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CONSENSUS DOCUMENT ON THE BIOLOGY OF MOSQUITO AEDES AEGYPTI

Series on Harmonisation of Regulatory Oversight in Biotechnology
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No. 65

Consensus Document on the Biology of Mosquito *Aedes aegypti*

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ABOUT THE OECD

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The Environment, Health and Safety Division publishes free-of-charge documents in twelve different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides; Biocides**; **Risk Management; Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds**; **Chemical Accidents**; **Pollutant Release and Transfer Registers**; **Emission Scenario Documents**; **Safety of Manufactured Nanomaterials**; and **Adverse Outcome Pathways**. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/chemicalsafety/).

This publication is available electronically, at no charge.

For the complete text of this and many other Biosafety publications, consult the OECD's World Wide Web site (www.oecd.org/science/biotrack/)

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Foreword

The Consensus documents prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology contain information for use during the regulatory assessment of the environmental safety (or 'biosafety') of a particular product. In the area of plants, these are being published on information on the biology of certain species of crops and trees, selected traits that may be introduced into plant species, and biosafety issues arising from certain general types of modifications made to plants. The first Consensus document dealing with the biology of an animal species, the Atlantic salmon, was issued as No. 64 of the Series in 2017.

This document addresses the biology of mosquito *Aedes aegypti*.

Mexico, Brazil and the ILSI Research Foundation served as the co-leads in the preparation of this document, and the draft has been revised based on the input from other member countries and stakeholders.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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Abbreviations and acronyms

°C	Degree Celsius
µl	Microlitre
Ae.	*Aedes*
Bti	*Bacillus thuringiensis* var. *israelensis*
CDC	Centres for Disease Control and prevention
CHIKV	Chikungunya virus
CI	Cytoplasmic incompatibility
CRISPR	Clustered regularly interspaced short palindromic repeats
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue virus
DENV-1	Dengue 1 virus
DENV-2	Dengue 2 virus
DENV-3	Dengue 3 virus
DENV-4	Dengue 4 virus
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
EIP	Entrinsic incubation period
ELISA	Enzyme-linked immunosorbent assay
FISH	Fluorescence in-situ hybridisation
g/ha	Gramme per hectare
Gb	Gigabases (= 10^9 base pairs; genome size unit)
GE	Genetically engineered
HEGs	Homing endonuclease genes
ICTV	International Committee on Taxonomy of Viruses
IGR	Insect growth regulator
IIT	Incompatible insect technique
IRM	Insecticide resistance management
IRS	Indoor residual spraying
ISS	Indoor space-spraying
IVM	Integrated vector management
kdr	Knockdown resistance
km	Kilometre
LGT	Lateral gene transfer
LLIN	Long-lasting insecticidal netting

m	Metre
Mb	Megabases (= 10^6 base pairs; genome size unit)
MDT	Mean distance travelled
MEB	Midgut escape barrier
MG	Midgut
MIB	Midgut infection barrier
MITE	Miniature inverted repeat transposable element
ml	Millilitre
mm	Millimetre
NADH	Nicotinamide adenine dinucleotide
ND4	NADH dehydrogenase subunit 4
NVA	Neovolcanic axis
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
piRNA	Piwi-interacting RNA (ribonucleic acid)
QTL	Quantitative trait locus [plural form: Q. t. loci]
RAPD	Random amplified polymorphic DNA (deoxyribonucleic acid)
RFLP	Restriction fragment length polymorphism
RNAi	RNA (ribonucleic acid) interference
SIT	Sterile insect technique
SNP	Single nucleotide polymorphism
STS	Sequence-tagged site
TE	Transposable elements
UBH	Uriah Butler Highway (in Trinidad Island, Trinidad and Tobago)
VC	Vector competence
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme
YFV	Yellow fever virus
ZIKV	Zika virus

Executive summary

This OECD consensus document on the biology of mosquito *Aedes aegypti* is published in the Series on Harmonisation of Regulatory Oversight in Biotechnology. The Series relates to the environmental risk/safety assessment of transgenic organisms, also called "biosafety" assessment. The document provides a useful tool to national authorities and scientists involved in the evaluation of the safety of genetically-engineered mosquitoes when released in the environment.

The mosquito *Aedes aegypti* is of major public health concern, being the main vector of viruses responsible for diseases such as yellow fever, dengue fever, Zika fever and chikungunya. Its development in tropical and sub-tropical areas is intrinsically linked to human habitats and activities that offer the insect its adequate living conditions and the blood meal it needs for reproduction. This mosquito species is subject to biotechnological research and applications (including genetic engineering), aiming to contribute to the control of its population and thus limiting its drastic impact on human health.

Considering the rising spread of related epidemics in many parts of the world, together with the development of genetically-engineered mosquitoes contemplated for use in integrated control management, the OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology (WG-HROB) decided to develop this document on *Ae. aegypti* biology. The project, launched in 2014, was co-led by Mexico, Brazil and the ILSI Research Foundation, with additional expertise provided by Australia, France, India, Kenya, the United States and the industry sector. Other countries and observer organisations involved in the WG-HROB activities also participated in the preparation of the document.

Modern biotechnologies are applied to plants species (crops, flowers, trees), animals and micro-organisms. The safety of the resulting transgenic organisms when released in the environment for their use in agriculture, forestry, the food and feed industry or for other applications represents a challenging issue. Genetically-engineered products are rigorously assessed by their developers during their elaboration, and by governments when ready for release, to ensure high safety standards. This remains essential with new biotechnology developments using insects to fight against disease outbreaks: engineered mosquitoes need to be evaluated through a scientifically sound approach to risk/safety assessment that will inform biosafety regulators and support the decision concerning the release of these novel organisms in the environment.

The OECD offers a long-standing recognised expertise in biosafety and contributes to facilitating a harmonised approach. The WG-HROB, established in 1995, gathers national authorities responsible for the environmental risk/safety assessment of products of modern biotechnology in OECD countries and other economies. International organisations and experts involved in biosafety activities are associated with this programme.

The environmental risk/safety assessment of transgenic organisms (biosafety assessment) is usually based on the information collected on the characteristics of the host organism, the introduced traits, the environment into which the organism will be released, the interaction between these, and the intended use of the organism. The OECD consensus documents elaborated by the WG-HROB identify parts of this information which could be commonly used in countries when conducting biosafety assessment, aiming to encourage information sharing and prevent duplication of effort among countries. They offer practical tools which compile science-based information relevant for this purpose. They are not a substitute for national requirements and locally-available data should also be taken into account, but they can contribute to the risk/safety assessment process. These documents are publicly available and considered worldwide as sustainable references for use in biosafety evaluation.

To conduct biosafety assessment of *Aedes aegypti*, a deep knowledge of the mosquito species is required to fully consider its potential interaction with the environment of release. Useful information can go from accurate taxonomic nomenclature, the origin of the species and its current distribution in the world, up to the life cycle of the mosquito in its successive forms (eggs, larvae, pupae, and male/female adults). The reproductive biology is also essential to understand its behaviour: what are its breeding sites and reproduction features (mating, physiological aspects, fecundity), and the potential effect of *Wolbachia* bacteria. The *Ae. aegypti* genetics is also of great value, including genetic linkage map, population genetics and phylogeography, susceptibility to insecticides and resistance mechanisms, as well as genetic variability in the mosquito competence to transmit virus infection. Then, it is crucial for biosafety assessors to acquire extensive knowledge of the ecology of this mosquito, i.e. its interactions with the other species in the environment: ecological niche it occupies; the climatic parameters influencing its development; its anthropic habitats in strong connection with human population; the abiotic requirements in terms of water and food availability; and the fitness to local conditions including its dispersal, population distribution and modelling.

Additional information regarding human and animal health affected by mosquitoes, as well as current strategies and integrated management set up to control *Ae. Aegypti*, can be found in the annexes to this publication.

To prepare this document, experts have shared their knowledge and summarised key elements from a vast range of solid science-based publications, selected for their potential interest during biosafety assessment and carefully referenced. This information is intended to benefit-risk assessors that may need to consider potential effects on the environment when releasing engineered *Ae. aegypti* in the context of mosquito control programmes, and therefore may contribute in facilitating the decision-making process.

Another mosquito, *Anopheles gambiae*, is currently considered by the WG-HROB for developing a similar biology document. The *A. gambiae* complex of species includes the most important vectors of malaria disease, and biotechnological solutions for its control are being explored. The future document will constitute a useful complement to this publication by enlarging the scope of insects covered by the OECD Biosafety consensus documents.

Chapter 1. Taxonomy, description and distribution of the mosquito *Ae. aegypti*

Classification and nomenclature of *Aedes aegypti*

Classification (Taxonomy)

The family Culicidae is divided into three subfamilies: Toxorhynchitinae, Anophelinae and Culicinae, within which only subfamilies Anophelinae and Culicinae have medically-important mosquito species. The subfamily Culicinae includes over 3 050 species, belonging to 109 genera, of which the most important regarding health issues are the genera *Aedes*, *Culex*, *Mansonia*, *Haemagogus*, *Sabethes*, and *Psorophora* (Service, 2012; Tyagi, Munirathinam and Venkatesh, 2015).

The systematic classification of *Aedes aegypti* is presented in Table 1.1 and localises this species within the order Diptera, family Culicidae, subfamily Culicinae, tribe Aedini, genus *Aedes*, subgenus *Stegomyia*, and species *Aedes aegypti* (ITIS, 2014; WRBU, 2014).

Table 1.1. Standardised taxonomic hierarchy and nomenclature for *Ae. aegypti* (Linnaeus, 1762)

TAXON	NOMENCLATURE (Authority)
Kingdom	Animalia (Margulis and Schwartz, 1998)
Subkingdom	Bilateria (Hatschek, 1888)
Infrakingdom	Protostomia (Grobben, 1908)
Superphylum	Ecdysozoa (Aguinaldo et al., 1997)
Phylum	Arthropoda (Latreille, 1829)
Subphylum	Hexapoda (Latreille, 1825)
Class	Insecta (Linnaeus, 1758)
Subclass	Pterygota (Lang, 1888)
Infraclass	Neoptera (Martynov, 1923)
Superorder	Endopterygota (Sharp, 1898)
Order	Diptera (Linnaeus, 1758)
Suborder	Nematocera (Berthold, 1827)
Infraorder	Culicomorpha (Wood and Borkent, 1989)
Family	Culicidae (Stephens, 1829)
Subfamily	Culicinae (Meigen, 1818)
Tribe	Aedini (Neveu-Lemaire, 1902)
Genus	*Aedes* (Meigen, 1818)
Subgenus	*Stegomyia* (Theobald, 1901)
Species	*Aedes aegypti* (Linnaeus, 1762)

Source: ITIS (2014), *Aedes aegypti*, Integrated Taxonomic Information System (database), www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=126240; WRBU (2014), Mosquito Classification Comparison, 2013, The Walter Reed Biosystematics Unit.

Subspecies. Human population increase and extension to wild habitats, in addition to the evolution of vector behaviour, are important phenomena that greatly influence the "domestication" and the constitution of subpopulations of many mosquitoes (Powell and Tabachnick, 2013). *Ae. aegypti* presents two subspecies or subpopulations:

- The first subspecies, *Ae. aegypti formosus*, is the ancestor of the domestic form of *Ae. aegypti* and still lives in forests and vegetated ecotones in sub-Saharan Africa (Lounibos, 1981). In addition to its attraction to tree holes for breeding habitats and egg laying, it has a preference for non-human blood as sources of blood meals (required by females for egg production) and feeds on wild animals. Morphologically, this form is much darker than the form adapted to human habitats (McClelland, 1974).
- The second subspecies, *Ae. aegypti aegypti* (often designated by the shorter name *Ae. aegypti*), is found globally in tropical and subtropical regions, typically in association with humans, but is absent from the interior of Africa south of the Sahara (Moore et al., 2013; Powell and Tabachnick, 2013). In contrast to the first subspecies, *Ae. aegypti aegypti* predominantly breeds in artificial containers provided by humans, also breeds indoors, and has a preference for feeding on human blood (Moore et al., 2013).

A third subspecies was previously thought to exist, *Ae. aegypti queenslandensis*, described as a light-coloured form found in the Mediterranean Basin (Mattingly, 1967). However, recent analysis suggests that *Ae. aegypti queenslandensis* is genomically identical to the second subspecies *Ae. aegypti aegypti* (Rašić et al., 2016).

Nomenclature

Common names. The usual common name for *Ae. aegypti* is the "yellow fever mosquito", as it is a principal vector for yellow fever. The closely-related species *Ae. albopictus* is often referred to as "Asian tiger mosquito". In colloquial language, "tiger mosquito" is sometimes used for naming both species indistinctly, drawn from the observation of their striped-colour abdomen.

Synonyms. If two or more names are found to apply to the same species, they are considered synonyms. The name *Ae. aegypti* (Linnaeus, 1762) is now in general use and has been for more than five decades. However, this species has appeared under many other names in the past, among the most cited are (ITIS, 2014; WRBU, 2014):

- *Culex aegypti* (Linnaeus, 1762)
- *Culex excitans* (Walker, 1848) and
- *Culex taeniatus* (Weidemann, 1828).

Recent studies have resulted in a number of generic and subgeneric changes to the classification of the tribe Aedini in Europe and other regions of the world. Among other changes, the subgenus *Stegomyia* was elevated to the category of genus for the species *Ae. aegypti* and *Ae. albopictus* (*Stegomyia aegypti* and *St. albopicta*, respectively) (Reinert and Harbach, 2005). In practice, it is rarely called *St. aegypti* and is still commonly referred to as *Ae. aegypti*.

Systematics

Ae. aegypti and *Ae. albopictus* populations seem to have different evolutionary histories, the former originated from Africa and the latter from South-East Asia. For *Ae. aegypti*,

the general structure of the phylogenetic trees based on mitochondrial genes showed that most populations from South America were found to be genetically similar to populations from South-East Asia (Thailand and Viet Nam), except for one sample from Boa Vista (northern Amazonia), which was more closely related to samples from Africa (Côte d'Ivoire and Guinea). This suggests that African populations of *Ae. aegypti* introduced during the slave trade have persisted in Boa Vista, resisting eradication campaigns (Mousson et al., 2005).

Over the past 50 years, many population genetic studies of *Ae. aegypti* have documented large genetic differences among worldwide populations. Phylogenetic analyses, including through studies involving population genetics of *Ae. aegypti* s.1. using mitochondrial DNA markers, have shown that global collections fell into two clades (Tabachnick and Powell, 1979; Powell, Tabachnick and Arnold, 1980; Tabachnick, 1982, 1991; Lorenz et al., 1984; Wallis, Tabachnick and Powell, 1984; Tabachnick et al., 1985; Muñoz et al., 2013; Moore et al., 2013). One clade contained *Ae. aegypti* from East Africa, South America and the Caribbean, suggesting that these New World populations were derived directly from East African populations. The other clade contained Asian and south-eastern United States *Ae. aegypti*, along with a basal branch containing subspecies *Ae. aegypti formosus* from both East and West Africa, suggesting an independent introduction of *Ae. aegypti* to Asia (Moore et al., 2013; Powell and Tabachnick, 2013). Further support for the existence of two principal clades worldwide is provided from studies in Africa (Brown et al., 2011; Delatte et al., 2011) as well as the New World (Bracco et al., 2007; Scarpassa, Cardoza and Cardoso Junior, 2008).

Morphology

Morphologic features have been used in many studies to describe variations among populations of the same species. Morphological characteristics of *Ae. aegypti* life stages are described in greater detail in the following sub-sections.

Eggs

Eggs of *Ae. aegypti* are long, smooth, more or less ovoid shaped, and approximately 1 mm long. They are white in colour when freshly laid but turn black as a result of melanisation about two hours after oviposition (this colour change is not exclusive to *Aedes* mosquito species) (Nelson, 1986; Service, 2012).

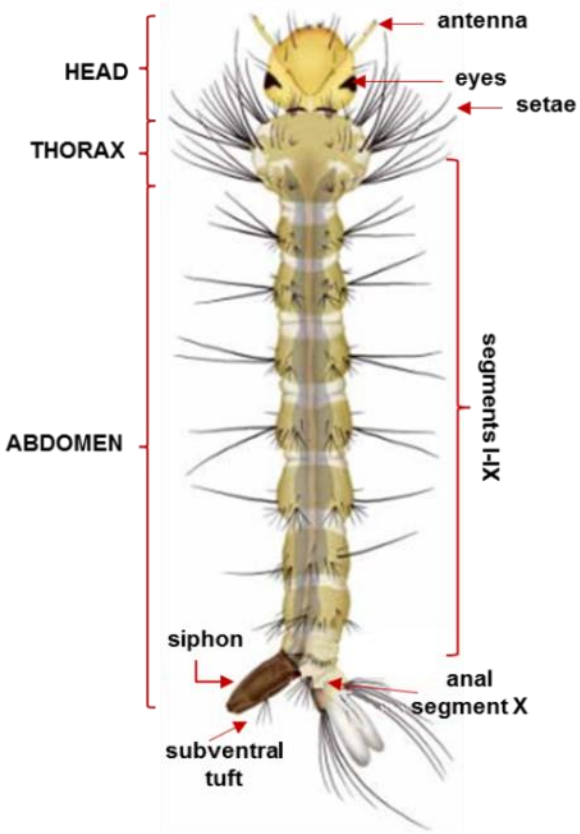
Aedes females lay individual eggs in artificial collections of water, often placed at varying distances from the water line. In addition, a female will preferably not lay the entire clutch at a single site, but rather spread the eggs over two or more sites in a practice known as "skip oviposition". Thus, the eggs stand a better chance of survival (Mogi and Mokry, 1980; Chadee, 1997; Harrington and Edmann, 2001; Foster and Walker, 2002). It was observed that eggs may be laid on successive occasions on the same site (Gillet, 1962) or in different sites (Fay and Perry, 1965; Chadee and Corbet, 1987). The practice of skip oviposition indicates the tendency of a female to avoid laying on surfaces that already bear her own eggs or those of conspecifics (Chadee, Corbet and Greenwood, 1990).

Ae. aegypti eggs can dry, survive desiccation, remain intact for several months and hatch when submerged with water. More details relating to their survival under different temperature and humidity conditions are given under the "Life cycle" section in Chapter 2.

Larvae

Ae. aegypti larvae resemble other mosquito larvae in their morphology; in general, they have an ovoid head, thorax, and abdomen of nine segments. The posterior segment (anal) has four lobed gills for osmotic regulation and a short barrel-shaped siphon bearing a single pair of subventral tufts for breathing at the water surface (Figure 1.1) (Nelson, 1986; Clements, 2000; Service, 2012). Additional morphologic characteristics include at least three pairs of setae in the ventral brush, antennae that are not greatly flattened, and a lack of enormous setae on the thorax. These characteristics are sufficient in distinguishing *Aedes* larvae from most others belonging to family Culicidae and subfamily Culicinae (Service, 2012).

Figure 1.1. Dorsal view of *Ae. aegypti* larva

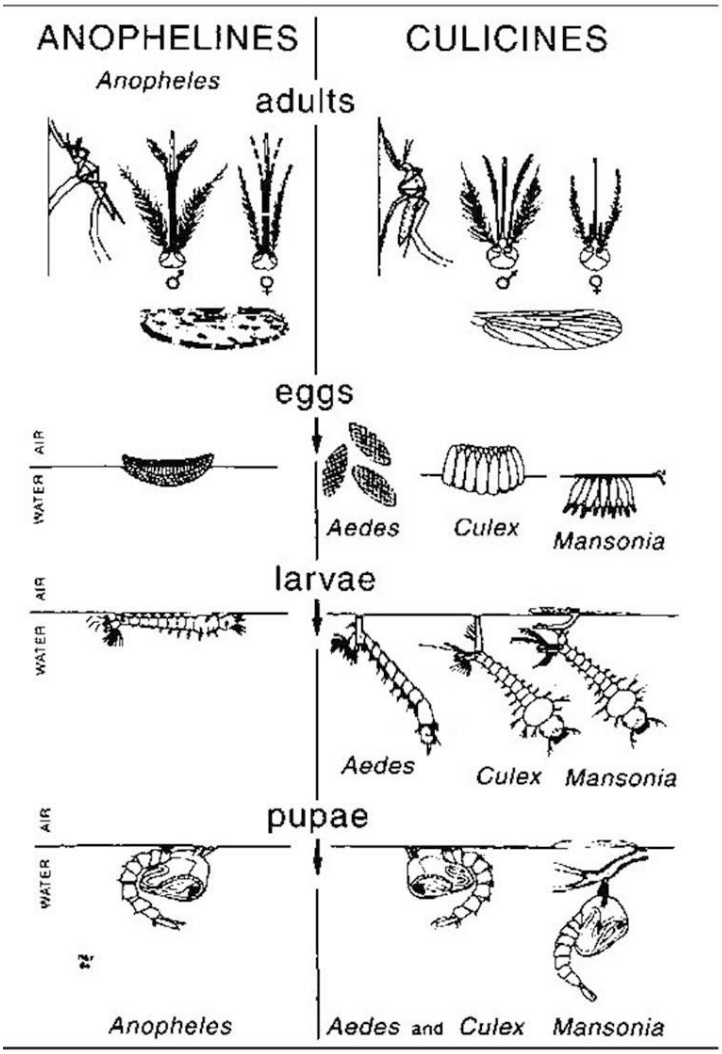


Source: Modified from Rueda, L. (2004), "Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission", in *ZOOTAXA 589*, Magnolia Press, Auckland, pp. 60.

The resting position at the water surface is also different among the various mosquito species: *Anopheles* larvae lay parallel to the water surface, *Culex* larvae rest at an angle and *Aedes* larvae hang almost vertically (Figure 1.2). The larvae pass through four instars (I, II, III, and IV respectively) with growth and changes in form and size occurring during their development. The first instar *Ae. aegypti* larva is only about 1 mm in length, whereas in the fourth instar stage it reaches a length of approximately 8 mm (Schaper and Hernandez-Chavarria, 2006; Bar and Andrew, 2013a). Growth and development of larval instars is temperature dependent, however, complex interactions with other factors such as resource availability and intraspecific density also contribute to variation in

development rate (Couret and Benedict, 2014). At cool environmental temperatures (around 15°C), *Ae. aegypti* larvae can remain in a particular instar for months, so long as the water supply is sufficient (Foster and Walker, 2002; Bar and Andrew, 2013a; Brady et al., 2013).

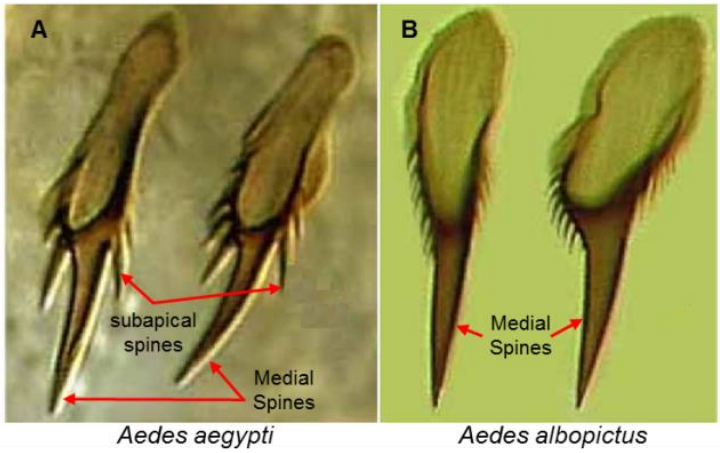
Figure 1.2. Comparison of the adults, eggs, larvae and pupae of mosquito genera *Anopheles*, *Aedes*, *Culex* and *Mansonia*



Source: Modified from Warrell, D.A. and H.M. Gilles (eds.) (2002), *Essential Malariology, 4th Ed.*, Hodder Arnold, London, pp. 350.

The most distinguishing characteristics facilitating the differentiation of *Ae. aegypti* larvae from many other species of the *Aedes* genus are the 2 lateral spines on each side of the thorax and the straight row of 7 to 12 comb scales on the 8th abdominal segment. *Ae. aegypti* exhibits a medial spine with stout, subapical spines (Figure 1.3, panel A) which are absent in *Ae. albopictus* (Figure 1.3, panel B) (Nelson, 1986).

Figure 1.3. Comb scales of *Ae. aegypti* exhibiting a medial spine with stout, subapical spines and of *Ae. albopictus* without subapical spines



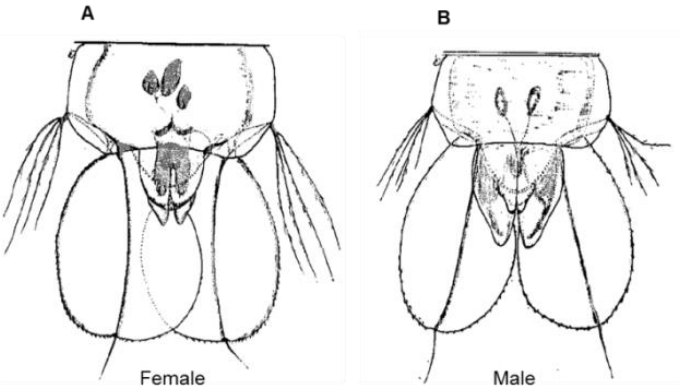
Source: Modified from Rueda, L. (2004), "Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission", in *ZOOTAXA 589*, Magnolia Press, Auckland, pp. 60.

Pupae (sexual dimorphism)

The pupa is the stage of the life cycle of mosquitoes that follows the last larval instar and precedes the adult stage. Pupae are comma-shaped, composed of two main sections, cephalothorax (head and thorax fused) and abdomen (Nelson, 1986; Service, 2012). At the base of the cephalothorax of the pupa is a pair of breathing tubes or "trumpets" that pierce the water surface to allow breathing (Nelson, 1986). At the tip of the abdomen there is a pair of oars or paddles used for swimming, which in the female (Figure 1.4, panel A) are wider and overlap, but in the male (Figure 1.4, panel B) are narrow and separated (Vargas, 1968).

Another morphologic difference between female and male pupae is their overall size, with the female usually being larger than the male (Figure 1.4). Since the range in body size between female and male pupae overlaps considerably and can be affected by both biotic and abiotic, including environmental factors such as diet, temperature, rearing conditions, overcrowding, it is deemed necessary to select additional sexually dimorphic characteristics such as the differences in paddles in order to determine the sex of pupae (Vargas, 1968).

Figure 1.4. Anal segments of *Ae. aegypti* pupae - ventral view, showing dimorphism characters between females and males

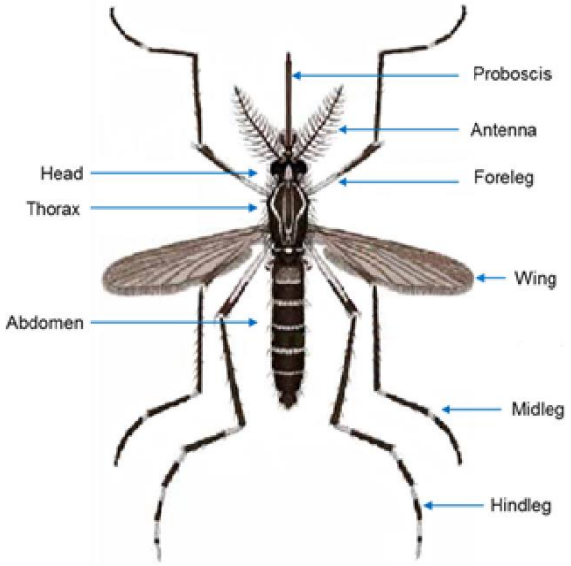


Source: Modified from Vargas, V.M. (1968), "Sexual dimorphism of larvae and pupae of *Ae. aegypti* (Linn.)", *Mosquito News*, Vol. 28, pp. 374-379.

Adults (male and female)

The body of an adult *Ae. aegypti* mosquito is composed of head, thorax, and abdomen (Figure 1.5). *Ae. aegypti* males and females are similar in appearance except for the differences in size and form of the antennae (males have plumose antennae), maxillary palps (females have shorter palps), abdomen, claws and in scale markings (Bar and Andrew, 2013b). These differences are described in detail below.

Figure 1.5. Dorsal view of the female mosquito *Ae. aegypti*



Source: Modified from Rueda, L. (2004), "Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission", in *ZOOTAXA 589*, Magnolia Press, Auckland, pp. 60.

Head

In both male and female *Ae. aegypti*, dorsally the head is globular in shape and laterally convex with a vertex that has silvery-white flat scales. The female clypeus has two silvery white dots, whereas the male has no dots. Females have a larger head capsule (0.55 ± 0.09 mm) than males (0.53 ± 0.06 mm) (Bar and Andrew, 2013b). The head bears several structures critical to the mosquito's ability to feed as well as to act as a vector of human diseases.

Mouthparts. The mouthparts in these mosquitoes include a pair of maxillary palps, which have five white scale bands and are longer (0.77 ± 0.06 mm) and more developed in males than in females (0.53 ± 0.06 mm) (Bar and Andrew, 2013b). The proboscis is longer in males (0.76 ± 0.04 mm) than in females (0.66 ± 0.03 mm) (Bar and Andrew, 2013b). However, only in females is this structure adapted for skin penetration to enable blood feeding, even though they may survive in nature by sucking plant juices. The male proboscis is adapted to feed on nectar and plant juices rich in carbohydrates (Clements, 1992).

Antenna. Each antenna of *Ae. aegypti* arises from a globular pedicel, has 13 flagellar segments and a greatly reduced scape. Males have longer antennae (0.57 ± 0.03 mm) than females (0.52 ± 0.07 mm). The antennal hairs are bushy and plumose in males whereas in females they are smaller and less dense (Nelson, 1986; Bar and Andrew, 2013b).

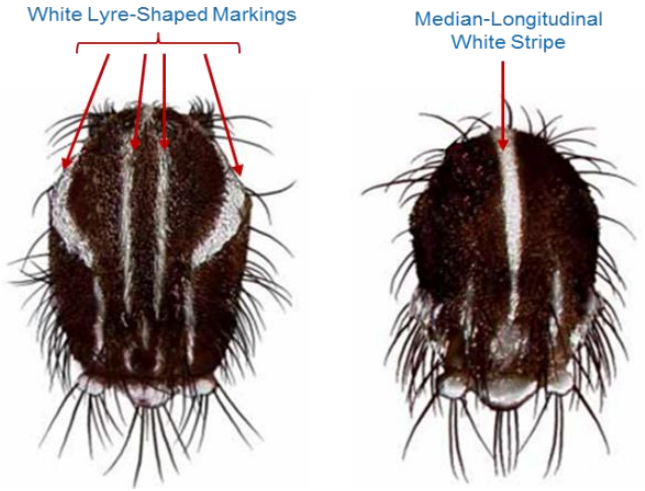
Thorax

Females of *Ae. aegypti* have a larger thorax measuring 0.50 ± 0.08 mm in length and 0.35 ± 0.07 mm in width while the shorter male thorax is 0.41 ± 0.06 mm in length and 0.29 ± 0.02 mm in width. The thorax of *Ae. aegypti* is black or dark brown coloured and consists of the pro-, meso-, and metathoracic segments, which together bear the wings (one pair), legs (three pairs), and halteres (one pair) (Bar and Andrew, 2013b).

Many, but not all, *Aedes* adults have conspicuous patterns on the thorax formed by white or silver coloured scales (Service, 2012), and these patterns vary between species. An example of the difference across species is the case of *Ae. aegypti* with its typical, white, lyre-shaped markings (Figure 1.6, panel A), compared to *Ae. albopictus* with its median-longitudinal white stripe (Figure 1.6, panel B) (Nelson, 1986). The scutellum in *Ae. aegypti* is three-lobed with each lobe having silvery white scale patches, and a few dark scales at the apex of the midlobe (Bar and Andrew, 2013b).

Figure 1.6. Comparative dorsal view of thoracic scutum of *Ae. aegypti* and *Ae. albopictus*

Figure 1.6A: *Ae. aegypti* Figure 1.6B: *Ae. albopictus*



Source: Modified from Rueda, L. (2004), "Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission", in *ZOOTAXA 589*, Magnolia Press, Auckland, pp. 60.

At the same time, adults of *Aedes* and other Culicinae may be distinguished from adult *Anopheles* mosquitoes by their shorter palps and their resting position which is more horizontal or parallel to the resting surface (Nelson, 1986; Service, 2012).

Abdomen

The abdomen consists of eight segments covered with black and white scales forming distinctive patterns in both males and females. In females, the eighth segment is greatly reduced. The tergites (dorsal portion of each abdominal segment) are dark brown in colour and the first abdominal segment has a patch of pale, median scales. The dorsal side of abdominal segments II through VII has transverse white bands. The size of abdomen in males is larger (length 3.03 ± 0.18 mm and width 0.51 ± 0.07 mm) than in females (length 2.94 ± 0.20 mm and width 0.41 ± 0.06 mm) (Bar and Andrew, 2013b).

The posterior tip of the abdomen is narrow in males while in females it has a broad round shape. *Ae. aegypti* can be differentiated from most of the other Culicinae by their pointed abdomen and the absence of spiracular bristles (Service, 2012).

With age, the lyre-shaped markings on the thorax may disappear, but the distinctive white scales on the pedicel, clypeus, and tip of the palps, and the pattern of white scales on abdominal sternites (ventral plate on each abdominal segment) III-V, usually remain. These characteristics are essential for the identification of *Ae. aegypti* females with damaged morphological structures and to differentiate them from *Ae. albopictus* females (Nelson, 1986; Savage and Smith, 1995).

Origin and current geographic distribution

The likely origin of *Ae. aegypti* is the Ethiopian region of the tropical belt in Africa, from which it has spread to tropical and subtropical regions throughout the world in association with humans (Nelson, 1986; Powell and Tabachnick, 2013). *Ae. aegypti* was probably carried to other continents via trading and transport ships that resupplied in African ports

during the 15th century through to the end of the 17th (Christophers, 1960; Reiter, 1998). These ships carried freshwater reservoirs on board and could maintain breeding colonies of *Ae. aegypti* (Christophers, 1960), so it is probable that the species was introduced to the rest of the world via this means (Tabachnick, 1991).

To date, *Ae. aegypti* is an invasive tropical species worldwide with a cosmopolitan habitat from 40° N to 40° S latitude (a range extending across all or most of the world in appropriate habitats).

Ae. aegypti is usually tolerant to temperatures ranging from 14°C to 30°C (Hemme et al., 2010; Brady et al., 2013, 2014). Under optimal conditions of temperature and humidity, the embryo needs two to three days for full development from oviposition to the next stage of the life cycle. The definition of physiological embryonic parameters within this temperature range correlates with the presence of *Ae. aegypti* in tropical and subtropical regions of the world (Farnesi et al., 2009). Larval development in *Ae. aegypti* is a function of temperature, and these effects have been well studied. Temperature also impacts on adult size, dry weight, and ovariole number, all of which decrease as the temperature increases (Christophers, 1960; Rueda et al., 1990). High extreme temperatures alone (> 40°C) are unlikely to limit the species, but low temperatures are a limiting factor. Below 15°C, adult *Ae. aegypti* mosquitoes become torpid, unable to fly, and can move their limbs only slowly (Christophers, 1960; Rowley and Graham, 1968; Yang et al., 2009). Lower temperatures can slow development to such a degree (where egg-to-adult cycles are longer than 45 days) that the species is prevented from establishing itself in the environment, although human habitations may afford some seasonal protection.

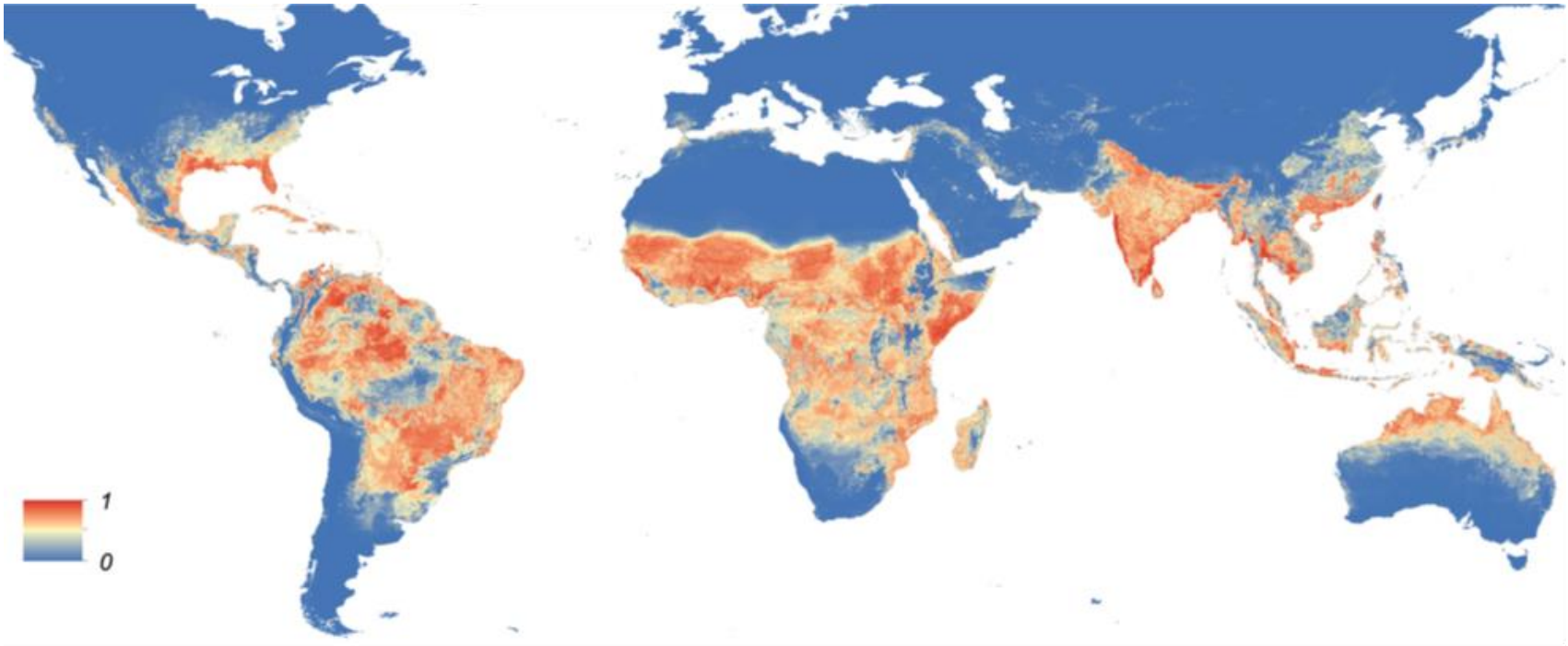
Rain quantity and frequency (precipitation level) is another factor which, combined with temperature, affects the sustainable establishment of the species in a given area.

Global historical collections and laboratory experiments on this well-studied vector have suggested its distribution is limited by the 10°C winter isotherm[1] (Christophers, 1960), while a more recent and complex stochastic population dynamics model analysis suggests the temperature's limiting value to be more towards the 15°C yearly isotherm (Otero, Solari and Schweigmann, 2006). Scholte et al. (2010) indicated that *Ae. aegypti* could not survive winter temperatures in Northern Europe. The predicted global distribution of *Ae. aegypti*, based on occurrence data as well as environmental and land-cover variables, is shown in Figure 1.7 (Kraemer et al., 2015).

Notes

[1] An isotherm is a line on a map or chart of the earth's surface connecting points having the same temperature at a given time or the same mean temperature for a given period.

Figure 1.7. Global map of the predicted distribution of *Ae. aegypti*



Note: The map depicts the probability of occurrence.
• Blue (dark grey) = 0
• Red (light grey) = 1

Source: Kraemer, M.U.G. et al. (2015), "The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*", *eLife*, Vol. 4: e08347.

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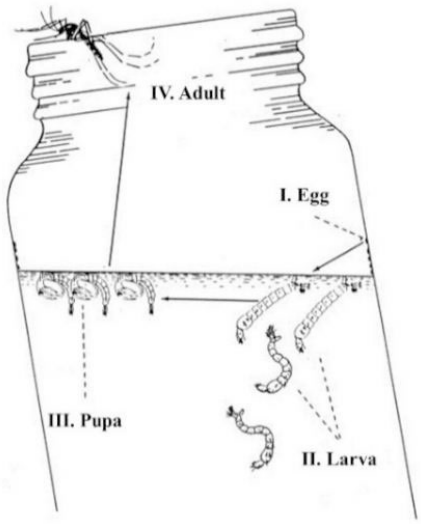
Chapter 2. Reproductive biology of the mosquito *Ae. aegypti*

Life cycle

Four life stages

The life cycle of all species of mosquitoes, including *Ae. aegypti*, corresponds to the holometabolous type (Gordh, 2001) which is basically characterised by complete metamorphosis and four distinct life stages: egg; larva; pupa; and adult (Figure 2.1). The development cycle depends directly on the presence of water and ambient temperature. In warm days with temperatures averaging 25°C, development of eggs into adults is completed in a little more than 1 week. In the case of cool days, development may occur over a period of months (Foster and Walker, 2002). The stages are described below.

Figure 2.1. Life cycle of *Ae. aegypti*



Source: NCDENR (n.d.), *Mosquitoes... Some Facts: Information Pamphlet*, www.alamance-nc.com/envhealth/wp-content/uploads/sites/9/2013/10/Mosquitoes_Facts.pdf.

Breeding sites

The females lay individual eggs above the water level within fresh water held in natural breeding sites including holes in trees, bamboo trunks, hollow rocks, plant axilla, coconut shells, and leaves. Females will also oviposite on the inner wall of various artificial containers such as tanks, vases, jars, tires, drums, buckets, pots, cans, scrap metal and gutters (Nelson, 1986; Ulloa et al., 2010; Pilger et al., 2011), distributed inside houses or in their yards (Kampen and Schaffner, 2008).

The variability in the preference of the different types of containers as sites for oviposition by female *Ae. aegypti* depends on the availability of artificial containers, the degree of urbanisation and the season (Mogi and Mokry, 1980; García-Rejón et al., 2011; Rubio, Cardo and Vezzani, 2011).

Egg stage and embryonic development

Eggs can survive dry conditions for months and hatch once submerged in water, thus enhancing dissemination during the rainy periods. This survival ability of *Ae. aegypti* populations to dry seasons, combined with their intensive spread during rainy seasons, makes the control of *Ae. aegypti* very difficult (Nelson, 1986; Service, 2012).

There has been some research on the correlated effects of temperature and humidity on the eggs of *Ae. aegypti*. Experimental studies indicate that 20% of eggs remain viable after 6 months in 98% humidity (Luz et al., 2008). In Japan, Sota and Mogi (1992) measured survival times of eggs from several *Aedes* species including *Ae. aegypti* and *Ae. albopictus* under 3 different humidity conditions (42%, 68% and 88% of relative humidity) at 25°C, showing that *Ae. aegypti* survived longer than *Ae. albopictus* at all humidity conditions. Sota and Mogi (1992) attributed this to egg volume, with *Ae. aegypti* having the greatest egg volume and thus the greatest ability to resist desiccation. Juliano et al. (2002) also found the effects of temperature and humidity on egg mortality significantly different between the two species, with *Ae. albopictus* experiencing much higher mortality at all combinations except at the highest humidity. The maximum temperature limit for embryogenesis is 35°C and the minimum 12°C and below; and for egg viability optimal temperature ranges between 16-31°C, and with relative humidity above 80% (Farnesi et al., 2009). In a recent study, Thomas et al. (2012) found that eggs of a tropical strain of *Ae. aegypti* could survive at a threshold of 2°C for 24 hours only before hatching ceased. Egg survival at temperatures below freezing is therefore extremely unlikely.

The first 48 hours of embryonic development are critical and microclimatic factors are crucial for embryo survival (Thirión, 2003; Farnesi et al., 2009). The eggs of aedine mosquitoes usually enter a diapause-like state (suspension of development or quiescence) in unfavourable weather conditions (such as low temperature and humidity). They will hatch asynchronously several weeks or even months after being deposited with the return of more favourable conditions (Gillett, Roman and Phillips, 1977; Jeffery et al., 2012). In a natural setting, flooding from rainfall induces a physicochemical stimulus that results in egg hatching. Similarly, eggs are stimulated to hatch when submerged as the water level rises in water storage containers which are in everyday use (Koenraadt and Harrington, 2008). Additionally, other types of stimuli have been associated with hatching, for example, the low concentration of oxygen dissolved in water (Judson, 1960) and the presence of some water-soluble compounds or organisms in the water as a result of microbial activity (Gillett, Roman and Phillips, 1977; Ponnusamy et al., 2011).

Larval and pupal stages

The larval and pupal stages are strictly aquatic. Larval development begins with the first of four instars, each larger than the last. Passing from one larval stage to the next is accomplished by the moulting of chitinous skin that is shed, allowing growth and development of the next instar. Complete larval development typically lasts five to seven days and ends when the fourth instar larva develops and reaches the pupal form (Thirión, 2003). Larvae are omnivorous and spend most of their time feeding with the help of oral

silks arranged in a fan which is used to filter particles of suspended organic matter and microorganisms in the water. They also graze organic matter on the bottom and sides of the flooded container (Colvard, 1978). The larvae feed in the water on protozoa, bacteria, yeasts and algae, both at the bottom of the habitat as well as in the water column (Ponce, 1999).

The duration of the aquatic phase of *Ae. aegypti* from first instar larvae to adult emergence, in the laboratory with water temperature at 24-27°C and no interspecific competition, is 8.42 days on average, with a range of 7.9-9.0 days. However, for both *Ae. aegypti* (Hancock et al., 2016) and *Ae. albopictus* (Sánchez-Hernández, 2011), the development time of larvae is significantly increased by competition for the limited amount of food in containers where the time to pupation can extend up to eight weeks.

Larval development is also favoured by the high prevalence of bacteria such as *Aeromonas hydrophila/caviae*, *Klebsiella oxytoca*, *Pseudomonas* sp., and *Enterobacter cloacae* in artificial breeding sites (tires, tanks, others) (Ulloa, 1996). These bacteria are potential food sources for larvae of *Ae. aegypti*. This study also revealed that discarded tires were the most important in terms of persistence in mosquito density and production of larvae. In this regard, Manrique-Saide et al. (1998) reported that the average time for immature stages of *Ae. aegypti* to develop in used tires was 11.15 to 12.95 days. Temperature, diet, density and their two-way interactions are all significant factors in explaining development rate variation of the larval stages of *Ae. aegypti* mosquitoes (Couret and Benedict, 2014).

The pupa is the last aquatic developmental stage, usually lasting between 2.0 and 3.6 days under optimal conditions (Focks et al., 1981; Nelson, 1986; Manrique-Saide et al., 1998). This stage is mobile (although non-feeding), and swims actively within the container in response to external stimuli such as vibrations and changes in light intensity.

Arrivillaga and Barrera (2004) determined the duration of the whole aquatic development phase (from first larval instar to adult) of *Ae. aegypti* in the laboratory associated with different levels of starvation for the immature stages. Development times varied between 8.5 days and 18.5 days with faster growth associated with increased food, highest water levels, and reduced density of larvae. Moreover, a comparative study between an *Ae. aegypti* wild type strain and a genetically engineered (GE) line[1] showed a shorter time of pupation for the GE line (one day on average) as compared to the wild type strain, with this difference being more pronounced for females (1.4 days) than for males (0.9 day) (Bargielowski et al., 2011).

Adult stage

The adult or imago of the genus *Aedes*, like other groups of mosquitoes, is the reproductive and dispersal stage. Emergence of adult *Ae. aegypti* is usually crepuscular with adults released from the pupal exuviae performing an initial flight to a dry, resting place. The initial 24-hour period post-emergence is the teneral period, a physiological state during which the exoskeleton hardens and sexual maturation occurs (Clements, 2000). The teneral phase results in a fully mature aerial adult capable of flight and mating. Males are the first to emerge and a balanced sex ratio is produced, although sex ratios can be skewed by the presence of other competing species (Sánchez-Hernández, 2011).

The adult life expectancy varies from 10-35 days for female mosquitoes (Goindin et al., 2015) and 3-6 days for male mosquitoes (Clements, 2000) although this is highly

dependent on temperature, being shorter in tropical regions and longer in more temperate climates, etc.

The dispersal range of adults is variable and is influenced by a variety of factors including the sex of the mosquito, density of human hosts, availability of breeding sites, abundance of plants in houses, as well as composition and configuration of ecological landscape (Reiter et al., 1995; Martinez-Ibarra et al., 1997; Rubio, Cardo and Vezzani, 2011). More information is given under Dispersal sub-section in Chapter 4.

Reproduction

Mating

Mosquitoes utilise sexual reproduction to produce new generations. Within 2-3 days after emergence, both sexes mate, and females can take a blood meal which is required for egg development (Lehane, 1991). These two activities often occur simultaneously because males are attracted to both the vertebrate host and the females, thus facilitating mating (Nelson, 1986).

The sound emitted by the flight frequency of females is used by males to locate and copulate with them (Brogdon, 1994). A source of attraction of a male to a female is the sound made by the beating of her wings during flight (Cator et al., 2009; Cator and Harrington, 2011). However, mating after engorgement of the females is rare because once the female has taken a blood meal, she must beat her wings more rapidly to carry her increased weight and the wing-beat frequency is no longer attractive to the male (Nelson, 1986; Cator et al., 2009).

During mating, the male clasps the tip of the female abdomen with his terminalia and inserts his aedeagus into the genital chamber. The female bursa copulatrix becomes filled with male sperm that passes within two minutes to the spermatheacae where they are stored prior to fertilisation of the eggs (Nelson, 1986).

Ae. aegypti females generally mate only once, since a single insemination event allows sufficient sperm to be stored within the spermatheacae to fertilise all the eggs that a female will develop during her lifetime. In addition, the seminal fluid proteins transferred from the male during mating render females unreceptive and refractory to further copulation (Sirot et al., 2008; Avila et al., 2011; Helinski et al., 2012). Thus, once mated, *Ae. aegypti* females are generally not responsive to additional matings for the duration of one or more egg-laying cycles (Cator et al., 2009). They may remate, however, if the spermatheacae is not adequately filled. Results from laboratory studies have revealed that 14% of females are involved in multiple matings (polyandry) within a 48-hour period (Helinski et al., 2012). Polyandry in a natural population of *Ae. aegypti* is low (6.25%), but also likely an underestimate and is within the range of polyandry estimates in other mosquito species (Richardson et al., 2015).

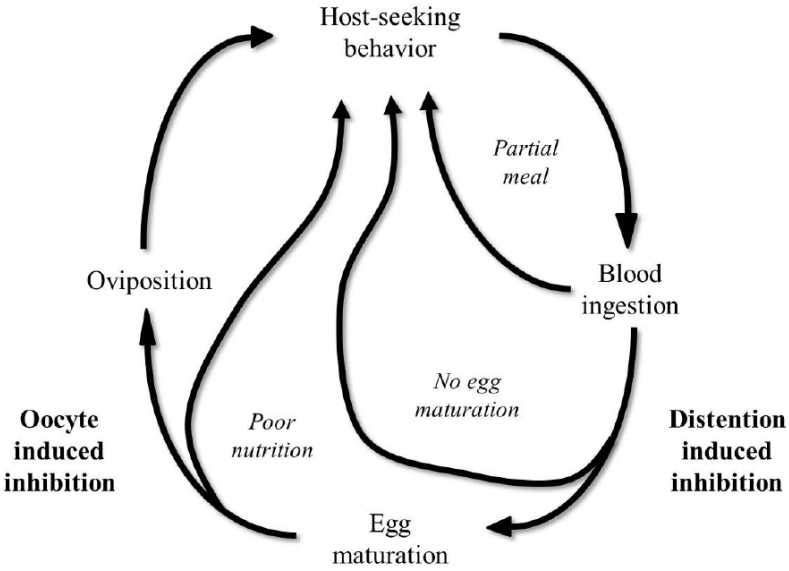
Laboratory studies have determined that the body size of male *Ae. aegypti* is a major predictor of total spermatozoa number, with significantly greater sperm numbers in larger males (2.27 mm wing length) versus smaller males (1.85 mm wing length) within the same age group (Ponlawat and Harrington, 2007). Other studies have shown that under field conditions, larger males inseminated females with more sperm than smaller ones and that older males transferred the greatest number of sperm to females (1 152 sperm by 1-day-old males compared to 1 892 sperm by 10-day-old males). At the same time, larger

females successfully mated with males more often than smaller females, especially with older males (> 25-day-old) (Ponlawat and Harrington, 2009).

Physiology of reproduction

From the biological point of view, the physiological condition and the time required for females to carry out the digestion of a blood meal, maturation of the follicles, and subsequent oviposition constitutes a strategy of reproductive competition; a strategy for competition between females for resources required for reproduction (Wheeler, 1996). The gonotrophic cycle includes the search for the host, the ingestion of a blood meal, the digestion of the blood, the maturation of ovaries. It is completed with the laying of eggs once females have found an appropriate oviposition site (Beklemishev, 1940). Figure 2.2 graphically describes the integration of the physiological processes (summarised as host-seeking, ingestion and digestion of blood, egg maturation and oviposition) associated with feeding and reproduction of *Ae. aegypti* as suggested by Klowden (1994).

Figure 2.2. *Ae. aegypti* gonotrophic cycle, taking into account the factors that can cause host-seeking behaviour to return after an initial blood meal



Source: Klowden, M.J. (1994), "Endogenous regulation of the attraction of *Aedes aegypti* mosquitoes", *Journal of the American Mosquito Control Association,* Vol. 10, pp. 326-332.

The host-seeking behaviour of *Ae. aegypti* is closely associated with anthropogenic environments, in and around homes and other places that people frequent. During host-seeking behaviour in mosquitoes, visual, thermal and olfactory stimuli all contribute to host location, but olfaction is probably the dominant sensory mode used for this purpose (Bowen, 1996).

The visual capacity of *Aedes* mosquitoes to distinguish between various optical stimuli such as luminous reflectance, vertical contrast, and movement (Muir, Kay and Thorne, 1992; Hoel, Kline and Allan, 2009), as well as their preference of resting on black, stationary objects and non-reflective surfaces such as clothing, are characteristics that have served in the development of various entomological sampling devices and traps,

e.g. the BG Sentinel trap, ovitraps, gravid Aedes Traps and BDV tent trap (Fay and Prince, 1970; Muir, Kay and Thorne, 1992; Edman et al., 1997; Kroeckel et al., 2006; Silver, 2007; Casas Martínez et al., 2013; Eiras, Buhagiar and Ritchie, 2014).

With regard to the role of olfaction in host-seeking behaviour, carbon dioxide (CO_2) is involved in both short-range and long-range attraction. Olfactory cues that are primarily involved in long-range attraction include skin emanations, exhaled air and urine. Each of these is attractive to all mosquito species. Attraction is caused by a mixture of several host emanated compounds (Takken, 1991). Lactic acid in the presence of CO_2 is attractive, and lactic acid-sensitive neurosensilla are present on the antennae of *Ae. aegypti*. Other host-produced chemicals are also attractive. The plant-derived odorant linalool oxide, in combination with CO_2 is also an effective long-range attractant (Nyasembe et al., 2015).

The amount of blood ingested by a female *Ae. aegypti* mosquito (> 2.5 µl on average) can affect its host-seeking response. The suspension of host-seeking behaviour is caused by abdominal distension due to the ingested blood, or due to hormonal inhibition (Klowden and Lea, 1978).

To meet the adult female's energy and reproductive needs this species has adopted a strategy that includes reduced consumption of plant carbohydrates, highly focused blood feeding on humans, and frequently engaging in multiple blood feedings (Scott and Takken, 2012). *Ae. aegypti* almost exclusively fed on humans (99%) as a single host species, and 97% of multiple-host blood meals included at least one human host. A low frequency of other hosts, including bovine, swine, cat, rat and chicken were detected, but they represented less than 1% of blood meals (Ponlawat and Harrington, 2005). Both males and females can feed on plant juices (nectar), damaged fruits, damaged and intact vegetative tissue, and homopterans (aphids) which act as an energy source for their physiological maintenance and locomotion (Clements, 2000). Carbohydrate consumption rates (fructose) ranged from 1% to 27% for females and 9% to 65% for males (males are not hematophagous) (Van Handel et al., 1994; Martínez-Ibarra et al., 1997). Sugar feeding in *Ae. aegypti* is believed to be facultative because studies indicate that in the absence of human hosts, females showed higher fructose feeding rates, up to 74% (Van Handel et al., 1994).

The usual gonotrophic cycle of *Ae. aegypti* is described above. However, lack of association between blood feeding and ovogenesis, a term known as gonotrophic discordance, is fairly common in *Ae. aegypti*. This concept is defined as the need for multiple blood meals during a single gonotrophic cycle. The occurrence of multiple partial meals for a gonotrophic cycle (Feinson and Spielman, 1980; Clements, 1992) and reduced feeding success may be due to host defensive behaviour, body size of females and local female *Ae. aegypti* mosquito abundance (Klowden and Lea, 1978; Clements, 1992). The habit of feeding on blood twice during one gonotrophic cycle depends greatly on the size and hence stored energy reserves of the teneral female (Takken et al., 1998).

Some field studies with *Ae. aegypti* females demonstrated that 88% of all detectable meals were identified as being from a single host (human) and only 7% of all the females had taken multiple meals (Scott et al., 1993). Engorged females in Thailand revealed that half to one-third imbibed two or more blood meals in a 36-hour time period. On average, the human biting rate was high, with 0.63-0.76 blood meals per day (Scott et al., 2000). Multiple blood meals were also recorded using histological examination.

Protein obtained from the blood meal supplies the amino acids needed for vitellogenin synthesis, which is a protein critical for egg production in the female *Ae. aegypti* mosquito. In general, the post-ingestion digestion of blood takes about 38-48 hours in the midgut (MG) of *Ae. aegypti* (O'Gower, 1955; Gaio et al., 2011) and is dependent upon temperature and, to a lesser extent, humidity (Shlenova, 1938; West and Eligh, 1952).

Many bacteria live and multiply in the MG of *Ae. aegypti*, contributing to digestion, nutrition, and development of their host. The reduction in these symbiotic MG bacteria (primarily *Enterobacter* sp. and *Serratia* sp.) can affect the lysis of red blood cells, subsequently retarding protein digestion, depriving the mosquito of essential nutrients and eventually affecting oocyte maturation resulting in the production of fewer viable eggs (Gaio et al., 2011).

The gonotrophic cycle duration is operationally defined as the average number of days that gravid mosquitoes took to oviposit after taking a blood meal. From a human health perspective, the gonotrophic cycle is one of the most important physiological processes in the life of mosquitoes vectoring dengue and represents an essential epidemiological component in the model of vectorial capacity. It is a significant and determining biological aspect in the population dynamics of *Ae. aegypti* and *Ae. albopictus*, both of which can coexist in urban, suburban, and rural regions with endemic dengue and other arboviral diseases. Bacon (1916) in West Africa found that the first meal was taken one to two days after emergence and subsequent meals taken after each oviposition at about three-day intervals. The development of follicles from stage I to V (Christophers, 1911), takes 1.67 days during the first gonotrophic cycle of *Ae. aegypti* females when fed with a blood supply to repletion, and maintained at an average temperature of 28.9°C. The maturation of eggs can extend up to 2.7 days with an average temperature of 26.2°C (Tamayo-Domínguez, 2011). Additionally, female *Ae. aegypti* took 2.8 days to complete the first gonotrophic cycle when the average temperature was 26.2°C (Tamayo-Domínguez, 2011).

Because the processes of feeding and reproduction are closely related in most anautogenous (requiring a blood meal) anthropophilic mosquitoes like *Ae. aegypti*, therefore larval nutritional regimen, body size of newly-emerged adults, and the quantity and quality of blood ingested by females are key considerations (Macdonald, 1956). In mosquitoes, egg production is a cyclic process; therefore, with each successive reproductive or gonotrophic cycle a batch of oocytes matures and a new set of follicles forms within the germaria, separates and starts development. In *Ae. aegypti*, secondary follicles appear when the primary follicles enter the previtellogenic resting stage (Clements, 2000).

Once ovogenesis, which is asynchronous (Clements, 1992), is complete (or reaches Christophers' stage V), the priority of a female *Ae. aegypti* is to search for an oviposition site. Typically, eggs are deposited in naturally occurring collections of fresh water (such as coconut shells, leaves and axils of plants, tree holes, hollows of rocks) and various artificial containers made of plastic, glass, ceramic or metal, while holding temporal (e.g. tires, vases, bottles, kitchenware, scrap metal) and/or permanent water sources (pools, drums, tanks, etc.) that provide both habitat and food for immature life stages (Thavara et al., 2001; Vezzani and Schweigmann, 2002; García-Rejón et al., 2011). Oviposition sites may be located inside and outside human habitations, as well as in non-residential places such as cemeteries, workshops, junkyards, tire repair facilities and vacant plots. There have been reports of *Ae. aegypti* larvae being found in the surface

clear water layer of septic tanks (Burke et al., 2010), but this is not frequent and usually occurs where the lid is cracked or broken, providing the female access; nonetheless, septic tanks can be prolific producers (Barrera et al., 2008). Breeding sites also can include those that might contain brackish water such as boats or man-made containers at coastal edges or on beaches (Ramasamy et al., 2011). Waste material containers that are situated in areas with overhanging vegetation provide more favourable habitats as the breeding site is both shaded from intense sunshine and the build-up of heat and provides a ready source of detritus and bacteria for larval consumption. These containers are usually breeding sites for mosquitoes only during the rainy season in countries with wet and dry seasons, but the eggs are resistant to desiccation and can remain in suitable containers until rains of the following season. These desiccated eggs form what is known as the egg bank.

The choice of an egg-laying site by *Ae. aegypti* is influenced by the presence of conspecific larvae and pupae, the container fill method, container size, lid and sun exposure (Wong et al., 2011). Surprisingly, egg-laying females were most attracted to sites containing other immature *Ae. aegypti*, rather than to sites containing the most food. Physical attributes of oviposition sites, such as size, light-dark contrasts and specular reflectance from water surfaces, also play a significant role in oviposition site selection (Harrington et al., 2008). Characteristics of oviposition sites can vary according to the geographic and sociocultural context such as region, country and location. The degree of landscape modification (urban-rural) is also a factor (Kittayapong and Strickman, 1993; Honório et al., 2009), as well as intra- and interspecific competition (Chadee, Corbet and Greenwood, 1990; Braks et al., 2004; Sánchez-Hernández, 2011).

Behaviour of reproduction

Ae. aegypti is recognised as a highly anthropophilic, endophilic, endophagic, and day-biting species (Scott and Takken, 2012; Brown et al., 2014; McBride et al., 2014). These designations are based on activity patterns exhibited by this mosquito around the world. An important aspect of the bionomics of *Ae. aegypti* that contributes to its efficiency as an epidemiological vector is the close association with domestic habitats (Scott et al., 2000). Adult mosquitoes frequently reside indoors in human dwellings, most commonly in bedrooms (60.3% to 63.5%) followed by living/dining rooms (9.3% to 18.4%), kitchens (7.5% to 9.7%) and bathrooms (6.6% to 11.5%) (García-Rejón et al., 2008; Casas-Martínez, 2013). Immature forms develop primarily in artificial containers such as cans, jars, tires and buckets (Winch et al., 1992; García-Rejón et al., 2011). In Chennai, one of the major metropolitan areas in India, intradomiciliary cement tubs containing water for multi-purpose works were mostly preferred by the *Ae. aegypti* immature forms for development (Arunachalam et al., 2010). More details are given under Chapter 4.

Mosquitoes are exposed to daily changes in environmental light-dark cycles along with variations in humidity and temperature. Adaptation to these changes is seen in the form of specific behaviours, which are in turn linked to the expression of specific endogenously-controlled genes. *Ae. aegypti* is a major vector of arbovirus in many countries,[2] therefore the ethological study of this mosquito is crucial to better understand their behaviour, the dynamics of transmission of the viruses, as well as to optimise the entomological surveillance and increase the efficiency of vector control (Lima-Camara, 2010; Sivagnaname and Gunasekaran, 2012).

There are two significant copulation peaks in indoor housing, an early-morning peak between 6h00 and 8h00 (25% of events) and a pre-sunset peak from 16h00 to 18h00 (24% of events). The outdoors copulation periodicity presents almost the same pattern in the timing, with 30% of events during the early morning peak and 25% of events during the pre-sunset peak. Observations in insectary have shown similar copulation patterns. Studies indicated that 38.6% of copulating females collected in and around breeding sites were nulliparous and not inseminated, whereas over 85% of the copulating females found indoors were parous, suggesting that successful insemination encounters occur at alternative sites (such as around the human host). Furthermore, males may not be able to detect the difference between virgin and mature, parous female mosquitoes (Chadee and Gilles, 2013).

Oviposition is also diurnal and bimodal, both indoors and outdoors, with consistent peaks at 6h00-8h00 and 16h00-18h00 (Chadee and Corbet, 1989, 1990; Corbet and Chadee, 1989). The oviposition activity intensifies during the rainy season due to increased availability of water filled containers and mosquito population abundance.

Visual observations of the mating behaviour of *Ae. aegypti* have shown that males swarm around the feet and lower legs of a sitting/standing human host, flying in a horizontal figure of eight pattern. Mating was usually initiated in flight at a height of not more than one metre from the ground. Copulating pairs have been observed in flight, on human bodies, on their trousers and on the ground (Hartberg, 1971). Mating also occurs near adult oviposition sites and resting sites. Tests carried out by Jong and Knols (1996), demonstrated that *Ae. aegypti* prefers to bite the head and upper part of the trunk of persons lying in prone or supine position but will often bite on the lower legs beneath tables and when the host is seated.

The host-seeking behaviour and biting activity of *Ae. aegypti* are closely related, therefore, both events describe overlapping biorhythms. Many authors have documented that males and females show a bimodal flying and landing activity and that the periodicity is the same for nulliparous, parous, inseminated or uninseminated females, all activity being predominantly diurnal, with sharp peaks at post-sunrise and pre-sunset (as reported above) in intra-, peri- and extradomiciliary sites (Trpis et al., 1973; Corbet and Smith, 1974; Casas-Martínez et al., 2013). Landing activity patterns of *Ae. aegypti* are influenced by environmental factors (for example electrical lighting in and around houses), both indoor and outdoor, and in urban and rural areas (Chadee and Martinez, 2000).

Fecundity and fertility

Some mosquito strains or species are able to lay eggs without taking a blood meal, a trait named autogeny. This may allow populations to persist through times or places where vertebrate hosts are scarce. Environmental and genetic factors determine whether the mosquito *Ae. aegypti* lays eggs without a blood meal (Ariani et al., 2015). Autogeny is increased by growth at a temperature of 28°C (compared with 22°C), good nutrition of larval stages and feeding on higher concentrations of sugar solution during the adult stage. There appears to be a genetic component to autogeny which allows adult females from some strains to utilise amino acids from fat stores from the larva stage instead of obtaining these nutrients from a blood meal. Genetic differences associated with autogeny also affect fecundity in autogenous *Ae. aegypti* strains as shown by blood feeding behaviour (Christophers, 1960), quantity and quality of blood acquired (Klowden and Lea, 1978; Clements, 1992) and insemination status (Lavoipierre, 1958).

Mosquito body size has been linked to longevity, the number of eggs per batch and vector competence, and it is therefore an important measure of mosquito fitness (Siegel et al., 1992). The average body size corresponding to a wing length of 2.6 mm was associated with 61.23 ± 29.15 eggs per batch (Tamayo-Domínguez, 2011).

Life table analysis, under natural (and laboratory) conditions

A life table of aquatic phase *Ae. aegypti* grown under favourable laboratory conditions (temperature maintained at 28 ± 1°C, humidity 70 ± 10%, app. 50 larvae in a 6-inch x 8-inch tray, yeast + dog biscuit powder, or an alternative, as food on alternate days) suggests that natural mortality during development of larval stages is initially low (1%, stage I) and then increases (9%, stage II; 34%, stage III; 34%, stage IV). Mortality then decreases during the pupal stage (6%). When grown in the laboratory, approximately 48% of eggs survive to adults (Sánchez-Hernández, 2011).

Observed patterns of coexistence/exclusion of *Ae. albopictus* and *Ae. aegypti* in the field (Murrell and Steven, 2008) may be due to variation in detritus type. Experimental trials confirm competitive asymmetry in favour of *Ae. albopictus* with oak, pine, rubber (Tyagi et al., 2006) or insect detritus. Certain detritus types may eliminate interspecific competition among the larvae of these species (Murrel and Steven, 2008), thereby allowing for stable coexistence. Desiccation and thermal tolerance of eggs are also factors affecting co-existence (Juliano et al., 2002). More details on the biotic interactions in the landscape are given in the related section of Chapter 4.

In general, the effects of microclimatic factors (temperature, humidity and rainfall) and other environmental variables (food source, breeding sites and shelters) in the life cycle of the mosquito *Ae. aegypti* and generation time have been well documented, in either natural or controlled conditions in an insectarium or laboratory. Environmental changes affect all life stages of the mosquito, influence their survival and thus their ability to transmit pathogens. Low humidity, for instance, can negatively affect adult survival and may decrease the vector population. Frequency and host type of blood meal influence fecundity and female survival (Christophers, 1960; Nelson, 1986; Rueda et al., 1990; Day, Edman and Scott, 1994; Carrington et al., 2013).

Interspecific breeding

Harper and Paulson (1994) examined the dynamics of interspecific and intraspecific mating between Florida strains of *Ae. aegypti* and *Ae. albopictus*. In non-choice experiments where conspecific males were not available, dissection of the spermatheacae showed that interspecific insemination was an infrequent event. Few eggs were produced from interspecific crosses and all were non-viable. The frequency of interspecific mating was not increased when the hind tarsi of females were removed, eliminating a significant mechanism for fending off unwanted courtship. When held with males of both species, females mated with conspecifics and oviposited without regard to the presence of other species. In low-density experiments in which a single female of either species is caged with an excess of males of the other species, the conspecific male always located and inseminated the female. However, the presence of females of the other species has some negative influence on the intraspecific mating success in male *Ae. aegypti*, most likely due to misdirected courting or mating efforts (Bargielowski, Blosser and Lounibos, 2015).

Additional studies further suggest that matings of *Ae. aegypti* with *Ae. albopictus* do not produce viable offspring in the laboratory (Harper and Paulson, 1994; Nazni et al., 2009). Forced matings in the laboratory between wild-type *Ae. aegypti* and *Ae. albopictus* yielded eggs but they were not viable, and when bleached were shown to have no embryos (Nazni et al., 2009). More recently a study showed that there is cross-species insemination in the field between *Ae. aegypti* and *Ae. albopictus* (Tripet et al., 2011), but these interspecific matings encounter many barriers and occur at low frequencies (a single *Ae. albopictus* was found to have *Ae. aegypti* sperm in this study, and three *Ae. aegypti* females were inseminated by *Ae. albopictus*), resulting in no viable progeny.

These results indicate that significant reproductive isolation exists between *Ae. aegypti* and *Ae. albopictus*. This occurs at both the prezygotic level (very low mating frequency) and the postzygotic level (non-viable progeny).

In rare cases, viable hybrids resulting from cross-mating between *Ae. aegypti* females and *Ae. albopictus* males have occurred in laboratories (Martínez-López et al., 2014). Eggs obtained from this cross-mating were viable, and the larvae and pupae showed development in seven days. Therefore, it is possible that viable hybrids can be produced experimentally, but this is rare and may be restricted to matings of only particular strains of each species. As reported in the previous section, there are important reproductive barriers existing between these two species living in sympatry in natural environments (Harper and Paulson, 1994; Nazni et al., 2009) and the possibility of hybridisation is unlikely, given the probable sterility of F1 hybrids (Haldane's rule).

Effect of *Wolbachia* on reproduction

Wolbachia pipientis is a monophyletic group of maternally inherited, gram-negative, endosymbiotic bacteria, related to the *Ehrlichia*, *Anaplasma* and *Neorickettsia* genera, all being members of Alphaproteobacteria (O'Neill et al., 1992; Lo et al., 2007). In recent years, evidence has been accumulated that shows *Wolbachia* infections affect several aspects of host biology, physiology, immunity, ecology, evolution and reproduction (Bourtzis, Braig and Karr, 2003; Bourtzis and Robinson, 2006; Werren, Baldo and Clark, 2008; Saridaki and Bourtzis, 2010). This bacterial group is widespread and abundant among insect species and has been associated with the induction of a number of reproductive outcomes including the death of males (Hurst et al., 2000), feminisation (Rousset et al., 1992), parthenogenesis (Stouthamer, Breeuwer and Hurst, 1999) and, most commonly, cytoplasmic incompatibility (Yen and Barr, 1973; O'Neill et al., 1997; Nirgianaki et al., 2003).

The cytoplasmic incompatibility (CI) results in the generation of unviable offspring when an uninfected female mates with a *Wolbachia*-infected male (McGraw et al., 2001). In contrast, *Wolbachia*-infected females can produce viable progeny when they mate with both infected and uninfected males, resulting in a selective reproductive advantage over uninfected females (Hoffmann and Turelli, 1997). This CI phenotype is induced by *Wolbachia* in mosquito species and allows the maternally-transmitted *Wolbachia* to efficiently invade host populations without being infectious or moving horizontally between individuals (Hoffmann and Turelli, 1997).

The ability of *Wolbachia* to manipulate diverse functional systems of its hosts (Bourtzis et al., 2014), particularly reproduction, has led to the proposal and the development of promising symbiont-based strategies aimed at the control of insect pests and disease vectors including mosquito species. Different *Wolbachia* species/strains can be naturally

found in *Aedes* mosquitoes, for example, *Ae. albopictus*, *Ae. polynesiensis* and *Ae. scutellaris*, but not in *Ae. aegypti*. Thus, the use of *Wolbachia* for *Ae. aegypti* control via CI has required its transinfection from naturally infected insect species (Ye et al., 2013; Joubert et al., 2016) and currently includes the wAlbB strain (from *Ae. albopictus*) and the wMelPop-CLA (cell-line-adapted) and wMel strains (from *Drosophila melanogaster*). More information on the use of *Wolbachia* as a biological control for virus transmission is given under Annex A.

Notes

[1] This GE line was carrying a tetracycline repressible, lethal positive feedback system.

[2] See Annex B. Human and animal health affected by mosquitoes, Table A B.1 on arbovirus definition and important infections.

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Chapter 3. Genetics of the mosquito *Ae. aegypti*

Linkage map organisation of *Ae. aegypti*

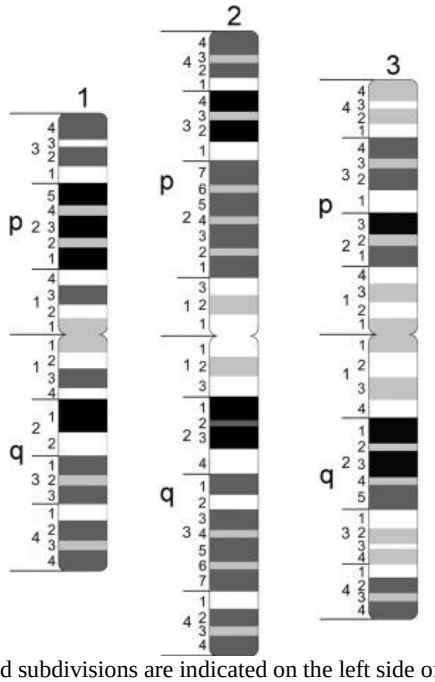
Ae. aegypti was the first mosquito species for which a detailed genetic linkage map was constructed and linked to the physical map (Craig and Hickey, 1967; Munstermann and Craig, 1979). Sequence-tagged site (STS) markers were developed for two different strategies, both based on physical maps using fluorescence in-situ hybridisation (FISH). The first mapping strategy used cosmids (8 RFLP markers) and the second strategy used cDNAs (21) (Brown et al., 2001). Recently, a band-based approach was used to perform a physical mapping of the *Ae. aegypti* genome to its mitotic chromosomes (Sharakhova et al., 2011; Timoshevskiy et al., 2013). The mitotic chromosome complement of *Ae. aegypti* consists of three pairs of metacentric chromosomes (Rai, 1963) that are numbered 1 (smallest), 2 (largest) and 3 (intermediate) (McDonald and Rai, 1970). *Ae. aegypti* sex determination alleles have been linked to the smallest homomorphic autosome 1 (McClelland, 1962). The three chromosomes of *Ae. aegypti* have been subdivided into a total of 23 regions and 94 subdivisions based upon staining of early metaphase chromosomes using YOYO-1 iodide (Timoshevskiy et al., 2014) (Figure 3.1). In addition to 100 genetic markers and 183Mb of genomic sequence, a marker linked with sex determination (Severson et al., 2002) and 12 quantitative trait loci (QTL) associated with pathogen transmission (Severson et al., 1995; Bosio, Fulton and Salasek, 2000; Gomez-Machorro, Bennett and Muñoz, 2004; Zhong et al., 2006) have been also anchored to the chromosomes (Timoshevskiy et al., 2014).

Although whole-genome sequencing has been undertaken (genome size of *Ae. aegypti* = 1.376 Gb), sequence compilation is still in progress due to the abundance of transposable elements (TEs) that cover approximately 50% of the *Ae. aegypti* genome (Nene et al., 2007; Severson and Behura, 2012; Timoshevskiy et al., 2014). TEs are extremely important to genome function and evolution (Arensburger et al., 2011) and may be key factors in mosquito genome plasticity. Low levels of polyteny resulting in poor quality of chromosome preparations add to the difficulty of using polytene chromosomes for physical mapping purposes in *Ae. aegypti* (Sharma et al., 1978; Campos, Andrade and Recco-Pimentel, 2003). Furthermore, the abundance of TEs complicates FISH experiments which require the use of unlabelled repetitive DNA fractions to block non-specific hybridisation. A physical map for *Ae. aegypti* corresponding to 13.3% of the genome was developed using FISH markers on mitotic chromosomes (Severson et al., 1993), genetic linked map (RFLP cDNA-based map) (Brown et al., 1995, 2001; Sharakhova et al., 2011), and QTL (Severson et al., 1994, 1995; Bosio, Fulton and Salasek, 2000; Gomez-Machorro, Bennett and Muñoz, 2004; Zhong et al., 2006). More recently, a more detailed physical map of the mosquito was constructed and a total of 624 Mb covered approximately 45% of the *Ae. aegypti* genome (Timoshevskiy et al., 2014).

Though this mosquito has a high TE load including miniature inverted-repeat transposable elements (MITEs) and piRNA biogenesis genes, its genome has a low

proportion of transposon-specific piRNAs (Biryukova and Ye, 2015). This is important in preserving overall genome stability because the small RNA pathway controls TE mobilisation and movement (Saito and Siomi, 2010; Senti and Brennecke, 2010). Unregulated movement of active elements or non-autonomous sequences can lead to insertional mutagenesis through the genome resulting in a decrease in genetic fitness. Arensburger (2011) also stated that the stability of the transposons in *Ae. aegypti* is the result of a low proportion of transposon-specific piRNAs.

Figure 3.1. A physical map of the *Ae. aegypti* genome



Note: Chromosome regions and subdivisions are indicated on the left side of the idiograms.
Source: Timoshevskiy, V.A. et al. (2013), "An integrated linkage, chromosome, and genome map for the yellow fever mosquito *Aedes aegypti*", *PLOS Neglected Tropical Diseases*, Vol. 7, No. 2, pp. 2052; Timoshevskiy, V.A. et al. (2014), "Genomic composition and evolution of *Aedes aegypti* chromosomes revealed by the analysis of physically mapped supercontigs", *BMC Biology*, Vol. 12, pp. 27.

RNA interference (RNAi) is an important anti-viral defence mechanism. Although the *Ae. aegypti* genome encodes RNAi component orthologs, however, most populations of this mosquito are readily infected by, and subsequently transmit, arboviruses (Nene et al., 2007; Arensburger et al., 2011).

Population genetics and phylogeography of *Ae. aegypti*

The two *Ae. aegypti* subspecies: *Ae. aegypti formosus*, a wild mosquito apparently limited to sub-Saharan Africa, and *Ae. aegypti aegypti*, found globally in tropical and subtropical regions typically in association with humans (Moore et al., 2013), are described with their characteristics under the Classification (Taxonomy) section in Chapter 1. In addition, the Systematics section reports on the existence of two principal clades of *Ae. aegypti* collections worldwide.

In Mexico, local patterns of gene flow among *Ae. aegypti* populations were assessed using random amplified polymorphic DNA (RAPD) markers. Large genetic distances

were observed, suggesting reduced gene flow among the mosquitoes (García-Franco et al., 2002; Gorrochotegui-Escalante et al., 2002; Muñoz et al., 2013a). The populations are panmictic along the Pacific coast, isolated by distance in northeast Mexico, and exhibit moderate gene flow across the Yucatan peninsula (Muñoz et al., 2013a). In the southern Pacific coast region reduced gene flow may result from sampling at altitudes greater than 1 500 m, which is close to the altitudinal limit for *Ae. aegypti* in Mexico (Lozano-Fuentes et al., 2009; Navarro et al., 2010), the mosquito being unable to survive at altitudes greater than 2 000 m (Lozano-Fuentes et al., 2012).

Sequence variation in the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene, has been used to describe patterns of gene flow among *Ae. aegypti* s.l. collections within and among countries outside Africa (Gorrochotegui-Escalante et al., 2000, 2002; Bosio et al., 2005; Costa-da-Silva, 2005; Herrera et al., 2006; Bracco et al., 2007; Ribeiro et al., 2007; Paduan and Ribolla, 2008; Paupy et al., 2008; Urdaneta-Marquez et al., 2008; Dueñas et al., 2009; Hlaing et al., 2009; Lima and Scarpassa, 2009; Lozano-Fuentes et al., 2009; Paupy et al., 2012; Muñoz, 2013a; Moore et al., 2013). In Mexico, novel ND4 haplotypes were discovered and used to assess the amount of gene flow among breeding sites and to possibly predict the degree to which dengue virus (DENV) is transferred among sites (Gorrochotegui-Escalante et al., 2000, 2002; García-Franco et al., 2002).

To date 96 novel ND4 haplotypes have been discovered and three phylogenetic patterns have been consistently noted: either mtDNA haplotypes were distributed as two well-supported clades (Gorrochotegui-Escalante et al., 2000; Bosio et al., 2005; Lima and Scarpassa, 2009), or as a basal group similar to outgroup subspecies from which a second derived clade arises (Gorrochotegui-Escalante et al., 2002; Bracco et al., 2007; Paduan and Ribolla, 2008; Dueñas et al., 2009; Hlaing et al., 2009; Lozano-Fuentes et al., 2009; Paupy et al., 2012; Muñoz et al., 2013a). The broad distribution of specific haplotypes in Venezuela (Urdaneta-Marquez et al., 2008), Brazil (Bracco et al., 2007; Paduan and Ribolla, 2008; Lima and Scarpassa, 2009), Guatemala (Bracco et al., 2007), and Peru (Costa-da-Silva, 2005) demonstrates efficient mosquito dispersion in Central and South America.

Control practices are implicated as a major cause of genetic drift in *Ae. aegypti*. This was the conclusion of a study investigating 19 *Ae. aegypti* collections in Thailand, from Chiang Mai in the north to Songkhla province in the south (Bosio et al., 2005). That study found seven mitochondrial ND4 haplotypes, no evidence of isolation by distance, and low gene flow estimates among collections. They also concluded that these patterns are consistent with genetic drift arising from vector control efforts. Furthermore, polymorphisms were examined at 10 isoenzyme loci among 15 *Ae. aegypti* collections from Chiang Mai (Mousson et al., 2002). Low gene flow was also detected among these collections. These authors also concluded that this pattern was related to insecticide treatments. Additional studies further demonstrate the contribution of insecticide exposure to genetic drift in Martinique (Marcombe et al., 2009, 2012, 2013), Phnom Penh (Paupy et al., 2004) and French Guiana (Failloux et al., 2002). More on natural factors and human activities affecting gene flow or distribution is given under Chapter 4.

In summary, because *Ae. aegypti* is the primary global vector of severe viral diseases to humans (see Annexes A and B), it is crucial to study its population genetics in order to develop strategies to control the dispersion of the mosquito. Studies over the past 50 years have shown large differences among global populations of this species. Past studies based on morphological polymorphisms and allozymes were recently completed by the use of molecular genetic markers (including microsatellites and mitochondrial markers).

Phylogenetic analyses consistently resolved two clades. In addition, phylogenetic analyses showed that populations of *Ae. aegypti* outside Africa consist of mosquitoes arising from two ancestral clades; one is basal and primarily associated with West Africa while the second arises from the first and contains primarily mosquitoes from East Africa. Across these many studies on population genetics, and those based on the distribution of the mosquito haplotypes around the world, it can be suggested that mosquito dispersion is very efficient, most likely due to commercial transportation and human movements.

Genetics of insecticide susceptibility and development of insecticide resistance

Resistance to insecticides

According to the World Health Organization (WHO, 2012a, 2012b), insecticide resistance is defined as the ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of mutation and natural selection. Appropriate tools (biological, biochemical and/or molecular) are needed to identify the mechanisms involved in developing resistance and to conduct surveillance at individual and/or population levels.

Such resistance has been observed in more than 500 insect species worldwide, including more than 20 *Aedes* species (Diptera: Culicidae). Over 400 scientific reports worldwide document insecticide resistance in *Ae. aegypti*.

The large use of insecticides, and the resultant selection pressure on insect populations, has led to widespread resistance to all classes of insecticides among many invertebrate pests, making control difficult. Frequent applications of the same insecticide will select for those individuals in a population that are able to survive the recommended rates of the compounds owing to a genetically-fixed difference. Over time, this selection pressure will lead to a resistant population becoming established. In such cases, other compounds within the same class of chemistry are most often also affected; for instance, resistance to one pyrethroid type usually confers resistance across the whole group of pyrethroids, a phenomenon known as cross-resistance. Sometimes, depending on the nature of the resistance mechanism, multi-resistance can occur between different chemical classes, for example organophosphates and carbamates. The frequent treatments of crops with similar synthetic insecticides may also indirectly affect the susceptibility of insects of public health importance, with insect vectors additionally exposed when in the vicinity of agricultural sprays (Brogdon and McAllister, 1998; Hemingway and Ranson, 2000; Liu et al., 2006).

The portfolio of insecticides available for management of arthropod vectors (PAHO, 1994; WHOPES, 2005) is very limited and unlikely to increase dramatically in the near future. Development of resistance to commonly-used insecticides is therefore a serious threat to human ability to combat mosquito-borne diseases. Insecticide susceptibility must be viewed as a valuable "natural resource" at risk for being depleted. This underscores the critical importance of monitoring insecticide resistance through development and implementation of relevant management schemes. More information is contained in the section on "Prevention and management of insecticide resistance" of Annex A.

Insecticides, mode of action and resistance mechanisms

Vector control programmes include activities to control both immature and adult stages of *Ae. aegypti*. Chemical or biological larviciding and physical source reduction of container habitats are intended to control larvae, but house-to-house larval control is too laborious

for sustainable implementation by vector control programmes or community participation (Reiter and Gubler, 1997; WHO, 2009; Horstick et al., 2010). During dengue outbreaks, outdoor and indoor spraying of insecticides is used to kill adults (WHO, 2009; Esu et al., 2010). Control measures are explored with more details in Annex A.

The four chemical classes of insecticides (organochlorides, organophosphates, carbamates, pyrethroids) used for larvae and adult mosquito control have their biochemical target sites in the insect central nervous system, which makes them fast-acting killing agents. They act on only two different molecular target sites in the central nervous system, leading the insect to over-excitation and death. Organophosphates and carbamates both inhibit acetylcholinesterase (AChE), an enzyme of crucial importance in terminating nerve impulses by cleaving the natural neurotransmitter acetylcholine (Eto, 1974). In contrast, synthetic pyrethroids (and DDT, representing the organochlorides) modulate voltage-gated sodium channels, resulting in rapid knockdown properties (Khambay, 2002). It is important to note that these four chemical classes address only two different modes of action, so there is much less target-site diversity involved in the control of adult mosquitoes compared with the agricultural sector, which can rely on many more modes of action to date (Nauen and Bretschneider, 2002; Nauen, 2006). Insect growth regulator (IGR), pyriproxyfen is a juvenile hormone analogue that can be considered as an alternative to conventional insecticides because of its specific activity against immature insects, low persistence in the environment and virtually non-toxic to mammals (Madhu and Vijayan, 2009).

The various mechanisms that enable insects to resist the action of insecticides can be grouped into four distinct categories as follows: metabolic resistance, target-site resistance, reduced penetration, and behavioural avoidance.

Metabolic resistance

Metabolic resistance is the most common resistance mechanism that occurs in insects. This mechanism is based on the enzyme systems, which all insects possess to help them to detoxify naturally-occurring xenobiotics and insecticides. It is commonly accepted that insect detoxification systems derived from the plant-insect evolutionary arms race, and several insect detoxification enzymes have been associated with the detoxification of plant toxins and all types of chemicals, including insecticides (Despres, David and Gallet, 2007). Over-expression of enzymes capable of detoxifying insecticides or amino acid substitutions within these enzymes, which alter the affinity of the enzyme for the insecticide, can result in high levels of insecticide resistance (Hemingway et al., 2004; Flores et al., 2005, 2006). Increased expression of the genes encoding the major xenobiotic metabolising enzymes is the most common cause of insecticide resistance in mosquitoes. Over-expression of detoxifying enzymes can occur as the result of gene amplification (e.g. duplication) or due to changes in either transacting regulator elements or the promoter region of the gene (Guillemaud et al., 1997; Hemingway and Ranson, 2000; Hawkes and Hemingway, 2002). The consequence is a significant increase in enzyme production in resistant insects that enables them to metabolise or degrade insecticides before they are able to exert a toxic effect.

Three enzyme families (with a variable number of gene members), the cytochrome P450 monooxygenases (P450s), glutathione transferases (GST), and carboxyl/cholinesterases (CCE) are implicated in insecticide metabolism. Each of these catalyses a wide range of detoxification reactions. They are the primary enzymatic defence against xenobiotics, are responsible for the removal of many by-products of metabolism, play essential roles

in multiple biosynthetic pathways and are involved in chemical communication (Feyereisen, 2005; Oakeshott et al., 2005; Ranson and Hemingway, 2005). Some individual enzymes also have structural roles instead of, or in addition to, their catalytic activity. This diversity in the function of each enzyme family is accomplished by a mixture of highly specialised enzymes, often with specific substrates and strictly regulated expression profiles, and more generalist, ubiquitously expressed enzymes. Many insect species show an amazing diversity of detoxification enzymes. As insect genomes have been sequenced, and the detoxification genes annotated, it has become apparent that these detoxification gene families are very rapidly evolving and each insect has a unique complement of detoxification genes, with very few orthologs across insect species (Ranson et al., 2002; Claudianos et al., 2006). The rapid expansion and diversification of detoxification genes likely facilitated the adaptation of insects to their particular ecological niches, and, on a more recent evolutionary timescale, has enabled them to survive various man-made xenobiotics, including insecticides. A small subset of the detoxification genes has been previously described in *Ae. aegypti* (Sieglaff, Duncan and Brown, 2005; David et al., 2006; Lumjuan et al., 2007).

Target-site resistance

Target-site resistance is the second most common mechanism of resistance to insecticides encountered in insects. Insecticides (e.g. organophosphates, carbamates, DDT and pyrethroids) generally act at a specific site within the insect, typically within the nervous system. The site of action can be modified in resistant insect strains such that the insecticide no longer binds effectively.

Reduced sensitivity of the target receptors to insecticide results from non-silent point mutations in the gene encoding the protein constituting the target site. For example, the target site for organophosphate and carbamate insecticides is AChE in the nerve cell synapses. Several mutations in the AChE gene have been found in insects (Fournier, 2005), which result in reduced sensitivity to inhibition of the enzyme by these insecticides (Weill et al., 2003).

Alterations in the target site that cause resistance to pyrethroids and DDT are often referred to as *knockdown resistance* (kdr), in reference to the ability of insects with relevant alleles to withstand prolonged exposure to insecticides without being 'knocked-down'. The kdr is conferred principally by non-synonymous mutations in the voltage-gated sodium channel gene that reduce insecticide binding to this channel in the insect nerve sheath, thereby preventing the loss of co-ordinated activity and paralysis in the insect (Soderlund and Knipple, 2003; Davies et al., 2007; Rinkevich, Du and Dong, 2013).

Worldwide, numerous kdr-conferring voltage-gated sodium channel allele mutations (e.g. S989P; I1,011M; I1,011V; V1,016I; V1,016G; F1,534C; and D1,794Y) have been described in *Ae. aegypti* (Vontas et al., 2012; Rinkevich, Du and Dong, 2013). Multiple kdr mutations had been reported from Mexico, the Caribbean, and Central America (Saavedra-Rodriguez et al., 2007). Subsequent studies reported the presence of kdr conferring mutations in *Ae. aegypti* collections from Brazil (Martins et al., 2009a, 2009b; Lima et al., 2011; Belinato, Martin and Valle, 2012), Mexico (Ponce-Garcia et al., 2009; Siller et al., 2011; Aponte et al., 2013) and the Caribbean (Marcombe et al., 2009, 2012, 2013; Harris, Rajatileka and Ranson, 2010; Bariami et al., 2012; McAllister, Godsey and Scott, 2012; Maestre-Serrano et al., 2014; Alvarez et al., 2015).

Reduced penetration

Reduced penetration (and behavioural resistance by reduced penetration) occurs when insects develop a heritable mechanism(s) that reduces or prevents the entry of a toxin into the insect's body. Modifications in the cuticle or digestive tract linings that prevent or slow the penetration/absorption of insecticides can be found in some resistant insects. This resistance mechanism is non-specific and can affect the effectiveness of a broad range of insecticides. Reduced uptake of insecticide, often referred to as cuticular resistance, is frequently described as a minor resistance mechanism. Certainly, for pests where the major route of insecticide delivery is via ingestion, this is likely to be the case. However, for dengue control, where insecticides are typically applied spatially or on wall surfaces, uptake of insecticides is primarily through the appendages. An increase in the thickness of the tarsal cuticle, or a reduction in its permeability to lipophilic insecticides, could have a major impact on the bioavailability of an insecticide *in vivo*.

Reduced cuticle penetration is the least understood resistance mechanism. Though it may have a primary role in resistance (Valles, Dong and Brenner, 2000; Ahmad, Denholm and Bromilow, 2006; Puinean et al., 2010), it more often acts in combination with the other mechanism(s).

Behavioural avoidance

Insecticide resistance in mosquitoes may also be conferred by behavioural changes in response to prolonged exposure to an insecticide. Behavioural avoidance does not have the same importance as physiological resistance but may be considered to be a contributing factor, leading to the avoidance of lethal doses of an insecticide (Chandre et al., 2000; Grieco et al., 2007). This type of response can be further divided into direct contact excitation (sometimes referred to as "irritancy"), and non-contact spatial repellency when insects move away from the insecticide-treated area before making direct contact (Chareonviriyaphap et al., 1997; Grieco et al., 2007).

To better approximate insect behaviour in natural field settings, numerous experiments have been made over many decades using specially constructed experimental huts (Smith, 1965; Rozendaal et al., 1989; Roberts and Alecrim, 1991; Bangs, 1999; Grieco et al., 2000, 2007; Polsomboon et al., 2008; Malaithong et al., 2010). Most experimental hut studies have been conducted to observe the behaviour of *Anopheles* mosquitoes; however, Grieco et al. (2007) successfully demonstrated that chemical actions could be observed in experimental huts using *Ae. aegypti* as a model system. The results obtained from both laboratory and field studies can help facilitate the choice of the most effective chemicals and measures to control house-frequenting adult mosquitoes.

Conclusion on resistance to insecticides

Insecticide resistance develops in an insect population when individuals carrying genes that allow them to survive exposure to the insecticide survive, mate and pass these genes onto the next generation. Thus, any activities that control the individuals with the resistance trait will delay the spread of the resistance genes in the population. Some elements on prevention and management of insecticide resistance are given in Annex A.

Genetics of vector competence in *Ae. aegypti*

Vector competence, a key factor

The vector competence (VC) is defined as the intrinsic permissiveness of an arthropod vector for infection, dissemination and transmission of a pathogen (Black et al., 2002; Dickson et al., 2014). The full competence of a vector is determined not only by its ability to become infected, but also by its ability to transmit a pathogen.

VC in *Ae. aegypti* is an element of primary importance to consider because of this mosquito's public health impact as main potential transmitter of dengue, yellow fever, Zika and chikungunya viruses. In a few restricted areas, *Ae. aegypti* is also a vector of *Wuchereria bancrofti* and *Brugia malayi*, both of which cause lymphatic filariasis or elephantiasis (Service, 2012; Powell and Tabachnick, 2013)

Consequently, *Ae. aegypti* has been the subject of numerous vector competence and population genetic studies (Aitken, Downs and Shope, 1977; Gubler et al., 1979; Tabachnick and Powell, 1979; Rosen et al., 1985; Tabachnick et al., 1985; Tardieux et al., 1990; Miller and Mitchell, 1991; Apostol, Reiter and Miller, 1996; Bosio and Beaty, 1998; Vazeille-Falcoz et al., 1999; Bosio, Fulton and Salasek, 2000; Bennett et al., 2002b; Gorrochotegui-Escalante et al., 2002; Mercado-Curiel, Black and Muñoz, 2008; Lozano-Fuentes et al., 2009; Sylla et al., 2009; Lambrechts, 2011; Lambrechts et al., 2011; Guo et al., 2013; Muñoz et al., 2013b; Chepkorir et al., 2014; Diagne et al., 2014; Dickson et al., 2014; Gonçalves et al., 2014; Vega-Rúa et al., 2014).

Susceptibility of Ae. aegypti *populations to viruses*

Infection with dengue virus

Ae. aegypti becomes infected with a viral disease, dengue for example, when the mosquito bites and acquires a blood meal from a dengue virus (DENV)-infected human, the primary host of the virus. The mosquito infection depends on factors such as DENV virulence, physical barriers and innate immunity that can confer resistance or susceptibility of an *Ae. aegypti* population to viruses.

The relationships between DENV and its arthropod vector *Ae. aegypti* are crucial, and an analysis of host cell responses to flavivirus infection of mosquito vectors is very important for understanding the maintenance and transmission of the disease.[1] Mosquito populations differ in their susceptibility to flavivirus development (i.e. VC), reflecting the different barriers encountered by the virus from its entry into the mosquito to its release in the saliva. Factors such as specific mosquito epithelial cells receptors, as well as differential viral replication in the mosquito, are critical for VC - as are other genes as exhibited by quantitative trait loci (QTL) studies (Gomez-Machorro, Bennett and Muñoz, 2004).

Three *Ae. aegypti* strains with different susceptibilities to DENV infection have been reported (namely DS3, DMEB and IBO-11), and these have been used to study whether midgut cell receptors for DENV may be markers for VC (Bennett and Beaty, 2005). *Ae. aegypti* susceptibility to DENV, attributable to multiple genetic factors, is found to be usually very high, particularly against DENV-2,[2] as elicited by DS3 and DMEB strains.

It has been observed that the three strains showed a difference in the degree of infection of their midgut (MG) cells, depending on their susceptibility to DENV. For example, the IBO-11 strain expressed almost no infection remaining after 26 days post-infection.

DMEB strain showed increase in infection up to 26 hours in all the three MG regions, having the maximal virus accumulation in the posterior MG which then diminished by 14 days post-infection, compared to the other susceptible strain DS3 that has maximal virus accumulation in anterior MG at 14 days post-infection. These results also display a statistically-significant MG infection increase from the first five hours post-infection to 26 hours in DS3 and DMEB strains ($p < 0.05$). Moreover, IBO-11 strain exhibited a significant decrease ($p < 0.05$) of MG infection from 5 to 336 hours post-infection. Virus infection of IBO-11 strain was almost completely abolished ($p < 0.05$) from 13 to 336 hours post-infection (Mercado-Curiel, Black and Muñoz, 2008). The susceptibility, resistance and refractoriness depend on multiple genetic factors (Miller and Mitchell, 1991).

Anatomic barriers to infection (midgut cells)

The VC for arboviruses is associated with a number of anatomic barriers to productive vector infection. These include a midgut infection barrier (MIB), a midgut escape barrier (MEB) and a salivary gland barrier (Black et al., 2002). In potential vectors provided with an MIB, a virus cannot infect and/or replicate in the mosquito MG cells. This may be due to a lack of specific cell surface receptors for the virus or to MG cells being non-permissive for infection with the virus (Mercado-Curiel, Black and Muñoz, 2008). Potential vectors provided with an MEB may allow virus replication in the MG, even to high titres (concentrations), but the virus is then unable to exit the MG to cause a disseminated infection. The VC for flaviviruses in *Ae. aegypti* is thought to be controlled by at least two genes or sets of genes, one controlling the MIB and the other controlling the MEB (Miller and Mitchell, 1991; Bosio and Beaty, 1998; Bosio, Fulton and Salasek, 2000). A study by Bennett et al. (2002b) also concluded that these barriers are probably major determinants of VC to DENV in nature and during experimental infections.

Bosio and Beaty (1998) proposed a significant additive genetic effect in MIB and demonstrated that the DENV titre in the mosquito MG and head did not correlate with the rate of infection. They also showed that the heritability for virus titres in tissues (MG or head) were almost identical in different strains of *Ae. aegypti formosus* and showed that the amount of virus in the MG did not determine if the virus was disseminated, which hypothetically may be due to the presence or absence of DENV receptors (in the MG, in particular).

Barriers to infection can vary widely in prevalence among *Ae. aegypti* populations, leading to large intraspecific variation of *Ae. aegypti* VC that may influence the epidemiology of DENV and other flaviviruses (Black et al., 2002).

Climatic factors affecting the infection susceptibility

Susceptibility of *Ae. aegypti* mosquito to DENV varies geographically and can be influenced by climatic factors such as temperature, which affect the incidence, seasonality and distribution of vector-borne diseases.

The VC has shown to be affected by temperature, which impacts biological processes of mosquitoes including their interaction with viruses (Watts et al., 1987; Lambrechts et al., 2011). Chepkorir et al. (2014) demonstrated a significantly higher infection rate at high temperatures for mosquitoes collected in Nairobi and Kilifi (Kenya), which is consistent with previous results (Watts et al., 1987). The 2014 study showed that the Nairobi *Ae. aegypti* population is a relatively inefficient vector for DENV-2 compared to that from Kilifi with the former showing high infection, but low dissemination rates

in low- and high-temperature settings. These results also suggested a weak MIB and a strong MEB for the Nairobi population, and a moderate MIB but weak MEB for the Kilifi population.

Genetic variability and geographical variations in Ae. aegypti *vector competence*

A number of genetic studies on VC were conducted worldwide, which demonstrated a great VC variability. The results of some of these works performed in different countries are presented hereinafter. All in all, they indicate that vector control strategies should be adapted to the available data for each region. Further analysis should be conducted to better understand the reasons for this large variability in VC and how these parameters correlate with epidemiological findings (Gonçalves et al., 2014).

Ae. aegypti populations exhibit considerable genetic variability in VC for flaviviruses, including DENV-2 viruses. The range of VCs shown suggests that the ability to overcome the MIB and MEB to transmit DENV-2 JAM1409 is a quantitative trait with multiple genes that likely condition VC and collectively determine the infection rates of mosquito populations. This theory has been studied using crosses of susceptible and refractory mosquito lines (Miller and Mitchell, 1991; Bosio and Beaty, 1998).

Significant genetic variation in *Ae. aegypti* on a smaller scale has been demonstrated in Puerto Rico (Apostol, Reiter and Miller, 1996) and in Mexico (Gorrochotegui-Escalante et al., 2000). Subsequently, the potential variation in VC on a regional geographic scale was addressed in Mexico. The major aim of this research was to determine if genetic variability in *Ae. aegypti* populations conditions the incidence and severity of dengue fever and dengue haemorrhagic fever outbreaks. Such study can help identify genetic biomarkers for mosquito populations that pose undue risk for severe diseases and allow control programmes to focus their resources on areas at greatest risk.

Several studies have shown that *Ae. aegypti* has a continuous variation in its competence to transmit flavivirus (Bennett et al., 2002a; Black et al., 2002; Severson et al., 2004; Gorrochotegui-Escalante et al., 2005). *Ae. aegypti* from 24 collections in Mexico and the United States were challenged orally with DENV-2 JAM1409, and the VC of the populations ranged from 24% to 83%. In general, the *Ae. aegypti* collections from throughout Mexico exhibited considerable variability in VC, and collections from the Yucatan Peninsula were generally more competent than those from other geographic regions (Bennett et al., 2002b). Lozano-Fuentes et al. (2009) showed that the Neovolcanic Axis (NVA) in Mexico is a natural barrier to *Ae. aegypti* VC for DENV, as a much lower VC (20%) prevails for mosquito populations from south of the NVA compared to mosquitoes collected from north of the NVA (55%).

Ae. aegypti populations from Belo Horizonte, Brazil, exhibited wide variation in VC to transmit dengue. Most Brazilian states are infested with *Ae. aegypti* and are consequently at risk of dengue transmission (Figueiredo et al., 2008). Moncayo et al. (2004) studied populations from various geographical locations and showed that *Ae. aegypti* from Galveston, Texas (United States) were more susceptible than those from Bolivia but were less susceptible than mosquitoes from Thailand. This concurred with the observations made by Bennett et al. (2002b) on *Ae. aegypti* collected from various locations in Mexico and by Chepkorir et al. (2014) on populations from two different Kenyan sites, that all differed significantly in their MG susceptibility to infection.

The VC studies on *Ae. aegypti* from West Africa have shown that mosquitoes are more refractory for both DENV (Tabachnick et al., 1985; Bosio and Beaty, 1998) and yellow fever virus (YFV) (Tabachnick et al., 1985; Miller and Mitchell, 1991) compared to *Ae. aegypti* collected from the Americas or Asia (Diallo et al., 2005, 2008).

In studies directly comparing collections within Senegal, wide variation in VC was shown for both high-passage (Sylla et al., 2009) and low-passage field isolates of DENV-2 (Diallo et al., 2005, 2008). Especially, sylvatic collections from south-eastern Senegal were more refractory than other collections from throughout the country. However, it should be noted that the study by Sylla et al. (2009) only examined the highly passaged DENV-2 Jam1409 isolate (reinforcing the importance of using strains circulating in the geographic area of study). Previous studies demonstrated that geographically-distinct collections of *Ae. aegypti* from Senegal are genetically diverse and documented the great variability in VC for both DENV-2 and YFV across the country. The northwest-southeast decline in the susceptibility to YFV BA-55 is very similar to that seen with DENV-2 JAM1409 (Huber et al., 2008; Sylla et al., 2009).

Furthermore, it has been demonstrated that VC of *Ae. aegypti* for DENV is dependent on the interactions between the mosquito strain and virus genotype in natural collections (Lambrechts et al., 2009). Using viruses and vectors that are geographically proximate and genetically diverse is important in order to make strong conclusions about VC between collections. Assessing VC in *Ae. aegypti* with a viral isolate collected in proximity seems to be the most informative approach (Lambrechts et al., 2009). Diallo et al. (2005, 2008) followed this process by examining the VC of *Ae. aegypti* from Senegal with multiple local isolates of DENV-2. The 2008 study reported low levels of MG infection (0.0–26.3%) and variable disseminated infection (0-100%) in six collections from Senegal regardless of geographic location. Both studies demonstrated variability in infection rates based on the isolate of DENV-2 and the collection site, confirming the local adaptation between the virus and the mosquito vector. They also showed that although collections of sylvatic *Ae. aegypti* presented lower infection rates than sylvatic *Aedes* from other species, some sylvatic *Ae. aegypti* mosquitos developed nevertheless a disseminated infection.

Similarly, Lambrechts et al. (2009) demonstrated that differences in VC among three *Ae. aegypti* collections from Thailand infected with three genotypes of DENV-1 was a result of mosquito and virus genotypes interactions. Dickson et al. (2014) also highlighted interactions between mosquito and virus genotypes in sylvatic *Ae. aegypti*, and with YFV in West Africa. Overall, VC was dependent upon both viral and vector strains. Importantly, and contrary to previous studies, the study by Dickson et al. (2014) reported that sylvatic collections of *Ae. aegypti* showed high levels of disseminated infection for local isolates of both DENV-2 and YFV.

Recently, VC of *Ae. aegypti* for chikungunya virus (CHIKV) has been investigated and it was found that *Ae. aegypti* populations from Cape Verde and Kedougou (Senegal) were competent for CHIKV, but *Ae. aegypti* from Dakar (Senegal) presented a low susceptibility to the virus. The virus strains belonging to the West African lineage were the only ones disseminated by the domestic population of *Ae. aegypti* from Dakar and transmitted by those from Cape Verde (Diagne et al., 2014). And as previously demonstrated in Kerala, India (Kumar et al., 2012), it has been also observed that *Ae. albopictus* was a better vector for this virus (CHIKV) than *Ae. aegypti* (Diagne et al., 2014).

In addition to DENV, CHIKV and YFV, *Ae. aegypti* is also a vector for Zika virus (ZIKV), a single-stranded RNA virus belonging to the Flaviviridae family (Faye et al., 2014; Abushouk, Negida and Ahmed, 2016). More details on ZIKV and related infection are given in Annex B, section "Virus infection vectored by mosquitoes". The VC for ZIKV in *Ae. aegypti* is variable, depending on the source of the mosquitoes and the virus strain. *Ae. aegypti* collected from French Polynesia displayed high ZIKV infection rate, but late ability to transmit the virus (Richard, Paoaafaite and Cao-Lormeau, 2016). *Ae. aegypti* from Singapore infected with ZIKV demonstrated high MG infection, resulting in the salivary glands of more than half of the mosquitoes being tested positive for ZIKV (62%) by Day 5, and all mosquitoes potentially infective by Day 10 (Li et al., 2012).

Summary on vector competence

In summary, *Ae. aegypti* is a most efficient vector for several deadly (e.g. dengue, yellow fever) and debilitating (e.g. chikungunya, Zika) arthropod-borne diseases. The vector competence (VC) for arboviruses is associated with a number of anatomic barriers to productive vector infection. The VC for flaviviruses in *Ae. aegypti* is thought to be controlled by at least two genes or sets of genes, one controlling the midgut infection barrier (MIB) and the other controlling the midgut escape barrier (MEB). The mosquito susceptibility to DENV, attributable to multiple genetic factors, is usually very high, particularly against DENV-2 as elicited by DS3 and DMEB strains. The ability to overcome the MIB and MEB to transmit DENV-2 JAM1409 is a quantitative trait with multiple genes that likely condition VC and collectively determine the infection rates of mosquito populations. The rate of midgut infection, midgut escape and salivary gland infection generally increases at higher temperature, though may vary amongst different populations. Natural geographic features may also act as a barrier to gene flow in varied *Ae. aegypti* populations (for VC) for DENV-2.

VC differences among different populations infected with genotypes of DENV result from interactions between mosquito and virus genotypes. At subspecies level, *Ae. aegypti aegypti* is generally far more efficient in transmitting DENV in urban agglomerations than the sylvatic *Ae. aegypti formosus*, albeit with a variation in VC.

While *Ae. aegypti* is unquestionably a much stronger potential transmitter for dengue, yellow fever and Zika viruses, it seems that *Ae. albopictus* would be a more efficient vector for CHIKV, especially in sylvatic and rural settings. Both mosquitoes are day biters, multiple feeders and capable to transmit several pathogens.

Notes

[1] More information on flavivirus infection can be found in Annex B. Human and animal health affected by mosquitoes.

[2] The four viral serotypes of DENV are explained in Annex B. Human and animal health affected by mosquitoes.

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Chapter 4. Ecology of the mosquito *Ae. aegypti*

Ecological niche/species distribution modelling of *Ae. aegypti*

The ecological niche of a species can be defined as the range of environmental and biotic conditions within which its populations can persist without immigration (Hutchinson, 1957). The range of environmental and biotic conditions can be assessed through niche modelling, providing evidence for geographic isolation between populations (either based on conserved or divergent ecological niches). By mapping the spatial distribution of environmental suitability of climatic variables (Raxworthy et al., 2007), the niche modelling provides a much stronger case for geographic isolation for populations isolated by intervening unsuitable regions reducing gene flow.

The population structure of *Ae. aegypti* is complex, varies by region and scale, and can be influenced by environment and geography (Yan, Chadee and Severson, 1998; Urdaneta-Marquez et al., 2008). Urban estimates of genetic differentiation have varied in part due to environmental conditions and dispersal patterns (Huber et al., 2002; da Costa-Ribeiro, Lourenço-de-Oliveira and Failloux, 2006). *Ae. aegypti* population dynamics in urban areas are subject to daily as well as seasonal meteorological variability (Halstead, 2008). Effects of seasonal climatic factors on mosquito life-history traits are well documented, particularly on adult distribution, survival and availability of oviposition sites. Several supportive studies have also been made on physiologic aspects such as decreased embryonic (e.g. Trpis, Haufe and Shemanchuk, 1973) and larval (e.g. Teng and Apperson, 2000) development times as well as decreased size of adults (e.g. Rueda et al., 1990) being associated with higher temperature.

It may be an oversimplified assumption that climate change will independently lead to an increased range for this species and a concomitant expansion of the risk of dengue infections around the world. A range of dynamic factors must be considered when predicting future global distribution trends. Constraining the focus of models to a local and/or regional scale rather than aspiring for global models may increase their predictive capacity. In light of climate change, the major drivers of future dengue susceptible areas will likely include unprecedented population growth, particularly in urban areas in the tropics; an increase in the movement of both vector and virus reservoirs via modern transport; and a lack of effective mosquito management (Mackenzie, Gubler and Petersen, 2004). More details are given in the "Abiotic requirements and tolerance" section below.

Ae. aegypti niche and trophic interactions

The mosquito *Ae. aegypti* has a relatively narrow niche with limited trophic interactions. The anthropophilic form of *Ae. aegypti*, *Ae. aegypti aegypti*, utilises flooded artificial containers as habitat for larvae and pupae. It is this form that has become established in most tropical and subtropical areas globally and is the primary vector of dengue, Zika and several other viruses. The original sylvan form of *Ae. aegypti*, *Ae. aegypti formosus*,

occurs in sub-Saharan Africa where natural containers such as flooded tree holes are the dominant larval habitat (Lounibos, 1981). A plethora of man-made objects composed of plastic, rubber, metal, concrete, masonry and ceramics have been shown to hold water, capture nutrients and produce *Ae. aegypti* (Ritchie, 2014). Within these flooded containers, larvae graze on the surface of the container, feeding on fallen detritus (typically leaves) and bacteria and algae that have grown on it. Protein sources such as insects, seeds, fruit and even dead conspecific mosquito larvae are fed upon. However, many of these containers are nutrient poor, especially covered containers such as water storage tanks, and typically produce stunted adults. The restriction of nutrients, coupled with high larval populations beyond the carrying capacity of the container, reduce larval growth and pupation via density-dependent regulation (Hancock et al., 2016). Indeed, it is the sudden input of protein from, for example, a cricket that falls into and drowns within the container, which can lead to a surge in larval growth, pupation and adult emergence.

There is a very limited number of species known to feed upon *Ae. aegypti* larvae and pupae. Most artificial containers are small to medium in size, only intermittently flooded and thus do not maintain populations of predaceous aquatic insects or vertebrates such as fish and amphibians. While many of these aquatic predators can eat mosquito larvae, they are uncommon in most *Ae. aegypti* habitat. In some larger containers, dytiscid beetles and dragonfly naiads can occur and feed upon mosquito larvae, while fish and tadpoles have been propagated and released in large water storage containers to control *Ae. Aegypti*. Among the many kinds of mosquito that do not consume blood, mosquitoes of the genus *Toxorhynchites* oviposit in artificial containers and selectively feed upon mosquito larvae (Trpis, 1973) being *Ae. aegypti* larvae as well as from other container mosquito species such as *Ae. albopictus* and *Ae. notoscriptus*. Copepods of the genus *Mesocyclops* will actively predate first instar larvae of *Ae. aegypti*.

Adult *Ae. aegypti* are also restricted to largely "artificial habitats" created by man. This "cockroach of mosquitoes", as it is often called, prefers to harbour inside buildings and houses in urban areas where it has ready access to humans for blood feeding. In some instances, all life stages of *Ae. aegypti* (egg, larvae, pupae and adult) can occur inside, especially in areas where water is stored indoors for domestic use. However, in many areas *Ae. aegypti* adults do spend considerable time outdoors where they seek flooded containers in which to oviposit. Predation of adult *Ae. aegypti* is poorly studied. Spiders, especially saltidae (jumping spiders), are known to actively stalk and feed upon adult mosquitoes indoors (Sulaiman et al., 1990) and can be a major predator in semi-field cages (S. Ritchie personal observation). Most other animals purportedly linked to adult mosquito predation, such as bats, geckoes and dragonflies, often feed either crepuscularly or at night, and would likely miss day active *Ae. aegypti*. Ants and cockroaches are known to feed upon *Ae. aegypti* eggs in containers (see below the section on "Biotic interactions in the landscape"), and mites and booklice often predate eggs in laboratory colonies and thus potentially would in the field. Ants readily consume dead adult mosquitoes on the ground and even stranded larvae in recently dried containers. As *Ae. aegypti* occurs in relatively low numbers (generally < 10 adults per house), the biomass of this mosquito is small (an estimated 2 g/ha in Cairns, Queensland, Australia [S. Ritchie, unpublished data]) and it is usually considered that it does not make a large trophic contribution.

In summary, urbanised *Ae. aegypti* (*Ae. aegypti aegypti*) is largely restricted to artificial, man-made habitats in geographic areas outside of its native range. Endemic species within "natural" tropical ecosystems are not trophically connected with

Ae. aegypti aegypti, or in a limited way. Thus, it is assumed that they are at minimal risk should the species be eliminated from those areas.

Anthropic habitats

Increase in the size and population density of major cities place increasing demands on infrastructure and essential services, particularly in developing countries. The response to these demands may dramatically alter the suitability of a locality for urban mosquito breeding. An absence or irregularity of water supply will lead to an increase in domestic water storage practices which, in turn, will alter the landscape of potential *Ae. aegypti* habitat, perhaps providing a far more regular or abundant supply of larval sites.

The effects of topographic features of urban environments on *Ae. aegypti* behaviour are not fully understood; however, Reiter et al. (1995) noted that buildings were not an impediment to *Ae. aegypti* flight. Certain results indicate that urban landscape does contain barriers to dispersal (Reiter et al., 1995; Chadee, 2004; Valerio et al., 2012), and this affects the mosquito population structure.

Such information can be useful to agencies in charge of vector control for better targeting mosquito populations and areas of higher risk within control zones. Understanding the role of landscape features on population dispersal is likely critical to achieving success with any *Ae. aegypti* control strategy (more information is given in Annex A. Control of the mosquito *Ae. aegypti*).

Abiotic requirements and tolerance

Considerable variation in adult size occurs as a result of habitat conditions such as water quality, food availability, and crowding during mosquito larval breeding (Nasci, 1991). The adult size strongly influences various aspects of mosquito life history: survivorship (Pumpuni and Walker, 1989), mating success (Yuval, Wekesa and Washino, 1993), blood meal size (Xue, Edman and Scott, 1995), parous rate (Haramis, 1983), fecundity (Packer and Corbet, 1989), dispersal (Renshaw, Service and Birley, 1994) and longevity (Feinson and Spielman, 1980). Among abiotic and biotic factors, high temperature and low nutrition in the developing stages of mosquitoes generally result in small adults. While temperature, humidity and rainfall have overt impacts on mosquito adult survival and ecology, other climatic factors such as photoperiod and wind velocity may also be influential. Importantly, it is necessary to consider that these meteorological conditions have a combined effect on the survival and development of mosquitoes and that it is difficult to examine the potential impact of these factors independently as a consequence (Jansen and Beebe, 2010).

Aquatic

Ae. aegypti prefers clean water found in many types of domestic containers inside or near human dwellings (Nazri et al., 2013). The *Aedes* mosquito larvae require standing water to complete their growth cycle, therefore, any body of standing water represents a potential *Aedes* mosquito breeding site for mosquito larvae to mature. Water quality affects the productivity of a potential mosquito breeding habitat. Typically, greater numbers of mosquitoes are produced in water bodies with poor circulation, higher temperatures and higher organic content than in water bodies having good circulation, lower temperatures and lower organic content (Focks et al., 1993; Murrell and Steven, 2008).

Aquatic habitats for *Ae. aegypti* are containers in which eggs develop into adult mosquitoes. Mosquitoes lay eggs on the walls of water-filled containers in or around the house. The eggs hatch when submerged in water and can survive desiccation for months (see section on Morphology in Chapter 1). There is a great variety of man-made containers on backyards or patios that collect rainwater or that are filled with water by people. Artificial or natural water containers (water storage containers, flower pots, discarded tires, plates under potted plants, cemetery vases, flower pots, buckets, tin cans, clogged rain gutters, ornamental fountains, drums, water bowls for pets, birdbaths, etc.) that are within or close to places where humans live are ideal larval habitats for this mosquito.

Terrestrial

Studies of associations between climate parameters and *Ae. aegypti* are complicated by the dependence of the mosquito on humans, especially its preference for human blood and its adaptation to use artificial containers as larval development sites (Focks and Alexander, 2006; Tun-Lin et al., 2009).

Ae. aegypti is the major urban vector of DENV worldwide. Over the last 25 years, there has been a global increase in both the distribution of *Ae. aegypti* and epidemic DENV activity (Mackenzie, Gubler and Petersen, 2004). Historically, *Ae. aegypti* has been thought to be able to establish in regions between the northern January and southern July 10°C isotherms, while more recent studies suggest that the 15°C yearly isotherm is a better estimate (see Chapter 1 section on "Origin and current geographic distribution").

Although *Ae. aegypti* is generally considered a tropical mosquito (Christophers, 1960), it should be noted that its distribution in some temperate regions of the world does appear to be influenced by climate variables (Liu-Helmersson et al., 2016).

The potential effects of climate and environmental change on *Ae. aegypti* and DENV transmission have generated much debate (Jetten and Focks, 1997; Patz et al., 1998; Hales et al., 2002; Barclay, 2008; Beebe et al., 2009; Ooi and Gubler et al., 2009; Banu et al., 2011; Brady et al., 2013, 2014). Part of this controversy relates to modelling future climate-driven change for the vector or disease without accounting for human-related factors, which also impact the vector itself (e.g. availability of water-filled artificial containers as larval development sites) or DENV transmission dynamics (e.g. serotype-specific susceptibility of the human population). Several reports consider that the domestic nature of this species probably exerts more influence on its distribution than climate variables. These confounding factors can, thus, modulate the effects of climate change on the mosquito distribution. It is also recognised that the effects of climate and environmental change are location-specific and likely to impact *Ae. aegypti* and, potentially, also DENV transmission to a greater extent in some geographic areas than others (Lozano-Fuentes et al., 2012). Studies in Australia suggest that future changes in *Ae. aegypti* distribution in the country may not be directly caused by climate change but rather, by human response to changing rainfall patterns by increased or decreased use of water storage containers (Beebe et al., 2009; Russell et al., 2009; Williams et al., 2010, 2014, 2015; Bannister-Tyrrell et al., 2013).

Biotic interactions in the landscape

Biological interactions between species occupying similar niches may also influence the distribution and abundance of *Ae. aegypti*. Whilst a number of underlying processes

including interspecific larval resource competition has been suggested (Lounibos et al., 2002; Juliano and Lounibos, 2005), it is most likely that multiple factors determine the current distributions of each species. Examples of these interconnected factors include the potentially asymmetrical effects of abiotic factors (including climate) on different life cycle stages as underlined above, apparent competition induced by parasites, mating interference and variation between the microclimates in given locations (Lounibos et al., 2002; Juliano and Lounibos, 2005).

In the aquatic environment, the larvae have a number of predators including other invertebrates, tadpoles and fish. Aquatic invertebrate predators from the Coleoptera (beetles), Diptera (flies including the predaceous mosquito *Toxorhynchites* spp.), Hemiptera (true bugs) and Odonata (dragonflies and damselflies) orders prey on all mosquito larvae in the same environment (Shaalan and Canyon, 2009). Because *Ae. aegypti* usually uses man-made containers as breeding sites, it does not seem to have specific predators but rather "opportunistic" ones that feed on larvae if encountering them, as detailed under a previous section dealing with trophic interactions. Predators can significantly affect the survival, development, and recruitment levels of mosquitoes in their aquatic breeding sites. There is also some evidence that the occasional presence of predators in vessels can favour oviposition by *Ae. aegypti*, the mosquitoes being attracted to predator kairomones[1] (Albeny-Simões et al., 2014). Mogi (2007), however, reviewed mosquito invertebrate predators and concluded that they are usually absent or sparse in man-made containers in residential areas.

Russell, Kay and Shipton (2001) placed filter-paper strips containing *Ae. aegypti* eggs within flooded telecommunication pits and surface containers in Charters Towers (Australia), and found that no subterranean eggs and only 1% of surface-placed eggs, respectively, survived the 4-month dry season despite the egg capacity to survive desiccation for months (see Chapter 2, section on Life cycle). In this case, predation was primarily by cockroaches. Attack by a fungus (*Penicillium citrinum*) also resulted in high mortality within the flooded subterranean site. The high mortality of eggs in subterranean sites led the authors to conclude that subterranean egg refugia were not responsible for the reintroduction of *Ae. aegypti* into surface containers at the onset of the wet season.

Ants are also a significant predator of *Ae. aegypti* eggs in colonies, and probably also in the field (Focks et al., 1993; Russell, Kay and Shipton, 2001; Ritchie, 2014).

Life history traits and fitness

The body size of mosquitoes can influence a number of bionomic factors, such as their blood-feeding ability, host attack rate and fecundity (Klowden and Lea, 1978; Xue, Edman and Scott, 1995; Farjana and Tuno, 2012). All of these traits are important determinants of their potential to transmit diseases (Farjana and Tuno, 2013).

Ae. aegypti, the container-breeding mosquito, is closely associated with humans and highly anthropophilic, tending to predominate in densely populated urban areas. They are commonly found indoors, breeding in artificial containers, with female needing to feed on blood to produce eggs, as described above. Studies have demonstrated high anthropophily, with over 90% of the ingested blood being human, and the rest from pets, such as dogs and cats (Scott et al., 1993). Multiple feeding in a gonotrophic cycle can increase the risk of disease transmission by increasing the frequency of contact with hosts (Garrett-Jones, 1964; Garrett-Jones and Shidrawi, 1969; Dye, 1986). Two types of multiple feeding have been recognised: supplementary feeding owing to nutritional

reserve depletion in teneral females (Scott et al., 1993; Xue, Edman and Scott, 1995; Scott et al., 2000; Reyes-Villanueva, 2004) and interrupted feeding owing mainly to host defence (Clements, 1999). For more detailed information, see Chapter 2 section on "Physiology of reproduction".

Dispersal

Landscape fragmentation and human demography can influence dispersal patterns of mosquitoes. The degree and nature of modification can affect the flow of genes conditioning vector competence and insecticide resistance (Hemme et al., 2010). Generally anthropic habitats minimise climatic variation where *Ae. aegypti* distribution is dependent on human behaviour (Jansen and Beebe, 2010).

Mosquito dispersal patterns are non-random and influenced by environmental factors as reported by Sheppard et al. (1969) and Hausermann, Fay and Hacker (1971) in *Ae. aegypti* mosquitoes using mark-release-recapture method. Ecological features including accessible water, vegetation patterns, humidity, contribute to determining the mosquito distribution. The range of dispersal is dependent upon a mosquito's ability to remain in flight and the availability and abundance of shelter, food sources, hosts for blood meals and suitable oviposition sites (Sheppard et al., 1969). Suitable host availability may reduce dispersal as reported by Suwonkerd et al. (2006) where fewer *Ae. aegypti* mosquitoes exited a hut when a human host was present compared to controls with the presence a dog, or with no human host.

Given that dispersal range is an important aspect of dengue transmission, much research has been conducted attempting to determine how far *Ae. aegypti* adults travel. A characteristic feature of *Ae. aegypti* is that they rarely disperse far from where they eclose (i.e. emergence as an adult from the pupa) (Getis et al., 2003), therefore, the presence of adult forms is for practical purposes an accurate indication of the proximity of breeding sites. Adults only disperse further when a vital requirement is limiting or absent or there is a disturbance. Typically, adult *Ae. aegypti* mosquitoes travel relatively short distances of up to 100 m, although longer dispersal estimates of about 800 m have been observed, particularly when host density is low and female mosquitoes are starved (McDonald, 1977; Honório et al., 2003; Harrington et al., 2005).

Overall, most studies show a very short dispersal distance for *Ae. aegypti*. This species has been reported to usually fly from 50 m to 300 m during its lifetime, with mean dispersal distances of 28 m to 199 m (Harrington et al., 2005). Experiments in different parts of the world involving the release and recapture of adults suggest that most are recovered within 20 m to 50 m of the release point, with a small percentage reaching distances greater than 170 m and not more than 200 m (Morlan and Hayes, 1958; Sheppard et al., 1969; McDonald, 1977; Trpis and Häusermann, 1986; Rodhain and Rosen, 1997; Muir and Kay, 1998; Ordoñez-Gonzalez et al., 2001; Harrington et al., 2005; Russell et al., 2005; Maciel-de-Freitas, Codeço and Lourenço-de-Oliveira, 2007a, 2007b; Valerio et al., 2012).

Even if important variations in mosquito daily and lifetime dispersal rates have been reported, however, the examination of the mean distance travelled (MDT) and the flight range of mosquitoes, as opposed to the maximum distance travelled, may be a more epidemiologically-important parameter (Harrington et al., 2005). Many studies using mark-release-recapture methods (above-mentioned) have reported a flight range for *Ae. aegypti* shorter than the largest observed dispersal of 800 m. And the majority of re-captured mosquitoes were collected at the house of release or neighbouring houses,

suggesting females are rarely expected to visit more than two or three houses in their lifetime. In a Kenyan village, McDonald (1977) recaptured a majority of mosquitoes within the house where they were released over 12 days. Marked mosquitoes released in a tire dump in New Delhi, India, dispersed with maximum distances from 50 m to 200 m, but most were recaptured within 50 m of the release point (Reuben, Yasuno and Panicker, 1972). Similarly, Muir and Kay (1998) reported females having a MDT of 56 m.

It has also been observed that females are less likely to disperse from houses with a large number of available oviposition sites (Edman et al., 1998). Given that most *Ae. aegypti* do not disperse very far, containers in close proximity to other productive vessels are more likely to be oviposition sites and to receive a large number of eggs. Holding other attributes constant, containers in areas of dense larval habitat will have a greater probability of being productive with a greater abundance of pupae than areas where suitable wet containers are rare and thus have a spatially-dispersed distribution. This low dispersal is a limit to the use of the autodissemination technique[2] for control in large areas, which would require a high density of dissemination stations (Devine et al., 2009).

In some studies, released mosquitoes tended to cluster around houses with some dispersal towards adjacent houses, and mosquitoes released on the perimeter of villages moved towards the centre of the village (Sheppard et al., 1969; Trpis and Hausemann, 1986; Getis et al., 2003; Harrington et al., 2005; Maciel-de-Freitas et al., 2006). The relatively large numbers and duration of DENV infected females captured in houses with confirmed dengue cases in Merida, Mexico may further indicate high fidelity between *Ae. aegypti* mosquitoes and place of pupal emergence (García Rejón et al., 2008).

The rate at which *Ae. aegypti* spreads to new areas outside of its native range is highly correlated with human activities that aid in its dispersal, including modes of transport. Boats, planes and terrestrial vehicles (e.g. cars, trucks, buses) also play a role on long-range human-mediated dispersal of adults and eggs. *Ae. aegypti* can "hitch a ride" in these vehicles, resulting in long-distance transport (Ritchie, 2014). In the Peruvian Amazon the incidence of *Ae. aegypti* coincides with interconnecting roads and highways and to a lesser extent, routes of boat traffic between ports (Guagliardo et al., 2014). Abandoned bottles, tires and other containers resulting from human activities along these travel routes provide a favoured habitat for the larval development of *Ae. aegypti* (Flores et al., 2005) and likely play a role in expanding its range. Furthermore, Chadee, Doon and Severson (2007) indicated that prevailing weather patterns may potentially influence dispersion.

Results from two classes of markers (SNPs) show strong evidence of limited gene flow across Uriah Butler Highway (UBH) in Trinidad island (Trinidad and Tobago), effectively fragmenting the populations on the east and west side of the highway (Hemme et al., 2010). Although the distance across the highway is well within dispersal estimates for *Ae. aegypti*, lack of cover and shade may have made the UBH a harsh environment for mosquitoes to transect. This is supported by Tun-Lin, Kay and Barnes (1995) who reported shade as a significant factor impacting the presence of *Ae. aegypti* in premise surveys and Russell et al. (2005) confirmed that released *Ae. aegypti* dispersal patterns were non-random with more mosquitoes being recaptured along a corridor with heavy shading from trees and vegetation. Furthermore, oviposition sites were most likely minimal, even along peripheral ditches and absence of blood meal hosts may have dissuaded migration across the UBH and prevented a stepping stone model of colonisation from occurring over UBH.

Population density and distribution

A primary determinant of adult mosquito population density concerns the types and number of containers in a given environment. Adult production is unevenly distributed across potential larval development sites.

In most cases, a few key types of containers are responsible for a large proportion of the pupal, and thus adult, production (Morrison et al., 2004; Focks and Alexander, 2006; Koenraadt et al., 2008). Protective measures such as lids, larvicide, removal of discarded and unused containers or biological agents have reduced adult vector population density (Kay and Nam, 2005; Morrison et al., 2008). Container capacity, water temperature, source of water and container location, all of which can vary seasonally (Strickman and Kittayapong, 2002; Lenhart et al., 2006; Koenraadt et al., 2008), have been cited as important ecological factors affecting the production of adult *Ae. aegypti* (Morrison et al., 2004; Barrera, Amador and Clark, 2006a). Access to humans for blood feeding is additionally important for the production of *Ae. aegypti* adults (Ritchie, 2014).

A number of studies have also found that *Ae. aegypti* abundance is not homogeneous among households, with disproportionate numbers of immature and adult mosquitoes clustered in key premises (Tun-Lin, Kay and Barnes, 1995; Getis et al., 2003; Barrera, Amador and Clark, 2006b). A study of *Ae. aegypti* production in American Samoa found that containers were more productive on average in houses with a large number of containers (Lambdin et al., 2009). To this point, the relationship between productivity and the spatial distribution of containers has not been rigorously examined.

Population modelling

Spatial models of *Ae. aegypti* could provide an important advance toward model-guided vector control and risk assessment (Williams et al., 2008; Xu et al., 2010). One of the key challenges in modelling *Ae. aegypti* is the lack of adequate data for validation. Most models seek to represent the temporal dynamic response to climate and endogenous forces (Focks et al., 1993; Ferreira and Yang, 2003; Otero, Solari and Schweigmann, 2006; Williams et al., 2013), while others consider the spatial-temporal dynamic by introducing dispersal mechanisms (Otero, Schweigmann and Solari, 2008; Magori et al., 2009; Almeida et al., 2010).

Models describing the population dynamics of *Ae. aegypti* are either deterministic (Ferreira and Yang, 2003) or stochastic (Otero, Solari and Schweigmann, 2006) and share a common structure based on the framework of System Theory (Bertalanffy, 1975). Few available computational models simulate *Ae. aegypti* spatial-temporal dynamics. Otero, Schweigmann and Solari (2008) proposed a stochastic spatially-explicit model, based on their previous temporal model (Otero, Solari and Schweigmann, 2006), in which space is modelled as cells which are occupied by autonomous mosquito populations interconnected by flying individuals. Dispersal between cells is modulated by the availability of breeding sites. A similar approach considered both the spatial distribution of breeding sites and the dynamics of the aquatic stage of the mosquitoes (larvae and pupae) (Focks et al., 1993; Magori et al., 2009).

Notes

[1] Kairomones are semiochemicals similar to pheromones but differing by the fact that they send signals between different species.

[2] See more information on this technique in Annex A. Section: Chemical control.

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Annex A. Control of the mosquito *Ae. aegypti*

Current control strategies

Mosquitoes can be vectors (transmitters) of several infectious diseases to humans and animals and are thus of significant importance to public health. The aim of mosquito control, in general, is to prevent mosquito bites, to maintain mosquito populations at "acceptable" densities, to minimise mosquito-host contact and to reduce the longevity of female mosquitoes (Foster and Walker, 2002).

Vector control is any method to limit or eradicate mosquitoes that transmit disease pathogens. Disease control is the reduction in the incidence, prevalence, morbidity or mortality of an infectious disease to a locally acceptable level or, if possible, its elimination or eradication. In order to be sustainable, a vector control strategy must limit the spread of resistance to insecticides within target mosquito populations.

Aedes aegypti control is generally performed in the context of public health because it is the vector of Zika, dengue, chikungunya and yellow fever, and a number of other diseases. Particularly for Zika, dengue and chikungunya, there are no vaccines, therapeutic treatments or cure. Preventing or reducing Zika, dengue and chikungunya virus transmission depends entirely on control of the mosquito vectors or interruption of human-vector contact (WHO, 2009b). Eradication of *Ae. aegypti* populations may be achievable, but is rarely sustainable, therefore, the present paradigm is to reduce mosquito density below disease transmission threshold levels rather than eliminate entire populations (McCall and Kittayapong, 2006).

Ae. aegypti control largely depends on organised control programmes at the community level administered by ministries of health undertaken together with some self-protection measures. Because *Ae. aegypti* lives in close affinity with humans and human-made ecosystems, it is an ideal candidate for integrated control (utilisation of multiple methods to provide control), which is summarised in the following Table A A.1 and briefly described in the following sections.

Chemicals for mosquito control may only be used in accordance with national legislation and approval of the products. Some of the chemicals mentioned as examples in Table A A.2 might be allowed in some countries but not in others.

Detailed information on the mosquito ecology, dispersal and the distribution of human habitats (see the chapter on Ecology) can be useful to vector control agencies for better targeting populations for suppression. Control programmes can be built on an urban area divided into zones of control along landscape features that are large enough to impede mosquito dispersal. This technique allows for the possibility of local elimination of *Ae. aegypti* mosquitoes, barring or at least minimising re-infestation due to the active transportation of the mosquito. Furthermore, during outbreaks, control agencies can more accurately target areas of higher risk along these same control zones. Understanding the

role of landscape features on population dispersal is likely critical to achieving success with any *Ae. aegypti* control strategy.

Chemical control

Immature stages: The control of *Ae. aegypti* larvae and pupae can be effected by treating containers holding water (specifically those that are productive breeding-sites and cannot otherwise be eliminated or managed) with insecticides (larvicides). Larvicides such as diflubenzuron, novaluron pyriproxyfen, fenthion, pirimiphos-methyl, temephos and spinosad (approved by WHOPES) target the immature mosquitoes living in water before they become biting adults.

Table A A.1. Summary of control tools/strategies available for *Ae. aegypti*

Method	Description	Examples
CHEMICAL CONTROL	Immature stages	Treating containers (breeding-sites) with for e.g. Temephos 1% Sand Granule; biorational larvicides; insect growth regulators (IGR) such as methoprene and pyriproxyfen, spinosad
	Adult in medium/large areas or houses	Aerial treatments, indoor spraying, surface treatments
	Personal protection	Domestic insecticides, repellents (natural or synthetic), insecticide-treated materials and paints
BIOLOGICAL CONTROL	Immature stages and adults (the whole population)	Fish, dragonflies, copepods, *Bti*, *Toxorhynchites*, *Wolbachia*
GENETIC CONTROL (self-limiting)	Immature stages and adults (the whole population)	Self-limiting insects, sterile insect technique, others
GENETIC CONTROL (population replacement)	Forces genes/organism through the whole population	Gene drive systems (i.e. HEGs and CRISPR), *Wolbachia*
ENVIRONMENTAL MANAGEMENT	Modification: permanent transformations in some characteristics to the vector breeding habitats Manipulation: temporal changes (management) to affect the breeding sites (key) behaviour Structural changes in human habitation and human behaviour	Draining/cleaning/recycling/disposal of breeding-sites or potential larval habitats Installation of reliable piped water supply to communities, comprehensive coverage and proper disposal of solid waste collection, filling, draining public spaces Public sensitisation to reduce the availability of breeding sites (source reduction) Installing mosquito screening on windows, doors and other entry points. Using mosquito nets Paints, peridomestic veneering to contribute eliminating natural habitats

Source: Modified from PAHO (1994), *Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control*, PAHO Scientific Publication 548, Pan American Health Organization, Washington, DC, and McCall, P.J. and P. Kittayapong (2006), "Control of dengue vectors: Tools and strategies", in *Report of the Scientific Working Group Meeting on Dengue*, World Health Organization, Geneva, WHO/TDR 2007, pp. 110-119.

The application of larvicides can also be done by ground or aerial treatments. However, the high density of small habitats (< 200 mL) makes it very difficult to treat a reasonable

proportion of highly disseminated breeding sites. It has been proposed recently to use auto-dissemination of pyriproxifen by adult females themselves to their breeding sites, after their contamination using dissemination stations (Devine et al., 2009). This approach is very efficient but has a short range of action because of low rates of adult dispersal. It has thus been proposed to release sterile males contaminated with pyriproxifen to contaminate the females through venereal transfer, an approach called the "boosted sterile insect technique" (Bouyer and Lefrançois, 2014). This control method has been successfully demonstrated recently in a field trial against *Ae. albopictus* at a very small scale (Mains, Brelsfoard and Dobson, 2015), and it is a major research axis to improve larvicidal control at the moment.

Adult: The control of adult vectors with insecticides (adulticides), applied either as residual surface treatments or as space treatments (thermal fogging and ultra-low volume aerosol sprays), is expected to impact mosquito densities, longevity and other transmission parameters. Insecticides from three chemical groups, namely pyrethroids, organophosphates and carbamates, are recommended by WHOPES both for indoor and outdoor spraying (WHO, 2003). The application of adulticides can be done by ground or aerial treatments but has a very short-term and local action.

Indoor residual spraying (IRS) involves the spraying of an insecticide on all the walls inside the house. This is usually done only once or twice a year because the effect is lasting and continues to kill mosquitoes for many months after treatment. Targeted indoor residual spraying involves spraying dark shady areas used by adult *Ae. aegypti* as resting places, such as under beds and tables, inside closets and dark objects such as plastic crates and suitcases. This method uses less pesticide and has been successfully used to protect residences from dengue transmission (Vazquez-Prokopec et al., 2017).

Indoor space-spraying (ISS) involves delivery of an insecticidal fog inside houses. However, space sprays do not leave a residual layer providing long-term control and have found to be ineffective for dengue control (Esu et al., 2010).

Outdoor fogging is the method commonly used in many parts of the world. The insecticide is usually sprayed from vehicles as a cloud of "fog" outside houses, targeting the flying female mosquitoes. Vector populations can be suppressed over large areas by the use of space sprays released from low-flying aircraft, especially where gaining access with ground equipment is difficult and extensive areas must be treated rapidly. It is generally ineffective against *Ae. aegypti* populations that have access to indoor harbourage sites.

Personal protection: *Ae. aegypti* exposure can be avoided with chemical products such as domestic insecticides, repellents (natural or synthetic) and insecticide-treated materials and paints including spatial repellents such as metofluthrin (Ritchie and Devine, 2013).

In general, pyrethroids are the main active ingredients in household aerosol products available to the public. Where indoor biting occurs, household insecticide aerosol products, mosquito coils or other insecticide vaporisers may reduce biting activity (WHO, 2009a).

Numerous insect repellent products are available commercially in a variety of formulations. Some of these products contain active ingredient(s) from botanical origin and some are synthetic organic products, with a vast majority available as sprays. Repellents may be applied to exposed skin or to clothing. Repellents recommended contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N butyl]-

aminopropionic acid ethyl ester) or Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1 methylpropylester) (WHO, 2009a).

Long-lasting insecticidal netting (LLIN) is factory-produced mosquito netting pre-loaded with synthetic pyrethroid insecticide that is intended to retain its biological activity for at least 20 standard washes under laboratory conditions, and three years of recommended use under field conditions (WHO, 2013). Deployed as bed nets, LLIN potentially can reduce human biting rates and vector longevity at both household and community levels (McCall and Kittayapong, 2006). In Latin America, encouraging results for *Ae. aegypti* control have also been obtained when LLIN are deployed as window or door screens, curtains or as container covers (Vanlerberghe et al., 2011; Rizzo et al., 2012; Manrique-Saide et al., 2015).

Biological control

Biological control is based on the introduction of organisms that prey upon, parasitise, compete with or otherwise reduce populations of the target species. *Bacillus thuringiensis* var. *israelensis* (*Bti*) is an entomopathogenic bacterium that has demonstrated high efficacy against *Ae. aegypti* larvae and is commercially available in different formulations that can be utilised in a variety of breeding habitats (Lacey, 2007; Boyce et al., 2013). Its strain AM65-52 in a water-dispersible granulated formulation is recommended by WHOPES (2016).

Other biological control agents that have been used for larval control of *Ae. aegypti* include species of larvivorous fish (WHO/EMRO, 2003) e.g. *Poecilia reticulata*, dragonflies (Sebastian et al., 1980, 1990; Venkatesh and Tyagi, 2013) and predatory copepods (Copepoda: Cyclopoidea) (Kay et al., 2012) which have proved effective in operational contexts in specific container habitats, but seldom on a large scale.

Wolbachia *as a biological control method for virus transmission*

Uses of Wolbachia *in control methods*

Wolbachia bacteria can be used to control *Ae. aegypti* and the diseases it spreads in two different ways, population reduction or population replacement:

a) Population reduction: *Ae. aegypti* males infected with *Wolbachia* are released. When the infected males mate with wild females, no offspring are produced, and with such release renewed over a period of time, the mosquito population can be reduced. It is important with this approach that no infected females be released as that could potentially lead to failure of the control programme; the infected females can pass *Wolbachia* onto their offspring, which survive and can spread into the environment. To date, there have been no successful suppression trials using *Wolbachia* for population reduction with *Ae. aegypti*.

b) Population replacement: *Wolbachia* can also be used in a population replacement strategy approach, similar to gene drive systems. In the wild, Wolbachia can spread through a species by a process known as cytoplasmic incompatibility (CI). CI is similar to a gene drive mechanism, which kills any offspring that are not infected with *Wolbachia*, effectively selecting for only offspring that are infected and hence spreading the *Wolbachia* through a population. The following paragraphs detail population replacement strategies being tested in *Wolbachia* and *Ae. aegypti*.

Introducing the *Wolbachia* strain wMelPop into wild populations of *Ae. aegypti* can shorten the adult mosquito lifespan, thereby theoretically reducing but not eliminating the transmission of dengue since it has not fully proven to reduce mosquito longevity shorter to the extrinsic incubation period for dengue virus (DENV). However, high fitness costs have prevented wMelPop from being successfully established in wild populations of *Ae. aegypti* in Australia and Viet Nam (Nguyen et al., 2015).

Two *Wolbachia* strains (wMel and wMelPop-CLA) have shown to confer antiviral properties to *Ae. aegypti* and limit DENV-2 infection in the mosquito by reducing the virus' ability to disseminate from the midgut (MG) into mosquito saliva and affected mosquito fitness for disease transmission. A major open field trial was conducted in which about 300 000 *Wolbachia* wMel-infected *Ae. aegypti* mosquitoes raised under laboratory conditions were deliberately released in 2011 at 2 locations near Cairns, Australia. The frequency of *Wolbachia*-infected *Ae. aegypti* initially increased to more than 15% in both locations at two-week post-release. After additional releases, frequencies increased to > 60% and reached near fixation levels 5 weeks after releases were terminated, and these high frequencies were maintained through 2017. These observations suggest that *Wolbachia* could potentially become a powerful bio-control agent to suppress DENV transmission by *Ae. aegypti* in endemic areas, though field data demonstrating reduction of DENV transmission has not been shown.

Wolbachia *transfer into* Ae. aegypti *mosquitoes*

Although *Wolbachia* infections are relatively common in mosquitoes (Kittayapong et al., 2000; Ricci et al., 2002) including *Culex pipiens* (Yen and Barr, 1973), *Cx. quinquefasciatus*, *Ae. fluviatilis* (Moreira et al., 2009) and *Ae. albopictus* (Sinkins, Braig and O'Neill, 1995), the main vectors for dengue fever (*Ae. aegypti*) and malaria (*Anopheles* spp.) are not naturally infected by *Wolbachia*. Approaches that use *Wolbachia* for the control of diseases transmitted by uninfected, naive insects rely on the successful establishment of stable *Wolbachia* infections, usually by embryonic microinjection of *Wolbachia*-infected cytoplasm or *Wolbachia* purified from infected insect hosts.

To create stably transinfected lines, embryo injections must target the region near the pole cells in pre-blastoderm embryos in order to incorporate *Wolbachia* into the developing germline and favour the transmission of *Wolbachia* to offspring. Several *Wolbachia* strains have been transferred across sometimes phylogenetically distant insects and, importantly, the phenotypes induced by these strains in their native hosts are generally also expressed in the newly infected hosts. *Wolbachia* transinfection experiments are more likely to be successful when the donor and recipient organisms are closely related.

In line with this, the transfer of wMelPop from its natural host, *Drosophila melanogaster*, into the dengue fever vector *Ae. aegypti* was achieved in the laboratory after *Wolbachia* was first maintained by continuous passage in *Ae. albopictus in vitro* cell culture for almost four years (McMeniman et al., 2008). *Wolbachia* adapted to a mosquito intracellular environment, facilitating transinfection *in vivo*. After microinjection of thousands of *Ae. aegypti* embryos, two stable wMelPop-CLA (cell-line-adapted) lines with maternal transmission rates of approximately 100% were generated (McMeniman et al., 2009). The wMelPop-CLA-infected mosquitoes showed an approximately 50% reduction in adult lifespan, compared with their uninfected counterparts (McMeniman et al., 2009). The halving of adult mosquito lifespan and the high *Wolbachia* maternal transmission rates were also maintained in more genetically diverse outbred mosquitoes

and larval nutrition did not affect the life-shortening ability of the wMelPop-CLA strain (Yeap et al., 2010).

The wMelPop-CLA infection is widespread in *Ae. aegypti* tissues, with high bacterial densities in the head (brain and ommatidia), thorax (salivary glands, muscle) and abdomen (fat tissue, reproductive tissues and malpighian tubules) (Moreira et al., 2009). Wide distribution across tissues has been found in other transinfected mosquitoes, such as *Ae. aegypti* infected with the wAlbB strain from *Ae. albopictus* (Bian et al., 2010). By using quantitative PCR and western blot analyses, this strain was also found in reproductive tissues, MG, muscles and heads, in both native *Ae. albopictus* (Dobson et al., 1999) and the transinfected *Ae. aegypti* (Bian et al., 2010), although the densities are not as high as those found in *Ae. aegypti* infected with wMelPop-CLA.

In addition, there is evidence that *Wolbachia* infection can result in permanent genetic modification of its insect hosts in a process called Lateral gene transfer (LGT). LGT of fragments of the *Wolbachia* genome (total size approximately 1.2 Mb), ranging from 500 base pairs to more than 1 Mb, have been observed in many invertebrates, including beetles (Nikoh et al., 2008), grasshoppers (Funkhouser-Jones, 2015; Toribio-Fernández et al., 2017), wasps (Dunning-Hotopp et al., 2007), fruit flies (Dunning-Hotopp et al., 2007; Klasson et al., 2014; Choi, Bubnell and Aquadro, 2015; Morrow et al., 2015), tsetse flies (Brelsfoard et al., 2014; Nakao et al., 2016), butterflies and moths (Ahmed et al., 2016), kissing bugs (Mesquita et al., 2015), mosquitoes (Klasson et al., 2009; Hou et al., 2014), filarial nematodes (Fenn et al., 2006; Dunning-Hotopp et al., 2007; Keroack et al., 2016) and spiders (Baldo et al., 2008).

Next step

The ability of some *Wolbachia* strains to reduce the lifespan of *Ae. aegypti*, invade mosquito populations through the induction of CI and, in particular, interfere with the replication of a variety of pathogens has distinct implications for disease control. There is some evidence that the *Wolbachia* can spread through a mosquito population as predicted, and the next phase is to prove that this leads to disease reduction.

Genetic control

Many trials have been conducted using classical sterile insect technique (SIT) and self-limiting insects (OX513A transgenic line) (Alphey, 2014). Classical SIT pilot projects have been tested in Indonesia, Malaysia, Mexico, Sri Lanka and Thailand. This technology is based on the mass-rearing production of male mosquitos sterilised under X-rays or by irradiation (Gamma). This technology is very well applied on agricultural pests and other vector species like the tsetse fly (Dicko et al., 2014; Vreysen et al., 2014), and can be very powerful on insect population suppression or even eradication. However, successful population suppression for *Ae. aegypti* using SIT has yet to be demonstrated. In China, *Ae. albopictus-Wolbachia* IIT/SIT strategies that use the introduction of infected males (IIT) and sterile females (SIT) are tested to reduce wild populations (Zhang et al., 2016). In Europe, the classical SIT is considered as a biological control technique and exempted from the "GMO" regulation, unlike self-limiting insects (EFSA Panel on Genetically Modified Organisms (GMO), 2013).

Self-limiting insects are engineered with a gene that causes offspring to die before reaching functional adulthood, a species-specific control approach that has been developed for *Ae. aegypti* but which is applicable to a wide range of insects. Released mosquitoes die along with their offspring and therefore do not persist in the environment

(Gorman et al., 2016). Additionally, the self-limiting OX513A mosquitoes and their offspring contain a fluorescent marker (DsRed2) that allows identification of OX513A larvae and pupae under laboratory conditions. Deployment of this technology through the release of self-limiting OX513A mosquitoes has achieved effective population suppression of wild *Ae. aegypti* in multiple trials in Brazil, the Cayman Islands and Panama (Harris et al., 2012; Carvalho et al., 2015; Gorman et al., 2016), and has been positively reviewed by regulatory bodies in Brazil, the European Union and the United States.

Environmental management

Environmental management seeks to change the environment in order to prevent or minimise vector propagation and human contact with the vector of pathogen by destroying, altering, removing or recycling non-essential containers that provide larval habitats. Such actions should be the mainstay of vector control and require important efforts for public sensitisation. Three types of environmental management are defined as follows (WHO, 1982; PAHO, 1994; Erlanger, Keiser and Utzinger, 2008; McCall, Lloyd and Nathan, 2009).

Environmental modification: Long-lasting physical transformations to reduce vector larval habitats such as the installation of reliable piped water supply to communities, including household connections.

Environmental manipulation: Temporary changes to vector habitats involving the management of "essential" containers, such as frequent emptying and cleaning by scrubbing of water-storage vessels, flower vases and desert room coolers, cleaning of gutters, sheltering stored tires from rainfall, recycling or proper disposal of discarded containers and tires, management or removal from the vicinity of homes of plants such as ornamental or wild bromeliads that collect water in the leaf axils. There are a great variety of man-made containers in backyards or patios that collect rainwater or that are filled with water by people. Disposing of unused containers, placing useful containers under a roof or protected with tight covers, and frequently changing the water of animal drinking pans and flower pots will greatly reduce the risk of dengue infections. Water storage containers should be kept clean and sealed so mosquitoes cannot use them as aquatic habitats (CDC, 2010).

Changes to human habitation or behaviour: Actions to reduce human-vector contact, such as installing mosquito screening on windows, doors and other entry points, and using mosquito nets while sleeping during daytime.

Integrated control management

Integrated vector management (IVM) is the strategic approach to vector control promoted by the World Health Organization (WHO, 2008) and includes control of the vectors of dengue. Defined as "a rational decision-making process for the optimal use of resources for vector control", IVM considers five key elements in the management process, namely (McCall, Lloyd and Nathan, 2009):

1. *Advocacy, social mobilisation and legislation* – the promotion of the IVM principles in development policies of all relevant agencies, organisations and civil society; the establishment or strengthening of regulatory and legislative controls for public health; and the empowerment of communities.

2. *Collaboration within the health sector and with other sectors* – the consideration of all options for collaboration within and between public and private sectors; planning and decision-making delegated to the lowest possible administrative level; and strengthening communication among policy-makers, managers of programmes for the control of vector-borne diseases, and other key partners.
3. *Integrated approach to disease control* – ensuring the rational use of available resources through the application of a multi-disease control approach; integration of non-chemical and chemical vector control methods; and integration with other disease control measures.
4. *Evidence-based decision-making* – adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation.
5. *Capacity-building* – the development of essential infrastructure, financial resources and adequate human resources at national and local levels to manage IVM programmes, based on a situation analysis.

Prevention and management of insecticide resistance

The evolution and spread of resistance to insecticides is a major concern for the control of the dengue vector *Ae. aegypti*. The reliance by most dengue control programmes on just two classes of insecticide (pyrethroids and organophosphates) available for use in public health, poses additional selection pressure on the mosquito vectors (Ranson et al., 2010).

Alterations in the molecular target sites of insecticides, which reduce the binding of insecticides, are the most understood resistance mechanisms. Several mutations in the sodium channel, the target site of DDT and pyrethroid insecticides, have been reported in *Ae. aegypti* (Brengues et al., 2003). Two alternative substitutions at one of the polymorphic sites, residue 1 016, have been linked to pyrethroid resistance and recently, methodologies to detect these mutations (often referred to as kdr mutations) in individual mosquitoes have been reported (Saavedra-Rodríguez et al., 2007; Rajatileka et al., 2008).

Resistance management strategies generally recommend the rotation of chemicals with different modes of action and the use of non-chemical methods of control. The implicit assumption is that resistance to a chemical will disappear from a population once the selection pressure is removed. Effective IVM will be possible only through an important development of available biological control tools, to be combined with insecticide and physical control.

In order to successfully develop and implement any resistance management strategies based on rotations, mosaics, mixtures or combinations, knowledge of the mode of action, chemical properties and residual life of the available insecticide products is crucial. Focusing on surveillance wherever possible is essential in order to react proactively once a regional population manifests a shift in its susceptibility towards synthetic insecticides.

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Annex B. Human and animal health affected by mosquitoes

Pathogens and diseases

An arthropod-borne virus or arbovirus is defined as a virus that is maintained in nature principally through biological transmission between susceptible vertebrate hosts by haematophagous arthropods; arboviruses multiply and produce virus in the vertebrate host, multiply in arthropod tissues, and are passed on after a period of extrinsic incubation to other vertebrates once again by the bites of an arthropod (PAHO, 1979). Most arboviruses fulfil the criteria laid down in this definition, but the group is very heterogeneous, containing viruses which, because they have not been fully classified on morphological or physicochemical grounds, are included among the arboviruses for convenience. There are currently 490 known arboviruses and this very large group contains representatives from several different viral families, the most important of which are the families Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae and Rhabdoviridae (Bishop et al., 1980; Rehle, 1989).

The extrinsic incubation period (EIP) is the time necessary for the development of arbovirus in the arthropod host. If the female mosquito longevity is lower than the viral EIP, then the potential for vector transmission is reduced. Average EIP is 15 days at 25˚C and 6.5 days at 30˚C (Chan and Johansson, 2012).

By definition, arboviruses have at least two different hosts, a vertebrate and an invertebrate arthropod, although many arboviruses have complex life-cycles involving several different vertebrates, and some are capable of transmission by more than one species of vector. All arboviruses, with perhaps very few exceptions, are current or potential zoonoses maintained in nature principally by wild animals and birds.

They have evolved to a state of mutual tolerance or symbiosis with their reservoirs. Since arboviruses rely only on virus production in the vertebrate host for successful transmission, disease in this host would be a disadvantage. Therefore, arboviruses seldom cause recognisable disease in maintenance hosts, and when disease is apparent in man or domesticated animals, it is only overt sign of the presence of these viruses.

Over 80 viruses produce significant human disease which ranges from mild febrile illness, which may or may not be accompanied by a skin rash and sometimes by polyarthritis, to severe and often fatal encephalitis or haemorrhagic fever. The same virus may produce different disease patterns in different subjects and illness often has a biphasic pattern. Mild fever, often not recognised, occurs during the initial viraemic stage. This may be followed by more serious symptoms, at which stage viraemia may have ceased and immunological responses, including antibody formation, have occurred. Frequently, only a small proportion of persons infected with potentially encephalitogenic arboviruses in epidemics develop encephalitis in this second phase. The great majority of infections do not develop past the first phase, which may even be asymptomatic.

Virus infection vectored by mosquitoes

There are 66 members in the flavivirus group, of which 31 are mosquito-borne. 26 flaviviruses can cause human disease but several of them have produced only laboratory-acquired infections or isolated cases of disease in man (Table A B.1). The range of clinical manifestations produced by flaviviruses is similar to those of the alphaviruses – febrile illnesses with or without a rash, or encephalitis. In addition, yellow fever, Kyasanur Forest disease, Omsk haemorrhagic fever, and dengue virus can cause haemorrhagic symptoms. Only those viruses which produce substantial prevalence are discussed in detail.

The International Committee on Taxonomy of Viruses (ICTV) has assigned the dengue virus (DENV) to the genus *Flavivirus*, of the Flaviviridae family. Based upon biological, immunological and molecular criteria, there are four viral serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4, which have different antigenic characteristics and serology (Boshell, 1995; Klungthong et al., 2004). Each serotype creates specific lifelong immunity against homologous reinfection, as well as short-term cross-immunity against the other serotypes, which can last several months (Leitmeyer et al., 1999; Monath, 2004). Each serotype has been subdivided into several genotypes (clades): three genotypes for DENV-1 (I, II and III) although two other clades named IV and V have been proposed, six genotypes for DENV-2 (American, Asian/American, Asian I, Asian II, Cosmopolitan and Sylvatic), four for DENV-3 (I, II, III and IV) although a fifth has also been proposed (V) and finally four for DENV-4 (I, II, III and Sylvatic) (Holmes, 2006).

Classic dengue fever affects both adults and older children. Following an infective mosquito bite, there is an incubation period of five to eight days followed by the sudden onset of acute fever, which often becomes biphasic, with a severe headache, pain behind the eyes, backache, chills and generalised pain in muscles and joints. A maculopapular rash generally appears on the thorax between the third and fifth day of illness and may spread later to the face and extremities. Lymphadenopathy, anorexia, constipation and altered taste sensation are common. Occasionally, petechiae are seen on the dorsal surfaces of the feet and the legs, hands, axillae and palate late in the illness. In young children, upper respiratory tract symptoms predominate and dengue fever is rarely suspected. The illness generally lasts for about ten days, after which recovery is usually complete, although convalescence may be prolonged. Laboratory findings reveal leukopenia, a mild thrombocytopenia, and slight lymphocytosis (Brathwaite et al., 2012).

Concerning the dengue haemorrhagic syndrome, fever, upper respiratory symptoms, headache, vomiting and abdominal pain may be present in the initial phase of the disease. Myalgia and arthralgia are uncommon. These symptoms (which are not severe enough for confinement) may last two to four days and many recover without any further symptoms. However, in a proportion of these cases, the initial phase is followed by an abrupt systemic collapse with hypotension, peripheral vascular congestion, petechiae, and sometimes a rash. Different degrees of shock may be evident, with the patient often restless, sweating, and febrile, clammy extremities, and a hot, feverish trunk. The fourth and fifth days are critical and purpura, ecchymoses, epistaxis, haematemesis, melaena, coma, convulsions and severe shock indicate a poor prognosis. Should the patient survive this period, however, recovery is usually complete. Laboratory studies often reveal thrombocytopenia, a prolonged bleeding time, an elevated prothrombin time, a raised haematocrit, hyperproteinemia and a positive tourniquet test. The liver is often enlarged, soft, and tender (Brathwaite et al., 2012). Several hypotheses have been proposed to explain why DENV now causes devastating epidemics, although it previously caused

relatively mild illness. The two principal proposals are that either there is an unusual response to infection in the host, or there is an increase in the virus' virulence. Haemorrhagic manifestations are thought to be due to secondary infection with different DENV, with a critical interval of six months between the two infections. The first infection probably sensitises the patient, whereas the second appears to produce an immunological catastrophe (WHO, 2009).

Table A B.1. Some important arbovirus infections of humans in geographic regions of the world

Disease	Geographic region(s)	Vectors	Vertebrate host(s)	DISEASE FEATURES IN HUMANS			
				Disease pattern	Description of diseases	Diagnosis	Control measure
Yellow fever urban	New World and Africa	*Ae. aegypti*	Man	Epidemic	Acute onset, high fever, prostration, later jaundice, proteinuria; fatalities common, although ratio of inapparent/apparent infection is high	Virus isolation, CF, HI, N, ELISA test	Vaccination with 17D vaccine, *Ae. aegypti* control
Yellow fever jungle	New World and African tropics	Mosquitoes haemagogus and aedines	Forest primates	Endemic	As above; cases occur sporadically in people exposed in forested regions in Africa and New World	Virus isolation, CF, HI, N, ELISA test	Vaccination with 17D vaccine, mosquito control not practicable
Dengue	New World and Old World tropics and subtropics	*Ae. aegypti* and other aedines	Man, possibly a jungle cycle in primates	Endemic and epidemic	Acute onset with rash in many cases and joint pains; simulates as influenza-like syndrome	Virus isolation, CF, HI, N, ELISA test	Vaccination with Dengvaxia, under conditions[1] Mosquito control and protection against mosquito bites
Dengue haemorrhagic fever	Southeast Asia and South America	*Ae. aegypti*	Man	Endemic and epidemic	Serious illness with haemorrhagic complicates, shock syndrome and high mortality almost exclusively in children and following a second infection with a different DENV	CF, HI, N, ELISA test, cell-culture system	Mosquito control
Japanese encephalitis	Korea to India and East Indies	*Culex tritaeniorhynchus* and other culicines	Wild birds, pigs can serve as amplifying host	Endemic and epidemic	Infection usually mild but encephalitic complications can be serious in young and in elderly, very important disease in the Orient	CF, HI, N, ELISA test	Mosquito control, vaccination with an inactivated vaccine
Murry Valley encephalitis	Australia	*Culex annulirostris*	Birds	Endemic, sporadic, over wide areas	Infection usually mild but encephalitis may occur with greatest probability in children and high fatality rates in the young	CF, HI, N test	Mosquito control measures and protection against mosquito bite

Disease	Geographic region(s)	Vectors	Vertebrate host(s)	DISEASE FEATURES IN HUMANS			Control measure
				Disease pattern	Description of diseases	Diagnosis	
Chikungunya	Africa and Asia, tropics and subtropics Cases of autochtonous transmission in Europe	*Ae. Aegypti* and *Ae. albopictus*	Possibly primates	Epidemic	Acute onset often with rash, rarely with haemorrhagic manifestations; joint aching and swelling are prominent features	CF, HI, N, ELISA test and virus isolation	Mosquito control
Kyasanur Forest disease	India (Mysore State)	Ticks mainly *Haemaphysalis*	Monkey, possibly also small mammals	Endemics and epidemic	Sudden onset, fever, headache, severe myalgia; there may be a diphase course with second phase	Virus isolation, CF, HI, N ELISA test	Protection against tick bite
Crimean-Congo haemorrhagic fever	Southern former USSR, Bulgaria, Central and South Africa, Pakistan, Iraq	Ticks - *Hyalomma marginatum*	Probably small mammals	Endemics	Sudden onset, chills, fever, headache, nausea, vomiting; haemorrhagic manifestations common; mortality rate 5%-10%	Virus isolation, CF test	Protection against tick bite
Venezuela equine encephalitis	Central and South America and southern United States	Mosquito of several species	Horses, possibly small mammals	Probably endemic, sharply epidemic	Fever, encephalitic signs, usually mild fatalities rate	Virus isolation, CF, HI, N, ELISA test	Mosquito control and protection against mosquito bites; attenuated vaccine exists for equines

Note: [1]WHO recommends that vaccine against dengue should only be used after testing on individuals to assess whether they have ever been exposed to the infection. (WHO Website, 2018)

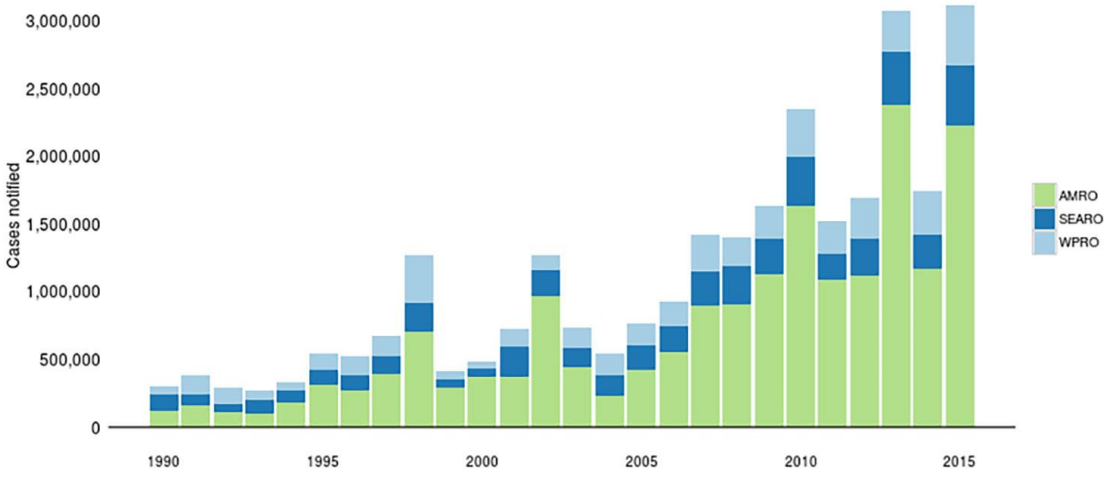
Source: Adapted from Evans, A.S. (Ed.) (1982), Viral Infections of Humans: Epidemiology and Control. Second Edition, Plenum Medical Book Company, New York and London.

Dengue virus serotypes, health effects and epidemics

The dengue disease may be endemic (which is often undiagnosed) or epidemic. In the Americas, there have been four epidemics during the 1963-83 period. The first epidemic in 1963 was caused by DENV-3 in the Caribbean and Venezuela. The second was in 1969, caused by DENV-2, affecting the Caribbean islands and also Colombia. The third epidemic began in 1977 in Jamaica, was caused by DENV-1 and affected more than 60 000 inhabitants, spreading to other Caribbean islands, Mexico, Central America, and Venezuela (Figueroa et al., 1982). In 1981 the fourth epidemic, resulting from DENV-4, began in Saint Barthélemy (French Antilles) and spread to other Caribbean Islands and Belize (PAHO, 2005).

Puerto Rico was seriously affected during all four epidemics, and after relatively high dengue activity in 1981 and 1982, the first epidemic of dengue in Brazil in 50 years began. Most countries reported only sporadic cases during 1983, however, Colombia, El Salvador and Mexico had significant localised outbreaks in 1983 (PAHO, 2005). *Ae. aegypti* reinfestation (1971-99) was caused by the failure of eradication programmes leading to increased dispersal of the mosquito and DENV circulation and a corresponding clear increase in the number of outbreaks over the 2000-10 period. During 2010, more than 1.7 million dengue cases were reported, with 50 235 severe cases and 1 185 deaths (Brathwaite et al., 2012). The epidemic seemed to continue extending globally in the following years; in 2015, the total number of suspected or laboratory-confirmed dengue cases notified to WHO for the Americas, South-East Asia and Western Pacific regions, exceeded three million (Figure A B.1).

Figure A B.1. Number of suspected or laboratory-confirmed dengue cases notified to WHO, 1990-2015



Note: a) AMRO: WHO Regional Office for the Americas
b) SEARO: WHO Regional Office for South-East Asia
c) WPRO: WHO Regional Office for the Western Pacific
Source: WHO (2018), Programmes – Dengue Control – Epidemiology Page, Website, www.who.int/denguecontrol/epidemiology/en/.

Zika virus infection

Zika virus (ZIKV) belongs to *Flavivirus* genus of the Flaviviridae family and it is transmitted to humans by mosquitoes (Gould and Solomon, 2008). However, sexual transmission between humans is another potential form of infection (Moreira et al., 2017). In 2015, ZIKV was shown to be associated with microcephaly and birth defects in children exposed *in utero* following infection of mothers during their pregnancy in Brazil (Zanluca et al., 2015; Calvet et al., 2016; Mlakar et al., 2016). Other studies evidenced the link between ZIKV infection during pregnancy and congenital cerebral malformations in newborns as microcephaly and other dysfunctions (Besnard et al., 2016; Driggers et al., 2016), and this was experimentally supported (Cugola et al., 2016). Moreover, the infection consequences in newborns can cause a range of different pathologies, which were described as the congenital Zika syndrome (Martines et al., 2016). ZIKV may additionally be associated with other neurological complications affecting adults, such as Guillain-Barré Syndrome (Dos Santos et al., 2016). Beyond to newborn disorders, the main symptoms of ZIKV infection are maculopapular rash, fatigue, lethargy, asthenia, fever, arthritis, arthralgia, myalgia, conjunctivitis and headache. The suspected patients can be submitted to RT-PCR assays or serological tests to confirm the ZIKV infection (Musso and Gubler, 2016).

The recent burden of Zika virus outbreaks in many countries is alarming. Although the virus is known since 1947 when it was first isolated from a sentinel *Rhesus* monkey exposed in the Zika Forest (Uganda) (Dick, Kitchen and Haddow, 1952), fewer reports of human infections were described until 2007. In that year, a ZIKV outbreak was first registered in Yap Islands, Federated States of Micronesia and since then, subsequent epidemics were reported in several islands in different Pacific regions between 2013 and 2014. This fast geographic expansion of the viral distribution was achieved in the Americas in 2015, causing important epidemics, mainly in Brazil. Currently, autochthonous transmission of ZIKV is occurring in many countries around the world where potential mosquito vectors are endemic (Musso and Gubler, 2016). The ZIKV emergent scenario caught the attention of the main health authorities mainly because congenital microcephaly and other neurological disorders in newborns were correlated with ZIKV infection in pregnant women, as described above. The WHO declared a state of public health emergency of international concern during almost the entire year of 2016 and launched a document named "Zika Strategic Response Plan" to guide the viral prevention and management by the national governments and communities where activities related to detection, prevention, research, care and support were recommended. This strategical document is constantly updated to provide the key information and progress achieved against ZIKV infections (WHO, 2016).

Entomological studies have demonstrated that Brazilian and other American populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes are competent to ZIKV, but they present different levels of susceptibility (Chouin-Carneiro et al., 2016). Moreover, well-known laboratory strains of *Ae. aegypti* also show vector competence to this pathogen, which can sustain vector-pathogen studies to clarify the interactions between this virus and its invertebrate host (Costa-da-Silva et al., 2017). Recently, a field study demonstrated the occurrence of naturally-infected *Ae. aegypti* in the city of Rio de Janeiro (Brazil), confirming the species potential to transmit ZIKV to humans (Ferreira-de-Brito et al., 2016). The entomological surveillance in endemic regions is an essential activity to monitor the circulation of ZIKV and the potential of new outbreaks to occur.

Ae. Aegypti other characteristics

Transmission to animals

In addition to being a vector for human pathogens, *Ae. aegypti* is capable of spreading disease among animal species that associate with humans, such as cattle and dogs. *Ae. aegypti* female mosquitoes are capable of the mechanical transmission of lumpy skin disease virus (LSDV) from infected to susceptible cattle (Chihota et al., 2001). Canine heartworm is transmitted by *Ae. aegypti* to dogs, which are companion animals frequently associated with the home environment.

Vertical transmission

The virus is transmitted to humans through the bite of the mosquito *Ae. aegypti* as principal vector and *Ae. albopictus* as a secondary vector. The mechanism of transmission of the virus that occurs most commonly involves the human-to-mosquito-to-human cycle.

However, it has been observed that vertical transmission of the virus can occur whereby infected females naturally transmit the virus to their progeny (transovarial transmission), the virus being in this case transmitted to the next generation without an intervening human host. Vertical transmission allows the virus to persist in nature during adverse weather conditions that limit mosquito reproduction, resulting in the appearance of virus-infected mosquitos once desiccated eggs hatch following a subsequent rainfall. Thus, vertical transmissions in vectors could play a role in the endemic maintenance of the viruses. Vertical transmission of dengue viruses in *Ae. aegypti* is documented by several studies (see below) and appears to vary with the vector geographical strains and virus serotypes (Rodhain and Rosen, 1997).

The first findings suggesting that transovarial transmission of DENV can occur in nature was reported by Khin and Than (1983). In this study, DENV-2 serotype was recovered from three of 123 pools of *Ae. aegypti* larvae (6 200 specimen) collected from water containers in Rangoon, Myanmar; the virus was also isolated from two of the 76 pools (7 730 mosquitoes) of male *Ae. aegypti* collected as larvae and reared in the laboratory to adults. In Trinidad and Tobago, the isolation of DENV-4 from adult *Ae. aegypti* reared from eggs and larvae collected in nature was documented by Hull et al. (1984): the virus was recovered in one out of the 158 mosquito pools tested from 10 different localities (10 957 adults processed for virus isolation), giving further evidence that transovarial transmission of DENV occurs in nature. In southern India, DENV-2 and DENV-3 were detected in vertical transmission to males in summer months when dengue infections were high in humans, suggesting how DENV adopted a novel strategy of surviving adverse climatic conditions (Thenmozhi et al., 2000). In Juchitán and Tuxtepec, Oaxaca, Mexico, vertical transmission of DENV in *Ae. aegypti* mosquitoes was recorded in two endemic localities. Although the presence of DENV in larvae could not be demonstrated, DENV- 2, - 3 and -4 serotypes were detected in four out of 43 pools of in-cage born mosquitoes (Günther et al., 2007). In Acapulco, Guerrero, only two (0.9%) of 226 pools of *Ae. aegypti* adults (one pool of adults emerged from field-collected larvae, and another of indoor-collected adults) were positive for DENV-1. This appears to be the first report of evidence on the vertical and transovarial transmission of DENV-1 in field-caught *Ae. aegypti* in Mexico (Martínez et al., 2014).

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