetized guinea pig 10 days after emergence, to ensure a strong diapause induction in SD temperate females. All females had access to a water-filled oviposition cup containing a Whatman paper sheet from the fifth to the tenth days following the blood feeding. About 1,000 to 3,000 eggs were laid on each paper sheet, stored for 5 to 10 days at 70% humidity rate to complete embryonation. For each of the 3 experimental groups (temperate LD, temperate SD and tropical LD), four paper sheets of eggs were used, from the first two gonotrophic cycles of two different cages.

Each paper band with embryonated eggs was stored in an open and weighted plastic cup placed inside a 3 L plastic bucket filled with 0.2 L of tap water (figure IV.9). Each bucket was closed with a tight insect-proof net, to prevent egg laying and insect activity, and placed in a rack, protected from direct sunlight and rainfalls by a plastic roof. The rack was positioned outdoor along a wall of the EID Méditerranée main facility in Montpellier, France (43°36' N 3°52' E). The outdoor transfer of egg batches was realized during the second half of October, corresponding to the end of *Ae. albopictus* egg laying activity in the field, when the diapause is fully initiated in eggs of the overwintering generation (Lacour *et al.* 2015).

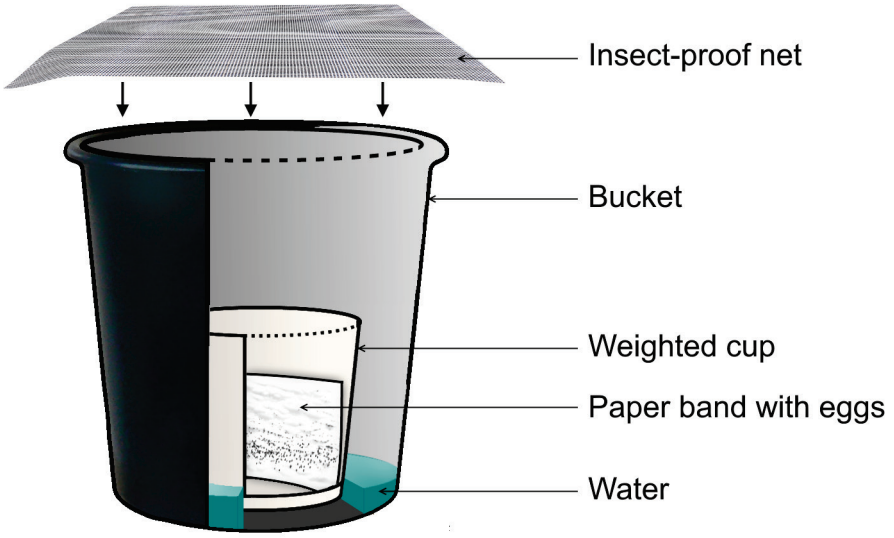


Figure IV.9. Experimental overwintering container for *Aedes albopictus* eggs. A bucket closed by an insect-proof net, containing a little of water and an open dry plastic cup with the paper band with eggs. This device protects the eggs from rain, drought and insects.

Eggs winter survival

All egg batches were brought into the laboratory on March the 11th 2013, period where the first larvae are usually found in the field (Lacour *et al.* 2015). Eggs were immediately submitted to a standardized hatching protocol: each test group of eggs was immersed in 150 ml of oxygenated tap water with 60 mg of milled dog food during 30 minutes. A dose of 15 mg of ascorbic acid was added in water to suppress the quiescence. The next day, eggs were brought out and let to dry during 2 hours, and were submitted to the hatching protocol a second time. Hatched eggs were counted and removed from the paper band. Unhatched eggs were stored under a light:dark cycle of 16h:8h at 21°C and 70%RH. The hatching protocol was repeated for 48 hours every fortnight.

After 4 egg hatching replicates, 44 days after the return of egg batches in laboratory, unhatched eggs were bleached in a bath of Trpiš solution to determine their embryonation status. Unhatched eggs were considered to be embryonated only when embryos were fully developed and without

deformity. Egg hatching rates of each test group and temporal replicate were calculated with embryonated and hatched eggs:

Hatch % = (hatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)

Larval survival

The early survival rate of immature stages was estimated on larvae issued from the first hatching. Larvae were transferred and reared in pans with 2 L tap water and 3.5 g of milled dog food. Three days after egg hatching, the first to third instars living larvae were counted and the survival rate was calculated as:

Early larval survival rate = 3-day old living larvae/hatched eggs

Wing shape and size measurement

To determine whether the egg diapause affected the individual development after diapause, the wing shape and size was investigated as marker of complete development in the temperate strain (Klingenberg 2010). The tropical strain has a genotype different from the temperate strain; consequently it was not used because it would have been a biased control group in this experiment. After the counting of the 3-day old living larvae, 4 replicates of 30 larvae per maternal photoperiod were transferred in plastic cups with 150 ml of water and 60 mg of food.

Emerging adults of *Ae. albopictus* were frozen. The first 39 females to emerge were sampled and their right wings removed. These wings were shaved by friction in a 50% ethanol solution, fixed on microscopic slides with Euparal and flattened under cover glasses. A digital camera Leica DFC425 (5 mega pixels) was used to capture the wing pictures. Then, a set of 20 landmarks located at veins and wing margin intersections were digitized with tpsDIG2 2.18 software (University of New York, Stony Brook, NY 11794-5245, available at http://life.bio.sunysb.edu/morph) (figure IV.10). The morphometric analyses were performed using TET and MOG modules of the CLIC package (Dujardin, available at http://life.bio.sunysb.edu/morph). The landmark configurations were scaled, translated, and rotated against the

consensus configuration by the GLS Procrustes superimposition method (Rohlf 1990). The partial warps obtained were used to extract principal components to compare both experimental groups. To assess the degree of similarity between groups, pairwise Mahalanobis distances were calculated and tested by nonparametric permutation tests with 1000 iterations each. Centroid size, defined as the square root of the sum of squared distances of a set of landmarks from their centroid, was analyzed as a size estimator using a nonparametric Wilcoxon-Mann-Whithney test or Kruskal Wallis test.

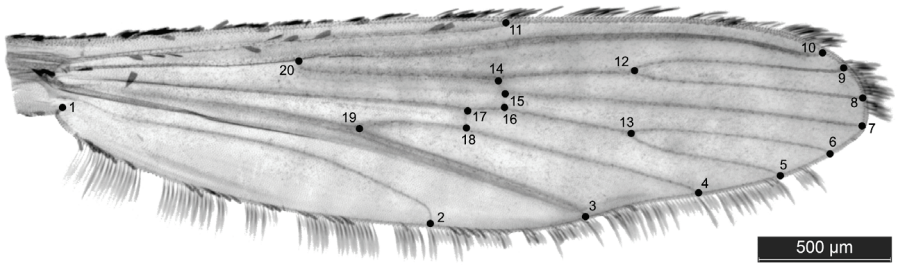


Figure IV.10. Set of 20 landmarks used on shaved right wing of *Aedes albopictus* female.

Meteorological data

The daily minimum (T_{min}) and maximum (T_{max}) air temperatures (°C) and daily precipitations (mm) were obtained from the Montpellier airport meteorological station, at 10.2 km from the study area. Daily data were pooled to calculate the average maximum temperature of the warmest month (M), the average minimal temperature of the coldest month (m), and the annual rainfalls (P). The Emberger coefficient (Q) was calculated according to this formula:

$$Q = 2000P / ((M+m+546.4).(M-m))$$

The data were reported on pluviothermic climograph to qualify the Mediterranean climatic conditions during the experiment (Daget 1977).

Development and validation of dynamics models of Aedes albopictus populations with diapause ability or inability

We used a mechanistic model of mosquito population dynamics (Cailly *et al.* 2012) adapted to *Ae. albopictus* (Tran *et al.* 2013). This model succinctly considers the effects of meteorological variables (temperature and rainfall) in a temperate urban environment (with water artificially supplied or conserved in a fixed numbers of breeding sites) on the abundance of all the life stages of the Asian tiger mosquito. The model was modified to better represent progressive egg diapause initiation, differential egg survival during favorable and unfavorable periods and progressive egg diapause termination (Annexe 1). The additional model parameters and functions were based on the results of the previously described overwintering experiment.

The model was run using daily meteorological data from Nice airport (France), obtained from the national meteorological service “Météo France” from 2007 to 2013. The simulated abundances of eggs newly laid were compared to the observed average number of eggs per trap in six different sites close to Nice, where entomological field surveys took place between 2008 and 2012 (see Tran *et al.* 2013). The degree of association between observed and simulated number of eggs at the time of ovitrap collection was assessed for each collection site by calculating the Bravais–Pearson correlation coefficient.

The comparative simulations of population dynamics between a diapausing and a theoretical non-diapausing populations were realized with the 6 years meteorological data of the Nice airport. Both *Ae. albopictus* populations ran as “naturally” diapausing for the first 3 years to have a mosquito density equal to well-established populations, then a diapause shut-down was simulated in the non-diapausing population for 3 next years, corresponding to the switch off of the previously described diapause parameters.

Results

Climatic and meteorological conditions

The study was realized during semi-arid years (Q_{2012}= 55.6; Q_{2013}= 63.6) with a fresh winter ($m_{2012\text{-}2013}$ = 1.55°C). During the winter 2012-2013, there were 30 days with negative T_{min}, among them 25 days with T_{mean}<5°C (figure IV.11). There was only one cold day (*i.e.*, T_{min} was lower than -5°C and the

T_{mean} negative). The winter was marked by 3 cold periods longer than a week (T_{mean}<10°C): a first for 16 consecutive days in December, the second for 23 consecutive days in January and the last but longest for 27 consecutive days in February. At the end of January, there was a 4-day period combining conditions for ice formation: rainfall and negative T_{min}. One episode of important rainfall occurred during winter (58 mm the 10/11/2012).

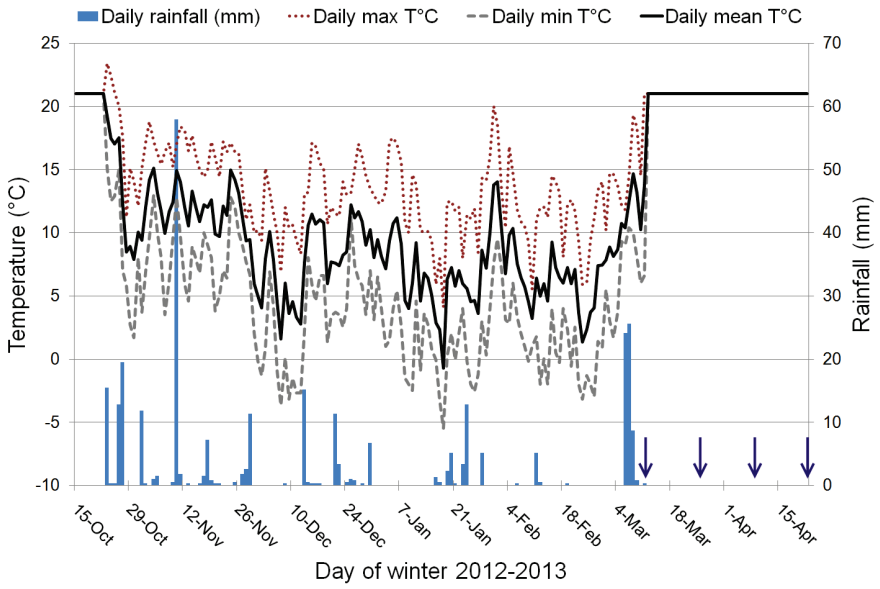


Figure IV.11. Meteorological conditions endured by *Aedes albopictus* eggs during the winter 2012-2013 in Montpellier, France. Minimal (dashed line), maximal (dotted line) and average (bold line) temperatures are represented. Eggs were protected from rainfalls (bars) during the experiment. The first plateau of temperature represents the embryonation at 21°C in laboratory; the second one is the return of eggs in insectarium for egg hatching stimulation (arrows) and life history traits characterization.

Winter survival of eggs

Diapausing temperate group has a temporal pattern of egg hatching relative rate significantly different than non-diapausing and tropical groups (Wilcoxon test, p<0.05) (figure IV.12A). Almost all of the living eggs of the tropical strain hatched at the first stimulus (mean ± s.d.: 98.8 ± 0.3%). The LD temperate egg hatching rate is decreasing too but more progressively (63.0 ± 21.0% of

hatching after the first hatching stimulus, 91.0 ± 6.6% 2 weeks later). The variability between the different bands of LD temperate eggs is very high. In contrast, the hatching rate of the SD temperate strain is very low after the first stimulus, at 12.3 ± 10.1%. It supports the claim that eggs were returned in laboratory at the date of diapause termination, when first larvae are usually found in the field. A fortnight later, 90.4 ± 6.6% of SD eggs had hatched, and the cumulated hatching rate raised 99.4 ± 0.5% a month after being back in the insectarium. These results suggest diapause termination can occur as fast as 15 days in *Ae. albopictus* egg bank.

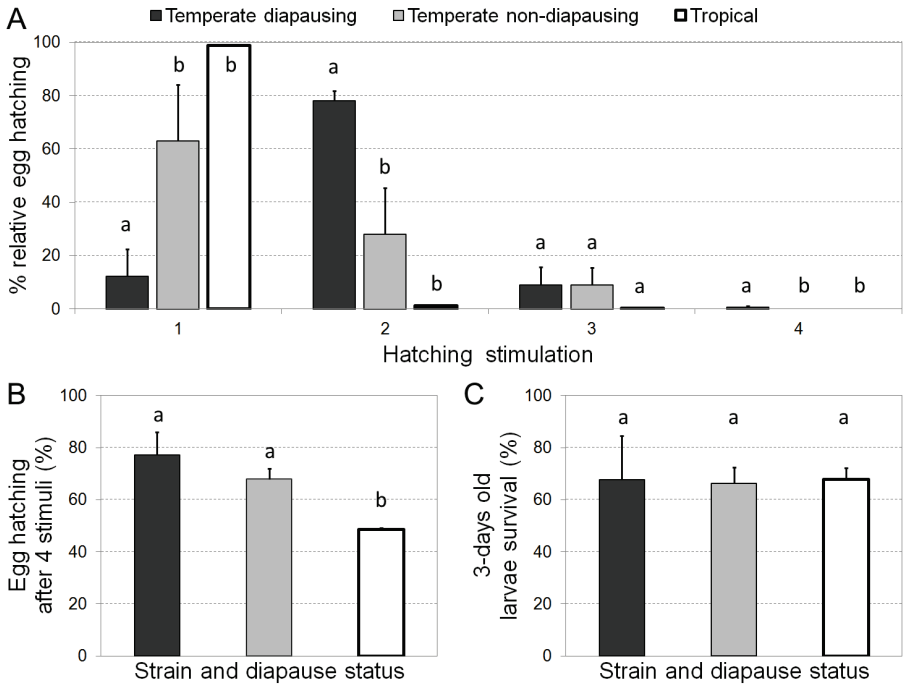


Figure IV.12. Diapause influence on *Aedes albopictus* of a temperate and a tropical strain overwintering in Mediterranean area. (A) Relative hatching and (B) total survival of eggs, after 4 hatching stimuli with an interval of 2 weeks between each. (C) Early survival of larvae hatched at the first hatching stimulus. Different letters indicate statistically different groups (Wilcoxon test, $p<0.05$).

The global survival rate was 77.1 ± 8.9% for SD temperate and 67.9 ± 4.1% for LD temperate (figure IV.12B). There was no significant difference of winter egg survival between temperate groups (Mann-Whitney; p=0.2), however tropical eggs have a significantly lower survival rate compared to the temperate strain with only 48.5 ± 0.6% of survival (Mann-Whitney; p= 0.02). Survival rate was based on egg hatching during 44 days and is assumed to not underestimate the survival of diapausing eggs, even as there were still few egg hatchings on day 44 (equivalent to 0.6% of the total egg hatching).

Larval survival rate at day 3 was 67.6 ± 17.0% in SD temperate strain, 66.3 ± 6.2% in LD temperate strain and 67.9 ± 4.2% in LD tropical strain (Figure IV.12C). There is no significant difference of survival between groups (KW; p=0.67).

Wing size and shape

The wing shape did not differ statistically between the temperate groups (figure IV.13). Mahalanobis distances between both groups (p>0.05) and the centroid size (diapausing group = 3.24 ± 0.20; non-diapausing group = 3.15 ± 0.17) showed no differences linked to the diapause (Wilcoxon-Mann-Whitney; p=0.117).

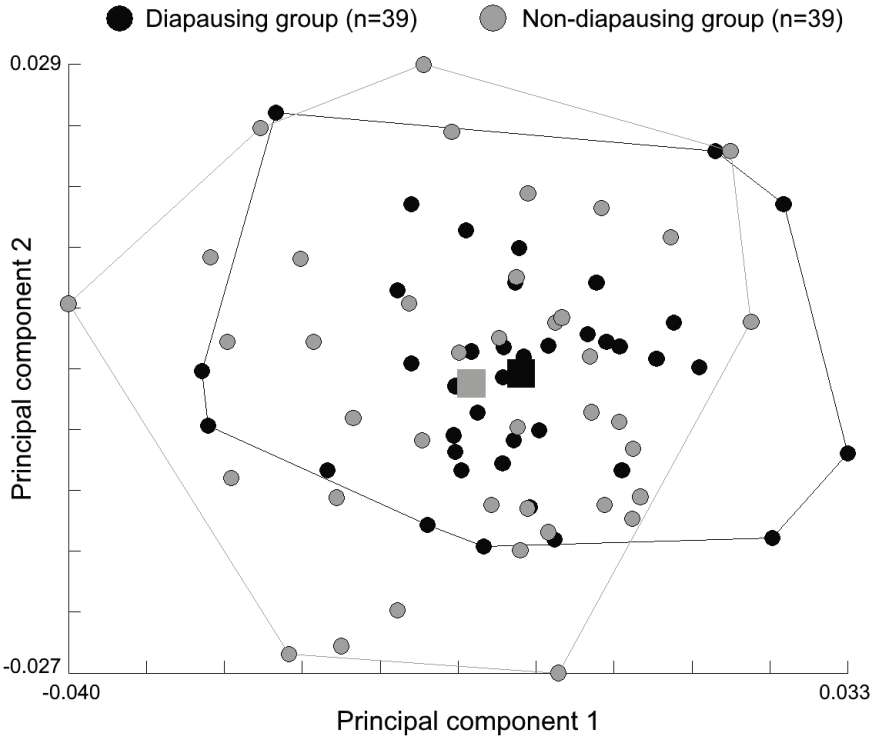


Figure IV.13. Wing shape adults of a temperate local strain of *Aedes albopictus* exposed to winter in semi-field condition at the stage of diapausing egg (in black) or non-diapausing eggs (in grey). Squares represent mean centroid sizes.

Model validation and simulations

The results of the semi-field experiment were used to define the period of the transition function from diapause to favorable season in the model, and to define the daily egg mortality rates of diapausing and non-diapausing temperate eggs during the unfavorable season (table IV.1).

Table IV.1. Parameters modified into the published model of Asian tiger mosquito population dynamics (Tran *et al.* 2013).

Parameter	Definition	Value
μ_{E1}	Egg mortality rate during favorable season (day^{-1})	0.05
μ_{E2}	Egg mortality rate during unfavorable season (day^{-1})	0.015
d_1	Earliest date for the start of favorable season (Julian day)	day 67
d_2	Latest date for the start of favorable season (Julian day)	day 81
d_3	Earliest date for the start of unfavorable season (Julian day)	day 230
d_4	Latest date for the start of unfavorable season (Julian day)	day 312

The model 2 correctly predicted the field egg abundance (table IV.2). The variability of the cross correlation coefficient is admittedly bigger among the model 2 simulations than the previous model, but the correlation seems mostly improved on the area with a high-density ovitraps. Consequently, the improved model can be used to test a theoretical shut-down of *Ae. albopictus* diapause in the Mediterranean area.

Table IV.2. Correlations between field and simulated population dynamics of *Aedes albopictus* egg laying. City networks are classed by ovitrap density, with higher densities at the bottom of the table (Tran *et al.* 2013).

Campaign		Cross-correlation coefficient
City	Years of trapping	
Nice-1	2008-2011	0.76
Cagnes-sur-Mer	2010-2012	0.69
Biot	2010	0.80
La Gaude	2010	0.91
Villeneuve-Loubet	2011	0.88
Nice-2	2011	0.96

The diapause shut-down was simulated for 3-years. The photoperiodically-induced diapause is completely initiated in the natural strain at the same period of the year (October). Immature stages of the diapause-free strain continue logically their development during the autumn until December

(figure IV.14A). The following years, the larval population recovery was strongly delayed by 3 months. Indeed, the egg bank density for the diapause free strain is dramatically low after winter.

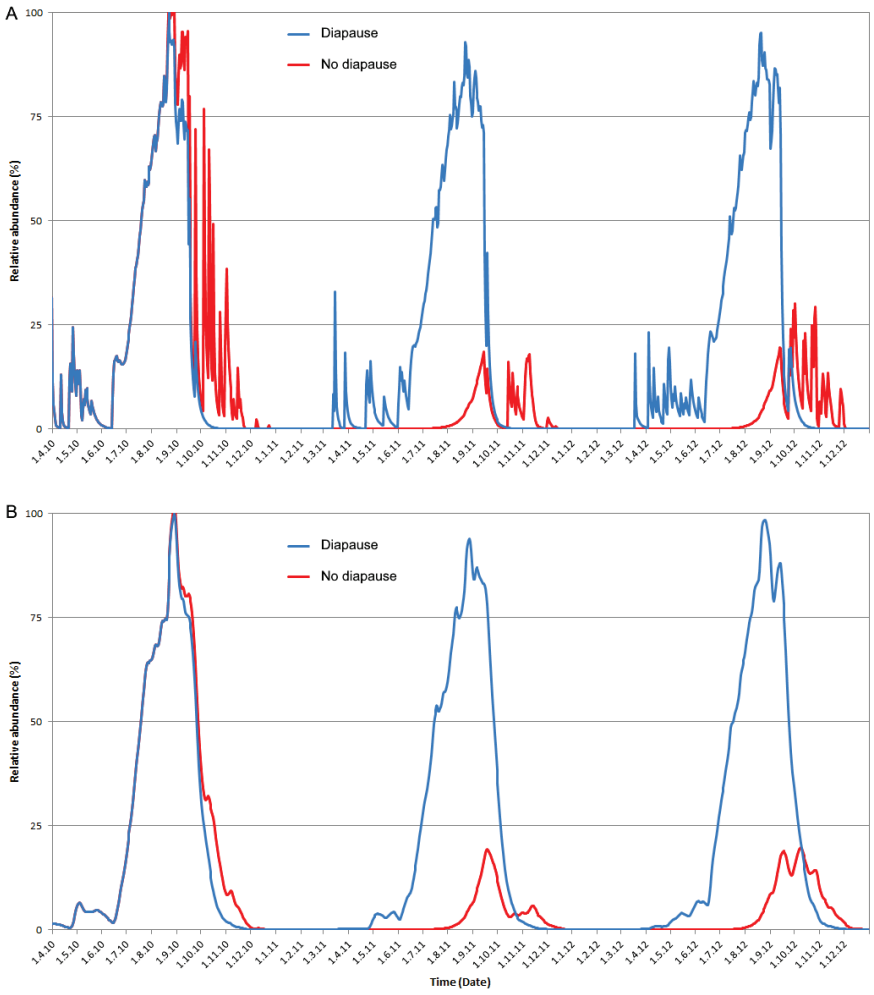


Figure IV.14. Simulated population dynamics of the Asian tiger mosquito in a Mediterranean climate during 3 years. Comparison between the relative density of *Aedes albopictus* larvae (A) and host-seeking females (B) able (in blue) or unable (in red) of egg diapause.

The annual maximal density of diapause-free female adults is decreased by 80% compared to a diapausing population (figure IV.14B). The following years, the increase of the population density begins 3 months later than a diapausing strain. The seasonal period of activity of the diapause-free strain ends one month later, when the environmental conditions become harsh for survival. However, the population density keeps low but stable from year to year at a large scale, even if local extinctions are possible.

Discussion

The diapause did not improve or reduce *Ae. albopictus* egg survival or spring larval survival, neither than resulting different female's wing phenotype. The only life-history trait that was greatly modified by diapause was the egg hatching response, with the majority of diapausing eggs insensitive to hatching stimulus until the second half of March. In the light of these results, it appears that egg diapause should have an unique role during Mediterranean winters: to prevent early egg hatching under unfavorable conditions for larval development.

Even if our study was based only on two strains of different generations, our results confirmed the conclusion of a quite similar experiment realized in Nagasaki, Japan (Mori *et al.* 1981). Indeed, non-diapausing eggs are sensitive to environmental hatching stimulus during the unfavorable season and risks to hatch before the arrival of lethal low temperature for larvae. The threshold temperature for larval development is around 11°C (Hawley 1988); the time needed for the complete development of larvae (Waldock *et al.* 2013) is extremely unlikely to happen before the occurrence of a lethal cold period. Even the buffer effect of aqueous medium on temperature would not be sufficient to prevent from a cold period lasting almost one month in our study. The possibility of winter larval activity was tested in Nagasaki, but none of *Ae. albopictus* larvae developed or survived in the field from December to February (Tsuda and Takagi 2001). Furthermore, fourth instar larva is very sensitive to cold temperature: twenty hours at 5°C or less are enough to kill all larvae of a tropical strain from Taiwan, when more cold-resistant first instars larvae underwent 50% of mortality after 5 days at 10°C (Chang *et al.* 2007). Winter hatched larvae are exposed to others meteorological dangers which characterized the Mediterranean climate, as container drying or the opposite, heavy rainfalls (as for one day in November 2012) sufficient to flush out *Ae. albopictus* larvae from small containers (Dieng *et al.* 2012).

In Nagasaki, Japan, no difference of survival was found between non-diapausing and diapausing eggs (Mori *et al.* 1981). The same observation was made in our experiment. With almost similar meteorological conditions, both study areas (Nagasaki and Montpellier) presented a relatively mild climate allowing a high survival rate of eggs with or without diapause. The diapausing status of eggs plays a crucial role for survival in Northern regions, such as in Indiana State, USA: 59% of diapausing eggs of a Japanese temperate strain survived until March to cold winter compared to 7% of non-diapausing eggs (Hawley *et al.* 1989). Actually, lethal temperature is quite difficult to precisely determine for *Ae. albopictus* eggs. In laboratory, a 4 hours exposure to 0°C killed half of non-diapausing eggs of a non-acclimated Italian strain (Thomas *et al.* 2012). However, a prolonged cold-conditioning can strongly improve the cold-resistance of non-diapausing and diapausing eggs of *Ae. albopictus* (Hawley *et al.* 1989, Hanson and Craig 1995b). Hanson and Craig observed 50% of mortality in temperate eggs after a 24 hours exposure at -8°C in non-diapausing and non-acclimated eggs, at -11°C in non-diapausing and acclimated eggs, and -12°C in diapausing and acclimated eggs (Hanson and Craig 1995b). Our experimental eggs had at least one month of acclimation with decreasing temperatures before the first day of negative temperature on December the first. The acclimation must be considered before applying laboratory results in a field context. Erguler and his collaborators arrived at a similar conclusion on the increased resistance of eggs to cold in the field than in laboratory with a modeling approach (Erguler *et al.* 2016).

Until 2016, the winters following the study were warmer than the winter 2012-2013, with a mean minimal temperature of the coldest month from 1.2 to 4.2°C higher than $m_{2012\text{-}2013}$. The probable direct consequence of this warming is an increase of the egg survival. In the future, the climate change scenarii predict an increase of the average winter temperature from 2.25 to 3.7°C until year 2100 (Giorgi and Lionello 2008). The biological response to climate change in mosquitoes is expected to be an adaptation of the diapause induction and termination to adjust to shorter winters, but also a decrease of the diapause incidence in the Southerners' mosquito populations.

Understanding the course of diapause may be significant to develop a new strategy to mosquito population control, by inhibiting diapause and foiling winter survival (Tauber *et al.* 1986, Hanson *et al.* 1993). It can be realized via photoperiodic inhibition of diapause, a technique usually tested toward pest species of agricultural interest (Barker *et al.* 1964, Tauber *et al.* 1986), but also on the rock pool mosquito *Ae. atropalpus* (Beach and Craig 1979). Other diapause disruption tools are going to be available in the future, based

on chemicals (Suman *et al.* 2015), or on genetic alteration of the presumably existing “universal diapause gene set” in female adults (Poelchau *et al.* 2013a).

The high survival rate of tropical eggs during our experiment is quite surprising. However, it was demonstrated that tropical and subtropical eggs of *Ae. albopictus* could survive a few days in an average temperature of -5°C, and the absolute T_{min} registered during the winter 2012-2013 was only -5.5°C (Mogi 2011). In a Japanese field experiment, less than 15% of tropical eggs survived when the absolute T_{min} was under 0°C (Hanson 1995). Tropical strain survived at the same rate than temperate eggs in Nagasaki (Hanson 1995). These Japanese eggs having passed one more month than ours in field conditions, the age of eggs could explain the survival difference, which is not only caused by cold but mostly by desiccation (Mogi 2011).

The fact that a lot of tropical eggs can survive under Mediterranean winters is a bad omen for the future. Tropical species could partially offset the handicap of diapause inability by overwintering in hibernacula sheltered from winter cold or flooding. This hypothesis is backed up by genetic evidence of a year-to-year maintenance of a population of *Ae. aegypti* in Washington D.C., USA (Lima *et al.* 2016). This region is colder than the Mediterranean area but *Ae. aegypti* persists here since about 4 years, supposedly in subterranean premises. Consequently, the temporary or long-term colonization of tropical species in temperate regions is likely to happen (Mogi 2011). It is especially feared for *Ae. aegypti*, at the gates of Europe since its establishment in the island of Madeira in 2004 (Medlock *et al.* 2015). This risk will magnify with global warming, and should be now considered by mosquito management organisms.

The primary role of egg diapause in warm temperate climate is to prevent fall and winter hatchings. The mechanistic modeling approach used in our study is not free of simplifications but is helpful to understand and quantify the advantages of diapause on the population dynamics of *Ae. albopictus*. Combined with experimental results, it is an efficient way to demonstrate that diapause is the winning asset to have a successfully infestation with invasive mosquito species in a Mediterranean climate.

Author Contributions

Conceived and designed the experiments: GL, CL, TH. Performed the experiments: GL, AT. Analyzed the data: GL, JP, TH. Wrote the paper: GL, TH.

Additional file 1: *Aedes albopictus* modified model

The model is based on a system of ordinary differential equations (ODE) describing the dynamics of mosquitoes in different stages. Following (Cailly *et al.* 2012, Tran *et al.* 2013), all of the steps of the mosquito life cycle are represented in the model: three aquatic stages (*E*, eggs; *L*, larvae; *P*, pupae), one emerging adult stage (A_{em}), three nulliparous stages (A_{1h}, A_{1g}, A_{1o}), and three parous stages (A_{2h}, A_{2g}, A_{2o}), considering females only in the adult stage.

To take into account a progressive egg diapause initiation (at the end of the favourable season), a progressive egg diapause termination (at the start of the favourable season), a transition function f_Z was defined as a trapezoidal-shaped membership function:

$$f_Z(t) = \begin{cases} 0 & \text{if } t \leq d_1 \text{ or } t \geq d_4 \\ (t - d_1)/(d_2 - d_1) & \text{if } t \geq d_1 \text{and } t \leq d_2 \\ 1 & \text{if } t \geq d_2 \text{and } t \leq d_3 \\ (d_4 - t)/(d_4 - d_3) & \text{if } t \geq d_3 \; t \leq d_4 \end{cases} \qquad \text{(Eq. 1)}$$

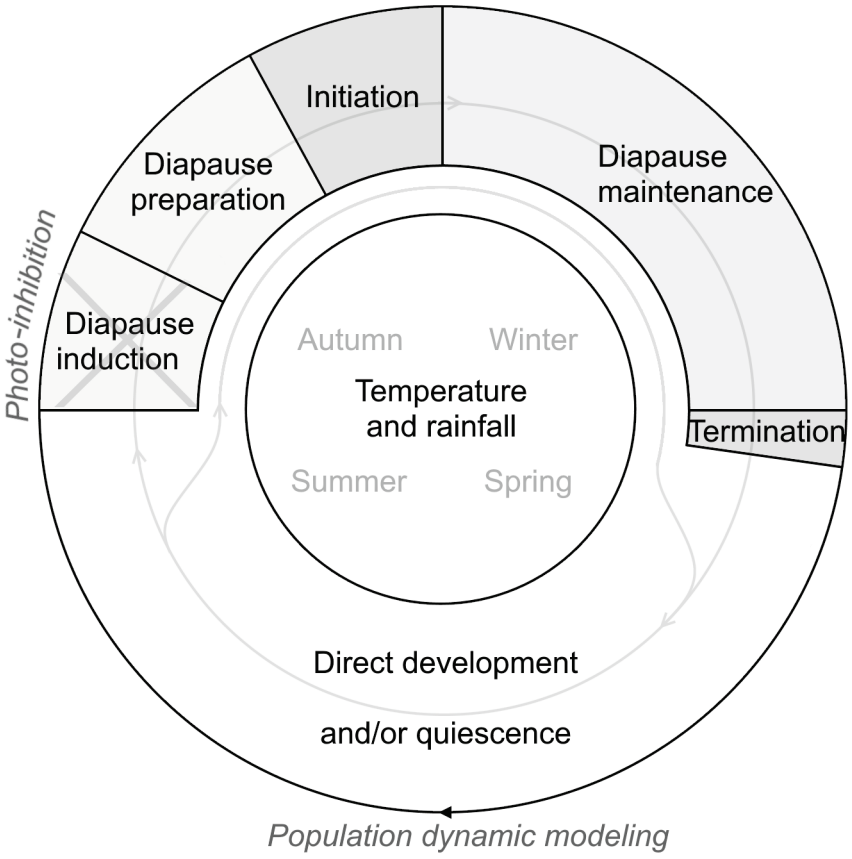
With d_1 and $_{d2}$ the earliest and latest dates for the start of the favourable season, respectively, and d_3 and d_4 the earliest and latest dates for the start of the unfavourable season, respectively.

In addition, two different egg mortality rates μ_{E1} and μ_{E2} were considered for the favourable and unfavourable seasons, respectively.

All other functions and parameters are the same as those described in (Tran *et al.* 2013), and the modified ODE system is:

$$
\begin{cases}
\dot{E} = \gamma_{Ao}\left(\beta_1 A_{1o} + \beta_2 A_{2o}\right) - \left[f_z * (\mu_{E1} + f_E) + (1 - f_z) * \mu_{E2}\right] E \\
\dot{L} = f_z * f_E E - \left[m_L\left(1 + L / k_L\right) + f_L\right] L \\
\dot{P} = f_L L - \left[m_P + f_P\right] P \\
\dot{A}_{em} = f_P P \sigma \exp\left[-\mu_{em}\left(1 + P / k_P\right)\right] - \left[m_A + \gamma_{Aem}\right] A_{em} \\
\dot{A}_{1h} = \gamma_{Aem} A_{em} - \left(m_A + \mu_r + \gamma_{Ah}\right) A_{1h} \\
\dot{A}_{1g} = \gamma_{Ah} A_{1h} - \left(m_A + f_{Ag}\right) A_{1g} \\
\dot{A}_{1o} = f_{Ag} A_{1g} - \left(m_A + \mu_r + \gamma_{Ao}\right) A_{1o} \\
\dot{A}_{2h} = \gamma_{Ao}\left(A_{1o} + A_{2o}\right) - \left(m_A + \mu_r + \gamma_{Ah}\right) A_{2h} \\
\dot{A}_{2g} = \gamma_{Ah} A_{2h} - \left(m_A + f_{Ag}\right) A_{2g} \\
\dot{A}_{2o} = f_{Ag} A_{2g} - \left(m_A + \mu_r + \gamma_{Ao}\right) A_{2o}
\end{cases}
$$

The previous paper demonstrated the adaptive value of diapause to the expansion of *Ae. albopictus* populations in Mediterranean area. Consequently, altering the diapause initiation of the Asian tiger mosquito population may be a way of control, by reducing winter survival. Indeed, it is theoretically possible to mislead photosensitive stages by using night-interrupted light (Barker *et al.* 1964, Beach and Craig 1979).
In the next paper, we tested with laboratory tests and modeling the possibility to control the introductions of invasive mosquito species on localized sites by photo-inhibition of diapause induction in temperate area.



Highlights (paper VI)

- If it confers an undeniable biological adaptation to winter temperature fluctuations, diapause is also an Achilles tendon for *Aedes albopictus* that could be used to combat it.
- Application of a one-hour night-interrupting light after 9 hours of scotophase inhibits diapause induction in *Ae. albopictus*.
- Diapause disruption decreases overwintering success.
- It could be a useful control tool against introduction of invasive mosquito species in used tires.
- This original approach can be seen as a practical application of our knowledge of the evolution of the species

Invaders in Tires: the Diapause Photo-inhibition Tool Against Introduced Invasive Mosquito Species

Paper VI

Guillaume Lacour[23,24], Annelise Tran[25,26], Grégory L'Ambert[23], Christophe Lagneau[23], Thierry Hance[24]

In preparation

Background

Since 2007, Europe is increasingly concerned by mosquito borne-disease formerly limited to tropical area, as chikungunya and dengue fever (Medlock *et al.* 2015). Outbreaks occurred because of the presence of three conditions: viruses are regularly introduced by viremic travelers, the population is immunologically naïve for arboviruses and some vectors species are established and increased their distribution range (Tomasello and Schlagenhauf 2013). The major way of introduction of invasive mosquito species (IMS) in temperate area is by international transport in infested used tires (Reiter 1998, Kuno 2012). The reintroduction of *Aedes aegypti* in El Salvador from United States, and the introduction of *Ae. albopictus* in 1985 in Texas, USA from Japan, are two examples of vector establishment caused by used tires importation (Kuno 2012). *Aedes* species easily survive long-distance transport inside tires under dormant desiccation-resistant egg stage, or develop in water puddles after rainfalls. The different control measures implemented as inspection and insecticide treatment of the used tire shipment are ineffective against dormant eggs (Reiter 1998, Kuno 2012). Continental transportation of used tires can also permit the expansion of the colonized area at a national scale, as tires constitute breeding sites for at least 32 species (Yee 2008); the spread of *Ae. albopictus* was facilitated by passive transport along the interstate highways in USA (Moore and Mitchell 1997) and in France (Roche *et al.* 2015).

[23] EID Méditerranée, Montpellier, France.
[24] Earth and Life Institute, BDIV, Louvain-la-Neuve, Belgium.
[25] CIRAD, UPR AGIRs, Sainte-Clotilde, Reunion Island.
[26] CIRAD, UMR TETIS, Montpellier, France.

In Europe, *Ae. albopictus* was successfully introduced in Italy in 1990-1991 in used tires from USA (Dalla Pozza *et al.* 1994). It quickly established and is currently colonizing all the South-Western area of Europe (Medlock *et al.* 2015). This event warned public health organisms of the reality of the risk and surveys of used tire companies were done in Europe. From 1999 to 2015, ten introductions of the Asian tiger mosquito in four sites of tires importation were detected in mainland France and systematically eradicated by insecticide treatments (Schaffner and Karch 2000, unpublished data) (figure IV.15). *Aedes albopictus* was detected in a used tire company of Vrasene, Belgium in 2000 and 2013 (Boukraa *et al.* 2013) and also further North in Netherlands in 2010 and 2011 in five sites (Scholte *et al.* 2012). Winter conditions and in some cases vector control operations eliminated the introduced populations (Deblauwe *et al.* 2015). Others IMS were unsuccessfully introduced in Europe in infested used tires. The most regularly detected IMS in Europe - after *Ae. albopictus* – was the American rock-pool mosquito *Ae. atropalpus*, discovered in Italy in 1996 (Romi *et al.* 1997), in France in 2003 and 2005 and in Netherlands from 2009 to 2011 (Scholte *et al.* 2012). *Aedes japonicus japonicus* and *Ae. triseriatus* were detected in France respectively in 2000 and 2004 (Schaffner *et al.* 2003). A population of *Ae. j. japonicus* was successfully introduced in Natoye, Belgium since 2002 (Versteirt *et al.* 2009) and maintained at least 10 years, until the first treatment in 2012 (Damiens *et al.* 2014). The Yellow fever mosquito *Ae. aegypti* was also introduced in Netherlands in 2010, but this tropical species is unable of diapause and could not survive to winter conditions of North Europe (Scholte *et al.* 2012). However *Ae. aegypti* could potentially established along Mediterranean area of Europe and must be carefully surveyed in southern sites (Medlock *et al.* 2015).

Early detection of the IMS, during the introduction stage, enables mosquito eradication by appropriate and rapid measures (Puth and Post 2005, ECDC 2012). The most efficient approach is preventing mosquito development by indoor storage of imported tires, secured from rainfalls. Nevertheless the construction can represent a substantial cost for companies and currently numerous storage areas are not covered. Even with a covered warehouse, prevention still depends of the human factor: product movements (arrival of tires) can generate temporary outdoor storage leading to flooding by rain and hatching of IMS eggs. The surveillance of these sites by public organisms is hence necessary, which generates an expenditure of public funds for private business. In addition, surveillance is limited by time and tools available for mosquito control operators, as adult traps and ovitraps are cheaper but less efficacy than larval prospection to detect IMS. In case of introduction, application of adult and larval insecticides is required. The treatment efficacy is driven by many factors (access to breeding sites,

meteorological conditions, etc.) and currently no insecticide with residual properties is commercially available in Europe (Baldacchino *et al.* 2015). Finally, treatments depend of the time of the season, because all the existing control methods are ineffective against diapausing eggs. The situation highlights the need of a complementary control tool against newly introduced IMS in used tire storages; a tool which would be efficient independently of IMS detection or human actions, which act against dormant eggs and preferentially affordable over time.

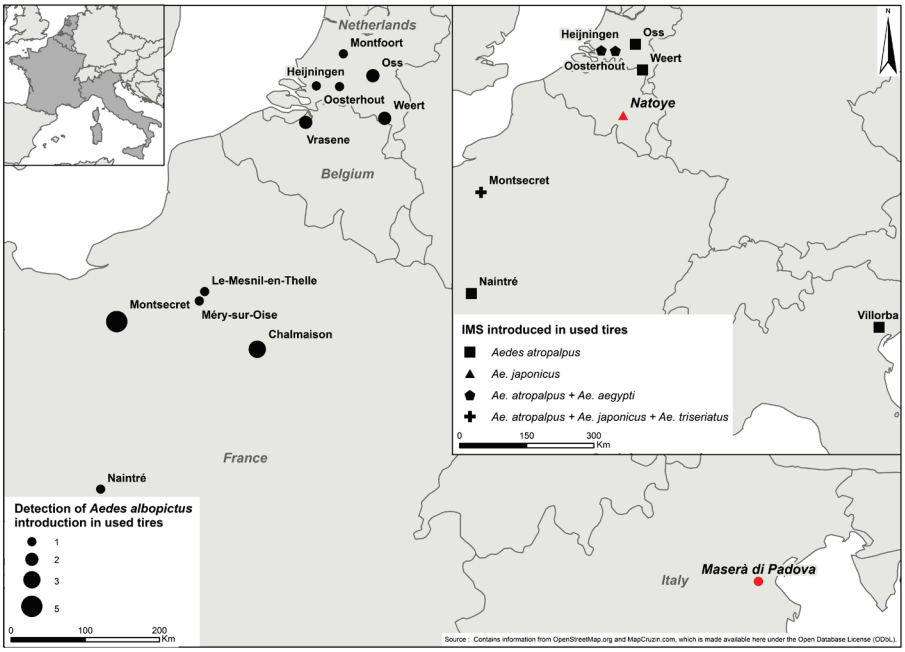


Figure IV.15. Review of the detections of invasive *Aedes* species introduced in used tires in Western Europe from 1999 to 2013. Italic city names (Masera di Padova, Italy and Natoye, Belgium) indicate successful establishment.

In temperate area, the naturalization of introduced mosquitoes depends of their ability to survive winter. The climatic synchronization is necessary to prevent the exposure of delicate stages at winter severe conditions and the consecutive mortality (Lacour *et al.* Paper V). The diapause state improves the physiological capacities of the dormant stage to overwinter, as cold and desiccation resistance among others (Denlinger and Armbruster 2014). The exposure of non-diapausing mosquitoes to severe winter conditions can result in failure to overwinter. In consequence, if diapause confers an

undeniable biological adaptation to winter temperature fluctuations, it is also an Achilles tendon for *Ae. albopictus* that can be used to control surviving of eggs in winter.

The diapause disruption was successfully tested on a temperate population of *Ae. albopictus* from United States mated with non-diapausing tropical males. It resulted in a low winter survival of the hybrid progeny (Hanson *et al.* 1993). An easier way than tropical male release to disrupt diapause induction is the use of artificial light during night (Barker *et al.* 1964). In *Aedes* species, the maternal exposure to short days induces usually diapause of the progeny, as in *Ae. albopictus* (Pumpuni *et al.* 1992) or *Ae. atropalpus* (Beach 1978). The annual variation in day length is the most reliable indicator of seasonality in temperate areas (Tauber *et al.* 1986). Insect endogenous circadian clocks can be misleading with altered photoperiod to prevent diapause induction (Barker *et al.* 1964). The photo-inhibition of diapause is an environmental method often experimented on pest species of agricultural interest, especially Lepidoptera (Tauber *et al.* 1986), but was also tested on *Ae. atropalpus* (Beach and Craig 1979).

In that context, our aim was to determine the efficiency of the photo-inhibition of diapause in the IMS *Ae. albopictus*. The optimal moment and duration of the light source during the scotophase were determined. The maximal efficacy obtained in laboratory was implemented in a deterministic population model. The theoretical efficacy of photo-inhibition on IMS is tested by modeling approach, and then the applicability of this control tool is discussed.

Material and method

Ethic statement.

We used the animal facility of the “EID Méditerranée” (Montpellier, France), which has received accreditation from the French Ministry of Agriculture to perform experiments on live guinea pig (permit number C34-172-29) in appliance of the French and European regulations on care and protection of Laboratory Animals.

Mosquito species and rearing protocol.

Experiments were made on the strain SPAM of *Ae. albopictus*, collected in 2007 in the coastal area of Nice, France (43°41′ N, 7°16′ E). The strain was reared in laboratory for 15 generations according to a standardized protocol under an environment of 21.5°C, 80% relative humidity, and a light:dark (L:D) cycle of 16:8 hours. Experimental batches of larvae were reared at a density of 500 larvae per pan in 2 L tap water and fed with 3.5 g of milled dog food. After the start of pupation, each pan was transferred in cages in photoperiodic chambers (described in Lacour *et al.* 2014). Pupae and adults were submitted to diapause-inducing short-days conditions of 9:15 L:D, without light transition between photophase and scotophase.

Moment and duration of night-interrupting light for efficient photo-inhibition of diapause induction.

In the experiment aiming to determine the optimal moment during the night to light exposure, three replicates of 7 groups of pupae short-day reared were daily exposed to a 1-hour light during scotophase, at 7 different times after the start of darkness (t): t+2h, t+4h, t+6h, t+8h, t+9h, t+10h and t+12h. In the “light exposure duration” experiment, three replicates of 7 batches of pupae were daily exposed at t+9h to a night-interrupting light of variable duration: 10, 20, 30, 60 or 90 minutes. In both experiments, emerged adults were supplied with a 10% sucrose solution. The blood meal was provided on anesthetized guinea pig 10 days after emergence, and then fed females were isolated into batches of 30 individuals for egg laying. All females had access to a water-filled oviposition cup containing a Whatman paper. Sheets of daily laid eggs were stored in darkness for 10 days at 70% humidity rate to complete embryonation.

Egg hatching protocol was done on 10-day old eggs. Sheets were immersed in oxygenated tap water during 30 min, and then a dose of 100 mg.L-1 of ascorbic acid was added to suppress egg quiescence. The next day, eggs were brought out and let to dry during 2 h, before being submitted a second time to the hatching protocol. Unhatched eggs were bleached for anatomic observation, and then considered to be in diapause when containing a fully developed first-instar larva (Lacour *et al.* 2014). Diapause rate was calculated with hatched and unhatched embryonated egg numbers:

Diapause incidence % = (embryonated unhatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)

Model simulation

We used a mechanistic model of mosquito population dynamics (Cailly *et al.* 2012) adapted to *Ae. albopictus* (Tran *et al.* 2013). This temperature and rainfall-driven model was developed to simulate temporal abundance of French local Asian tiger mosquito populations in a favorable temperate urban environment (with anthropogenic water supply). The diapause process development in the model is based on semi-controlled field experiments (Lacour *et al.* paper V) and the field phenology of French Mediterranean populations of *Ae. albopictus* (Lacour *et al.* 2015). The model was run using daily meteorological data from Nice airport, France, obtained from the national meteorological service “Météo France” from 2008 to 2013.

Firstly, we simulated an unique introduction of a small amount of *Ae. albopictus* eggs in used tires in order to have 100 active females in the beginning of July. The introduced population is submitted to a night-interrupted one-hour light since the 18th August during 6 years.

Secundly, we used the model to simulate the situation of a fully established population of *Ae. albopictus*. The comparative simulations of population dynamics between a diapausing population and a population submitted to night-interrupted one-hour light were realized with the 6 years meteorological data. *Aedes albopictus* populations developped normally for the first 3 years in the model to obtain a mosquito density equal to well-established populations; in a first simulation a diapause-inhibiting treatment was modeled at “Year 1” as applied from the last week of August until the end of the year. In a second simulation, the treatment was reproduced the next year.

Results

Moment and duration of light exposure for an efficient inhibition of diapause induction.

The diapause incidence in eggs is strongly reduced by a 1-hour light application 8 to 10 hours after the beginning of the scotophase (Wilcoxon test, $p<0.001$) (figure IV.16A). The diapause in *Ae. albopictus* is induced by quantification of the night length. The number of diapausing eggs decreased when the duration of the light increased until a plateau at 20% of diapause incidence from 60 minutes of light (figure IV.16B).

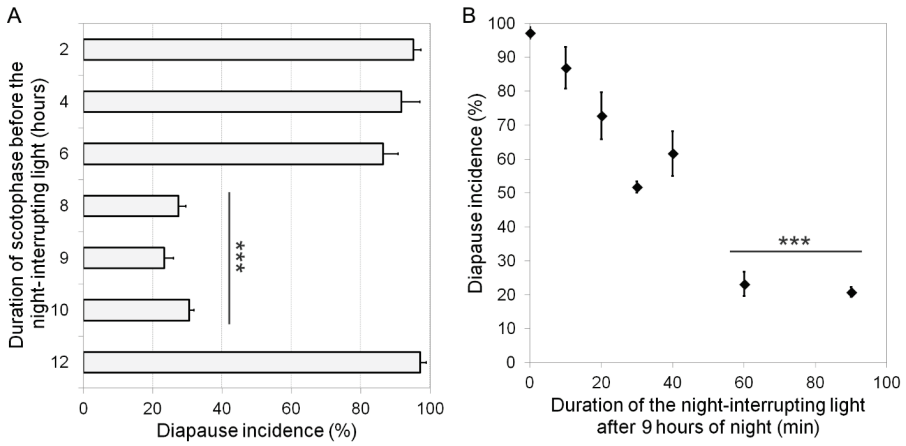


Figure IV.16. Efficacy of diapause photo-inhibition in *Aedes albopictus* eggs in function of the moment of application and the duration of a night light. (A) Application of a one-hour light at different time of the scotophase. (B) Duration of the night-interrupting light applied after 9 hours of scotophase (Mean ± standard-deviation). Significant difference are marked by asterisks (Wilcoxon rank sum test, $p<0.001$).

Model simulation

The model with photo-inhibition induced diapause in only 23% of the mosquito population. In the case of an unique introduction of a small population (400 blood-seeking females on the 18th August) of *Ae. albopictus* in a site treated by a night-interrupted one-hour light, the photo-inhibition elicited a complete eradication of the IMS during the first winter (when the 18th August during 6 years. In the presence of 700 blood-seeking females on the 18th August (and 4,300 larvae), the population persisted the year following the introduction but became extinct after 2 winters.

In the case of an effective photo-inhibition of 77% of a well established mosquito population, the maximal seasonal abundance of progenitors is decreased by 60% the year following the diapause photo-inhibition (figure IV.17). The maximal female abundance in the simulation (Year 1) is used as a reference for comparison. The population decrease resulted of the high mortality of non-diapausing immature stages during winter. Mosquito adult abundance increased significantly only since the end of July, because of the limited number of founder's individuals in spring. The photo-inhibition reduced the annual period of adult activity by one month (105 days with the females abundance >5% of the relative maximal abundance for photo-inhibited population in year 2), even if adults are active until December. The

arrest of the photo-inhibition treatment in “Year 2” enhanced the mosquito population to reach its natural abundance level the next year.

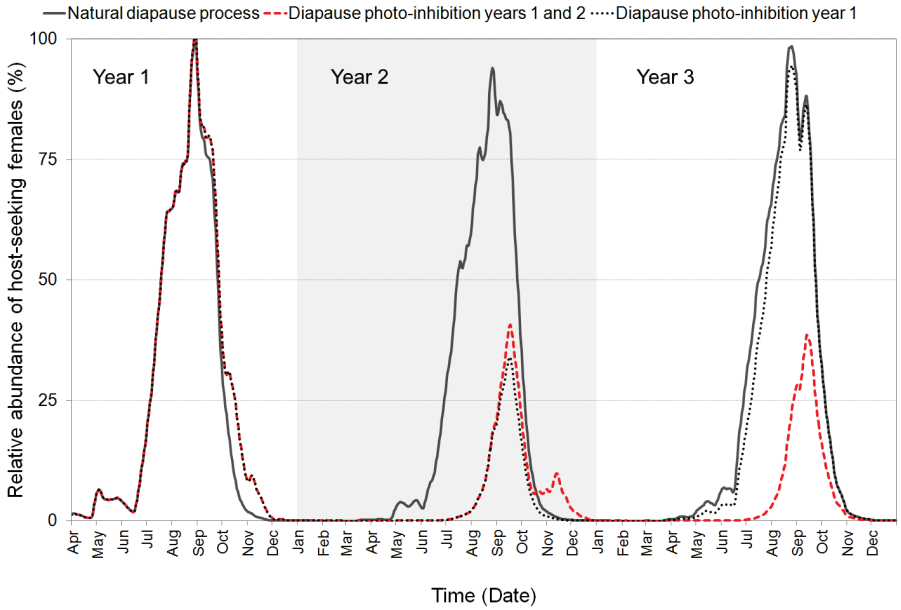


Figure IV.17. Modeling of the dynamic of established population of *Aedes albopictus* vector. Comparison of the natural field dynamics (grey line) with altered dynamics by application of a one-hour light after 8 hours of scotophase from mid-August to December during year 1 (dotted line) or years 1 and 2 (red dashes).

Discussion

The timing of effective diapause inhibition is similar among insect species (Shah *et al.* 2011). A one-hour light pulse done between hours 8 through 10 of the scotophase inhibited until 90% of diapause in the *Ae. atropalpus* strain (Beach and Craig 1979). The field experiment done in United States on the same species obtained only 12.5% of diapause in eggs deposited by females (Beach and Craig 1979). Consequently, laboratory results are repeatable with the same efficacy in the field. The efficacy of diapause inhibition in *Ae. albopictus* in laboratory experiments was slightly lower than results described in *Ae. atropalpus*, but the results are encouraging and demonstrated the potential of this method against *Aedes* IMS. The modeling approach done in this study used very favorable environmental conditions,

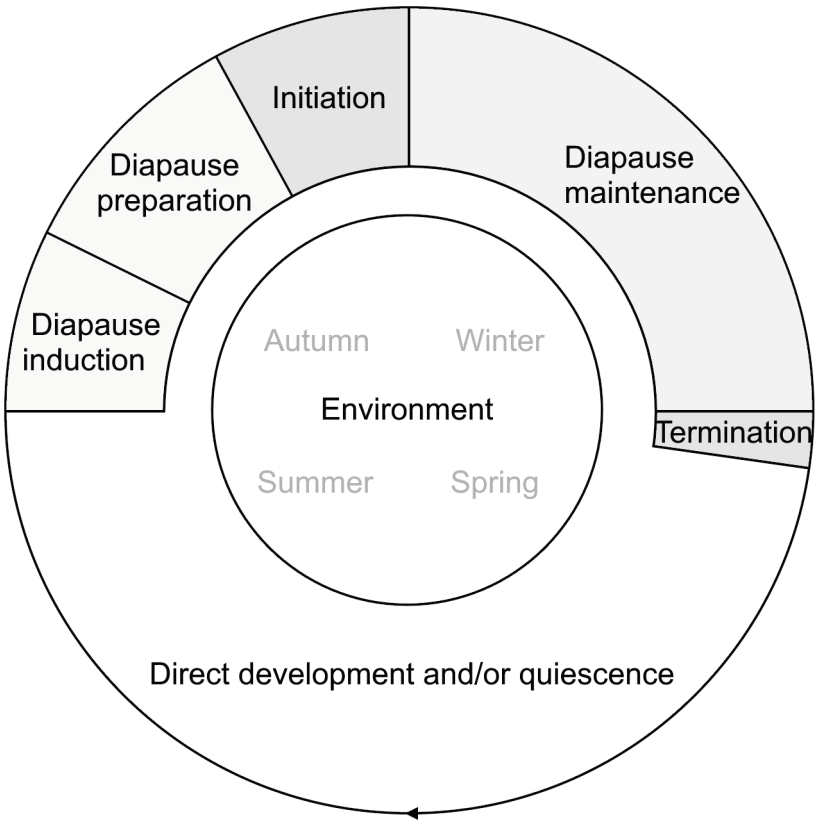
as a constant anthropogenic water supply of breeding sites and a very abundant number of mosquito founders which are not encountered in importation sites of used tires. Consequently it will not be surprising if the efficacy of diapause photo-inhibition in the field could be even better than modeled for *Ae. albopictus*. The method can be improved by determining the optimal light intensity and mosquito sensitivity to light spectrum, as the decrease of diapause incidence depends of the quality of the light (Shah *et al.* 2011). The sensitivity of the diapausing last instar larvae of *Chaoborus americanus* to the light spectrum was maximal at 540 nm, closed to the 500 nm registered in other insect species (Bradshaw 1972). The use of wavelengths from blue-green to yellow-green in the light spectrum would be recommended to limit possible adverse effect on non target wildlife. Indeed artificial lighting is known to alter a lot of biological process (reproductive physiology, migration, behavior, immunology, etc.) in a large part of organism (Longcore and Rich 2004). Insects are particularly sensitive to night lights, and for certain species light-traps are considered as the most efficient –and sometimes unique- control tool, as for the moth *Hylesia metabus*, responsible of Lepidopterism in French Guiana (Jourdain *et al.* 2012).

Photo-inhibition of IMS appeared to be a control method restrained to some specific situations, such as used tire outdoor storage, but should be particularly useful against Stegomyia species. Indeed, only pupae and adult stages are photosensitive and can induced diapause in *Ae. albopictus* (Pumpuni 1989). The mobility and the short range flight of the adults should expose them to the light, out of the shaded larval habitats. The prime use of this tool -complementary with usual tools as insecticide- is the eradication of newly introduced IMS. However its use in storage area of imported tires localized in colonized area may be recommended. Indeed, IMS introductions are a common occurrence, increasing the genetic diversity of the established invasive population. The successive introductions of large number of mosquito can avert the genetic bottleneck consecutive to foundation effect. This phenomenon was demonstrated in recent successful invasive populations of *Ae. albopictus* in Europe and USA (Urbanelli *et al.* 2000) and in *Ae. j. japonicus* in United States (Fonseca *et al.* 2010). It must not be underestimated in the invasive process, especially for IMS as *Ae. j. japonicus*. The introduced population in Belgium apparently did not spread from the site of introduction during at least 6 years (Damiens *et al.* 2014), reflecting probably a long process of naturalization. The photo-inhibition strategy could be interesting in northern part of Europe, where winters are severe and would impacts egg survival even in absence of complementary control tools.

Author Contributions

Conceived and designed the experiments: GL, GLA, CL, TH. Performed the entomological investigations and experiments: GL, GLA. Analyzed the data: GL. Wrote the paper: GL, CL, TH.

Chapter V. General discussion



The Asian tiger mosquito is a major concern related to vector-borne diseases, not only in tropical areas but also increasingly in areas with warm to temperate climates. It is an anthropophilic species for which a shift from a forest habitat to an urban one represents a major adaptation. The worldwide spread of this species is due to increasing international traffic and trade, and may be influenced by climate change allowing its settlement in new areas. International travelers who are in contact with diseases such as dengue, Chikungunya and Zika fevers risk bringing arboviruses to their country of origin. The conjunction of the two phenomena creates a new kind of sanitary risk in temperate area that is often difficult to estimate the extent. Yet, as we have shown with the following of the recent outbreak of Chikungunya, this risk for public health is real and must be anticipated.

This thesis is devoted to the study of particular biological properties of the Asian tiger mosquito that are some of the factors allowing the expansion of the species outside its natural distribution. One factor is the ability to enter diapause in the egg stage, through a signal maternally induced. This thesis also aims to contribute to the management of *Ae. albopictus* populations and by extension others invasive mosquito species. In this context, we used clearly a multidisciplinary approach to investigate diapause and other aspects of the vector biology: fundamental physiology and morphology, field population dynamic, modeling of natural and theoretical populations, and to a lesser extent sociological aspects. Our work was concluding by a practical proposition for mosquito control based on disruption of diapause in temperate area. This section aims to synthesize and discuss the principal findings of the thesis and to present perspectives to short and medium terms.

Scientific and methodological contribution

Prediapause

The influence of the prediapause stage on the embryogenesis kinetic and the egg morphology was investigated in the paper I (figure V.1). We demonstrated that the preparation stage increases the development time of embryo, contrary to previous observations of a strain from Manassa, in the United States (Reynolds *et al.* 2012). We stressed the role of the photoperiod underwent by the mother on the future development of the embryo. This helpful morphologic information will allow in the future to explore the molecular mechanisms of the diapause process in

Ae. albopictus. We have already gather proteomics data that are not present in this thesis. Indeed, an important work of interpretation of these data has still to be done. A clear understanding of embryologic development is needed because molecular and "-omics" studies have to compare similar developmental stages of diapausing and non-diapausing ovocytes and embryos, development that in fact did not occurs at the same moment after oviposition in the two physiotypes. In that context, the genome of *Ae. albopictus* was recently sequenced (Chen *et al.* 2015), which will help in further understanding of its biology even if annotation work is still needed. In the future, based on the amount of data available, the Asian tiger mosquito could become a reference model for diapause and developmental studies.

Conversely, we showed that the volume increase of diapausing eggs was not only caused by the diapause process as generally suggested (Reynolds *et al.* 2012), but depends on photoperiod (figure V.1). This observation is new and brings new insight in the diapause syndrome. Indeed, most of studies on mosquito egg size focused on egg length rather than egg width, but a study made on *Cx. pipiens* demonstrated than females exposed to short night of 3.5 h laid eggs with a smaller diameter than females exposed to night of 8 h (Honnen 2015). Egg diameter -and subsequently egg volume- seems negatively correlated to day length in several mosquito genera. Egg-size plasticity reflects the maternal influence on the offspring phenotypes and fitness. In insect species, egg size modification is often a response to environmental change or stress (Gillooly and Dodson 2000). However there is usually a tradeoff between egg size and egg number (Mousseau and Fox 1998).

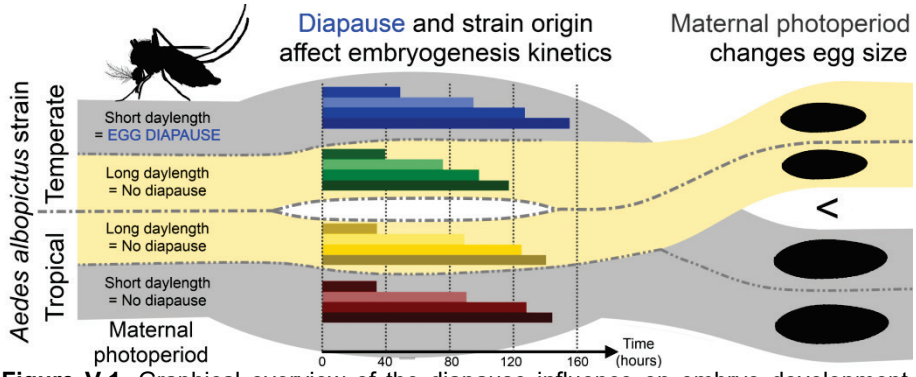


Figure V.1. Graphical overview of the diapause influence on embryo development timing and of the photoperiod on the egg volume in *Aedes albopictus*.

The analysis of the phenology of the Asian tiger mosquito showed ecological limitations in the efficiency of the maternal induction of diapause. The time of prediapause stage –from induction to initiation- took about 17 days, which is long in a mosquito life. It is mainly caused by the partial induction of diapause in the eggs of the first gonotrophic cycle (Pumpuni 1989). The timing of diapause induction allowed the complete development from one to two generations of progenitors. At a first glance, diapause induction could appear to be quite precocious, producing diapause eggs earlier than what would be predicted by adaptive optimality models. However the Mediterranean environment can not be considered as deterministic, with rainfall occurring scarcely and in highly variable proportions in autumn. *Aedes albopictus* presents a phenology in adequacy with a “bang-bang” strategy, but in a context of overlapping generations and in a stochastic environment (McNamara 1994). If the species was transferred in a more deterministic environment with regular rainfalls and egg hatching, we can predict that its CPP would likely adapt to lower day length.

The decrease of diapause incidence in eggs laid in late autumn suggests a seasonal selection process of non-diapausing individuals. These individuals are probably not capable of diapause due to genetic differences. It is consistent with the observation of a rapid loss of the maternally-induced diapause in laboratory strains reared under short-day conditions, in which diapause can be almost totally counter-selected in 6 generations (Pumpuni 1989). This seasonal shift in the population procured an advantage to colonize areas with warm climate, as observed during the spread of *Ae. albopictus* in Florida (Lounibos *et al.* 2011). This rapid adaptive phenomenon is expected to happen in the Mediterranean areas in the global warming context.

Modeling and field data

Modeling is a powerful approach to predict consequences of small changes in the biology or environment of a species on its future population growth. Furthermore, it is also a tool that can be useful for population management. In that context, we developed a deterministic model (Tran et al. 2013) to simulate the temporal evolution of abundance and dynamics of *Ae. albopictus* populations. The coupling of experimental and modeling approaches allows predicting the middle-term influence of the biological processes, from molecular to macroscopic scale. As suggested by the invasive history and the field surveys, modeling confirmed the importance of

human water supply in the rapid growth in abundance of the invasive population, passing in 3 years from the stage III (localized and numerically rare) to the stage V (widespread and dominant) of the invasive process (Colautti and McIsaac 2004).

The modeling approach also predicts that a non-diapausing population would have its phenology dramatically affected but would not obligatory being extinct despite winters (paper V). It demonstrates thus that diapause reduces the selection pressure due to winter conditions. Consequently diapause appears to be a non-essential but great adaptive advantage in areas at the margin of the temperate climate. Non-diapausing temperate and tropical eggs of *Ae. albopictus* survived to winter in Mediterranean climate if sheltered from rainfall. Diapause did not improve egg survival in field or life history traits in larval and adult stages in our study; the primal advantage of the diapause in Mediterranean area is to prevent early egg hatchings in a fluctuating winter environment. Precocious hatching would expose larvae to cold and result in high larval mortality rate. The absence of difference between diapausing and non-diapausing egg survival suggests either an absence of cold-hardy improvement in diapausing eggs, or a cold acclimation process in non-diapausing eggs as protective as diapause improvements in diapausing eggs (Hanson and Craig 1995b). Acclimation is also related to phenotypic plasticity; consequently the adaptive response to winter in Southern temperate climate would be either the diapause syndrome alone, or an association of diapause and cold acclimation.

Contribution for mosquito control application

The availability of the deterministic model of *Ae. albopictus* population dynamics (Paper IV) is of great interest for the development of integrated optimal control strategies. Indeed, until now deterministic models available are oriented to epidemiological aspects, concerned tropical and subtropical areas or are limited to some specific vector control parameters such as insecticide resistance (Andraud *et al.* 2012). The development of a deterministic model modulated by biological parameters of local populations helps mosquito control operators at their local scale. Model validation can be simply checked with survey data based on different traps (ovitrap, gravid trap, etc.). The efficiency of tool used can be simulated by entering in the model the tool efficacy measurements made in laboratory, semi-field controlled conditions or available in scientific literature. It provides a decision support for managers. The development of an user-friendly interface is still

considered for a larger use in French and European mosquito control organisms.

The possibility to mislead the photoperiodic control of the diapause induction was investigated in *Ae. albopictus* and other mosquito species (Paper VI) and tested with the model (Paper IV) improved to diapause simulation (Paper V). If diapause photo-inhibition appeared to be a quite more anecdotic method than other available control tools (Baldacchino *et al.* 2015), its use in specific situations (used tires storage areas for example) could be strategic to prevent or limit naturalization or genetic enrichment of invasive mosquito populations. The efficiency of the control by photoperiod alteration should present a clinal pattern, as the mortality rate of non-diapausing mosquito will increase with harsher winter conditions. Its application should be considered in northern areas of Europe which are not immune to invasive mosquito species (Scholte *et al.* 2012, Damiens *et al.* 2014).

No evidence was found of the overwintering ability of potential transovarial infection in *Ae. albopictus* collected in an area infested by CHIKV in the previous autumn (paper III). Seasonality exercises a major influence on the vector density (Tran *et al.* 2013) and the arboviral transmissions are highly dependent of the vector seasonality (Andraud *et al.* 2012). The winter and the associated diapause process of vectors are the major asset to avoid endemicity of mosquito-borne disease in temperate area. The surveillance by public health organisms of the infested cities in the spring following an outbreak is not necessary, as the risk of viral re-emergence is marginal.

Limitations of the study

This study is limited to the comparison of two laboratory strains (a temperate and a tropical) and one field population. Field experiments were made in the French Mediterranean area (from Nice to Montpellier), and the model was developed to be suitable to this geographical area. Analysis of other populations in other temperate area would strengthen and extend our conclusion. Moreover, we did not have the opportunity to test the photo-inhibition of diapause in the field on *Ae. albopictus* neither another IMS. Field test are still necessary to validate our conclusions (based on laboratory experiment and modeling) on the efficiency of this potential control method and to determine its feasibility.

Perspectives

The photoperiodic synchronization with climatic conditions is based on the sensitivity to a determined photoperiod. It results in a rapid selection of individuals with an appropriate critical photoperiod adapted to local environment (Urbanski *et al.* 2012). The photoperiod transition -the intrapopulational variability of day length around the critical photoperiod (CPP) which is effective to induce a part of diapause in the strain- appeared to be variable among the strains (figure V.2). The origin of this variability (plasticity or genetic selection) and its adaptive value are still unknown. Theoretically, a large photoperiod transition in an introduced population could increase the chance to have enough individuals quite well synchronized with their new environment. A bad photoperiodic synchronization can result in a huge decline in fitness of survivors, as shown in *Wyeomyia smithii* (Bradshaw *et al.* 2004). On the contrary, a short transition time may reflect an adaptation (by loss of plasticity or genetic selection) to a local environment but will be deleterious for the naturalization in another environment with early winter conditions. We expected to observe a latitudinal cline of photoperiod transition in recent invasive populations, as in USA, compared to old alien populations, as in Japan (figure V.2). If a cline positively correlated with transition time is observed, the correlation coefficient is too low to conclude to a selection or maintenance of large photoperiod transition in invasive species. More investigations on the invasive populations of *Ae. albopictus* in Europe are needed.

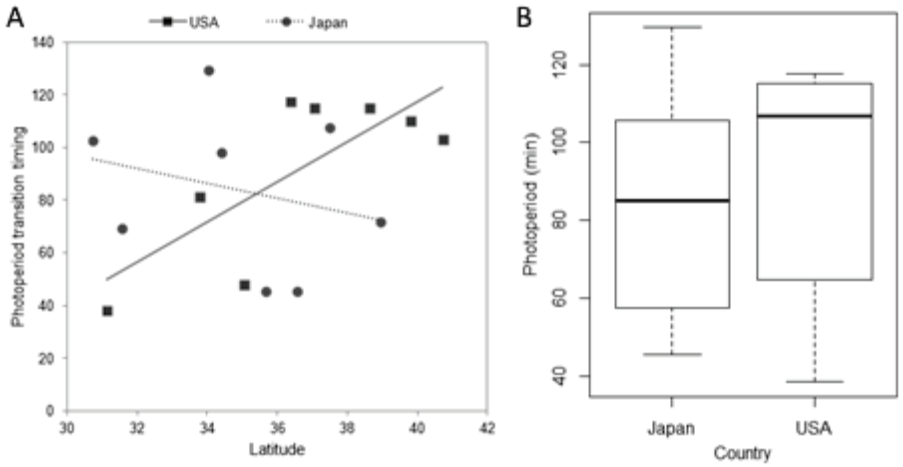


Figure V.2. Photoperiodic transition timing (in minutes) for diapause induction in *Aedes albopictus* strains from Japan and United States (A) in function of collection latitude. (B) Average duration of the photoperiod transition (Kruskal-Wallis; p-value =

0.43). Photoperiodic transition timing was calculated as the difference between the daylength inducing 10% of diapause and the day length inducing 90% of diapause. Data were calculated from (Urbanski *et al.* 2012).

The Northern situation of Europe could be a serious constraint for this invasive species. Western Europe, Eastern USA and Japan have roughly similar climatic conditions, but only Europe is above the 40°N parallel of latitude. As photoperiod variability increased with latitude, the advantage of particular photoperiodic transition timing will be reduced in Europe (figure V.3). It can be illustrated by an example: an increase of 30 minutes of the CPP would theoretically induced diapause 14 days early in USA, but only 7 days earlier under the European photoperiodic conditions. Consequently the sprawl of the maternal photoperiod sensitivity in founders may be a pre-adaptive necessity to a rapid colonization of Northern Europe. Otherwise, the European photoperiodic regime may have a negative influence on the Northward invasive kinetic of *Ae. albopictus*, limiting the distance of successful “jumps” from colonized area toward regions in northern latitude. Until recently, none of these “jumps” succeed to initiate a new invasion front in France (Roche *et al.* 2015). However in 2015 a population of *Ae. albopictus* introduced the previous year next to Strasbourg city (48°34’N) –suspected to originated from French Riviera– succeed to overwinter during the winter 2014-2015 and may be now established (stage III or IVb of the invasive process) (EID Méditerranée, personal communication). The study of the CPP of the invasive strains before and after the first winter in the introduction area would be very informative on the adequacy between founders’ biology and the environmental determinants of their new habitat.

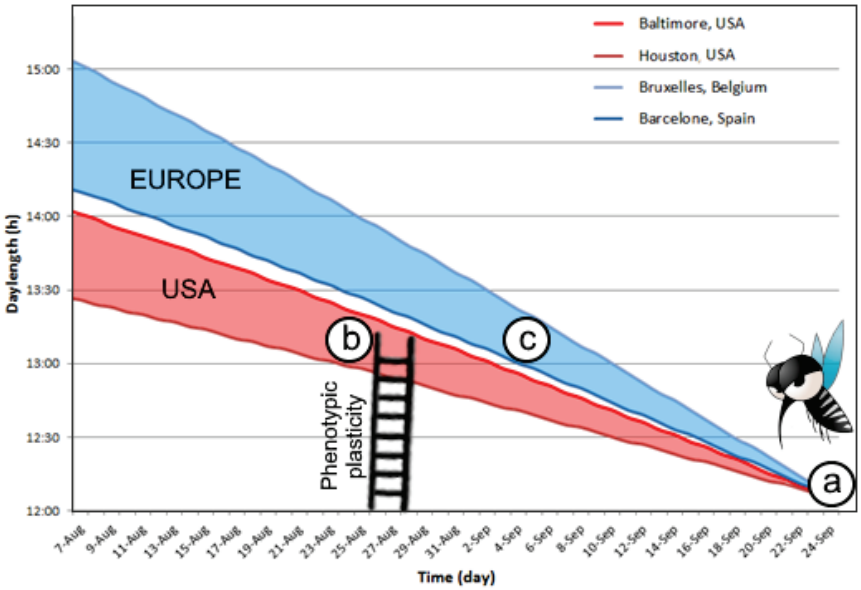


Figure V.3. Illustration of the northward expansion challenge for *Aedes albopictus* in temperate area. The seasonal daylength in August-September is represented relative to suitable areas for *Ae. albopictus* establishment in United States of America and Europe. The phenotypic plasticity of the critical photoperiod of the mosquito population is represented by the ladder. For a fixed value of phenotypic plasticity/length of the ladder, individuals of the mosquito population from (a) which would be introduced in Northern areas could have their critical photoperiod synchronized with the climatic conditions from areas (a) to (b) in USA, but only until the area (c) in Europe, due to photoperiodic amplitude. The survival of the introduced population depends of its seasonal synchronization to induce diapause before earlier winter arrival.

The Asian tiger mosquito is expected to continue to expand its range distribution in Europe (Caminade et al. 2012, Erguler et al. 2016). However, some extremely invasive species have known an unexpected and rapid decline, such as the green algae *Caulerpa taxifolia* in Mediterranean Sea. Two hypotheses were proposed by scientists to explain the decline of *Caulerpa taxifolia*: a genetic loss in the population only constituted by cloned males, or some pathogenic infections (Montefalcone *et al.* 2015). The occurrence of a similar scenario is an unlikely case for *Ae. albopictus*, but was also totally unexpected for *Caulerpa taxifolia*.

In the context of global warming, populations of *Ae. albopictus* established in Mediterranean area are expected to become partially homodynamic (development without diapause) at short or medium term. There are increasing evidences of this phenomenon with the monitoring of continuous

egg laying in Southern Spain and Italy (Collantes *et al.* 2015, Bonacci *et al.* 2015). This adaptive selection is likely directed by the advantage to expand its period of activity, but may be also linked to the suppression of a cost of diapause (Scheiner 1993). Consequently, Southern European alien mosquito populations may become fully homodynamic at long-term. This situation of cohabitation of diapausing and non-diapausing strains in the margins of the temperate area (Lounibos *et al.* 2003) and the repeated introductions of populations in colonized areas (Delatte *et al.* 2011, Kuno 2012) make the climatic speciation unlikely to occurred. This form of parapatric speciation may be generated by the seasonal disruptic selection of diapausing and non-diapausing populations (Tauber *et al.* 1986).

The findings made during this Ph.D. thesis open new questions and many research perspectives to explore. The analysis of the CPP and the mosquito fitness in populations in northward and newly colonized area in Europe would give information on the early evolutionary process in early time of invasion. The deterministic population dynamic model should be developed to better integrate the expected clinal adaptation of diapause induction and termination. An integration of a climatic dependent diapause process has been made recently in our model adapted to *Ae. albopictus* populations of China (Jia *et al.* 2016). If the improved-model was well correlated with Chinese mosquito population, the annual variability of the prediapause stage in some simulations did not match with the constancy of the diapause initiation observed in our field survey (Lacour *et al.* 2015). The development of an user-friendly interface for the model would allow mosquito control organisms to test the theoretical efficiency of their treatments.

The molecular mechanism of the diapause is under investigation. The last years many transcriptional studies were made (Poelchau *et al.* 2011, Reynolds *et al.* 2012), and the recent complete genome sequencing (Chen *et al.* 2015) will permit to develop the knowledge on the molecular basis of diapause. Based on our embryogenesis findings (Lacour *et al.* 2014), we made a proteomic experiment on the serosal cuticle and the egg burster development stages to compare the protein expression in both strain (tropical and temperate) reared under short-day and long-day conditions. Results are currently analyzed.

General conclusion

This work is a contribution to the scientific knowledge of the biology of insect populations. It brings improved methodologies in the study of the embryogenesis of diapause and proposed a new tool for the control of invasive mosquitoes. The multidisciplinary and integrative approach realized in this Ph.D. thesis involved ecophysiology, population biology and modeling skills and knowledge. It permits to highlight the causality and effects of the diapause process, and to put in evidence the adaptive value of diapause for temperate population of *Ae. albopictus*. Of course, a lot of work is still needed to understand the process of colonization of invasive species. In that context, a better understanding of the molecular mechanism of diapause is still needed along with its plasticity and how it can open the possibility to colonize new areas. On a more general point of view, some of our findings but also the methodological approach we developed may be useful for other species and other geographic areas, as invasive process is a worldwide phenomenon.

References

A

Abramides, G.C., Roiz, D., Guitart, R., Quintana, S., Guerrero, I., Gimenez, N., 2011. Effectiveness of a multiple intervention strategy for the control of the tiger mosquito (*Aedes albopictus*) in Spain. Transactions of the Royal Society of Tropical Medicine and Hygiene 105(5), 281-88.

Adhami, J., Murati, N., 1987. The presence of the mosquito *Aedes albopictus* in Albania. Revista Mjekësore 1, 13-16.

Adhami, J., Reiter, P., 1998. Introduction and establishment of *Aedes* (*Stegomyia*) *albopictus* Skuse (Diptera: Culicidae) in Albania. Journal of the American Mosquito Control Association 14(3), 340-43.

Akiner, M.M., Demirci, B., Babuadze, G., Robert, V., Schaffner, F., 2016. Spread of the Invasive Mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea Region Increases Risk of Chikungunya, Dengue, and Zika Outbreaks in Europe. PLoS Neglected Tropical Diseases 10, e0004664.

Alley, T.R., 1982. Competition theory, evolution, and the concept of an ecological niche. Acta Biotheoretica 31(3), 165-79.

Alto, B.W., Juliano, S.A., 2001. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): Implications for range expansion. Journal of Medical Entomology 38(5), 646-56.

Andraud, M., Hens, N., Marais, C., Beutels, P., 2012. Dynamic Epidemiological Models for Dengue Transmission: A Systematic Review of Structural Approaches. PLOS ONE 7(11), e49085.

Andreadis, T.G., 2009. Failure of *Aedes albopictus* to overwinter following introduction and seasonal establishment at a tire recycling plant in the northeastern USA. Journal of the American Mosquito Control Association 25(1), 25-31.

Andreadis, T.G., Armstrong, P.M., Bajwa, W.I., 2010. Studies on hibernating populations of *Culex pipiens* from a West Nile virus endemic focus in New York City: parity rates and isolation of West Nile virus. Journal of the American Mosquito Control Association 26(3), 257-64.

Anon, A., 1972. A system of world-wide surveillance for vectors. Weekly Epidemiological Record of the World Health Organization 47, 73-84.

Aranda, C., Eritja, R., Roiz, D., 2006. First record and establishment of the mosquito *Aedes albopictus* in Spain. Medical and Veterinary Entomology 20(1), 150-52.

B

Bailey, C.L., Eldridge, B.F., Hayes, D.E., Watts, D.M., Tammariello, R.F., Dalrymple, J.M., 1978. Isolation of St. Louis encephalitis virus from overwintering *Culex pipiens* mosquitoes. Science 199(4335), 1346-49.

Baldacchino, F., Caputo, B., Chandre, F., Drago, A., della Torre, A., Montarsi, F., Rizzoli, A., 2015. Control methods against invasive Aedes mosquitoes in Europe: a review. Pest Management Science 71(11), 1471-85.

Baqar, S., Hayes, C.G., Murphy, J.R., Watts, D.M., 1993. Vertical transmission of West Nile virus by Culex and Aedes species mosquitoes. The American Journal of Tropical Medicine and Hygiene 48(6), 757-62.

Barker, R.J., Cohen, C.F., Mayer, A., 1964. Photoflashes: A potential new tool for control of insect populations. Science 145, 1195-97.

Beach, R., 1978. The required day number and timely induction of diapause in geographic strains of the mosquito *Aedes atropalpus*. Journal of Insect Physiology 24(5), 449-55.

Beach, R.F., Craig, G.B., 1979. Photoinhibition of diapause in field populations of *Aedes atropalpus*. Environnemental Entomology 8(3), 392-96.

Bean, D.W., Dalin, P., Dudley, T.L., 2012. Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (Tamarix spp.). Evolutionary Applications 5(5), 511-23.

Becker, N., Huber, K., Pluskota, B., et al. 2011. *Ochlerotatus japonicus japonicus*—a newly established neozoan in Germany and a revised list of the German mosquito fauna. European Mosquito Bulletin 29, 88-102.

Becker, N., Geier, M., Balczun, C., Bradersen, U., Huber, K., Kiel, E., Krüger, A., Lühken, R., Orendt, C., Plenge-Bönig, A., Rose, A., Schaub, G.A., Tannich, E., 2013. Repeated introduction of *Aedes albopictus* into Germany, July to October 2012. Parasitology Research 112(4), 1787-90.

Bellini, R., Medici, A., Calzolari, M., Bonilauri, P., Cavrini, F., Sambri, V., Angelini, P., Dottori, M., 2012. Impact of Chikungunya Virus on *Aedes albopictus* Females and Possibility of Vertical Transmission Using the Actors of the 2007 Outbreak in Italy. PLOS ONE 7(2), e28360.

Berrigan, D., 1991. The Allometry of Egg Size and Number in Insects. Oikos 60(3), 313-21.

Bloch, G., Hazan, E., Rafaeli, A., 2013. Circadian rhythms and endocrine functions in adult insects. Journal of Insect Physiology 59(1), 56-69.

Boisson, P., 1967. La Démoustication Du Littoral Méditerranéen, Entente Interdépartementale pour La Démoustication Du L. M. ed.

Bonacci, T., Mazzei, A., Hristova, V.K., Ahmad, M.A., 2015. Monitoring of *Aedes albopictus* (Diptera, Culicidae) in Calabria, Southern Italy. International Journal of Scientific & Engineering Research 6(5), 1186-89.

Bonizzoni, M., Gasperi, G., Chen, X., James, A.A., 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends in Parasitology 29(9), 460-68.

Boukraa, S., Raharimalala, F.N., Zimmer, J.-Y., Schaffner, F., Bawin, T., Haubruge, E., Francis, F., 2013. Reintroduction of the invasive mosquito species *Aedes albopictus* in Belgium in July 2013. Parasite 20(54).

Bradshaw, W.E., 1972. Action spectra for photoperiodic response in a diapausing mosquito. Science 175(4028), 1361-62.

Bradshaw, W.E., Zani, P.A., Holzapfel, C.M., 2004. Adaptation to Temperate Climates. Evolution 58(8), 1748-62.

Brady, O.J., Golding, N., Pigott, D.M., Kraemer, M.U.G., Messina, J.P., Reiner, R.C., *et al.*, 2014. Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. Parasites & Vectors 7, 338.

Breteau, H., 1954. Yellow fever in French West Africa; an aspect of mass-preventive medicine. Bulletin of the World Health Organization 11, 453-81.

Brouard, C., Bernillon, P., Quatresous, I., Pillonel, J., Assal, A., De Valk, H., Desenclos, J.-C., workgroup "Quantitative Estimation of the Risk of Blood Donation Contamination by Infectious Agents," 2008. Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 48(7), 1333-41.

Brower, J.H., 1980. Irradiation of Diapausing and Nondiapausing Larvae of *Plodia Interpunctella*: Effects on Larval and Pupal Mortality and Adult Fertility. Annals of the Entomological Society of America 73(4), 420-26.

Bueno-Mari, R., Jiménez-Peydró, R., 2015. First observations of homodynamic populations of *Aedes albopictus* (Skuse) in Southwest Europe. Journal of Vector Borne Diseases 52(2), 175-77.

Buhagiar, J.A., 2009. A second record of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Malta. European Mosquito Bulletin 27, 65-67.

C

Cailly, P., Tran, A., Balenghien, T., L'Ambert, G., Toty, C., Ezanno, P., 2012. A climate-driven abundance model to assess mosquito control strategies. Ecological Modelling 227(24), 7-17.

Caminade, C., Medlock, J.M., Ducheyne, E., McIntyre, K.M., Leach, S., Baylis, M., et al., 2012. Suitability of European climate for the Asian tiger

mosquito *Aedes albopictus*: recent trends and future scenarios. Journal of the Royal Society Interface :rsif20120138.

Cancrini, G., Frangipane di Regalbono, A., Ricci, I., Tessarin, C., Gabrielli, S., Pietrobelli, M., 2003. *Aedes albopictus* is a natural vector of *Dirofilaria immitis* in Italy. Veterinary Parasitology 118(3-4), 195-202.

Capelli, G., Drago, A., Martini, S., Montarsi, F., Soppelsa, M., Delai, N., Ravagnan, S., Mazzon, L., Schaffner, F., Mathis, A., Di Luca, M., Romi, R., Russo, F., 2011. First report in Italy of the exotic mosquito species *Aedes* (*Finlaya*) *koreicus*, a potential vector of arboviruses and filariae. Parasites & Vectors 4, 188.

Carrieri, M., Albieri, A., Angelini, P., Baldacchini, F., Venturelli, C., Zeo, S.M., Bellini, R., 2011a. Surveillance of the chikungunya vector *Aedes albopictus* (Skuse) in Emilia-Romagna (northern Italy): Organizational and technical aspects of a large scale monitoring system. Journal of Vector Ecology 36(1), 108-16.

Carrieri, M., Angelini, P., Venturelli, C., Maccagnani, B., Bellini, R., 2011b. *Aedes albopictus* (Diptera: Culicidae) population size survey in the 2007 Chikungunya outbreak area in Italy. I. Characterization of breeding sites and evaluation of sampling methodologies. Journal of Medical Entomology 48(6), 1214-25.

Carrieri, M., Angelini, P., Venturelli, C., Maccagnani, B., Bellini, R., 2012. *Aedes albopictus* (Diptera: Culicidae) population size survey in the 2007 chikungunya outbreak area in Italy. II: Estimating epidemic thresholds. Journal of Medical Entomology 49(2), 388-99.

Carrieri, M., Bacchi, M., Bellini, R., Maini, S., 2003. On the Competition Occurring Between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. Environmental Entomology 32(6), 1313-21.

Carrieri, M., Masetti, A., Albieri, A., Maccagnani, B., Bellini, R., 2009. Larvicidal activity and influence of *Bacillus thuringiensis* var*. israelensis* on *Aedes albopictus* oviposition in ovitraps during a two-week check interval protocol. Journal of the American Mosquito Control Association 25(2), 149-55.

Carron, A., Duchet, C., Gaven, B., Lagneau, C., 2003. An easy field method for estimating the abundance of culicid larval instars. Journal of the American Mosquito Control Association 19(4), 353-60.

Cecílio, A.B., Campanelli, E.S., Souza, K.P.R., Figueiredo, L.B., Resende, M.C., 2009. Natural vertical transmission by *Stegomyia albopicta* as dengue vector in Brazil. Brazilian Journal of Biology 69(1), 123-27.

Chan, K.L., Ho, B.C., Chan, Y.C., 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 2. Larval habitats. Bulletin of the World Health Organization 44, 629-33.

Chang, L.-H., Hsu, E.-L., Teng, H.-J., Ho, C.-M., 2007. Differential Survival of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Larvae Exposed to Low Temperatures in Taiwan. Journal of Medical Entomology 44, 205-10.

Chen, S.C., Hsieh, M.H., 2012. Modeling the transmission dynamics of dengue fever: Implications of temperature effects. Science of the Total Environment 431, 385-91.

Chen, X.-G., Jiang, X., Gu, J., Xu, M., Wu, Y., Deng, Y., et al., 2015. Genome sequence of the Asian Tiger mosquito, *Aedes albopictus*, reveals insights into its biology, genetics, and evolution. Proceedings of the National Academy of Sciences 112, E5907–E5915.

Chima, R.I., Goodman, C.A., Mills, A., 2003. The economic impact of malaria in Africa: a critical review of the evidence. Health Policy 63, 17-36.

Chouin-Carneiro, T., Vega-Rua, A., Vazeille, M., Yebakima, A., Girod, R., Goindin, D., Dupont-Rouzeyrol, M., Lourenço-de-Oliveira, R., Failloux, A.-B., 2016. Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus. PLOS Neglected Tropical Diseases 10, e0004543.

Christophers, S.R., 1960. *Aedes aegypti* (L.) the Yellow Fever Mosquito: its Life History, Bionomics and Structure. Cambridge University Press, Cambridge. 1960. 739p.

Claeys, C., Mieulet, E., 2013. The Spread of Asian Tiger Mosquitoes and Related Health Risks Along the French Riviera: An Analysis of Reactions and Concerns Amongst the Local Population. International Review of Social Research 3(2), 151-73.

Clements, A.N., 1963. The Physiology of Mosquitoes. International series of monographs on pure and applied biology (17). Pergamon Press.

Clements, A.N., 2000. The Biology of Mosquitoes: Development; Nutrition and Reproduction; CABI Publishing: Eastbourne, UK.

Clemons, A., Haugen, M., Flannery, E., Tomchaney, M., Kast, K., Jacowski, C., Le, C., Mori, A., Simanton Holland, W., Sarro, J., Severson, D.W., Duman-Scheel, M., 2010. *Aedes aegypti*: an emerging model for vector mosquito development. Cold Spring Harbor Protocols, pdb.emo141.

Colautti, R.I., MacIsaac, H.J., 2004. A neutral terminology to define “invasive” species. Diversity and Distributions 10(2), 135-41.

Collantes, F., Delacour, S., Alarcón-Elbal, P.M., Ruiz-Arrondo, I., Delgado, J.A., Torrell-Sorio, A., Bengoa, M., Eritja, R., Miranda, M.Á., Molina, R., Lucientes, J., 2015. Review of ten-years presence of *Aedes albopictus* in Spain 2004–2014: known distribution and public health concerns. Parasites & Vectors 8.

Collantes, F., Delgado, J.A., Alarcón-Elbal, P.M., Delacour, S., Lucientes, J., 2014. First confirmed outdoor winter reproductive activity of Asian tiger mosquito (*Aedes albopictus*) in Europe. Anales de Biología. 36, 71-6.

Coluzzi, M., Di Deco, M., Gironi, A., 1975. [Effect of the photoperiod of the site of oviposition of *Aedes mariae* (Diptera,Culicidae)]. Parassitologia 17, 121-30.

Couret, J., Benedict, M.Q., 2014. A meta-analysis of the factors influencing development rate variation in *Aedes aegypti* (Diptera: Culicidae). BMC Ecology 14, 3.

D

Daget, P., 1977. Le bioclimat Méditerranéen: Analyse des formes climatiques par le systeme d'Emberger. Vegetatio 34(2), 87-103.

Dalla Pozza, G.L., Romi, R., Severini, C., 1994. Source and spread of *Aedes albopictus* in the Veneto region of Italy. Journal of the American Mosquito Control Association 10(4), 589-92.

Damiens, D., Ayrinhac, A., Van Bortel, W., Versteirt, V., Dekoninck, W., Hance, T., 2014. Invasive process and repeated cross-sectional surveys of the mosquito *Aedes japonicus japonicus* establishment in Belgium. PloS One 9(4), e89358.

Danilevskii, A.S., 1965. Photoperiodism and Seasonal Development of Insects. Edinburgh: Oliver & Boyd.

Darwin, C., 1859. On the origin of species. Murray, London, United Kingdom, p490.

Deblauwe, I., Demeulemeester, J., De Witte, J., Hendy, A., Sohier, C., Madder, M., 2015. Increased detection of *Aedes albopictus* in Belgium: no overwintering yet, but an intervention strategy is still lacking. Parasitology Research 114(9), 3469-77.

Delatte, H., Bagny, L., Brengue, C., Bouetard, A., Paupy, C., Fontenille, D., 2011. The invaders: Phylogeography of dengue and chikungunya viruses Aedes vectors, on the South West islands of the Indian Ocean. Infection, Genetics and Evolution 11(7), 1769-81.

Delatte, H., Dehecq, J.S., Thiria, J., Domerg, C., Paupy, C., Fontenille, D., 2008. Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a Chikungunya epidemic event. Vector Borne and Zoonotic Diseases 8(1), 25-34.

Delatte, H., Gimonneau, G., Triboire, A., Fontenille, D., 2009. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian ocean. Journal of Medical Entomology 46(1), 33-41.

Delaunay, P., Mathieu, B., Marty, P., Fauran, P., Schaffner, F., 2007. Historique de l'installation d'*Aedes albopictus* dans les Alpes-Maritimes (France) de 2002 à 2005. Médecine tropicale 67(3), 310-11.

Delisle, E., Rousseau, C., Broche, B., Leparc-Goffart, I., L'Ambert, G., Cochet, A., et al., 2015. Chikungunya outbreak in Montpellier, France, September to October 2014. Euro Surveillance 20(17).

Denlinger, D.L., 2002. Regulation of Diapause. Annual Review of Entomology 47, 93-122.

Denlinger, D.L., Armbruster, P.A., 2014. Mosquito diapause. Annual Review of Entomology 59, 73-93.

Denlinger, D.L., Yocum, G.D., Rinehart, J.L., 2005. in Hormonal Control of Diapause: Comprehensive Molecular Insect Science, eds Gilbert, L.I., Iatrou, K., Gill, S.S. Elsevier Press, Amsterdam, 3, 615-50.

Dhileepan, K., Azuolas, J.K., Gibson, C.A., 1996. Evidence of vertical transmission of Ross River and Sindbis viruses (Togaviridae: Alphavirus) by mosquitoes (Diptera: Culicidae) in southeastern Australia. Journal of Medical Entomology 33(1), 180-82.

Dhimal, M., Gautam, I., Joshi, H.D., O'Hara, R.B., Ahrens, B., Kuch, U., 2015. Risk Factors for the Presence of Chikungunya and Dengue Vectors (*Aedes aegypti* and *Aedes albopictus*), Their Altitudinal Distribution and Climatic Determinants of Their Abundance in Central Nepal. PLoS Neglected Tropical Diseases 9(3).

Dieng, H., Rahman, G.M.S., Abu Hassan, A., Che Salmah, M.R., Satho, T., Miake, F., et al., 2012. The effects of simulated rainfall on immature population dynamics of *Aedes albopictus* and female oviposition. International Journal of Biometeorology 56(1), 113-20.

Dumont, Y., Chiroleu, F., 2010. Vector control for the Chikungunya disease. Mathematical Biosciences and Engineering 7(2), 313-45.

E

ECDC, 2009. Development of *Aedes albopictus* Risk Maps. Stockholm: European Centre for Disease Prevention and Control. 52p.

ECDC, 2012. Guidelines for the Surveillance of Invasive Mosquitoes in Europe. Stockholm: European Center for Disease Control and Prevention. Available online: http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf (accessed on 12 March 2013).

ECDC, 2013. Environmental risk mapping: *Aedes albopictus* in Europe. Stockholm: European Center for Disease Control and Prevention.

ECDC VBORNET. European Network for Arthropod Vector Surveillance for Human Public Health. Available online: http://www.vbornet.eu/index.php?p=11 (accessed on 11 March 2013).

EID-Méditerranée, 2010. Surveillance du Moustique *Aedes albopictus* en France Métropolitaine: Bilan 2010; Entente Interdépartementale Pour la Démoustication du Littoral Méditerranéen: Montpellier, France.

Encinas Grandes, A., 1982. Taxonomía y Biología de los Mosquitos del área Salmantina (Diptera, Culicidae); University de Salamanca: Salamanca, Spain.

Erguler, K., Smith-Unna, S.E., Waldock, J., Proestos, Y., Christophides, G.K., Lelieveld, J., Parham, P.E., 2016. Large-Scale Modelling of the Environmentally-Driven Population Dynamics of Temperate *Aedes albopictus* (Skuse). PLoS ONE 11, e0149282.

Estrada-Franco, J.G., Craig, G.B., & Pan American Health Organization, 1995. Biology, disease relationships, and control of *Aedes albopictus*. Washington, D.C: Pan American Health Organization, Pan American Sanitary Bureau, Regional Office of the World Health Organization.

F

Fang, J., 2010. Ecology: A world without mosquitoes. Nature News 466, 432-34.

Farnesi, L.C., Martins, A.J., Valle, D., Rezende, G.L., 2009. Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influence of different constant temperatures. Memórias do Instituto Oswaldo Cruz 104, 124-26.

Flacio, E., Lüthy, P., Patocchi, N., Guidotti, F., Tonolla, M., Peduzzi R., 2004. Primo ritrovamento di *Aedes albopictus* in Svizzera. Bollettino della Societa Ticinese di Scienze Naturali 92, 141-42.

Focks, D.A., 2003. A review of entomological sampling methods and indicators for dengue vectors, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. ed, TDR/IDE/DEN/03.1. Geneva, Switzerland.

Focks, D.A., Haile, D.G., Daniels, E., Mount, G.A., 1993. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): Simulation results and validation. Journal of Medical Entomology 30, 1018-28.

Fonseca, D.M., Kaplan, L.R., Heiry, R.A., Strickman, D., 2015. Density-dependent oviposition by female *Aedes albopictus* (Diptera: Culicidae) spreads eggs among containers during the summer but accumulates them in the fall. Journal of Medical Entomology 52(4), 705-12.

Fonseca, D.M., Widdel, A.K., Hutchinson, M., Spichiger, S.-E., Kramer, L.D., 2010. Fine-scale spatial and temporal population genetics of *Aedes japonicus*, a new US mosquito, reveal multiple introductions. Molecular Ecology 19(8), 1559-72.

Fontenille, D., Failloux, A.B., Romi, R., 2007. Should we expect Chikungunya and Dengue in Southern Europe? In: Takken W, Knols BGJ. Emerging pests and Vector-borne diseases in Europe. 169-184.

Fritz, G.N., Washino, R.K., 1992. Evidence of Seasonal Eggs in *Anopheles punctipennis* (Diptera: Culicidae). Journal of Medical Entomology 29, 704-06.

Fulhorst, C.F., Hardy, J.L., Eldridge, B.F., Presser, S.B., Reeves, W.C., 1994. Natural vertical transmission of western equine encephalomyelitis virus in mosquitoes. Science 263, 676-78.

G

Ganushkina, L.A., Patraman, I.V., Rezza, G., Migliorini, L., Litvinov, S.K., Sergiev, V.P., 2016. Detection of *Aedes aegypti*, *Aedes albopictus*, and *Aedes koreicus* in the Area of Sochi, Russia. Vector-Borne and Zoonotic Diseases 16(1), 58-60.

Gatt, P., Deeming, J.C., Schaffner, F., 2009. First records of *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) in Malta. European Mosquito Bulletin 27, 56-64.

Geister, T.L., Lorenz, M.W., Hoffmann, K.H., Fischer, K., 2009. Energetics of embryonic development: effects of temperature on egg and hatchling composition in a butterfly. Journal of Comparative Physiology B 179, 87-98.

Giatropoulos, A., Emmanouel, N., Koliopoulos, G., Michaelakis, A., 2012. A study on distribution and seasonal abundance of *Aedes albopictus* (Diptera: Culicidae) population in Athens, Greece. Journal of Medical Entomology 49(2), 262-69.

Gillett, J.D., 1971. Mosquitos. Weidenfeld and Nicolson; 274 p.

Gillooly, J.F., Dodson, S.I., 2000. The relationship of egg size and incubation temperature to embryonic development time in univoltine and multivoltine aquatic insects. Freshwater Biology 44, 595-604.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Global and Planetary Change, Mediterranean climate: trends, variability and change 63, 90-104.

Giron, S., Rizzi, J., Leparc-Goffart, I., Septfons, A., Tine, R., Cadiou, B., et al., 2015. Nouvelles apparitions de cas autochtones de dengue en région Provence-Alpes-Côte d'Azur, France, août-septembre 2014. Bulletin Epidémiologique Hebdomadaire 13-14, 217-23.

Gjenero-Margan, I., Aleraj, B., Krajcar, D., Lesnikar, V., Klobučar, A., Pem-Novosel, I., et al., 2011. Autochthonous dengue fever in Croatia, August-September 2010. Euro Surveillance 16(9):pii=19805.

Grandadam, M., Caro, V., Plumet, S., Thiberge, J.M., Souarès, Y., Failloux, A.-B., et al., 2011. Chikungunya virus, southeastern France. Emerging Infectious Diseases 17(5), 910-13.

Grard, G., Caron, M., Mombo, I.M., Nkoghe, D., Mboui Ondo, S., Jiolle, D., et al., 2014. Zika Virus in Gabon (Central Africa) – 2007: A New Threat from *Aedes albopictus*? PLoS Neglected Tropical Diseases 8(2), e2681.

Gratz, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. Medical and veterinary entomology 18, 215-27.

Guo, X., Zhao, T., Dong, Y., Lu, B., 2007. Survival and Replication of Dengue-2 Virus in Diapausing Eggs of *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology 44, 492-97.

H

Hahn, D.A., Denlinger, D.L., 2011. Energetics of Insect Diapause. Annual Review of Entomology 56, 103-21.

Halstead, S.B. 2008. Dengue. First edition. London : Hackensack, NJ: Imperial College Press. p 508.

Hanson, S.M., 1995. Field overwinter survivorship of *Aedes albopictus* eggs in Japan. Journal of the American Mosquito Control Association 11, 354-57.

Hanson, S.M., Craig, G.B., Jr., 1995a. *Aedes albopictus* (Diptera: Culicidae) eggs: Field survivorship during northern Indiana winters. Journal of Medical Entomology 32, 599-604.

Hanson, S.M., Craig, G.B., Jr, 1995b. Relationship between cold hardiness and supercooling point in *Aedes albopictus* eggs. Journal of the American Mosquito Control Association 11, 35-38.

Hanson, S.M., Mutebi, J.P., Craig, G.B., Novak, R.J., 1993. Reducing the overwintering ability of *Aedes albopictus* by male release. Journal of the American Mosquito Control Association 9, 78-83.

Hardy, J.L., Houk, E.J., Kramer, L.D., Reeves, W.C., 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annual Review of Entomology 28, 229-62.

Harrat, A., Petit, D., 2009. Chronologie du développement embryonnaire de la souche « Espiguette » avec ou sans diapause de *Locusta migratoria* Linnaeus (Orthoptera : Acrididae). Comptes Rendus Biologies 332, 613-22.

Hawley, W.A., 1988. The biology of *Aedes albopictus*. Journal of the American Mosquito Control Association Suppl. 1, 1-39.

Hawley, W.A., Pumpuni, C.B., Brady, R.H., Craig, G.B., Jr., 1989. Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. Journal of Medical Entomology 26, 122-29.

Ho, B.C., Chan, K.L., Chan, Y.C., 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 3. Population fluctuations. Bulletin of the World Health Organization 44, 635-41.

Holstein, M., 1967. Dynamics of *Aedes aegypti* distribution, density and seasonal prevalence in the Mediterranean area. Bulletin of the World Health Organization 36: 541-43.

Holzapfel, C.M., Bradshaw, W.E., 1981. Geography of larval dormancy in the tree-hole mosquito, *Aedes triseriatus* (Say). Canadian Journal of Zoology 59, 1014-21.

Honnen, A.-C., 2015. Evolutionary implications of artificial night-time lighting for the mosquito *Culex pipiens.* PhD diss. Freien Universität Berlin. 124p.

I

Institut National de Veille Sanitaire (INVS) 2015. Surveillance sanitaire en Languedoc-Roussillon. Point épidémiologique au 4 Novembre 2015. [Medical surveillance in Languedoc-Roussillon. Epidemiological point 4 Nov 2015]. Paris: INVS. French.

J

Jetten, T.H.; Takken, W., 1994. Anophelism Without Malaria in Europe: A Review of the Ecology and Distribution of the Genus Anopheles in Europe; Wageningen Agricultural University Press: Wageningen, The Netherlands; p. 69.

Jia, P., Lu, L., Chen, X., Chen, J., Guo, L., Yu, X., Liu, Q., 2016. A climate-driven mechanistic population model of *Aedes albopictus* with diapause. Parasites & Vectors 9.

Jourdain, F., Girod, R., Vassal, J.M., Chandre, F., Lagneau, C., Fouque, F., Guiral, D., Raude, J., Robert, V., 2012. The moth *Hylesia metabus* and French Guiana lepidopterism: centenary of a public health concern. Parasite 19(2), 117-28.

Juliano, S.A., 2010. Coexistence, Exclusion, or Neutrality? A Meta-Analysis of Competition Between *Aedes albopictus* and Resident Mosquitoes. Israel Journal of Ecology and Evolution 56.

Juliano, S.A., O'Meara, G.F., Morrill, J.R., Cutwa, M.M., 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia 130, 458-69.

K

Kalan, K., Buzan, V., Ivović, V., 2014. Distribution of two invasive mosquito species in Slovenia in 2013. Parasites & Vectors 7, P9.

Kalan, K., Kostanjšek, R., Merdic´, E., Trilar, T., 2011. A survey of *Aedes albopictus* (Diptera: Culicidae) distribution in Slovenia in 2007 and 2010. Natura Sloveniae 13, 39-50.

Kampen, H., Kronefeld, M., Zielke, D., Werner, D., 2013. Further specimens of the Asian tiger mosquito *Aedes albopictus* (Diptera, Culicidae) trapped in southwest Germany. Parasitology Research 112(2), 905-07.

Kaufman, M.G., Fonseca, D.M., 2014. Invasion Biology of *Aedes japonicus japonicus* (Diptera: Culicidae). Annual Review of Entomology 59, 31-49.

Klingenberg, C.P., 2010. Evolution and development of shape: integrating quantitative approaches. Nature Reviews Genetics 11, 623-35.

Klobucar, A., Merdić, E., Benić, N., Baklaić, Z., Krcmar, S., 2006. First record of *Aedes albopictus* in Croatia. Journal of the American Mosquito Control Association. 22(1), 147-48.

Kobayashi, M., Nihei, N., Kurihara, T., 2002. Analysis of northern distribution of *Aedes albopictus* (Diptera: Culicidae) in Japan by geographical information system. Journal of Medical Entomology 39(1), 4-11.

Koch, L.K., Cunze, S., Werblow, A., Kochmann, J., Dörge, D.D., Mehlhorn, H., Klimpel, S., 2015. Modeling the habitat suitability for the arbovirus vector *Aedes albopictus* (Diptera: Culicidae) in Germany. Parasitology Research 115(3), 957-64.

Koplan, J.P., 2012. Eradication: Ridding the World of Diseases Forever? Emerging Infectious Diseases 18, 1215-16.

Koštál, V., 2006. Eco-physiological phases of insect diapause. Journal of Insect Physiology 52(2), 113-27.

Kuno, G., 2012. Revisiting Houston and Memphis: the background histories behind the discovery of the infestation by *Aedes albopictus* (Diptera: Culicidae) in the United States and their significance in the contemporary research. Journal of Medical Entomology 49, 1163-76.

Kutsuna, S., Kato, Y., Moi, M.L., Kotaki, A., Ota, M., Shinohara, K., et al. Autochthonous dengue fever, Tokyo, Japan, 2014. Emerging Infectious Diseases [Internet]. 2015

L

Labbe, P., Berticat, C., Berthomieu, A., Unal, S., Bernard, C., Weill, M., Lenormand, T., 2007. Forty years of erratic insecticide resistance evolution in the mosquito *Culex pipiens*. PLoS Genetics 3, e205.

Lachmi, I., 2009. Distribution and oviposition in time and space of the mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) in Western Galilee, and the effect of copper on site selection to oviposit and survival of larvae. MSc dissertation. 53p.

Lacour, G., Carron, A., Jeannin, C., Delaunay, P., Benoît, R., Perrin, Y., Lagneau, C., 2010. *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) in France: Gonotrophic Cycle, Fecundity and Longevity. In Proceedings of the International Conference "Emerging Vector-borne Diseases in a Changing European Environment", Montpellier, France, 10-12 May 2010.

Lacour, G., Chanaud, L., L'Ambert, G., Hance, T., 2015. Seasonal Synchronization of Diapause Phases in *Aedes albopictus* (Diptera: Culicidae). PLoS ONE 10(12), e0145311.

Lacour, G., Marquereau, L., Dujardin, J.P., Benoît, R., Chanaud, L., Viano, M., Boussès, P., Lagneau, C., Hance, T., 2012. Winter is Coming: Diapause Phenology, Wing Shape and Size Seasonality of a Temperate Population of *Aedes albopictus* (Diptera: Culicidae). In Proceedings of the 18th European Society of Vector Ecology Conference, Montpellier, France, 8–11 October 2012.

Lacour, G., Vernichon, F., Cadilhac, N., Boyer, S., Lagneau, C., Hance, T., 2014. When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Insect Physiology 71, 87-96.

La Ruche, G., Souarès, Y., Armengaud, A., Peloux-Petiot, F., Delaunay, P., Desprès, P., et al., 2010. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveillance 15(39), 19676.

Lee, K.-Y., Valaitis, A.P., Denlinger, D.L., 1997. Further evidence that diapause in the gypsy moth, *Lymantria dispar*, is regulated by ecdysteroids: a comparison of diapause and nondiapause strains. Journal of Insect Physiology 43, 897-903.

Leroy, E.M., Nkoghe, D., Ollomo, B., Nze-Nkogue, C., Becquart, P., Grard, G., Pourrut, X., Charrel, R., Moureau, G., Ndjoyi-Mbiguino, A., De Lamballerie, X., 2009. Concurrent Chikungunya and Dengue Virus Infections during Simultaneous Outbreaks, Gabon, 2007. Emerging Infectious Diseases 15, 591-93.

Lima, A., Lovin, D.D., Hickner, P.V., Severson, D.W., 2016. Evidence for an Overwintering Population of *Aedes aegypti* in Capitol Hill Neighborhood, Washington, DC. American Journal of Tropical Medicine and Hygiene 94, 231-35.

Linard, C., Poncon, N., Fontenille, D., Lambin, E.F., 2009. A multi-agent simulation to assess the risk of malaria re-emergence in southern France. Ecological Modelling 220(2), 160-74.

Linley, J.R., Craig, G.B., Jr, 1994. Morphology of long- and short-day eggs of *Aedes atropalpus* and *A. epactius* (Diptera: Culicidae). Journal of Medical Entomology 31, 855-67.

Lockwood, J.A., 2012. Insects as weapons of war, terror, and torture. Annual Review of Entomology 57, 205-27.

Longcore, T., Rich, C., 2004. Ecological light pollution. Frontiers in Ecology and the Environment 2(4), 191-98.

Lord, C.C., 1998. Density dependence in larval *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology 35, 825-29.

Lounibos, L.P., Escher, R.L., Lourenço-De-Oliveira, R., 2003. Asymmetric Evolution of Photoperiodic Diapause in Temperate and Tropical Invasive Populations of *Aedes albopictus* (Diptera: Culicidae). Annals of the Entomological Society of America 96, 512-18.

Lounibos, L.P., Escher, R.L., Nishimura, N., 2011. Retention and adaptiveness of photoperiodic egg diapause in Florida populations of invasive *Aedes albopictus*. Journal of the American Mosquito Control Association 27(4), 433-36.

Lourenco-de-Oliveira, R., Castro, M.G., Braks, M.A., Lounibos, L.P., 2004. The invasion of urban forest by dengue vectors in Rio de Janeiro. Journal of Vector Ecology 29, 94-100.

M

Marchand, E., Prat, C., Jeannin, C., Lafont, E., Bergmann, T., Flusin, O., et al., 2013. Autochthonous case of dengue in France, October 2013. Euro Surveillance 18(50), 20661.

Marcombe, S., Darriet, F., Tolosa, M., Agnew, P., Duchon, S., Etienne, M., Yp Tcha, M.M., Chandre, F., Corbel, V., Yebakima, A., 2011. Pyrethroid resistance reduces the efficacy of space sprays for dengue control on the island of Martinique (Caribbean). PLoS Neglected Tropical Diseases 5(6), e1202.

Marini, F., Caputo, B., Pombi, M., Tarsitani, G., Della Torre, A., 2010. Study of *Aedes albopictus* dispersal in Rome, Italy, using sticky traps in mark-release-recapture experiments. Medical and Veterinary Entomology 24(4), 361-68.

Martins, V.E.P., Alencar, C.H., Kamimura, M.T., de Carvalho Araújo, F.M., De Simone, S.G., Dutra, R.F., Guedes, M.I.F., 2012. Occurrence of Natural

Vertical Transmission of Dengue-2 and Dengue-3 Viruses in *Aedes aegypti* and *Aedes albopictus* in Fortaleza, Ceará, Brazil. PLoS One 7(7).

Matsuo, K., Yoshida, Y., Kunou, I., 1972. The scanning electron microscopy of mosquitoes (Diptera: Culicidae). I. The egg surfaces of five species of *Aedes* and *Armigeres subalbatus*. Journal of Kyoto Prefectural University of Medicine 81(7), 358-63.

McNamara, J., 1994. Timing of Entry into Diapause: Optimal Allocation to « Growth » and « Reproduction » in a Stochastic Environment. Journal of Theoretical Biology 168(2), 201-09.

Medley, K.A., 2010. Niche shifts during the global invasion of the Asian tiger mosquito, *Aedes albopictus* Skuse (Culicidae), revealed by reciprocal distribution models. Global Ecology and Biogeography 19(1), 122-33.

Medlock, J.M., Avenell, D., Barrass, I., Leach, S., 2006. Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. Journal of Vector Ecology 31(2), 292-304.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., van Bortel, W., 2012. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector Borne and Zoonotic Diseases 12(6), 435-47.

Medlock, J.M., Hansford, K.M., Versteirt, V., Cull, B., Kampen, H., Fontenille, D., Hendrickx, G., Zeller, H., Van Bortel, W., Schaffner, F., 2015. An entomological review of invasive mosquitoes in Europe. Bulletin of Entomological Research 105(6), 637-63.

Mersini, K., Velo, E., Tisseuil, C., Kadriaj, P., Shukullari, A., Simaku, A., Rogozi, E., Ducheyne, E., Gilbert, M., Bino, S., Reiter, P., 2015. Altitudinal Ecological Gradient Study of *Aedes albopictus* in Albania. Conference GERI "Genes, Ecosystems and the Risk of Infectious", Crete, Greece, April 2015.

Meuti, M.E., Denlinger, D.L., 2013. Evolutionary Links Between Circadian Clocks and Photoperiodic Diapause in Insects. Integrative and Comparative Biology 53(1), 131-43.

Ministère de la santé et des sports, 2010. Circulaire N°DGS/RI1 n° 2010-163 du 17 mai 2010 relative aux modalités de mise en œuvre du plan anti-dissémination du chikungunya et de la dengue en métropole.

Mitchell, C.J., 1995. Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. Journal of Vector Ecology 20(1), 44-58.

Mogi, M., 2011. Variation in Cold Hardiness of Nondiapausing Eggs of Nine *Aedes* (*Stegomyia*) Species (Diptera: Culicidae) from Eastern Asia and Pacific Islands Ranging from the Tropics to the Cool-Temperate Zone. Journal of Medical Entomology 48(2), 212-22.

Mogi, M., Armbruster, P., Fonseca, D.M., 2012. Analyses of the northern distributional limit of *Aedes albopictus* (Diptera: Culicidae) with a simple thermal index. Journal of Medical Entomology 49(6), 1233-43.

Montefalcone, M., Morri, C., Parravicini, V., Bianchi, C.N., 2015. A tale of two invaders: divergent spreading kinetics of the alien green algae *Caulerpa taxifolia* and *Caulerpa cylindracea*. Biological Invasions 17(9), 2717-28.

Moore, C.G., Mitchell, C.J., 1997. *Aedes albopictus* in the United States: ten-year presence and public health implications. Emerging Infectious Diseases 3(3), 329-34.

Mori, A., Oda, T., Wada, Y., 1981. Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. Tropical Medicine 23(2), 79-90.

Moulay, D., Aziz-Alaoui, M.A., Cadivel, M., 2011. The chikungunya disease: Modeling, vector and transmission global dynamics. Mathematical Biosciences 229(1), 50-63.

Mousseau, T A, Fox, C.W., 1998. The adaptive significance of maternal effects. Trends in Ecology & Evolution (Amst.) 13, 403-07.

Musolin, D.L., Saulich, A.K., 2012. Responses of insects to the current climate changes: from physiology and behavior to range shifts. Entomological Review 92(7), 715-40.

N

Nasci, R.S., Savage, H.M., White, D.J., Miller, J.R., Cropp, B.C., Godsey, M.S., Kerst, A.J., Bennett, P., Gottfried, K., Lanciotti, R.S., 2001. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. Emerging Infectious Diseases 7(4), 742-44.

Nawrocki, S.J., Hawley, W.A., 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. Journal of the American Mosquito Control Association 3(2), 314-17.

Nene, V., Wortman, J.R., Lawson, D., Haas, B., Kodira, C., et al., 2007. Genome Sequence of *Aedes aegypti*, a Major Arbovirus Vector. Science 316(5832), 1718-23.

Neteler, M., Roiz, D., Rocchini, D., Castellani, C., Rizzoli, A., 2011. Terra and Aqua satellites track tiger mosquito invasion: Modelling the potential distribution of *Aedes albopictus* in north-eastern Italy. International Journal of Health Geographics 10, 49.

Nijhout, H.F., 2003. The control of body size in insects. Developmental Biology 261(1), 1-9.

O

Oki, M., Yamamoto, T., 2013. Simulation of the probable vector density that caused the Nagasaki dengue outbreak vectored by *Aedes albopictus* in 1942. Epidemiology and Infection 141(12), 2612-22.

Oter, K., Gunay, F., Tuzer, E., Linton, Y.-M., Bellini, R., Alten, B., 2013. First record of *Stegomyia albopicta* in Turkey determined by active ovitrap surveillance and DNA barcoding. Vector Borne and Zoonotic Diseases 13(10), 753-61.

Otero, M., Solari, H.G., Schweigmann, N., 2006. A stochastic population dynamics model for *Aedes aegypti*: Formulation and application to a city with temperate climate. Bulletin of Mathematical Biology 68(8), 1945-74.

P

Pampiglione, S., Rivasi, F., Angeli, G., Boldorini, R., Incensati, R.M., Pastormerlo, M., et al., 2001. Dirofilariasis due to Dirofilaria repens in Italy, an emergent zoonosis: report of 60 new cases. Histopathology 38(4), 344-54.

Paupy, C., Delatte, H., Bagny, L., Corbel, V., Fontenille, D., 2009. *Aedes albopictus*, an arbovirus vector: From the darkness to the light. Microbes and Infection 11(14–15), 1177-85.

Petric, D., Pajovic, I., Ignjatovic Cupina, A., Zgomba., M., 2001. *Aedes albopictus* (Skuse, 1894), new mosquito species (Diptera: Culicidae) in entomofauna of Yugoslavia. Biljni lekar 29(6), 547-48.

Pluskota, B., Jöst, A., Augsten, X., Stelzner, L., Ferstl, I., Becker, N., 2016. Successful overwintering of *Aedes albopictus* in Germany. Parasitology Research 1-3.

Poelchau, M.F., Reynolds, J.A., Denlinger, D.L., Elsik, C.G., Armbruster, P.A., 2011. A de novo transcriptome of the Asian tiger mosquito, *Aedes albopictus*, to identify candidate transcripts for diapause preparation. BMC Genomics 12, 619.

Poelchau, M.F., Reynolds, J.A., Elsik, C.G., Denlinger, D.L., Armbruster, P.A., 2013a. Deep sequencing reveals complex mechanisms of diapause preparation in the invasive mosquito, *Aedes albopictus*. Proceedings of the Royal Society B. 280(1759), 20130143.

Poelchau, M.F., Reynolds, J.A., Elsik, C.G., Denlinger, D.L., Armbruster, P.A., 2013b. RNA-Seq reveals early distinctions and late convergence of gene expression between diapause and quiescence in the Asian tiger mosquito, *Aedes albopictus*. The Journal of Experimental Biology 216(21), 4082-90.

Poletti, P., Messeri, G., Ajelli, M., Vallorani, R., Rizzo, C., Merler, S., 2011. Transmission potential of chikungunya virus and control measures: The case of Italy. PLoS One 6(5): e18860.

Poncon, N., Tran, A., Toty, C., Luty, A.J., Fontenille, D., 2008. A quantitative risk assessment approach for mosquito-borne diseases: Malaria re-emergence in southern France. Malaria Journal 7, 147.

Prioteasa, L.F., Dinu, S., Fălcuţă, E., Ceianu, C.S., 2015. Established Population of the Invasive Mosquito Species *Aedes albopictus* in Romania, 2012-14. Journal of the American Mosquito Control Association 31, 177-81.

Pumpuni, C.B., 1989. Factors influencing photoperiodic control of egg diapause in *Aedes albopictus* (Skuse). PhD diss. University of Notre Dame, Notre Dame, IN. 296p.

Pumpuni, C.B., Knepler, J., Craig, G.B., Jr, 1992. Influence of temperature and larval nutrition on the diapause inducing photoperiod of *Aedes albopictus*. Journal of the American Mosquito Control Association 8(3), 223-27.

Puth, L.M., Post, D.M., 2005. Studying invasion: have we missed the boat? Ecology Letters 8(7): 715-21.

R

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratsitorahina, M., Harisoa, J., Ratovonjato, J., Biacabe, S., Reynes, J.-M., Zeller, H., Raoelina, Y., Talarmin, A., Richard, V., Soares, J.L., 2008. Outbreak of Dengue and Chikungunya Fevers, Toamasina, Madagascar, 2006. Emerging Infectious Diseases 14(7), 1135-37.

Reinert, J.F., Harbach, R.E., Kitching, I.J., 2004. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zoological Journal of the Linnean Society 142, 289-368.

Reiter, P., 1998. *Aedes albopictus* and the world trade in used tires, 1988-1995: the shape of things to come? Journal of the American Mosquito Control Association 14(1), 83-94.

Reynolds, J.A., Poelchau, M.F., Rahman, Z., Armbruster, P.A., Denlinger, D.L., 2012. Transcript profiling reveals mechanisms for lipid conservation during diapause in the mosquito, *Aedes albopictus*. Journal of Insect Physiology 58(7), 966-73.

Rezende, G.L., Martins, A.J., Gentile, C., Farnesi, L.C., Pelajo-Machado, M., Peixoto, A.A., Valle, D., 2008. Embryonic desiccation resistance in *Aedes*

aegypti: presumptive role of the chitinized Serosal Cuticle. BMC Developmental Biology 8, 82.

Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A.C., Panning, M., et al., 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370(9602), 1840-46.

Rinehart, J.P., Robich, R.M., Denlinger, D.L., 2006. Enhanced cold and desiccation tolerance in diapausing adults of *Culex pipiens*, and a role for Hsp70 in response to cold shock but not as a component of the diapause program. Journal of Medical Entomology 43(4), 713-22.

Roche, B., Léger, L., L'Ambert, G., Lacour, G., Foussadier, R., Besnard, G., et al. 2015. The Spread of *Aedes albopictus* in Metropolitan France: Contribution of Environmental Drivers and Human Activities and Predictions for a Near Future. PLOS ONE 10(5), e0125600.

Rochlin, I., Ninivaggi, D.V., Hutchinson, M.L., Farajollahi, A., 2013. Climate Change and Range Expansion of the Asian Tiger Mosquito (*Aedes albopictus*) in Northeastern USA: Implications for Public Health Practitioners. PLoS ONE 8(4), e60874.

Rohlf, F.J., 1990. Rotational fit (Procrustes) methods. In: Rohlf, F.J., Bookstein, F.L., eds. Proceedings of the Michigan Morphometrics Workshop. Number 2, Ann Arbor, Michigan: The University of Michigan Museum of Zoology, 1990; Special Publication No 2:227-36.

Roiz, D., Rosà, R., Arnoldi, D., Rizzoli, A., 2010. Effects of temperature and rainfall on the activity and dynamics of host-seeking *Aedes albopictus* females in northern Italy. Vector-Borne and Zoonotic Diseases 10(8), 811-6.

Roiz, D., Boussès, P., Simard, F., Paupy, C., Fontenille, D., 2015. Autochthonous Chikungunya Transmission and Extreme Climate Events in Southern France. PLoS Neglected Tropical Diseases 9(6), e0003854.

Romi, R., Sabatinelli, G., Savelli, L.G., Raris, M., Zago, M., Malatesta, R., 1997. Identification of a North American mosquito species, *Aedes atropalpus* (Diptera: Culicidae), in Italy. Journal of the American Mosquito Control Association 13(3), 245-6.

Romi, R., Severini, F., Toma, L., 2006. Cold acclimation and overwintering of female *Aedes albopictus* in Roma. Journal of the American Mosquito Control Association 22(1), 149-51.

Rosen, L., 1987. Overwintering mechanisms of mosquito-borne arboviruses in temperate climates. American Journal of Tropical Medicine and Hygiene 37(3), 69S–76S.

S

Sabatini, A., Raineri, V., Trovato, G., Coluzzi, M., 1990. [*Aedes albopictus* in Italy and possible diffusion of the species into the Mediterranean area]. Parassitologia 32, 301-04.

Saltelli, A., Chan, K., Scott, E.M., 2000. Sensitivity Analysis; John Wiley and Sons: New York, NY, USA.

Samanidou-Voyadjoglou, A., Patsoula, E., Spanakos, G., Vakalis, N.C., 2005. Confirmation of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Greece. European Mosquito Bulletin 19, 10-12.

Schaffner, F., Chouin, S., Guilloteau, J., 2003. First record of *Ochlerotatus* (*Finlaya) japonicus japonicus* (Theobald, 1901) in metropolitan France. Journal of the American Mosquito Control Association 19(1), 1-5.

Schaffner, F., Karch, S., 2000. Première observation d'*Aedes albopictus* (Skuse, 1894) en France métropolitaine. Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie 323, 373–375.

Schaffner, F., Van Bortel, W., Coosemans, M., 2004. First record of *Aedes* (*Stegomyia*) *albopictus* in Belgium. Journal of the American Mosquito Control Association 20(2), 201-03.

Scheiner, S.M., 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology, Evolution, and Systematics 24, 35-58.

Scholte, E.-J., Dik, M., Ibáñez-Justicia, A., Den Hartog, W., Schoelitsz, B., Brooks, M., Braks, M., Steeghs, M., 2012. Findings and control of two invasive exotic mosquito species, *Aedes albopictus* and *Ae. atropalpus* (Diptera: Culicidae) in the Netherlands, 2011. European Mosquito Bulletin 30, 1-14.

Scilab. Available online: http://www.scilab.org/ (accessed on 11 March 2013).

Šebesta, O., Rudolf, I., Betášová, L., Peško, J., Hubálek, Z., 2012. An invasive mosquito species *Aedes albopictus* found in the Czech Republic, 2012. Euro Surveillance 17(43): pii=20301. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20301

Seidel, B., Duh, D., Nowotny, N., Allerberger, F., 2012. First record of the mosquitoes *Aedes* (*Ochlerotatus*) *japonicus japonicus* (Theobald, 1901) in Austria and Slovenia in 2011 and for *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895) in Austria 2012. Entomologische Zeitschrift 122, 223-26.

Semenza, J.C., Sudre, B., Miniota, J., Rossi, M., Hu, W., Kossowsky, D., Suk, J.E., van Bortel, W., Khan, K., 2014. International dispersal of dengue through air travel: Importation risk for Europe. PLOS Neglected Tropical Diseases 8(12), e3278.

Setbon, M., Rode, J., 2008. Sociologie comparée de l'épidémie de chikungunya à La Réunion et à Mayotte. Bulletin Epidémiologique Hebdomadaire 38-39-40.

Shah, M., Suzuki, T., Ghazy, N.A., Amano, H., Ohyama, K., 2011. Night-interrupting light inhibits diapause induction in the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). Journal of Insect Physiology 57(9), 1185-89.

Shaman, J., Spiegelman, M., Cane, M., Stieglitz, M., 2006. A hydrologically driven model of swamp water mosquito population dynamics. Ecological Modelling 194, 395-404.

Shannon, R.C. Hadjinicalao, J., 1941. Egg production of Greek Anophelines in nature. Journal of Economic Entomology 34(2): 300-05.

Sinègre, G., 1974. Contribution à l'étude physiologique d'*Aedes* (*Ochlerotatus*) *caspius* (Pallas 1771) (Nematocera-Culicidae): éclosion, dormance, développement, fertilité. PhD diss. Université des Sciences et Techniques du Languedoc. 280p.

Sota, T., Mogi, M., 1992a. Survival time and resistance to desiccation of diapause and non-diapause eggs of temperate Aedes (Stegomyia) mosquitoes. Entomologia Experimentalis et Applicata. 63:155-61.

Sota, T., Mogi, M., 1992b. Interspecific variation in desiccation survival time of Aedes (Stegomyia) mosquito eggs is correlated with habitat and egg size. Oecologia 90, 353-58.

Soti, V., Tran, A., Degenne, P., Chevalier, V., Lo Seen, D., Thiongane, Y., Diallo, M., Guegan, J.F., Fontenille, D., 2012. Combining hydrology and mosquito population models to identify the drivers of rift valley Fever emergence in semi-arid regions of west Africa. PLOS Neglected Tropical Diseases 6, e1795.

Suman, D.S., Wang, Y., Gaugler, R., 2015. The Insect Growth Regulator Pyriproxyfen Terminates Egg Diapause in the Asian Tiger Mosquito, *Aedes albopictus*. PLoS ONE 10, e0130499.

Suter, T.T., Flacio, E., Fariña, B.F., Engeler, L., Tonolla, M., Regis, L.N., Santos, M.A.V. de M., Müller, P., 2016. Surveillance and Control of *Aedes albopictus* in the Swiss-Italian Border Region: Differences in Egg Densities between Intervention and Non-intervention Areas. PLOS Neglected Tropical Diseases 10, e0004315.

T

Talbalaghi, A., Moutailler, S., Vazeille, M., Failloux, A.-B., 2010. Are *Aedes albopictus* or other mosquito species from northern Italy competent to sustain new arboviral outbreaks? Medical and Veterinary Entomology 24, 83-87.

Tauber, M.J., Tauber, C.A., Masaki, S., 1986. Seasonal adaptations of insects. Oxford University Press, New York. 411p.

Theodor, O., 1925. Observations on Palestinian Anopheles. Bulletin of Entomological Research 15, 377-82.

Thomas, S., Obermayr, U., Fischer, D., Kreyling, J., Beierkuhnlein, C., 2012. Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae). Parasites & Vectors 5, 1-7.

Toma, T., Miyagi, I., 1990. Seasonal changes in the hatchability of *Aedes albopictus* (Diptera: Culicidae) eggs in Okinawajima, Ryukyu Archipelago, Japan. Japanese Journal of Sanitary Zoology 41, 195-204.

Toma, L., Severini, F., Di Luca, M., Bella, A., Romi, R., 2003. Seasonal patterns of oviposition and egg hatching rate of *Aedes albopictus* in Rome. Journal of the American Mosquito Control Association 19(1), 19-22.

Tomasello, D., Schlagenhauf, P., 2013. Chikungunya and dengue autochthonous cases in Europe, 2007-2012. Travel Medicine and Infectious Disease 11, 274–284.

Tran, A., L'Ambert, G., Lacour, G., Benoît, R., Demarchi, M., Cros, M., et al. 2013. A rainfall- and temperature-driven abundance model for *Aedes albopictus* populations. International Journal of Environnemental Research and Public Health 10(5), 1698-719.

Trpiš, M., 1970. A new bleaching and decalcifying method for general use in zoology. Canadian Journal of Zoology 48, 892-93.

Trpiš, M., Haufe, W.O., Shemanchuk, J.A., 1973. Embryonic development of *Aedes* (*O.*) *sticticus* (Diptera: Culicidae) in relation to different constant temperatures. The Canadian Entomologist 105, 43-50.

Tsuda, Y., Takagi, M., Wada, Y., 1991. Preliminary laboratory study on population growth of *Aedes albopictus*. Tropical Medicine 33, 41-46.

Tsuda, Y., Takagi, M., 2001. Survival and development of *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) larvae under a seasonally changing environment in Nagasaki. Japanese Environnemental Entomology 30, 855-60.

U

Unlu, I., Farajollahi, A., Strickman, D., Fonseca, D.M., 2013. Crouching tiger, hidden trouble: urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. PLoS ONE 8, e77999.

Urbanelli, S., Bellini, R., Carrieri, M., Sallicandro, P., Celli, G., 2000. Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity 84(3), 331-37.

Urbanski, J.M., Benoit, J.B., Michaud, M.R., Denlinger, D.L., Armbruster, P., 2010a. The molecular physiology of increased egg desiccation resistance during diapause in the invasive mosquito, *Aedes albopictus*. Proceedings of the Royal Society B 277, 2683-92.

Urbanski, J.M., Aruda, A., Armbruster, P., 2010b. A transcriptional element of the diapause program in the Asian tiger mosquito, *Aedes albopictus*, identified by suppressive subtractive hybridization. Journal of Insect Physiology 56, 1147-54.

Urbanski, J.M., Mogi, M.M., O'Donnell, D.D., DeCotiis, M.M., Toma, T.T., Armbruster, P.P., 2012. Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. The American Naturalist 179, 490.

V

Vafopoulou, X., Cardinal-Aucoin, M., Steel, C.G.H., 2012. Rhythmic release of prothoracicotropic hormone from the brain of an adult insect during egg development. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 161(2), 193-200.

Valéry, L., Fritz, H., Lefeuvre, J.-C., Simberloff, D., 2008. In search of a real definition of the biological invasion phenomenon itself. Biological Invasions 10(8), 1345-51.

Vargas, H.C.M., Farnesi, L.C., Martins, A.J., Valle, D., Rezende, G.L., 2014. Serosal cuticle formation and distinct degrees of desiccation resistance in embryos of the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*. Journal of Insect Physiology 62, 54-60.

Vazeille, M., Mousson, L., Martin, E., Failloux, A.-B., 2010. Orally Co-Infected *Aedes albopictus* from La Reunion Island, Indian Ocean, Can Deliver Both Dengue and Chikungunya Infectious Viral Particles in Their Saliva. PLoS Neglected Tropical Diseases 4(6).

Vega-Rua, A., Zouache, K., Caro, V., Diancourt, L., Delaunay, P., Grandadam, M., Failloux, A.-B., 2013. High Efficiency of Temperate *Aedes albopictus* to Transmit Chikungunya and Dengue Viruses in the Southeast of France. PLoS ONE 8(3), e59716.

Velo, E., Bino, S.A., Shukullari, A., Kadriaj, P., Simaku, A., Mersini, K., Qirjo, M., Rogozi, E., Caputo, B., Schaffner, F., Della Torre, A., Reiter, P., 2012. Introduction, Distribution and Altitudinal Ecological Gradient Study of *Aedes albopictus* in Albania. In Proceedings of the 18th European Society of Vector Ecology Conference, Montpellier, France, 8–11 October 2012.

Versteirt, V., Schaffner, F., Garros, C., Dekoninck, W., Coosemans, M., Van Bortel, W., 2009. Introduction and establishment of the exotic mosquito species *Aedes japonicus japonicus* (Diptera: Culicidae) in Belgium. Journal of Medical Entomology 46(6), 1464-67.

Vinogradova, E.B., 2007. Diapause in Aquatic Insects, with Emphasis on Mosquitoes, in: Alekseev, V.R., Stasio, B.T. de, Gilbert, J.J. (Eds.), Diapause in Aquatic Invertebrates Theory and Human Use, Monographiae Biologicae (84). Springer Netherlands, 83-113.

Vinogradova, E.B., 2012. Ecological prerequisites for spread of bloodsucking mosquitoes. Entomological Review 92(3), 291-95.

Vital, W., Rezende, G., Abreu, L., Moraes, J., Lemos, F.J., Vaz, I., Logullo, C., 2010. Germ band retraction as a landmark in glucose metabolism during *Aedes aegypti* embryogenesis. BMC Developmental Biology 10, 25.

W

Waldock, J., Chandra, N.L., Lelieveld, J., Proestos, Y., Michael, E., Christophides, G., Parham, P.E., 2013. The role of environmental variables on *Aedes albopictus* biology and chikungunya epidemiology. Pathogens and Global Health 107, 224-41.

Werner, D., Kampen, H., 2015. *Aedes albopictus* breeding in southern Germany, 2014. Parasitology Research 114(3), 831-34.

West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution. OUP USA.

Wheeler, W.M., 1893. A contribution to insect embryology. Journal of Morphology 8, 1-160.

Wilkerson, R.C., Linton, Y.-M., Fonseca, D.M., Schultz, T.R., Price, D.C., Strickman, D.A., 2015. Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. PLoS ONE 10(7), e0133602.

Willmer, P., Stone, G., Johnston, I., 2000. Environmental physiology of animals. Blackwell Science Ltd. Oxford, USA, 754 p.

Wong, P.-S.J., Li, M.I., Chong, C.-S., Ng, L.-C., Tan, C.-H., 2013. *Aedes* (*Stegomyia*) *albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLOS Neglected Tropical Diseases 7, e2348.

X

Xu, G., Dong, H., Shi, N., Liu, S., Zhou, A., Cheng, Z., et al., 2007. An Outbreak of Dengue Virus Serotype 1 Infection in Cixi, Ningbo, People's Republic of China, 2004, Associated with a Traveler from Thailand and High Density of *Aedes albopictus*. The American journal of tropical medicine and hygiene 76(6), 1182-88.

Y

Yee, D.A., 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. Journal of Medical Entomology 45(4), 581-93.

Yee, D.A., Juliano, S.A., Vamosi, S.M., 2012. Seasonal photoperiods alter developmental time and mass of an invasive mosquito, *Aedes albopictus* (Diptera: Culicidae), across its north-south range in the United States. Journal of Medical Entomology 49(4), 825-32.

Z

Ziegler, R., Van Antwerpen, R., 2006. Lipid uptake by insect oocytes. Insect Biochemistry and Molecular Biology 36(4), 264-72.

Zouache, K., Fontaine, A., Vega-Rua, A., Mousson, L., Thiberge, J.-M., Lourenco-De-Oliveira, R., Caro, V., Lambrechts, L., Failloux, A.-B., 2014. Three-way interactions between mosquito population, viral strain and temperature underlying chikungunya virus transmission potential. Proceedings of the Royal Society of London B: Biological Sciences 281(1792), 20141078.

Annexes

Poster ESOVE 2012 (prix du meilleur poster étudiant)

Photoperiod and diapause effects on chronology of embryonic development of *Aedes albopictus* (Diptera: Culicidae)

Lacour G.[1,4], Vernichon F.[1], Cadilhac N.[1], Boyer S.[2], Dehecq J.S.[3], Le Goff G.[2], Lagneau C.[1], Hance T.[4]

1: EID Méditerranée. 2: UMR-MIVEGEC, IRD, 911 avenue Agropolis, 34394 Montpellier cedex 5, France.
3: ARS Océan Indien, 2 bis avenue Georges Brassens, 97408 Saint-Denis cedex 9, France.
4: Centre de Recherche sur la Biodiversité, Université catholique de Louvain, Croix du Sud 5, 1348 Louvain-la-Neuve, Belgique.

18th E-SOVE Conference: European Society for Vector Ecology, Montpellier, France, Montpellier (France), October 8th-11th, 2012.

Background & objective

The Asian tiger mosquito *Aedes albopictus* is a very efficient invasive species. It rapidly spreads across the temperate countries by means of its egg diapause. The diapause strengthens the survival capacity of eggs to pass the winter, increasing notably its resistance to cold [1] and to desiccation [2]. Diapause is a crucial adaptation and a complex process [3], induced by the maternal photoperiod [4] (Fig 1.).

The preparative phase of diapause modifies the metabolism of ovocytes [5] up to the pharate first-instar larvae. These modifications should interfere with the velocity of the embryogenesis in *Aedes albopictus* eggs, such as egg volume [6]. This is the focus of this study.

Materials & methods

- Rearing under standardized protocol at **21°C**, under long-days **LD** (16h:8h Light:Dark photoperiod) or short-days **SD** (9:15 L:D).
- Oviposition of 5-days post blood-feeding females in nest-boxes during 30 minutes (A) or 60 minutes (B, C, D). The middle of the synchronous egglaying period determines the 0 HAE (Hour after Egglaying).

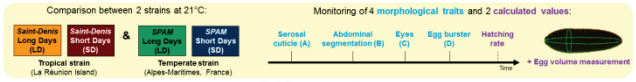


Fig 2. Experimental monitoring

→Hatching rate calculated on at least 10-days old eggs:
Hatch % = (hatched eggs x 100) / (embryonated unhatched eggs + hatched eggs)
→Presence of the serosal cuticle (A) determined by chlorine digestion (solution of 3.6% of chlorine during 30 minutes exposure) on 3 x 150 eggs/HAE.
→Presence of abdominal segmentation (B), eyes (C) and egg burster (D) noticed on 3 x 50 eggs/HAE bleached by Trpis solution (1h30).
→Egg length and width measured on 60 x 9-days-old eggs / experimental group. Egg volume calculated: ***Volume = 1/6.π.Length.Width²***

Results

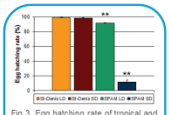


Fig 3. Egg hatching rate of tropical and temperate strains of *Ae. albopictus* under long days LD and short days SD (mean ± sd) (T test, **: P<0.01)

Under a short day length, the temperate strain only enters into diapause.

The tropical strain being incapable of diapause, it is a good negative control to discriminate between the effects of photoperiod and/or diapause.

- No difference in the tropical strain under different photoperiods.
- The **development of temperate diapause-destined embryos** is always **delayed** (A, B and D) and/or **slower** (A and C), and this slowing down increased with time.
- The delay of the median development time reaches 9h (A), 18h (B), 32h (C) and 38 hours (D) between temperate LD and SD eggs.

Diapause preparation clearly impacts embryogenesis duration.

- The timing of embryogenesis : **Temperate LD ≥ Tropical ≥ Temperate SD**
- The serosal cuticle (A) provides desiccation resistance → strong evolutionary pressure! It is the only trait acquired at the same speed in tropical eggs as in no-diapause temperate eggs.

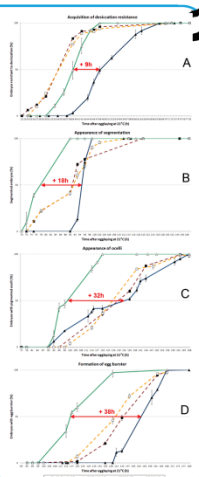


Fig 4. Differences in embryogenesis timing of tropical and temperate strains of *Ae. albopictus* under long days LD and short days SD (mean ± sd)

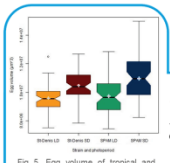


Fig 5. Egg volume of tropical and temperate strains of *Ae. albopictus* under long days LD and short days SD (crosses are means)

SPAM SD = St-Denis SD > SPAM LD = St-Denis LD

→ Egg volume varies according to photoperiod in each strain (T test, * : P<0.05, ** : P<0.01).

Photoperiod influences egg volume.

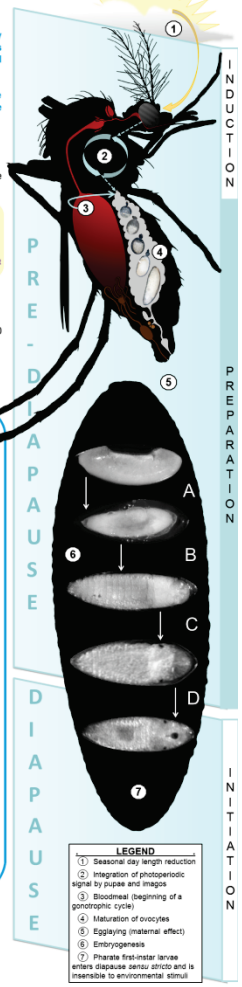


Fig 1. Process of egg diapause, from light to diapause *sensu stricto*.

These findings indicate that the diapause process modifies the timing of embryogenesis, in contradiction with results of a previous study [6]. This retardation is probably a consequence of the molecular responses occurring during diapause preparation. However, some modifications, such as the increase in egg volume, seems to be a direct response to photoperiodism rather than an exclusive result of diapause process. To conclude, the knowledge of embryonic development chronology is crucial for molecular studies on *Aedes albopictus* egg diapause preparation.

Bibliographic references: [1] THOMAS S. *et al.* (2012) Parasites & Vectors 5: 1-7. [2] URBANSKI J.M. *et al.* (2010). Proc. R. Soc. B. 277: 2683-2692. [3] KOSTAL V. (2006) J. Insect Physiol. 52: 113-127. [4] PUMPUNI C.B. (1990). PhD Thesis. 148p. [5] URBANSKI J.M. *et al.* (2010). J. Insect Physiol. 56: 1147-1154. [6] REYNOLDS J.A. *et al.* (2012) J. Insect Physiol. 58: 966-973.

ENTENTE INTERDEPARTEMENTALE POUR LA DEMOUSTICATION DU LITTORAL MEDITERRANEEN
Direction technique Pôle méditerranéen de l'environnement littoral
165 avenue Paul-Rimbaud 34184 Montpellier Cedex 4
Tél : 04 67 63 67 63 - Fax : 04 67 63 54 05 - E - mail : glacour@eid-med.org - http://www.eid-med.org

Modélisation de la dynamique temporelle des populations d'*Aedes albopictus* en milieu tempéré: un outil d'aide à la décision pour la démoustication

LIFE08 ENV/F/000488

Objectifs

La colonisation actuelle de l'Europe par le moustique tigre *Aedes albopictus* crée un nouveau contexte de nuisance et de risque sanitaire. Le contrôle de cette espèce est complexe et requiert de comprendre la dynamique de ses populations et d'adapter les moyens de lutte préventifs et curatifs. Nous présentons ici un modèle destiné aux opérateurs de lutte pour prédire l'abondance des moustiques tigre au cours du temps, déterminer les paramètres clés de sa dynamique de population à cibler afin, à terme, de simuler différentes stratégies de lutte et d'en estimer l'efficacité.

Fonctionnement du modèle

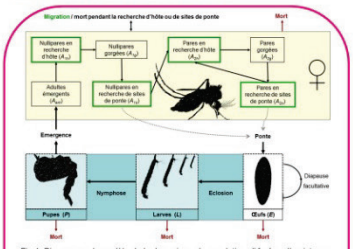


Fig 1. Diagramme du modèle de la dynamique de population d'*Aedes albopictus* en climat tempéré. Les stades aquatiques sont en bleu et les femelles adultes en jaune. Adapté de Cailly et al. (2007).

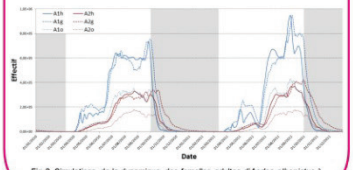


Fig 2. Simulations de la dynamique des femelles adultes d'*Aedes albopictus* à Nice (modèle 2). Les femelles dans leur premier cycle trophogonique sont en bleu, et les femelles pares en rouge.

Paramètres du modèle

Le modèle déterministe développé par Cailly *et al.* (2007)[1] est adapté à la biologie d'*Aedes albopictus* en France métropolitaine (fig 1 et 2) à partir de données de laboratoire et de terrain obtenues majoritairement sur des souches locales (table 1).

Ce modèle est basé sur un système d'équations différentielles ordinaires, décrites dans Tran *et al.* (2013)[2] →

Ce modèle utilise des paramètres biologiques prédéterminés (biologie du moustique, gîtes, etc.) et les paramètres météorologiques journaliers réels (température et précipitations).

Table 1. Valeurs des paramètres utilisés dans le modèle de la dynamique de population d'*Aedes albopictus* en climat méditerranéen tempéré.

Deux modèles:

Le modèle 1, plus simple, fonctionne avec un arrêt immédiat de l'éclosion au début de la période défavorable et un taux de survie des œufs constant (fig 3a), tandis que le modèle 2 considère une initiation et une terminaison de diapause progressives, couplées à une meilleure survie hivernale des œufs (fig 3b). La comparaison des sorties des 2 modèles montre que si le triple des œufs survit à l'hiver dans le cas du modèle 2 (fig 4a), cela ne modifie que très peu l'effectif de femelles actives au cours de l'année (fig 4b).

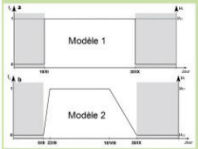


Fig 3. Différentes modulations du taux d'éclosion et de survie entre la période favorable (blanche) et défavorable (grise) dans le modèle 1 (a) et 2 (b).

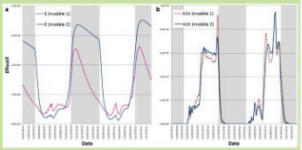


Fig 4. Comparaison des simulations de la dynamique de la banque d'œufs viables (a) et de femelles adultes en recherche d'hôte (b) entre le modèle 1 (tirets) et 2 (trait continu).

Validation du modèle

Les 2 modèles sont comparés avec la dynamique de ponte sur 6 sites de la Côte d'Azur suivis par pièges-pondoirs (fig 5 et 6) entre 2008 et 2011. **Les 2 modèles prédisent correctement les dynamiques de terrain** (table 2).

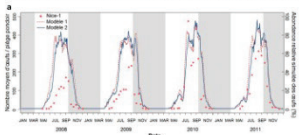


Fig 5. Comparaison entre les simulations et les données terrain de la dynamique de ponte d'*Aedes albopictus* à Nice (a) et sa périphérie (b).

Ville / r	Nice-1	Cagnes-sur-Mer	Biot	La Gaude	Villeneuve-Loubet	Nice-2
Modèle 1	**0,79**	**0,77**	0,73	0,87	**0,93**	0,92
Modèle 2	0,76	0,69	**0,80**	**0,91**	0,88	**0,96**

Table 2. Coefficients de corrélation de Pearson entre les simulations des 2 modèles et les données issues de 6 zones d'études de la Côte d'Azur.

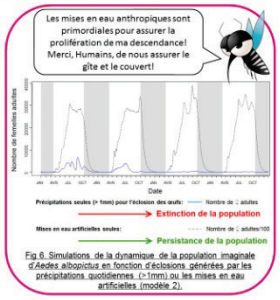


Fig 6. Simulations de la dynamique de la population imaginale d'*Aedes albopictus* en fonction d'éclosions générées par les précipitations quotidiennes (>1mm) ou les mises en eau artificielles (modèle 2).

Conclusion

Le modèle prédit correctement la dynamique de ponte sur une période de 4 ans. Il met en évidence le rôle essentiel des mises en eau d'origine anthropique dans la persistance des populations de moustiques tigre. Il est fonctionnel en France métropolitaine pour la zone biogéographique méditerranéenne et transposable aux autres zones biogéographiques sous condition de validation préalable par des données de terrain locales. Ce modèle représente un outil efficace pour prédire la dynamique de population de ce vecteur et pour modéliser l'efficacité potentielle des différentes stratégies de lutte. Couplé à une extension SIG, il permet de produire des cartes de risque saisonnières (cf. session « outil de prévision du risque entomologique »).

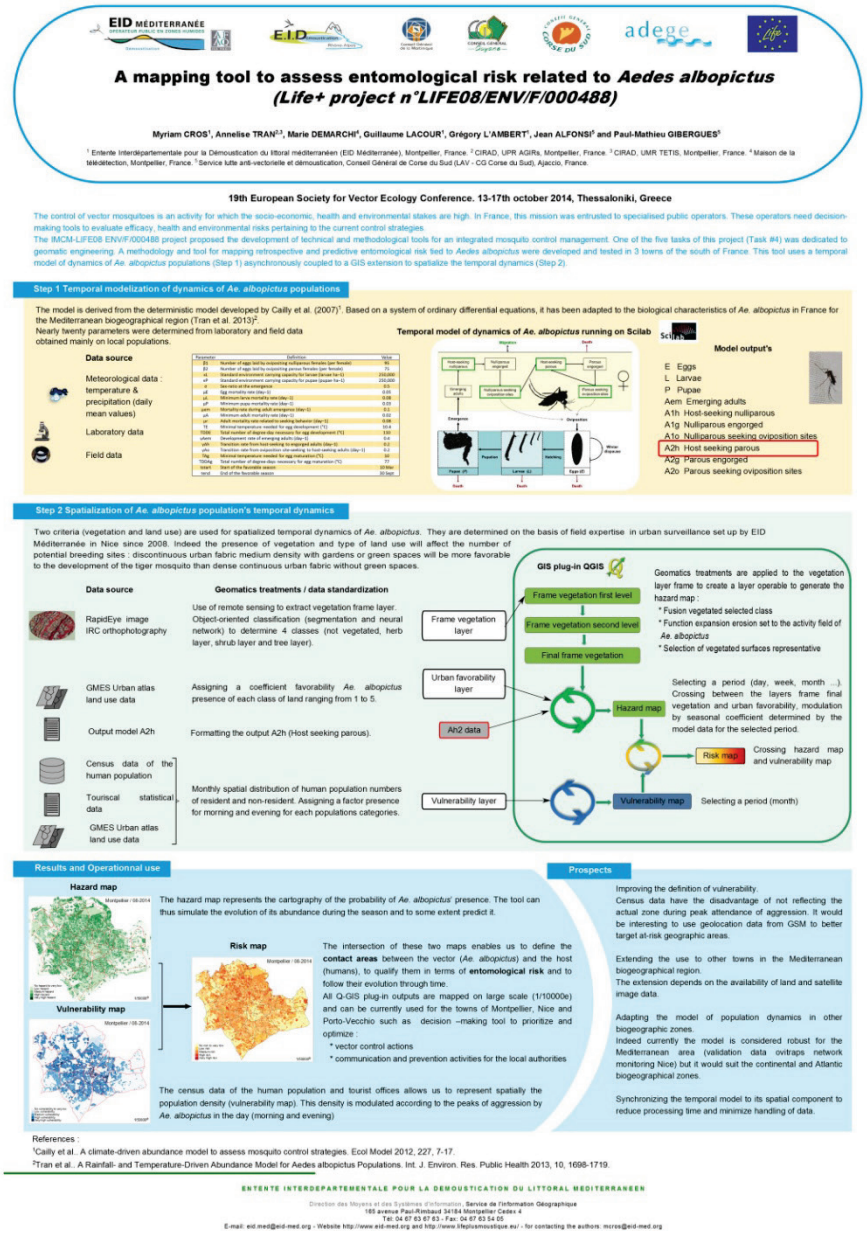
Références bibliographiques:

1) Cailly, P.; Tran, A.; Balenghien, T.; L'Ambert, G.; Toty, C.; Ezanno, P. A climate-driven abundance model to assess mosquito control strategies. Ecol Model 2012, 227, 7-17.
2) Tran, A.; L'Ambert, G.; Lacour, G.; Benoit, R.; Demarchi, M.; Cros, M.; Cailly, P.; Aubry-Kientz, M.; Balenghien, T.; Ezanno, P. A Rainfall- and Temperature-Driven Abundance Model for *Aedes albopictus* Populations. Int. J. Environ. Res. Public Health 2013, 10, 1698-1719.

Lacour G.[1,2], Tran A.[3], L'Ambert G.[1], Cros M.[1], Benoît R.[1], Chanaud L.[1], Viano M.[1], Demarchi M.[4]

1: EID Méditerranée, F-34184, Montpellier. 2: Earth and Life Institute, Centre de Recherche sur la Biodiversité, B-1348 Louvain-la-Neuve. 3: CIRAD, UPR AGIRs, UMR TETIS, F-34398 Montpellier. 4: SIRS, F-34093 Montpellier

Poster ESOVE 2014



ENTENTE INTERDEPARTEMENTALE POUR LA DEMOUSTICATION DU LITTORAL MEDITERRANEEN

Poster EMBO 2015

A retrospective review of chikungunya and dengue outbreaks in French Mediterranean area in view of the biology of the Asian tiger mosquito

LACOUR G.[1,2], L'AMBERT G.[1], CROS M.[1], JEANNIN C.[1], FERRE J.-B.[1], GION M.[1], TRAN A.[3,4], LAGNEAU C.[1]
1: EID Méditerranée, F-34184 Montpellier, France.
2: Biodiversity Research Center, Earth and Life Institute, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium.
3: CIRAD, UPR AGIRs, UMR TETIS, F-34398 Montpellier, France. 4: CIRAD, UMR TETIS, F-34398 Montpellier, France.

EMBO Conference: "Molecular and population biology of mosquitoes and other disease vectors", Kolymbari (Greece), July 24th-29th, 2015.

Background & objective

The Asian tiger mosquito *Aedes albopictus* is an invasive species extending its distribution range in Europe since 1990. The establishment of this vector represents a new health risk issue, which resulted in several chikungunya (CHIKV) and dengue fever (DENV) outbreaks since 2007. Within Western Europe, France is very exposed as shown by the frequently reported autochthonous cases (2 outbreaks of CHIKV (13 cases) and 5 outbreaks of DENV (7 cases)). These outbreaks occurred despite the system of surveillance and treatment of imported and autochthonous cases, set up by France and unique in Europe. We present here an integrated analysis of these cases, using biological, environmental and epidemiological parameters.

Where does trouble come from?

From an early local detection of *Ae. albopictus*:

- About **4-5 years are necessary** to reach the **maximum density of mosquitoes** [1].
- And 80% of the **autochthonous cases** (n=16) occurred **only 3 years** after the local detection.

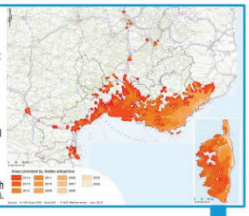


Fig 1. Spread of *Aedes albopictus* in the French Mediterranean area (2004-2014).

The modelled vectorial transmission risk depends on female mosquitoes' seasonal abundance [1] and the human population density. **August and September are the months of higher risks**.

All first autochthonous cases occurred in **houses with garden**, in urban and **suburban habitats** (high numbers of mosquito breeding sources). The autochthonous transmissions remained focal, thanks to the early treatment of cases.

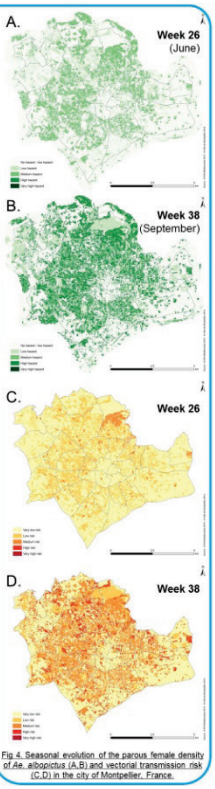


Fig 4. Seasonal evolution of the parous female density of *Ae. albopictus* (A,B) and vectorial transmission risk (C,D) in the city of Montpellier, France.

When does it break bad?

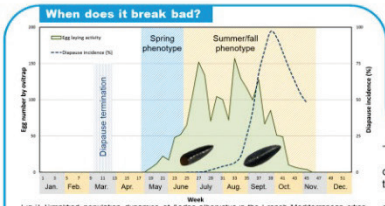


Fig 2. Simplified population dynamics of *Aedes albopictus* in the French Mediterranean cities.

A spatiotemporal risk

- A species with 2 seasonal wing phenotypes:
  → There is no difference in sensitivity to deltamethrin (insecticide used for antivectorial treatments) between both phenotypes.
  → The 2 phenotypes possess different values for biological parameters (fecundity, longevity, etc.); Do they also have a different vectorial competence?

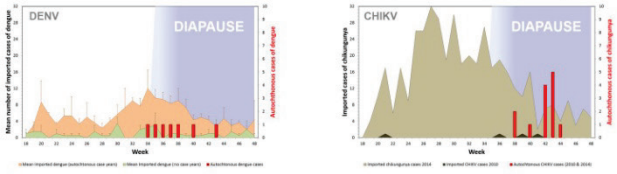


Fig 3. Imported and autochthonous cases of DENV and CHIKV from May to November in Metropolitan French areas colonized by *Ae. albopictus* (data: INVS).

- Each arbovirus has its own pattern of transmission potential:
  → DENV transmission is **density-dependent of imported cases**…
  → … contrary to CHIKV, where the transmission potential depends of the **CHIKV strain** (all cases were caused by the East / Central / South Africa (**ECSA**) genotype [2;3]) and the local *Ae. albopictus* populations [4].
- No indigenous case of DENV and CHIKV before mid-August (unlike the 2007 Italian outbreak of CHIKV [5]):
  → The risk of a large scale outbreak is decreased…
  → … but the outbreaks occurred during diapause initiation. The risk that arboviruses might be maintained in the vector populations during winter, through vertical transmission from the female to her progeny, is increased. This risk need to be better estimated.

Discussion

The recurrent autochthonous transmissions of CHIKV and DENV in France are linked to the vector competence and population dynamics. Indigenous transmission occurred less than 3 full years after a local introduction of *Ae. albopictus*. No outbreak began before August, partly due to the lower transmission risk. Early outbreaks are possible, which could lead at worst to a large scale outbreak. The possibility of reappearance of an outbreak from diapausing mosquitoes should also be considered. To avoid these scenari we recommend, a generalized spring larval source reduction by operators (preceding and/or following autochthonous transmission) and an increased vigilance beginning of summer by physicians. Hence the French surveillance and treatment process shows a relative efficiency, even though it is impossible to prove that actual outbreaks have been avoided.

References: [1] Tran et al., 2013; [2] Grandadam et al., 2011; [3] Delisle et al., 2015; [4] Zouache et al., 2014; [5] Rezza et al., 2007.

Acknowledgments: Marie-Claire PATY (INVS) and Nathalie BARRAS (EID-Med)

ENTENTE INTERDEPARTEMENTALE POUR LA DEMOUSTICATION DU LITTORAL MEDITERRANEEN
Direction technique Pôle méditerranéen de l'environnement littoral
165 avenue Paul-Rimbaud 34184 Montpellier Cedex 4
Tél : 04 67 63 67 63 - Fax : 04 67 63 54 05 - E - mail : glacour@eid-med.org - http://www.eid-med.org

Scientific communications list

Published articles

Lacour, G., Chanaud, L., L'Ambert, G., Hance, T., 2015. Seasonal Synchronization of Diapause Phases in *Aedes albopictus* (Diptera: Culicidae). PLoS ONE 10(12). doi: 10.1371/journal.pone.0145311

Roche, B., Léger, L., L'Ambert, G., **Lacour, G.**, Foussadier, R., Besnard, G., Barré-Cardi, H., Simard, F., Fontenille, D., 2015. The spread of *Aedes albopictus* in Metropolitan France: contribution of environmental drivers and human activities and predictions for a near future. PLoS ONE 10(5). doi: 10.1371/journal.pone.0125600

Lacour, G., Vernichon, F., Cadilhac, N., Boyer, S., Lagneau, C., Hance, T., 2014. When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Insect Physiology 71, 87–96. doi:10.1016/j.jinsphys.2014.10.008

Tran, A., L'Ambert, G., **Lacour, G.**, Benoît, R., Demarchi, M., Cros, M., Cailly, P., Aubry-Kientz, M., Balenghien, T., Ezanno, P., 2013. A Rainfall- and Temperature-Driven Abundance Model for *Aedes albopictus* Populations. International Journal of Environmental Research and Public Health 10(5), 1698-1719. doi:10.3390/ijerph10051698

Yssouf, A., Socolovschi, C., Flaudrops, C., Ndiath, M.O., Sougoufara, S., Dehecq, J.S., **Lacour, G.**, Berenger, J.M., Sokhna, C.S., Raoult, D., Parola, P., 2013. Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry: An Emerging Tool for the Rapid Identification of Mosquito Vectors. PLoS ONE 8(8): e72380. doi:10.1371/journal.pone.0072380

Posters

Lacour, G., L'Ambert, G., Cros, M., Jeannin, C., Ferre, J.B., Gion, M., Tran, A., Lagneau, C., 2015. A retrospective review of chikungunya and dengue outbreaks in French Mediterranean area in view of the biology of the Asian tiger mosquito. EMBO conference on molecular and population biology of mosquitoes and other disease vectors, Kolymbari, Greece, 24-29th july 2015.

Cros, M., Tran, A., Demarchi, M., **Lacour, G.**, L'Ambert, G., Alfonsi, J., Gibergues, P-M., 2014. A mapping tool to assess entomological risk related to *Aedes albopictus*. E-SOVE 19th conference, Thessaloniki, Greece, 13-17th october 2014.

Lacour, G., Tran, A., L'Ambert, G., Cros, M., Benoît, R., Chanaud, L., Viano, M., Demarchi, M., 2013. Modélisation de la dynamique temporelle des populations d'*Aedes albopictus* en milieu tempéré: un outil d'aide à la

décision pour la démoustication. LIFE+ "lutte intégrée contre les moustiques" final conference, Montpellier, France, 23-24th october 2013.

Mercier, A., Velo, E., Bino, S., Shukullari, A., Mersini, K., **Lacour, G.**, Reiter, P., 2013. Chikungunya virus transmission in Paris. EDENext 2013 Annual Meeting, Bellaterra, Spain, 19-22th march 2013.

Lacour, G., Vernichon, F., Cadilhac, N., Lagneau, C., Hance, T., 2012. Photoperiod and diapause effects on chronology of embryonic development of *Aedes albopictus*. E-SOVE 18th conference, Montpellier, France, 8-11th october 2012.

Lacour, G., Hance, T., 2010. Synthèse: la diapause de l'œuf chez *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae). 7ème conférence internationale francophone d'entomologie (CIFE), Louvain-la-Neuve, Belgique, 5-10th july 2010.

Fontaine, A., Pascual, A., Orlandi-Pradines, E., Bourdon, S., Bakkali, N., Coffinet, T., Gardella, F., Mouchet, F., L'Ambert, G., **Lacour, G.**, Remoue, F., Pages, F., Fusai, T., Rogier, C., Almeras, L., 2010. Relationship between exposure level of southeast French individuals to mosquito bites and their respective antibody response against mosquito salivary gland extracts. "Emerging vector-borne Diseases in a changing european ENvironment" (EDEN) conference, Montpellier, France, 10-12th may 2010.

Lacour, G., Carron, A., Jeannin, C., Delaunay, P., Benoît, R., Perrin, Y., Lagneau, C., 2010. *Aedes albopictus* (Skuse, 1894) (Diptera : Culicidae) in France: gonotrophic cycle, fecundity and longevity. "Emerging vector-borne Diseases in a changing european ENvironment" (EDEN) conference, Montpellier, France, 10-12th may 2010.

Oral communications

Lacour, G., 2015. When night comes closer: Interest and consequences of photoperiodic regulation of diapause for the invasive vector Asian tiger mosquito. EMBO conference on molecular and population biology of mosquitoes and other disease vectors, Kolymbari, Greece, 27th july 2015.

Lacour, G., 2014. Nouveaux contextes en épidémiologie vectorielle: influence du climat et des facteurs anthropiques sur la circulation de la dengue et du Chikungunya en métropole. Invited talk, premier colloque scientifique national « Santé et Biodiversité», Lyon, France, 27th october 2014.

Lacour, G., Tran, A., Prudhomme, J., L'Ambert, G., Hance, T., 2014. Revealing the necessity of winter diapause for temperate populations of *Aedes albopictus* (Diptera: Culicidae) by field tests and modelling. E-SOVE 19th conference, Thessaloniki, Greece, 16th october 2014.

Lacour, G., 2013. Lutte antivectorielle contre *Aedes albopictus* par photo-inhibition en milieu tempéré. Invited talk, IMMI, INSERM, Paris, France, 3rd july 2013.

Lacour, G., 2013. Winter is coming : phenology and phenotypic plasticity of the Asian tiger mosquito under temperate climate. Invited talk, BDIV, UCL, Louvain-la-Neuve, 7th februar 2013.

Lacour, G., Marquereau, L., Dujardin, J.P., Benoît, R., Chanaud, L., Viano, M., Boussès, P., Lagneau, C., Hance, T., 2012. Winter is coming: diapause phenology, wing shape and size seasonality of a temperate population of *Aedes albopictus* (Diptera: Culicidae). E-SOVE 18th conference, Montpellier, France, 8th october 2012.