Evolution of resistance to insecticide in disease vectors

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Summary

Control of infectious diseases is a major challenge of the century. Their arthropod vectors are prolifering, leading to increasing prevalences of deadly diseases (ex. malaria, dengue, yellow fever…) and putting at risk a large part of the World human population. In many countries, particularly the poorest ones, vector-control using insecticides is the only affordable way to fight these diseases. Unfortunatelly, resistance to these insecticides is often rapidly selected and is now widespread in many arthropod vectors.

The general aim of this chapter is to provide a global overview of insecticide resistance mechanisms, their evolution in disease vectors, and to explore some anti-vector strategies. After defining resistance, the first part will describe its discovery and its spread in the last century until today, in relation with the various types of insecticide used. The second part will describe the different mechanisms and genetic modifications leading to resistance, and their evolution for several insecticides in various species. In the third part, the different strategies implemented to prevent vector-borne diseases through insecticide treatment will be described, to show how resistance is taken into account to achieve sustainable control of arthropod vectors.

Overall, it appears that some changes in the treatment strategies are urgently required to manage the development of insecticide resistance in disease vectors. This includes carefully using available insecticides, using alternative tools and finally implicating the local population to establish a continuous survey of resistance. Clearly, the greatest challenge for successful vector and disease control is the coordination of the different actors, despite their divergent agendas. Beside its implications in public health, insecticide resistance is a powerful model to study the evolution of adaptation, these fundamental approaches concurring to design new vector-control strategies.

Key words: vector control, infectious diseases, insecticides, mechanisms of resistance, evolution of adaptation.

Table of content

Introduction

Part 1: Insecticide resistance: definition and history

1- Synthetic insecticides

2- Alternative insecticides

Part 2: Mechanisms of resistance

1- Metabolic Resistance

a- Glutathione S-transferases

b- Multifunctional monooxygenases

c- Carboxyl-esterases

2- Target site modification

a- GABA receptors

b- Voltage-dependent sodium channels

c- Acetylcholinesterase

3- Other resistances

a- Growth regulators

b- Toxin receptors

c- Other

4- Resistance generalities

Part 3: Treatment practices and resistance management strategies

1- Treatment practices

a- Adult spray

b- Larvicidal treatment

c- Indoor residual spraying (IRS)

d- Insecticide treated materials

e- Other treatments

2- Impact of agriculture

3- Sustainable resistance management

a- Resistance management strategies

b- Examples

Conclusion

Bibliography

Introduction

The control of vector-borne diseases represents one of the greatest global public health challenges of the 21st century. They contribute substantially to the global burden of infectious diseases (~17%) and their prevalence tends to increase. Human population growth in many areas has led to extensive deforestation, irrigation, and urbanization, and these environmental modifications have created conditions that favor the proliferation of many arthropod vectors, such as mosquitoes, ticks, flies, etc. More than a billion people, primarily in developing countries, are now at risk for contracting many new or re-emerging diseases (World Health Organisation, WHO 2006).

Mosquitoes are probably the most common vectors of infectious diseases (review in Tolle 2009). 3,500 species are found thoughout the World and, in almost all species, the female find the proteins she needs for developping eggs through blood-feeding on vertebrates. This makes mosquitoes particularly prone to transfer viruses and other parasites between humans and animals hosts.

Malaria is a human plague documented since Greek Antiquity (it is mentioned in the Illiade, or by Hippocrate, Horace and Tacite) and may have afflicted humanity even earlier, as indicated by Neolithic bones pathologic modifications (see Reiter 2001). It is responsible for 350 to 500 millions annual cases with more than 1 million deaths, mostly in children from Africa. It is caused by the parasitic protists (*Plasmodium sp.)* vectored by mosquitoes of the *Anopheles* genus.

Dengue, yellow fever and chikungunya are viral diseases vectored by mosquitoes of the genus *Aedes* (*Ae. aegypti* and *Ae. albopictus*) that recently expanded their range due to increased human population growth and travel, together with poor sanitary conditions. The importance of yellow fever, with 200 000 cases and 30 000 deaths reported annually, is probably underestimated. Dengue (50 million infections, >12 000 deaths per year, mostly children) is the most widespread arthropod-borne virus infection: nine countries were affected in 1970 and 60 in 1999. It is caused by four distinct viruses and is mainly vectored by *Ae. aegypti* (Lambrechts et al. 2009). Finally, chikungunya, a formerly obscure arbovirus endemic to East Africa, recently caught attention after several outbreaks in countries of the Indian Ocean and South-East Asia, where millions of cases were documented. A mutation of the original virus (making it better adapted to *Ae. albopictus* than *Ae. aegypti*, the original vector) is probably responsible for recent chikungunya outbreaks in La Réunion Island and in Italy (Rezza et al. 2007; Tsetsarkin et al. 2007; Vazeille et al. 2007). Over the past 20-30 years, the geograpgic distribution of *Ae. albopictus* has considerably increased through worldwide commerce of used tires and because of its capacity of diapausing and the

resistance of its eggs to dissecation (Enserink 2008). It is presently replacing *Ae. aegypti* in tropical regions and causes the diffusion of the chikungunya in temperate regions.

Other mosquito-borne diseases include the West Nile virus, now endemic in the USA (Campbell et al. 2002), the Japanese encephalitis virus, which is expanding in the Indian subcontinent and Australasia (both transmitted by *Culex* genus), and filarial nematodes, causing elephantiasis vectored by the *Culex* species and *Ae. polynesiensis*.

Other major vector-borne diseases are transported by non-mosquito arthropods. This is the case of the sleeping sickness, vectored by the tse-tse flies (*Glossina sp.*), a neglected disease that imposes a burden close to that of malaria on humans (>300 000 cases per year, but severe morbidity), mainly in African isolated and underserved rural areas, where it also affects the cattle, the main local resources (see Welburn et al. 2006). Other dipteran as the sand flies (Phlebotominae) and black flies (Simulidae) are vector of leishmaniasis and onchocercosis, respectively, as well as of several viruses (review in Alexander and Maroli 2003; Surendran et al. 2005); houseflies transmit diarrheal diseases (WHO 2006). Finally, other public health pests include fleas (plague, Bartonella, rickettsioses), ticks (Lyme disease, ehrlichiosis, babesiosis and anaplasmosis), lices (Bartonella, rickettsioses), cockroaches, bedbugs and Triatomine bugs (trypanosomiasis, Chagas disease).

In addition to an increase of "airport malaria" (Europe and USA, Guillet et al. 1998; Tatem et al. 2006), some tropical vector-borne diseases have recently been observed in developed countries: Chikungunya (Italy, La Réunion) or West Nile virus (Europe, USA). It is often assumed that this expansion of tropical vector-diseases could reflect the influence of climate change on vector range. However, if climate (temperature, rainfall and humidity) does influence disease transmission, expansion of disease range is mostly due to human factors such as forest clearing, increase travelling and transport activities. Overall, it seems that the main determinants of vector-borne diseases' prevalence are socio-economic (see Reiter 2001; Kay and Vu 2005; Ooi et al. 2006; Morrison et al. 2008). They disproportionately affect poor and underserved populations living in tropical and sub-tropical regions. For example, dengue vector is present in both Mexico and Texas, which have a similar climate but because of distinct human factors (air conditioning, layout of cities, building structures), dengue is frequent in Mexico but almost absent from Texas (Reiter 2001). Unfortunately, the burden that vector-borne diseases impose directly impairs the public health and socio-economic development of many of the poor areas. Controlling these diseases is thus a necessity. This ideally entails active case-detection and treatment of human infections (vaccines, anti-parasitic drugs). However, few vaccines are currently available (e.g. yellow fever, Japanese encephalitis) and many pathogens are now resistant to anti-parasitic drugs.

Moreover, populations from endemic countries struggle to get access to them due to economic impediments. Thus in many instances, the control of vectors is the only affordable measure.

The first documented attempts to control malaria by limiting the densities of vectors go back to the Roman times: in an attempt to control the “Roman fever” (the name of malaria at that time), Julius Caesar himself had the Codetan swamp around Rome drained and planted with trees (Varro approx. 40 BC, in Cheesman 1964; Kelly 2009). Environmental modifications aimed at reducing the number of breeding sites have shown great success: for example, the construction of the Panama Canal was possible only after US Army Surgeon General William Gorgas stopped yellow fever transmission among workers by eliminating *Ae. aegypti* breeding sites (in Morrison et al. 2008). However, today the most common and affordable way of fighting the major disease-vectors is the use of insecticides (Roberts and Andre 1994; Hemingway and Ranson 2000; Beier et al. 2008). Many scientific investigations and reports show that the use of synthetic insecticides can dramatically reduce the risk of insect-borne diseases. These approaches, combined with extensive use of drugs, have rapidly led to the eradication of many diseases (e.g. malaria) from most non-tropical areas of the world, but in spite of initial successes, eradication has proven more elusive in the tropics (Dialynas et al. 2009). However, mechanisms allowing survival to insecticide exposures have been selected in many species of arthropod vectors. Resistance to all classes of synthetic insecticides is now widespread among pests of public health importance, and it is considered to be the most important impediment in the successful control of vector-borne diseases.

The general aim of this chapter is to provide a global overview of insecticide resistance mechanisms, their evolution in disease vectors, and to explore some anti-vector strategies. The first part will describe various aspects of insecticide resistance from an historical point of view. The second part will describe the different mechanisms and genetic modifications leading to resistance, and their evolution for different insecticides in various species. In the third part, the different strategies implemented to prevent vector-borne diseases through insecticide treatment will be described, to show how resistance is taken into account.

Part 1: Insecticide resistance: definition and history

Insecticide resistance in pest populations affects both economy and public health at a worlwide scale: it decreases crop yields (and thus profitability), induces the need to increase the quantity of insecticide and to develop new insecticides (thereby having a strong impact on costs and on the environment), and finally it is responsible for higher incidence of human

or animal diseases (Georghiou and Lagunes-Tejeda 1991; Whalon et al. 2008). This general society problem however provides evolutionary biologists with a unique contemporary model, ideal for studying how new adaptations evolve by natural selection. The selecting agent is known (insecticides), evolution is recent and rapid (few years after insecticide selection) and the biological and genetic mechanisms are often known (see Part 2). This explains why it has been the subject of such a large body of work over the years.

Resistance is defined as a heritable decrease of the susceptibility to an insecticide (Nauen 2007). Three categories of resistance can be distinguished: behavioural (avoidance of contact with insecticide), physiological (e.g. increased cuticule thickness) and biochemical (enhanced insecticide detoxification and/or decreased insecticide target sensitivity). Only few examples of behavioural and physiological resistances have been reported because they provide weak protection (e.g. horn flies and Anophelines, Roberts and Andre 1994, *Triatoma infestans*, Pedrini et al. 2009), while biochemical resistances typically result in relatively high level of protection. Resistance arises from the selection of individuals able to survive and reproduce in presence of insecticide. Resistant individuals carry one or several genetic mutations that prevent insecticide disruption of the target functionning. As a result, the frequency of resistance gene(s) increases in the population over time. Insecticide resistance is confirmed by toxicological tests (bioassays) establishing resistance ratio (or RR corresponding to the number by which an insecticide dose must be multiplied in order to obtain the same mortality in resistant than in susceptible insects). It can be investigated at many levels, from the molecular characterization of genes conferring resistance and their biochemical products, to the effect of these genes on the fitness (i.e. reproductive capacity) of the individuals carrying resistance alleles, to the dynamics and evolution of these resistance alleles in natural vector populations and their effect on pests and disease control.

The first recorded attempt of insect pest control is found in the litterature of the XVIIIth century with the application of tobacco juice against sheep scabs (Lisle 1757, in Wood 1981). The first case of resistance was reported in 1908, in a population of San Jose scale (*Aspidiotus perniciosus*) resistant to lime sulphur (Melander 1914). A century later (2007), 553 arthropod species are reported as resistant to at least one insecticide, among which many disease vectors such as flies, mosquitoes, lice, bedbugs, triatomines, fleas, cockroaches and ticks. Some species can be resistant to a large array of compounds: *Tetranychus urticae* (a non-vector Acari that is a pest for crops) has been found resistant to 80 different compounds. More than 100 mosquito species are resistant to at least one insecticide (56 Anopheline species, 39 Culicine species); *Cx. pipiens pipiens* and *An. albimanus* are resistant to more than 30 different compounds (Whalon et al. 2008).

1- Synthetic insecticides

Originally only inorganic insecticides (such as lime sulphur) and natural products were available, for example flower-extracted pyrethrum for malaria control in the 30s (in Brooke et al. 2001). Today, four classes of organic (synthetic) insecticides are essentially used: the organochlorines (OC), the organophosphates (OP), the carbamates (CX) and the pyrethroids (PYR) (Fig. 1).

Insert Figure 1 here

The first synthetic insecticides, introduced during World War II for malaria control, belonged to the OC class. The first one was the dichlorodiphenyltrichloroethane or DDT (introduced in 1943), which target is the voltage-gated sodium-channels (Na-channels), the other one was the cyclodiene dieldrin, which targets the γ-aminobutyric acid (GABA) receptor, both targets being essential in the insect nervous system (see Part 2). In addition to their public health applications, enormous tonnages of DDT and dieldrin were used worldwide in agriculture. It was at first a great success with large WHO-led campaigns leading to reduction of morbidity and mortality from malaria in many endemic regions after World War II. Widely acclaimed, DDT and dieldrin rapidly selected resistance in insect vectors. In *An. gambiae*, resistance to DDT was first noted 11 years after its introduction (WHO 1957), while a population from northern Nigeria was reported resistant to dieldrin soon after (Davidson 1956). DDT resistance has now been reported in mosquitoes (*Aedes sp*., *Anopheles sp*. and *Culex sp*.), houseflies, sandflies, body lice and head lice, while resistance to dieldrin (60% of reported cases of resistance before 1990) has been detected in more than 277 arthropods, including mosquitoes (*Aedes sp*., *Anopheles sp*. and *Culex sp*.), fleas, ticks, biting flies, bedbugs, coackroaches and human lice (ffrench-Constant et al. 1993; Roberts and Andre 1994; ffrench-Constant et al. 2000; Hemingway and Ranson 2000; WHO 2006).

An important issue against these insecticides was their environmental impact. Rachel Carson's book “Silent Spring” (1962) was a seminal work publicizing and politicizing the toxic effects of the accumulation of DDT and its metabolites in the food chain. For vertebrates, DDT can interfer with reproduction, and in humans it can have neurologic, carcinogenic and reproductive effects, although the evidences remain debated. These insecticides are also extremely stable in the environment, contaminating groundwaters and remaining in soil samples long after their use. In the 70s, the Persistent Organic Pollution Treaty led to total banning of dieldrin and to the banning of DDT for all uses except malaria control when this disease is very frequent and there is no alternative. DDT use rapidly declined in the 70s (it is

no longer used in Latin America, van den Berg 2009), but it recently gained new advocates due to the development of resistance to the alternative insecticides, and to its low cost (Brooke et al. 2001; Rogan and Chen 2005; WHO 2006; Coleman et al. 2008; van den Berg 2009).

From the late 70s, OCs were replaced by the pyrethroids (PYR) class for vector control, and these became widely used in agriculture and public health, and more particularly against malaria vector (in Hemingway et al. 2004). As DDT, these insecticides target the Na-channels (i.e. neurotoxic effect). Their rapid popularity comes from their very low toxicity to human, their rapid knock down effect (i.e. mosquitoes are rapidly incapacitated, which diminishes their potential of blood-feeding and thus of transmitting pathogens) associated with an excito-repellancy effect (i.e. most of the mosquitoes fly away of the treated material, which diminishes their transmission potential). Finally, PYRs have also cyto- and genotoxic effects (i.e. they disturb the transmission of nervous influx, as well as the normal functioning of the cells). Pyrethroid-based indoor residual spraying (IRS) and insecticide-treated nets and curtains (ITNs) are currently advocated as standard malaria vector control strategies (WHO 2006).

Unfortunately, PYR resistance was reported in 1993, in *An. gambiae* populations from Côte d'Ivoire (Elissa et al. 1993) and later in *Cx. p. quinquefasciatus* also in West Africa (Chandre et al. 1998). Resistance is now widespread in mosquitoes (*Aedes sp*., *Anopheles sp*. and *Culex sp*., (see Liu et al. 2006 for a review), body and head lice, tick (ex. *Boophilus microplus*) and fleas (Roberts and Andre 1994; Hemingway and Ranson 2000; Hernandez et al. 2002; Thomas et al. 2006; WHO 2006). For example, resistance to PYRs in the *Cx. pipiens* complex is now found in Martinique, Cuba, USA, China, Saudi Arabia, Tanzania, West Africa and Tunisia (Hardstone et al. 2007). As PYR resistance developped, many control programs attempted to revert to DDT for disease control. However, because these insecticide classes share a common target site, there is cross-resistance to both insecticide classes in many locations (Brooke et al. 2001; Coleman et al. 2008).

Finally, two other classes of synthetic insecticides are used at a large scale worldwide: the organophosphates (OP) and the carbamates (CX), which were first used in the 40s and the 50s, respectively (Nauen 2006; WHO 2006). OPs and CXs target the synaptic acetylcholinesterase (AChE), an essential enzyme in the nervous system (Massoulié and Bon 1993).They are usually used as larvicids (although some are now considered for ITN impregnation, as an alternative to PYR, Kolaczinski et al. 2000; Sharp et al. 2007a; Oxborough et al. 2008), and are particularly well-suited for species with delimited breeding sites (most *Culex* and *Aedes* species). However, they have a short half-live and two

to three rounds of IRS are needed per year. This, combined in some instances with their high price, can make these insecticides too costly for most malaria control programs, despite fewer reports of resistance (Coleman et al. 2008). Early resistance to these insecticides has been detected shortly after their first application: for example, first OP treatments in the Montpellier area (Southern France) started in 1969, the first resistance being detected only 3 years later (Pasteur and Sinègre 1975). Resistance has now been recorded in mosquitoes (*Aedes sp*., *Anopheles sp*. and *Culex sp*.), biting flies (e.g. *Simulium damnosum*, vector of onchocerciasis), sandflies, houseflies and fleas (reviews in Roberts and Andre 1994; Hemingway and Ranson 2000; Oakeshott et al. 2005; WHO 2006).

Despite intense research to find insecticides with different mode of action, for agricultural use which is more profit-making, the molecules available today essentially belong to the four classes described above (OP, CX, PYR and OC), for which resistance is now widespread. Some species are even resistant to most or all classes: for example, *An. arabiensis* is resistant to OPs, dieldrin, PYRs and DDT in Sudan (Matambo et al. 2007; Abdalla et al. 2008), *Cx. p. quinquefaciatus* is resistant to DDT, dieldrin, OPs and PYRs in Côte d'Ivoire and Burkina Faso (Chandre et al. 1998) and *Cx. pipiens* is resistant to DDT, OPs, CXs and PYRs in China (Cui et al. 2006). A fifth class has been discovered in the 90s, the neonicotinoids that target the nicotinic acetylcholine (ACh) receptors in the central nervous system; however they are used mostly for agricultural pests, not for disease vectors (Nauen 2006; Whalon et al. 2008). More recently few new insecticides have been described (phtaleic acid diamides or anthranilic acid diamides): they target the ryanodine-sensitive intracellular Ca^{2+} release channels (that mediate many cellular and physiological activities), but again they are used only for agriculture so far (Nauen 2006). Finally, another type of synthetic insecticides is growth regulators (GR). It regroups synthetic products called juvenoids that mimic the juvenile hormone (JH) (review in Hollingworth and Dong 2008) and chitine inhibitors (see Hirose et al. 2010). By simulating a continuous high level of JH or limiting the chitine synthesis, they disrupt the insect development, particularly affecting the transitions between larval stages, the larva-to-pupa molt and the adult's emergence. So far, only few cases of resistance have been reported in houseflies and mosquitoes (e.g. resistance to methoprene, a JH analogue in the mosquito *Ochlerotatus nigromaculis*, Cornel et al. 2002).

In summary, most often only PYRs are available, essentially for economic cost reasons(Kelly-Hope et al. 2008): the most recent PYR has been introduced 30 years ago and no new synthetic insecticide has been found in the last 20 years (Kelly-Hope et al. 2008).

The shrinking availability of insecticides as a result of resistance is exacerbated by the removal from the market of insecticides that are no longer registered for public health use: some coumponds are too costly and insecticide use is restricted by regulatory agencies, due to environmental concerns. Consequently, new environment-proof products (higly specific, no effects on non-targets) are now required for sustainable vector control (Farenhorst et al. 2009). Some alternative mean of control are emerging through growth regulators and biological insecticides, but they represent only a small fraction of the insecticide market (Fig.1).

2- Alternative insecticides

Environmental pollution concerns and unresolved issues pertaining to the toxicity of synthetic insecticides to humans and non-target species have led the public and researchers' interest to investigate alternative "biological" insecticides (Mittal 2003; Scholte et al. 2003). Three main types of these alternative insecticides are documented: i) bacterial toxins, ii) essential oils and iii) fungi.

There are two main sources of bacterial toxins: *Bacillus sphaericus* (Bs) and *Bacillus thuriengiensis* (Bt). They kill insect larvae by binding to various receptors on midgut epithelial cells (review in Hollingworth and Dong 2008). Bs toxicity is due to a binary toxin whereas Bt toxicity is due to the interaction of four different toxins. Both Bs and Bt induce a cytolysis, although the involved mechanisms are different. Despite being called "biological", the usual form of these insecticides is a purified extract of the toxins. These larvicides are presented as highly specific and effective at low doses, and are thus expected to be safe for the environment. Toxins extracted from Bs and a variety of Bt (*Bacillus thuringiensis* var *israelensis* or Bti) are used for mosquito control. In these species, bacterial toxins show some differences in specificity: Bti is more effective against *Aedes* and *Culex* species than against *Anopheles*, whereas Bs is more effective against *Culex* than *Anopheles* species, and has no effect on *Aedes* species that lack receptors. While the presence of several toxins was expected to delay resistance apparition, Bs and Bti resistances have been detected in various mosquitoes (Nielsen-Leroux et al. 1997; Mittal 2003; Paul et al. 2005) and resistance to Bt has been detected in several agricultural pests (Tabashnik et al. 1997a; Gahan et al. 2001; Griffitts et al. 2001).

Although less documented, essential oils are investigated as potential biological larvicides. They are advocated to be more specific than synthetic insecticides, and biodegradable, thus with reduced impact on the environment. Three essential oils (*Satureja*

hortensis, *Thymus satureoides*, *Thymus vulgaris*) have showed relatively good efficacy at low dose against *Cx. p. quinquefasciatus* larvae: they appear to increase larval mortality and decrease adult's emergence and oviposition; they also show a repellent effect (review in Pavela 2009).

Finally, fungi can be used as biological insecticides: they target the adult stage of mosquitoes and are used essentially for malaria control. The fungus *Metarhizium anisopliae* has been shown to reduce *An. gambiae* adult life span in the laboratory and in the field in Tanzania (Scholte et al. 2003; Scholte et al. 2005), while *Beauveria bassiana* decreases the survival of another malaria vector, *An. stephensi* (Blanford et al. 2005). These agents have several advantages: they are cheap, easily stored for long term and specific to insects (thus innocuous to humans). They kill their host later than other insecticides, but before the time required by the malaria agents, *Plasmodium sp.*, to reach the infectious stage. Moreover, experiments have shown that they can decrease the female mosquito blood meal size, its feeding propensity and its fecundity (Scholte et al. 2006). Consequently these fungal insecticides have a direct effect on *Plasmodium* transmission and are expected to decrease malaria prevalence. Finally, their acting late in life is considered by several authors to be an important advantage, as it will decrease selective pressure and reduce the risk of resistance development (potentially "evolution-proof" insecticides, Farenhorst et al. 2009; Michalakis and Renaud 2009; Read et al. 2009).

To conclude this first part, it should be noted that all treated species do not develop insecticide resistance. This can be linked to the particular life cycle of the species or to molecular constraints preventing the evolution of resistance mechanism. For example, after decades of treatment, the tsetse flies (*Glossina sp*.) have not yet developed resistance to DDT or PYRs, probably due to their very small number of youngs, which limits their evolutionary reactivity (Hemingway and Ranson 2000; Welburn et al. 2006; WHO 2006). Similarly, *Ae. aegypti* did not develop the most efficient resistance mechanism to OPs and CXs because its particular codon usage prevents the apparition of the required mutation (Weill et al. 2004). This last example shows that understanding why resistance occurs or not also requires elucidating the mechanisms of insecticide resistance at the molecular and biochemical levels.

Part 2: Mechanisms of resistance

The target of most insecticides are critical molecules of the insect nervous system. Insecticides bind to specific sites on their target and disrupt its function. Any mechanism that decreases the insecticide effect will lead to resistance. This encompasses reduced prenetration of the insecticide, increased excretion or sequestration of the insecticide, increased metabolism of the insecticide and finally target modification that limits the binding of the insecticide.

The three first mechanisms are poorly documented and do not seem to play a prominent role in resistance. Reduced uptake of PYRs through the cuticule has been observed in the cockroach, inducing a 2-3x resistance (i.e. the PYR dose needed to be multiply 2-3 times to achieve the same mortality as in susceptibles). Similarly, reduced uptake through cuticule (9-10x) has also been identified in the housefly (Scott 1999). Transporters (efflux pumps) that excrete the insecticide have been identified in *D. melanogaster*, but they have only a limited effect on resistance (Hollingworth and Dong 2008). Finally, sequestration of the insecticide has been described as playing a role in resistance (see carboxyl-esterases below), although the extent of this role is poorly known.

Most studies aiming at understanding the mechanisms and the genetic bases of insecticide resistance focus on metabolic resistance and target site modification. Increased rates of insecticide detoxification and reduced sensitivity of insecticide targets may be due either to point mutations in structural genes, to gene amplification (i.e. increased number of gene copies leading to an increased number of produced proteins) or to transcriptional regulation (i.e. increased production of transcripts and then of proteins). Usually these resistances are explained by a limited number of mechanisms, most of the time monogenic.

In this chapter, we will present the various documented mechanisms of resistance. We will specifically focus on disease vector species, although many mechanisms are common to agricultural pests. We will insist on the evolutionary aspects of resistance, while the detailed mechanisms will be treated more succintly. More comprehensive reviews can be found (e.g. Hemingway et al. 2004; Liu et al. 2006; Hollingworth and Dong 2008).

1- Metabolic Resistance

Metabolic resistance regroups the various defense mechanisms that degrade the insecticide in less or non-toxic products, thus decreasing the quantity of toxic molecules that reach the target. Three major families of enzymes are involved in this type of resistance,

although recent genomic studies have suggested that other types of enzymes may be implicated (Oakeshott et al. 2003; Vontas et al. 2005; Waterhouse et al. 2008; Awolola et al. 2009).

a- Glutathione S-transferases

Glutathione S-transferases (GSTs) catalyse the reaction of the sulphydryl group of the tripeptide glutathione of various xenobiotics. This sulphydryl group reacts with electrophilic sites on xenobiotics, leading to formation of conjugates that are more readily excreted and typically less toxic than the parent insecticide. In addition to this direct detoxication, GSTs catalyse the secondary metabolism of compounds oxidised by other enzymes (see below).

GST enzymes are present in most insects. They represent a large family of generalist detoxifying enzymes (6 classes of GSTs have been identified in the genome of *An. gambiae*) and have thus broad substrate specificities. The GST family expands either by alternative splicing or by local gene duplication, the last leading to clusters of GST genes. Quantitative genetics analyses identified a quantitative trait locus (QTL) for resistance to DDT in *An. gambiae*, within which there is a cluster of 8 GSTs (Ortelli et al. 2003).

However, GSTs usually provide limited levels of resistance (~10x) and are primarily associated with resistance to OCs, particularly DDT, and OPs (Table 1). They are also suspected to play a role in the resistance to PYRs, although this issue is still debated.

GST-based resistance seems to be associated with an increased amount of enzyme resulting from gene duplication or, more often, increased transcription rates. For example, a constitutive GST overexpression has been found in resistant strains of *An. gambiae* (Hemingway and Ranson 2000). GST enzymes display specific activity : e.g. the enzyme GST2-2 shows a specific DDT dechlorinase activity in *An. gambiae* (Ortelli et al. 2003; Enayati et al. 2005). Finally, GST may have an indirect role in resistance to PYRs by detoxifying the lipid peroxidation products induced by this class of insecticides (i.e. an antioxidant effect, e.g. in the brown planthopper *Nilaparvata lugens*) (Vontas et al. 2001).

b- Multifunctional monooxygenases

The multifunctional monooxygenases (MFOs) catalysed by the cytochrome P450 enzymes (the terminal oxidases of the system) are found in all aerobic organisms. Cytochrome P450 activates an oxygen atom, which is then inserted in a large variety of

substrates. The oxidation products of MFOs are often unstable and breack down further. These P450 MFOs can oxidise a large variety of substrates and this defense mechanism is implicated in many reactions against a large array of xenobiotics, notably insecticides and toxins.

MFOs are the members of a very large family of enzymes in insects, with an average of about 100 genes in the different insect genomes analysed so far. They display high constitutive levels of expression in strategic tissues (midgut, fat body, Malpighian tubules), and are also inducible by the presence of xenobiotics. They are probably the most frequent metabolic resistance mechanism, although the level of resistance conferred is often low: the resistance ratio is usually around 5.

Cytochrome P450 associated MFOs have been reported as responsible for resistance to PYRs, OPs, CXs, OCs (DDT and CDs) and neonicotinoid insecticides (Table 1), mostly in *Drosophila melanogaster*, with indications that different genes of the cytochrome P450 (CYP) MFO family induce resistance to different classes of insecticides (Scott 1999; Brooke et al. 2001; Daborn et al. 2002; Hemingway et al. 2004; Daborn et al. 2007) . The implication of MFOs in insecticide resistance is usually detected by bioassays including a non-specific inhibitor of some cytochrome P450s, the synergist piperonyl butoxide (PBO). The observation that toxicity is significantly more reduced in a resistant than in a susceptible strain exposed to the insecticide associated with PBO is indicating a role of MFOs. Nevertheless the large diversity of MFOs and the less than perfect specificity of PBO impose further evidences to verify that MFOs have a role in the observed resistance, like for example gene silencing.

Resistance is generally associated with the over-expression of one or several P450 associated MFOs. This over-expression usually results of enhanced transcription rather than gene amplification, although the large diversity of MFOs makes it difficult to pinpoint the exact mechanisms at the origin of the observed resistance. The responsible genes are consequently rarely identified directly, although a certain specificity is reported: recent transcriptome analyses have shown that particular over-expressed CYP genes may have a direct role for resistance to some insecticides (e.g. *Cyp6g1* for resistance to DDT and *Cyp6g2* for resistance to diazon (OP) in *D. melanogaster*) but the results are often conflicting (Daborn et al. 2007). Similarly, using microarray and expression in *Escherichia coli*, the *Cyp6p3* gene has been shown to be involved in the resistance metabolism of permethrin and deltamethrin (PYRs) in *An. gambiae* (Muller et al. 2008). Interestingly, when a link between insectide resistance and particular CYP genes is suggested, most of time these genes belong to the *Cyp6* family (Hemingway et al. 2004). No common mutation has been

identified, but increased transcription might be due to the loss of function in some trans-acting suppressors: e.g. in *D. melanogaster*, the insertion of a retrotransposon in the regulatory area sequence (absent in susceptible but present in all resistant individuals) appears to be responsible for over-transcription of the *Cyp6g1* gene (Daborn et al. 2002). However, despite some knowledge of the genes that are over-expressed, the molecular basis for this over-expression and the final proof of their implication in resistance is most of the time lacking (Hollingworth and Dong 2008; but see Muller et al. 2008).

Finally, MFOs can play a role in activating certain insecticides, this is notably the case of OPs like diazon or malathion; in this case, resistance may be achieved by down-regulating the expression of a particular MFO.

c- Carboxyl-esterases

More than 30 genes coding carboxyl-esterases (COE) are found in insects (see detailed review in Oakeshott et al. 2005; Hollingworth and Dong 2008). Most COEs are serine esterases, i.e. they have a serine residue within a catalytic triad necessary for hydrolysis. COEs bind to an ester group and then break the ester bound by a process of acylation-deacylation. Multiple forms of COEs are found in insects, with broad and overlapping substrate specificities.

The majority of insecticides, including almost all CXs and OPs, most PYRs and some GRs are esters: in most cases, hydrolysis of the ester group leads to reduced toxicity of the insecticide. Consequently, COEs are often implicated in metabolic mechanisms leading to resistance, although the level of resistance conferred is often relatively low (~10x). As for MFOs, the role of COEs in resistance is usually diagnosed by the addition of a synergist, the S,S,S-tributyl phosphorotrithioate or DEF to bioassays. DEF is an inhibitor of the COEs (but also inhibits GSTs): if COEs are responsible of resistance, toxicity is expected to undergo a significantly higher decrease in resistant than in susceptible insects in presence of DEF (Pasteur et al. 1984). COEs have been detected in various species (Table 1) as a mechanism of resistance to OPs and to a lesser extent to PYRs (Hemingway and Ranson 2000; Nauen 2007).

Resistance in mosquitoes of the *Culex* genus is generally caused by an elevated COE protein quantity, up to 80 times the level found in susceptible individuals (Mouchès et al. 1987). This over-expression is usually caused by upregulation or by an increased gene number (amplification) coding for one or two different esterases, named A and B (Mouchès et al. 1986; Rooker et al. 1996; Guillemaud et al. 1997; Vaughan et al. 1997; Callaghan et al.

1998; Paton et al. 2000) . The loci coding for the esterases A and B (*Est-3* and *Est-2*, respectively) are very close (e.g. in the mosquito *Cx. pipiens*, less than 1% recombination, 2 to 6kb, Pasteur et al. 1981) and behave as a single locus named *Ester* (Wirth et al. 1990; Tomita et al. 1996; Guillemaud et al. 1997; Labbé et al. 2005). The number of genes within an amplification of the *Ester* locus can vary greatly, potentially in relation with the intensity of insecticide treatments (Pasteur et al. 1984; Guillemaud et al. 1999; Weill et al. 2000a).

However, qualitative changes may also be responsible for COE-mediated resistance to particular insecticides. For example, resistances specific to malathion (OP) in Anophelinae, *M. domestica* and *Lucilia cuprina* are due to a particular point mutation that induces a faster hydrolysis, with no elevation of the esterase quantity (Claudianos et al. 1999; Hemingway and Ranson 2000; Hemingway et al. 2004; Oakeshott et al. 2005).

Because over-expressed COEs can represent a large percentage of the total protein of the insect (up to 12% of the soluble proteins in some resistant mosquitoes, Fournier et al. 1987), it is difficult to disentangle their sequestration effect (i.e. when the esterase is bound to the insecticide, the insecticide cannot reach its target) from the direct hydrolysis of the insecticide. It depends on the species and the esterase allele: hydrolysis is major in aphid E4 esterase, while in mosquitoes the $Ester^{B1}$ and $Ester^{2}$ allele sequesters rapidly the insecticide and then degrades it slowly (Cuany et al. 1993; Feyereisen 1995; Karunaratne et al. 1995). This large over-expression can also affect pathogen transmission: in the mosquito *Cx. p. quinquefasciatus*, over-expressed COE have been shown to have a negative effect on the parasite burden of the filarial worm *Wucheria bancrofti* (McCarroll et al. 2000; McCarroll and Hemingway 2002).

COE resistance to OPs in *Cx. pipiens* is probably one of the best-studied cases. In this mosquito, several *Ester* alleles confer OP resistance: the $Ester^{1}$ allele results in over-production by transcription up-regulation of the *Est-3* gene (esterase A), whereas $Ester^{B1}$ results from a *Est-2* (esterase B) gene amplification, and $Ester^{2}$ and $Ester^{4}$ from the co-amplification of both A and B genes (Mouchès et al. 1986; Rooker et al. 1996; Guillemaud et al. 1997; Weill et al. 2000a). *Cx. pipiens*' resistance to OPs is monitored since their first application (1969) in Montpellier area, Southern France (Pasteur and Sinègre 1975; Pasteur et al. 1981; Chevillon et al. 1995; Guillemaud et al. 1998; Raymond et al. 2001; Labbé et al. 2009). This more than 40 years monitoring, showed that several *Ester* resistance alleles have been replacing each other: $Ester^{1}$ was the first detected allele in 1972, then $Ester^{4}$ in 1986, and finally $Ester^{2}$ arrived by migration in 1991. These alleles are selected in insecticide-treated areas (i.e. a selective advantage) as they survive better in this environment, but they are costly, i.e. they confer a fitness disadvantage (lower mating

success, lower survival, etc., Berticat et al. 2002a; Berticat et al. 2002b; Berticat et al. 2004; Bourguet et al. 2004; Duron et al. 2006b) in absence of treatment, and are thus selected against in nontreated areas (Lenormand et al. 1998b; Lenormand et al. 1999). Their frequencies follow a clinal shape, which has been recently used to quantify the fitness cost and advantage of the three alleles (Labbé et al. 2009). It has been shown that *Ester*^{4} was favoured over *Ester*^{1} because of a lower cost (selection for a generalist allele), while *Ester*^{2} is replacing the two first alleles thanks to its higher resistance in the current treatment practices, despite its relatively high cost (selection for a specialist allele).

The *Cx. pipiens* example shows that resistance is an evolutive dynamic process, as new mutations can appear that improve the previous adaptation. Several metabolic mechanisms can be present in the same species for resistance to a same class of insecticides, as for example in *Ae. aegypti* (Brengues et al. 2003; Strode et al. 2008) or in *An. gambiae* from Cameroon (Etang et al. 2007) where MFOs, GSTs and COEs contribute to the observed resistance to DDT and PYRs. However, these metabolic resistance mechanisms most often confer relatively low resistance levels, particularly when compared to target site modifications.

2- Target site modification

Resistance by target site modification is due to point mutations in the insecticide target that limits insecticide binding, rather than to a change in expression level. Because most insecticide targets are vital molecules, there is generally only a limited number of mutations in the target able to decrease insecticide affinity without impeding its original function to an unsustainable degree (see detailed review in Hollingworth and Dong 2008). A mutation conferring resistance while partly impairing the target's normal function leads to a fitness cost.

a- GABA receptors

γ-aminobutyric acid (GABA) is a major neurotransmitter in the insect central and peripheral nervous system and in neuromuscular junctions. The GABA receptors are linked to Cl^--gated channels, causing hyperpolarization that blocks the nervous influx. GABA receptors are the target of cyclodienes (CD). CDs are non-competitive inhibitors that bind to a site on the receptor close to the Cl^--gated channel, stabilizing it in an inactive closed state. This induces an over-excitation by removal of the inhibition, and leads to convulsions and death of the insect. GABA receptors have also secondary binding-sites for some PYRs or insecticides of the avermectin family (Hemingway and Ranson 2000).

Resistance to CDs is due to a decreased sensibility to insecticide of the GABA receptor A, through the mutation of a single amino acid in the receptor-coding gene. This gene, called *Rdl* (Resistance to dieldrin, the most used CD), has been first cloned in *D. melanogaster* (ffrench-Constant et al. 1993). It is composed of a 2kb open reading frame encoding one of the GABA receptor sub-units. In all *D. melanogaster* resistant individuals, the *Rdl* locus displays a similar mutation at postion 302 in the channel-lining domain sequence, changing an alanine into a serine (A302S). The role of this mutation in CD resistance was confirmed by directed mutagenesis. The serine residue occupies the insecticide-binding site of the GABA receptor and destabilizes its conformation, which in turn increases the opening time of the Cl^--gated channel and thus limits the nervous influx transmission (review in ffrench-Constant et al. 2000). The resistance allele (Rdl^R) is semi-dominant and can confer some cross-resistance (e.g. between dieldrin and fipronil, ffrench-Constant et al. 2000; Hemingway et al. 2004). Interestingly, the *Rdl* gene is duplicated in the greenbug *Myzus persicae* (Anthony et al. 1998).

Due to an extensive use of CDs before their banning in the 80s, resistance has been selected in several insect species (Table 2), which all display a mutation at the same position (A302S or A302G) (ffrench-Constant et al. 1993; Hemingway et al. 2004). For example, *An. gambiae* (A302G) became resistant to dieldrin everywhere in Africa in the 50s-60s (Chandre et al. 1999a). Whether these mutations are costly depends on species: a fitness cost associated with resistance has been shown both in the lab and in the field for *L. cuprina* (McKenzie 1996) and has been recently suggested for *Cx. pipiens* and *Ae. albopictus* (Tantely et al. 2010), but no cost has been found for *D. melanogaster* (ffrench-Constant et al. 2000).

b- Voltage-dependent sodium channels

Nerve action potentials are transmitted by a wave of depolarization along the neural axone. It is due to the movement of sodium ions (Na^+) accross the axonal membrane through the opening of voltage-dependent sodium channels (Na-channels), and stops when they are inactivated. Na-channels are glycoproteins with a pore for ion transport and can adopt three different states: resting, open or inactivated; the Na^+ ions pass only when they are open (Lund 1984).

Na-channels are the targets of DDT and PYRs. When these insecticides bind to the Na-channels, they slow their closing speed, prolonging the depolarization (Lund 1984; Vais et al. 2001; Soderlund and Knipple 2003). The intensity of the effect is dose-dependent, proportional to the number of Na-channels inactivated (Lund 1984). For PYRs, the

magnitude of the effect depends on the type of insecticide molecules, type I (ex. permethrin) or type II (ex. lambda-cyhalothrin and deltamethrin), which respectively lack or not a cyano group. During action potential, type II PYRs prolonge the sodium flux more than type I, and thus usually display a more intense effect (Vais et al. 2001). At the phenotypic level, Na-channels inactivation results in a rapid knockdown effect (KD), the insect being incapacited for some time, with an eventual recovery or death depending on species and development stages (in mosquitoes, the adults tend to recover, while larvae will drown).

One major mechanism, named knockdown resistance (kdr), is responsible for PYR and DDT resistance, by reducing the receptors sensitivity (binding capacity) to these insecticides and modifying the action potential of the channel (Soderlund and Knipple 2003; Hollingworth and Dong 2008). First discovered in *M. domestica*, this mechanism has been described in many agricultural pests and vectors (Table 2). This resistance mechanism has several consequences: it decreases the irritant and the repellent effect, and either cancels or reduces the KD effect (Chandre et al. 2000).

By extension, the gene encoding the Na-channels has been called *kdr*. By sequencing the Na-channel protein (>2000 amino acids), the two first mutations conferring the kdr phenotype were identified in *M. domestica,* both in the protein second domain. The first one (L1014F) is associated with moderate (10-30x) PYR resistance; the second (M918T, also called *super-kdr*) is always associated with the L1014F to confer a higher resistance (up to 500x) (Williamson et al. 1996). Substitution of the L1014 is found in a large variety of species (L1014F or L1014S, Table 2, and also L1014H in *H. virescens*) and corresponds to the kdr^R alleles (Martinez-Torres et al. 1999; Vais et al. 2001; Soderlund and Knipple 2003; Etang et al. 2009). The phenotype conferred by kdr^R is recessive or semi-recessive (Chandre et al. 2000; Hemingway and Ranson 2000; Corbel et al. 2004), with higher resistance to type I than type II PYRs (Chandre et al. 1999a). However, the various mutations show some specificity, as L1014F confers a high resistance to both DDT and permethrin (PYR), while L1014S confers a lower resistance to permethrin than to DDT (Martinez-Torres et al. 1999; Ranson et al. 2000). Some other mutations (about 30 in total) have also been described in various species, including super-kdr mutations (Vais et al. 2001; Soderlund and Knipple 2003). Some of these mutations are conserved over a large array of organisms, while others are more specific and unique. In *Ae. aegypti*, the kdr phenotype has been observed, but it appears that a codon bias does not allow the appearance of any L1014 mutation (Brengues et al. 2003). Several mutations were observed to be associated with resistance, the V1016I and V1016G mutations being particularly interesting candidates in Latin America and the Carribbean (Saavedra-Rodriguez et al. 2007; Garcia et al. 2009; Kawada et al. 2009;

Marcombe et al. 2009). However the causal effect of these mutations remains to be confirmed.

The role of the L1014F/S mutations (kdr^R) as the sole cause of the kdr phenotype is still discussed (Brooke 2008). kdr^R is clearly associated to PYR and DDT resistance in *Bl. germanica*, *Cx. pipiens*, houseflies, hornflies and some moths (review in Xu et al. 2006). In *An. gambiae*, although metabolic resistance is often present, high resistance to PYR and DDT is most of times associated with a high kdr^R frequency and resistant insects carry at least one kdr^R copy (Ranson et al. 2000; Awolola et al. 2007; Reimer et al. 2008; Dabire et al. 2009; Ramphul et al. 2009). Moreover, kdr^R frequency usually increases when PYRs are used (Stump, 2004 #170, but see Corbel et al. 2004). Similarly in West African *Cx. p. quinquefasciatus*, resistance frequency follows a gradient of treatment intensity (Chandre et al. 1998). A recent study showed that a *Cx. p. quinquefasciatus* strain resistant to PYR contained 87% of kdr^R heterozygotes, a surprising proportion that remained stable despite selection. It appears that alternative splicing and RNA editing could explain the discrepancies between kdr^R frequencies and PYR/DDT resistance level (Xu et al. 2006).

In the field, *An. gambiae* resistance to PYRs through *kdr* can lead to reduced repellent effect and decreased mortality. For example, kdr^R frequency is high in Benin and Côte d'Ivoire while no other PYR resistance mechanism is found: studies have shown strong diminution of vector control with PYR treated bednets in these countries (Kolaczinski et al. 2000; N'Guessan et al. 2007). In contrast, other studies have found that despite the high correlation between *kdr* mutations and PYR resistance, PYR-treated bednets remained somewhat efficient against resistant *An. gambiae* (Casimiro et al. 2007; Brooke 2008). This could be due to the ability of resistant mosquitoes to stay on a treated bednet longer than susceptibles, and thus absorb a high enough quantity of insecticide to be killed (Chandre et al. 2000). For example, in Kenya, the use of PYR-treated bednets increased kdr^R frequency, but had no impact on malaria and mosquito population densities, as both decreased in treated and un-treated villages (Stump et al. 2004). Similarly two studies found that *kdr* alone (i.e. in absence of metabolic resistance) did not reduce bednet efficiency against resistant *An. stephensi*, despite a reduced KD effect (Hodjati and Curtis 1997; Enayati and Hemingway 2006). The issue of the impact of *kdr* resistance on PYR-treated bednet efficiency to control malaria remains thus hotly debated.

Evolution of the kdr phenotype is best described in *An. gambiae* in Africa. The L1014F mutation was first detected in West Africa (Côte d'Ivoire, Burkina Faso) (Martinez-Torres et al. 1998), while only the L1014S mutation was observed soon after in East Africa (Kenya) (Ranson et al. 2000). These mutations appear to have spread from their center of

origin: L1014F is now present everywhere in West and Central Africa, from Senegal to Angola (it is almost fixed in Côte d'Ivoire and Burkina Faso, Dabire et al. 2009), while L1014S is absent from West Africa but present in Central and East Africa (Pinto et al. 2007; Santolamazza et al. 2008; Etang et al. 2009). However, analyses of the non-coding regions of the *kdr* gene suggest that the two alleles occurred several times independently (at least 3 times for L1014F and 2 for L1014S) (Weill et al. 2000b; Pinto et al. 2007; Etang et al. 2009).

c- Acetylcholinesterase

In the cholinergic synapses of invertebrate and vertebrate central nervous system, acetylcholinesterase (AChE) terminates the synaptic transmission by rapidly hydrolysing the neurotransmitter acetylcholine (ACh). AChE is the target of OPs and CXs insecticides, which are competitive inhibitors of ACh with a low turnover: when they bind to AChE, their very low release prevents hydrolysis of the natural substrate. Consequently, ACh remains active in the synapse and the nervous influx is continued, leading to the insect death by tetany (see Massoulié and Bon 1993).

In most insects there are two genes, *ace-1* and *ace-2,* coding for AChE1 and AChE2, respectively. In these species, AChE1 is the main synaptique enzyme, and the physiological role of AChE2 is unknown. Diptera of the Cyclorrapha group or "true" flies (such as *D. melanogaster* and *M. domestica*) possess a single AChE, which is encoded by the *ace-2* gene and is the synaptic enzyme in that case. Phylogenetic analyses have shown that the presence of two *ace* genes is probably the ancestral insect state (Weill et al. 2002; Huchard et al. 2006).

The first molecular studies on an insensitive AChE conferring resistance to OPs and CXs were carried out on *D. melanogaster*. Several mutations were identified, each giving a low resistance when alone, and a higher resistance when in combination (Fournier et al. 1989; Fournier and Mutéro 1994). Similar results were later found with other Diptera that have only the *ace-2* gene (e.g. *M. domestica*, Oakeshott et al. 2005).

In mosquitoes where AChE1 is the synaptique enzyme, the most common resistance mutation (G119S) in the *ace-1* gene is situated just near the catalytic site. In *Cx. pipiens*, G119S occurred at least 3 times independently, once in *Cx. p. pipiens* and twice in *Cx. p. quinquefasciatus* (Weill et al. 2003; Weill et al. 2004; Labbé et al. 2007a). However, two other mutations in *ace-1* have been identified, both close to the active site: (i) F331W has been observed only in *Cx*. *tritaeniorhynchus* (Nabeshima et al. 2004; Alout et al. 2007), (ii) F290V has been observed only in *Cx. p. pipiens* (Alout et al. 2009). The type of mutation is

highly constrained by the codon use: the G119S mutation has never been found in *Ae. aegypti*, *Ae. albopictus* or *Cx*. *tritaeniorhynchus* probably because it requires two mutation steps (Weill et al. 2004).

The *ace* mutations are responsible for a decreased inhibition of the AChE by the insecticides (Alout et al. 2008). There are only few resistance mutations observed in various species (Table 2), suggesting high constraints: those observed in the field are within the active gorge of the enzyme and cause steric problems with bulkier side-chains, while other substitutions (lab-engineered) often result in the inability of enzyme to degrade ACh (Oakeshott et al. 2005). In mosquitoes, these mutations confer a high resistance to OPs and CXs, respectively up to 100x (e.g. chlorpyrifos) and >9000x (e.g. propoxur); OP resistance conferred by *ace* alleles is usually higher than COE metabolic resistance (Raymond et al. 1986; Poirié et al. 1992; Severini et al. 1993).

The evolution of insensitive AChE1 has been studied in depth in the mosquitoes *Cx. pipiens* and *An. gambiae*. In *Cx. pipiens*, it was first detected in Southern France in 1978, nine years after the beginning of OP treatments (Raymond et al. 1986). The gene coding for this G119S mutated AChE1 (*ace-1*^{R}) rapidly spread in treated natural populations. However, its frequency remained low in adjacent untreated areas connected by migration, indicating a fitness cost associated with *ace-1*^{R} (Lenormand et al. 1999). The >60% reduction of AChE1 activity in G119S resistant mosquitoes (Bourguet et al. 1997) may probably explain, at least partially, this cost, which is expressed phenotypically through various developmental and behavioral problems in individuals carrying *ace-1*^{R} (Berticat et al. 2002a; Berticat et al. 2004; Bourguet et al. 2004; Duron et al. 2006b). Similarly, the F290V mutation is probably associated with a fitness cost, although it does not appear to be due to activity reduction (Alout et al. 2009). Recently, several independant duplications of the *ace-1* gene, putting a susceptible and a resistant copy in tandem (*ace-1*^{D}), have been identified in *Cx. p. pipiens* and *Cx. p. quinquefasciatus* (Table 2 Lenormand et al. 1998a; Labbé et al. 2007a). These alleles are thought to be selected because they reduce the cost of the *ace-1*^{R} allele, although not always successfully (Labbé et al. 2007b). Several other duplications have been observed recently in the Mediterranean area, with a F290V copy instead of a G119S copy (Alout et al. 2009). In *An. gambiae*, the recent occurrence of *ace-1*^{R} has been detected in West Africa, probably spreading from Côte d'Ivoire to Benin (Weill et al. 2003; Djogbénou et al. 2008). A duplication carrying a G119S copy has also been found, and appears to follow the same trajectory as in *Cx. pipiens* (Djogbénou et al. 2009).

3- Other resistances

a- Growth regulators

Juvenoids mimic juvenile hormone (JH) and disrupt the target insect development. Few resistances have been detected but they have been reported in various species (review in Hollingworth and Dong 2008). High resistance to methoprene has been described in the mosquito *Ochlerotatus nigromaculis* in California, potentially through target site mutation (Cornel et al. 2002), while a 7.7x resistance to the same insecticide has been reported in *Cx. p. pipiens* from New York (Paul et al. 2005).

b- Toxin receptors

Bt toxins have a complex mode of action not clearly understood. Moreover, Bt resistance is rare in the field (Griffitts et al. 2001; Mittal 2003). It has been mainly studied in the agricultural pest moth *Plutella xylostella*: in a lab strain from Hawaii, a single mutation confers resistance to at least 4 toxins by decreased binding on a common receptor, but it is not the only responsible, as strains from various places in the World show complementation which indicates the epistasis between several genes (Tabashnik et al. 1997a; Tabashnik et al. 1997b). In another pest moth, *Heliothis virescens*, QTL mapping showed that Bt resistance in a lab strain is probably due to a modification of the cadherin gene BtR-4 (Gahan et al. 2001). Presently, the only report of field resistance for a vector is a 33x resistance to Bti (Bt var. *israelensis*, the only Bt variety active on mosquitoes) detected in a natural population of *Cx. p. pipiens* from New York. However, the mechanism of this resistance is still unknown (Paul et al. 2005).

For Bs toxins, resistance has been described essentially in mosquitoes of the *Cx. pipiens* complex. It developed very rapidly within the first year of treatment in India (10 to 155x resistance Mittal 2003) and in Tunisia (*Sp-T* gene, > 5000x resistance, Nielsen-Leroux et al. 2002). Similarly, control using Bs toxins started in the early 90s in Southern France and first failure was reported in 1994 in Port-Louis (near Marseille). This resistance (> 10000x) was due to a recessive sex-linked gene, named *sp-1*. In 1996, Bs resistance was reported close to the Spain border (Perpignan, 200 km away from Port St Louis); it was due to a second gene, *sp-2*, which was recessive and sex-linked (Chevillon et al. 2001). Now Bs resistance has been observed worldwide in the *Cx. pipiens* complex (Mittal 2003). Two of the genes identified ($sp\text{-}2^R$ and a gene selected in a laboratory strain from California (Nielsen-Leroux et al. 1995) change the toxin receptor binding properties and were found to be due to "stop" mutations or mobile element insertion in the toxin receptor (Darboux et al. 2002; Darboux et al. 2007), while the effect of the others is unknown (Nielsen-Leroux et al. 2002). Bs resistance has also been selected in the lab in *An. stephensi* (Mittal 2003).

c- Other

Most studies focus on small set of genes. Recently developed genomic and transcriptomic techniques can however access mechanisms that had previously proven intractable. They allowed the deeper description of known resistance gene families and helped find new candidate genes (Daborn et al. 2002; Oakeshott et al. 2003; Vontas et al. 2005). For example in *An. funestus,* genomic positional cloning identified a major QTL including a cluster of 11 P450 MFOs for PYR resistance, two of them being overexpressed in a resistant strain (Wondji et al. 2009). Detox chips were designed (including P450 MFOs, GSTs, COEs, redox genes and partners of P450 in oxidative metabolic complex) for *An. gambiae* and *Ae. aegypti* (David et al. 2005; Strode et al. 2008). In *An. gambiae*, several GST and MFO genes showed overexpression in mosquitoes resistant to PYR and DDT, but also other candidate genes (COEs, transferases, Aldehyde dehydrogenase, NADH-cytochrome *b* reductase, NADH dehydrogenase, NADH-ubiquinone oxidoreductase, nitrilase thioredoxin peroxidase and cuticular genes, Vontas et al. 2005; Awolola et al. 2009). Genomic analyses in *Ae. aegypti* show an expansion of certain gene families (ex. MFO and COE) or increased alternative splicing (ex. GST) (Strode et al. 2008), and identified new candidates for resistance (aldehyde oxidase and xanthine deshydrogenase, Waterhouse et al. 2008). However, in most cases the causal role of the candidates remains to be formally validated.

4- Resistance generalities

Some general patterns can be identified from the variety of mechanisms observed for insecticide resistance.

A first characteristic is that resistance evolves rapidly, with fast selective sweeps in field populations. Although clear evidences are scarce, most of times resistance seems absent before insecticide treatments (Andreev et al. 1999), but there are some contradictory examples like malathion resistance in *L. cuprina* (Hartley et al. 2006) or PYR resistant *kdr* L1014S in Kenya in *An. gambiae* (Stump et al. 2004).

Secondly, resistance appears locally but can spread very rapidly (Brogdon and McAllister 1998). A single resistance gene may have a large distribution (ffrench-Constant et al. 2000; ffrench-Constant et al. 2004; Etang et al. 2009), e.g. the Worldwide migration of $Ester^2$ in *Cx. pipiens* (Raymond et al. 2001). Alternatively, other resistance genes have multiple origins: $ace\text{-}1^R$ mutations in *Cx. pipiens* (G119S, Weill et al. 2003; F290V, Alout et

al. 2009) or *kdr* mutations in *Ae. aegypti* (Saavedra-Rodriguez et al. 2007; Garcia et al. 2009; Kawada et al. 2009; Marcombe et al. 2009).

It also seems that resistance evolution is quite constrained. For target site resistance, most mutations are costly and compromise the performance of the native protein function, so that codon use may prevent resistance apparition (Brengues et al. 2003; Weill et al. 2004).

Another issue is the cross-resistance between different insecticides (Fig. 2). Cross-resistances between families of insecticides are associated with the sharing of target sites. For example, kdr^R causes cross-resistance between DDT and PYRs in *An. gambiae* (Chandre et al. 1999b), and $ace\text{-}1^R$ between OPs and CXs (Alout et al. 2008). In contrast, a particular metabolic resistance gene usually does not confer cross-resistance between insecticide families. However, different genes belonging to a same metabolic family can cause resistance to several insecticides of this family ("gene family cross-resistance"): for example, different COE and MFO genes cause resistance to DDT, others to PYRs, OPs and CXs in Anophelines (Brogdon and McAllister 1998; Brooke et al. 2001; Etang et al. 2007), and different MFO genes are responsible for resistance to DDT, neocotinoïds and GRs in *D. melanogaster* (Daborn et al. 2002). The consequences of these cross-resistances are to reduce the alternative insecticides available, thereby gravely endangering vector control.

Insert Figure 2 here

Finally despite advances, a full analysis of resistance remains challenging due to interactions and pleiotropy when several resistance mechanisms and/or resistance genes are present in the same insect (Hollingworth and Dong 2008). This is unfortunately a common case: for example almost all known resistance mechanisms are present in the Anopheline species from Sri Lanka (Perera et al. 2008), Latin America (Penilla et al. 1998), and from various parts of Africa (Vulule et al. 1999; Diabate et al. 2002; Hougard et al. 2003; Stump et al. 2004; East Africa Casimiro et al. 2006b; Enayati and Hemingway 2006; Brooke, 2001 #93, Central Africa Etang et al. 2006; Corbel et al. 2007; Dabiré et al. 2008; Okoye et al. 2008; Awolola et al. 2009; Ndjemai et al. 2009; Ramphul et al. 2009; Matambo, 2007 #6, West Africa Yadouleton et al. 2009). Multiple resistances with multiple mechanisms are also observed in *Cx p. quinquefasciatus* (Chandre et al. 1998; Kasai et al. 1998; Corbel et al. 2007; Hardstone et al. 2007), *Ae. aegypti* (Brengues et al. 2003), sandflies (Alexander and Maroli 2003; Surendran et al. 2005) and head lice (Thomas et al. 2006). Interactions between resistance loci have been studied in houseflies or mosquitoes, and most of them appear to be synergic. Such synergies have been observed between COE and *ace-1* for OP resistance and between *kdr* and P450 MFO for PYR resistance in *Cx. pipiens* (Raymond et al. 1989; Hardstone and Scott 2009), between repellents (DEET) and CXs in *Ae. aegypti*

(Pennetier et al. 2005; Bonnet et al. 2009) or between PYR resistance and susceptibility to fungus applications in three *Anopheles* species (Farenhorst et al. 2009). Moreover, these interactions may vary with environmental conditions (positive synergism for resistance in treated area but negative synergism for cost in nontreated areas) or with the genetic background of the insect (Hardstone and Scott 2009). For example, the presence of kdr^R decreases the cost of $ace\text{-}1^R$ in *Cx. pipiens* (Berticat et al. 2008).

In conclusion, any disease control strategy should take into account these various aspects of resistance as they can greatly impact its success (vector control failures) and may have a direct effect on pathogens transmission (McCarroll et al. 2000; McCarroll and Hemingway 2002; Vontas et al. 2004). The various strategies for vector control and how they deal with resistance will be the subject of the next part.

Part 3: Treatment practices and resistance management strategies

As mentioned before, disease control relies greatly on vector control. Vector control strategies are designed to reduce adult vector populations (and thus pathogen transmission), but are faced with many constraints. They must be environmentally, socially and politically acceptable (i.e. they must prevent any adverse effect on the environment, non-target species and humans) and must be economically realistic both on the short and long terms. To be successful, knowledge of the life history and ecology of vector species is critical (breeding sites, life cycle, preferred blood source, etc., Alexander and Maroli 2003; WHO 2006; Walker and Lynch 2007). Another key of success for these strategies is to prevent the development of insecticide resistance, by monitoring resistance genes and adapting the practices. Ideally, vector control strategies should be integrated into national health and community systems to be sustainable (Kay and Vu 2005; Ooi et al. 2006; Beier et al. 2008; Morrison et al. 2008).

In this part, we will review the current treatment practicies, their limits and constraints and how vector resistance is accounted for.

1- Treatment practices

a- Adult spray

Large scale adult spraying, where the insecticide is pulverised in the air, presents a low efficacity for a high operational cost, and is consequently recommended only in cases of

severe epidemic. Preferred insecticides for this type of treatments are OPs and PYRs (WHO 2006). They are used locally to control tsetse flies (Welburn et al. 2006) and *Ae. aegypti* for dengue control (Morrison et al. 2008; Luz et al. 2009). They are also used against *Anopheles* species, domestic flies, sandflies, midges (*Simulium* sp.) and fleas (Walker and Lynch 2007).

b- Larvicidal treatment

Other common control procedures are larvicidal treatments. Their efficacy is maximal when larvae are restricted to breeding sites accessible and limited in size and numbers. Various insecticides are used (oils, Bti, GRs, CX or OPs), mostly against *Aedes* and *Culex* mosquito species, but also against Anophelines, *Simulium* flies (midges), ceratopogonidae and Phlebotominae (see details in WHO 2006; Walker and Lynch 2007).

c- Indoor residual spraying (IRS)

IRS (on walls) has long been the preferred vector control, because of its simplicity. The first insecticide used (and still used in case of high malaria prevalence) was DDT. It has been presently replaced by PYRs and to a lesser extent by CXs, OPs (WHO 2006). PYRs are preferred because of their excellent contact action, their rapid KD effect (knockdown effect) and their safe use (low toxicity on mammalian). IRS is used against mosquitoes, houseflies, sandflies and tsetse flies (*Glossina sp.*) (Walker and Lynch 2007). It has contributed to eradication of malaria in several countries and is still an important tool for vector control. It is very efficient if the target vector populations retain their susceptibility to insecticide exposure and if the insecticides are used reliably, efficiently and sufficiently in terms of coverage (Okoye et al. 2008; Read et al. 2009). New insecticides as fungi have a high potential for IRS, because they infect through the insects legs when they are resting (Scholte et al. 2003; Blanford et al. 2005; Scholte et al. 2005; Scholte et al. 2006).

d- Insecticide treated materials

The use of insecticide-impregnated fabrics (bed nets, curtains, sheeting, hammocks) may also produce significant levels of protection against disease vectors, if appropriatly used with sufficient coverage on susceptible vector populations (Okoye et al. 2008). The use of insecticide-treated nets (ITN) impregnated with PYRs is the most common protective measure against malaria vectors (WHO 2006): they act as physical barriers, which combines with the repellent and killing effects of PYRs. ITN are always preferred to IRS by local users (Curtis et al. 1998). They can be highly cost-effective (Enayati and Hemingway 2006), and have a large effect if used appropriatly, by reducing the vectors and pathogen prevalence in populations (in these cases people who do not use a net are also protected (Stump et al. 2004). The problem is that ITN are often torn or poorly fitted (Roberts and Andre 1994; Pages et al. 2007). The necessity to regularly re-impregnate the nets with insecticide is a

major constraint: washing decreases their efficacy by removing the insecticides, so that their effective usable life is of 2-3 years in field conditions, after what they are dirty and holed (Curtis et al. 1998; Erlanger et al. 2004). In consequence, proper use of ITNs is often highly correlated to the socio-economic status (Fillinger et al. 2008; Goesch et al. 2008). In response to these constraints, long lasting impregnated nets (LLIN) have been conceived, for which there is no need for retreatment during five years. They showed good efficacy in rural Côte d'Ivoire where they were first tested, although some appear better than others. In particular, washing induces no significant decrease of their killing efficacy by contact (Kilian et al. 2008), although their repellent effect disappears quickly (N'Guessan et al. 2001; Dabiré et al. 2006; Kilian et al. 2008). Finally, new impregnated fabrics are being developed, e.g. plastic sheetings to cover ceilings or walls, or as tents to control malaria in refugee's camps (Graham et al. 2002; Diabate et al. 2006; Djenontin et al. 2009).

If ITNs have shown good efficacy against malaria vectors (Anophelines) and various flies (Walker and Lynch 2007), they have little impact on some species as the dengue vector *Ae. aegypti* which bites during the day, or sandflies which are active at sunset, before people are inside a net (Reiter 2001; Alexander and Maroli 2003). Moreover, ITNs are often perceived as inefficient by users, as, despite the protection they confer against *Anopheles* mosquitoes, they often fail at preventing bitting by other mosquito species as *Cx. p. quinquefasciatus*, which is more resistant and bites during the day (Fillinger et al. 2008).

e- Other treatments

IRS and ITNs are the most common means of vector control. They usually work well in countries with a developed health system (N'Guessan et al. 2007). In Tanzania both ITNs and IRS were efficient at decreasing vector densities and prevalence of *Plasmodium* in mosquitoes; however, no decrease in the cases of malaria was observed, which may show the limits of such strategies (Curtis et al. 1998).

Other methods are available to decrease vector populations (WHO 2006; Pages et al. 2007):

- environment management: this is probably the most efficient of all and has been used for centuries. It consists in modifying the habitat by eliminating potential breeding sites of vectors. These modifications can be permanent (drainage, land levelling and filling) or temporary (vegetation modification, intermittent flushing or irrigation, changing water salinity, using polystyrene beads). Modifying the habitat may also have secondary positive effects, for example sanitation, increase of agriculture lands and reduction of water-borne diseases. However, two problems limit these applications: first, environment modification can potentially be problematic for the ecology of non-target organisms, and second, these modifications, particularly the permanent ones, are often extremely costly. Nevertheless,

such a cheap procedure as vegetation clearing is very efficient against *Glossina* flies, and hygiene can greatly reduce domestic flies-borne diseases (Walker and Lynch 2007).

- biological control: one method of biological control is to use baits like ovitraps, which are cheap and efficient means of control for *Glossina* and domestic flies (Walker and Lynch 2007) and are frequently used to control *Ae. aegypti* and dengue (Morrison et al. 2008). Another method consists in rendering the potential breeding sites unsuitable for vectors with an aquatic larval phase by adding some predators (copepods or fishes). It has shown great success for controlling dengue and *Ae. aegypti* populations in Vietnam. Local copepods (*Mesocyclops sp.*) were identified in the field, reproduced and then shared at the community level. They were put into water storage which led to dengue disappearance in treated towns (while it was still present in close untreated areas) (Kay and Vu 2005). However, although also relatively efficient, the use of introduced fishes (*Gambusia sp.*) at a World scale is now criticized for its impact on local ecology (Walker and Lynch 2007).

- sterile males and genetically modified organisms (GMOs) releases: these methods may be used to carry out two objectives: (1) decreasing vector populations by massive introduction of sterile males or of GMOs with a dominant gene killing females (Alphey et al. 2007); when crossed with field females, they will produce no offspring or only male offsprings; (2) introducing genes conferring resistance to the pathogens in the vector natural populations (review in Sinkins and Gould 2006). These methods require a fitness advantage for the released insects, which could be provided by various gene-drives: transposable elements, homing endonucleases genes, meiotic drive, or endosymbiotic bacteria such as *Wolbachia* (Bourtzis and Robinson 2003; Sinkins and Gould 2006; Engelstadter and Telschow 2009). Release of *Culex pipiens* males incompatible with field females due to *Wolbachia* have been attempted in the early 1970 in France and India (Laven 1967; Curtis and Adak 1974; Curtis 1976; Curtis et al. 1982) but was short lasting, a fact that is not surprinsing since we know today that these endosymbionts have an extremely high genetic variability (Duron et al. 2006a; Duron et al. 2007). The only satisfactory results concern the American screwworm *Cochliomyia hominivorax*, a cattle parasite, which is controlled by releasing every year several hundred thousand of irradiated sterile males and has been eradicated from the USA, and the control of tse-tse flies and trypanosomiasis in Zanzibar, again using release of sterile males (review in Bowman 2006).

There is thus an array of methods available to control vector populations and, through these, the diseases they carry. Some are presently available, and others (sterile male and GMO releases) remain to be worked out, tested and validated. For maximising the chances of success of available methods, all authors agree that they should be implemented in

conjunction. For example, tsetse flies are controled by modifying the habitat (vegetation clearing), spraying insecticides (PYRs) locally and trapping adults (review in Welburn et al. 2006). They also emphasize the necessity of cohesion between the various actors of vector control (scientists, operationals, managers, politics and local populations) and of long-term plannings (Beier et al. 2008; Morrison et al. 2008). For example, apart from three examples (Singapore, Cuba and Vietnam), lack of cohesion between these different actors resulted in a global disaster in broad-scale dengue vector control, with an increase in dengue cases since the 60s (Kay and Vu 2005).

2- Impact of agriculture

Another pitfall awaiting control programs is the impact of agriculture. Urban agriculture is developing in many large cities across Africa, where it is necessary to prevent food shortage and to provide a balanced diet (Akogbéto et al. 2006; Yadouleton et al. 2009). However, it creates new breeding sites for vectors as mosquitoes or livestock parasites: e.g. in Accra (Ghana), urban agriculture development led to an increase in both quantities of vectors and malaria cases (Klinkenberg et al. 2008). Moreover, most agricultural pesticides are toxic for disease vectors: more than 90% of all insecticides produced have indeed been devised and used for agriculture, vector control being of very low rentability for chemical firms. When vectors are exposed to these pesticides, they can potentially develop a resistance, threatening the success of current and later control attemps (Georghiou 1990; Roberts and Andre 1994; Brogdon and McAllister 1998; Boyer et al. 2006; Poupardin et al. 2008). Several examples have been suggested in the litterature:

i) in SriLanka, CX resistance has been detected in *An. subpictus*, *An. nigerrimus* and *An. peditaniatus*, while these insecticides are only used for agriculture (Perera et al. 2008), i.e. resistance is present before insecticide use for public health. Similarly, a high frequency of the Rdl^R allele (resistance to OCs) has been recently found in La Réunion Island in *Cx. p. quinquefasciatus* and *Ae. albopictus*, while this insecticide has never been used for public health (Tantely et al. 2010).

ii) an other possible impact is the higher vector resistance in agricultural areas, as in Benin where *An. gambiae* resistance is present in urban vegetable areas where PYRs, OPs and OCs are used as agropesticides (Corbel et al. 2007; Yadouleton et al. 2009). This could result in malaria outbreaks in cities (Pages et al. 2007).

iii) several studies show a correlation between the quantities of insecticide for agriculture and the level of resistance. In Mexico, a reduction of *An. albimanus* insecticide resistance was observed after decrease in insecticide use for agriculture (Penilla et al. 1998). In Burkina Faso, a change from pure PYRs to use of a mixture of OPs and PYRs to control

H. armigera in cotton fields in the late 90's is probably the cause OP resistance increase in *An. gambiae* (Dabiré et al. 2008). Similarly, intensification of cotton cultivation and consecutive insecticide use was associated with an increased insecticide resistance in both *An. gambiae* and *An. arabiensis* (Dabire et al. 2009). Finally in Spain, the end of OP use in public health did not decrease OP resistance frequency in *Cx. p. pipiens* (despite its fitness cost) due to the large use of OPs in agriculture close to breeding sites (Eritja and Chevillon 1999).

iv) the impact of agropesticides on vector resistance can also be reveald by fluctuations of resistance frequency with period of crops spraying, as in Burkina Faso where PYR treatment intensification during cotton growth is correlated to an increase of *An. gambiae* resistance to these insecticides (Diabate et al. 2002).

Effects of agriculture and forestry on resistance spread have been reported also in Cameroon for *An. gambiae* (Ndjemai et al. 2009), and in China for *Cx. p. pipiens*, *An. siniensis*, *Ae. aegypti* and *Ae. albopictus* (Cui et al. 2006). However, a specific public health use of insecticides can also select for resistance in non-targeted but potential desease vector organisms: in Sri Lanka, CX and OP resistance in sand flies is believed to be due to their use in agriculture and for mosquito control, respectively (Surendran et al. 2005), and in Benin, *Cx. p. quinquefasciatus* may have become resistant to PYRs due to *An. gambiae* treatment for public health (Corbel et al. 2007).

3- Sustainable resistance management

All treatment practices tend to select for insecticide resistance, a selection that is reinforced by other pesticide uses (agriculture, forestry, etc.). In malaria control for example, as the use of IRS and ITN is scaling up, so will the selection pressure for insecticide resistance (Farenhorst et al. 2009). Moreover, due to these resistance mechanisms and to environmental concerns, the number of insecticide molecules available for vector control is decreasing and no new molecule is expected soon (Nauen 2007; Kelly-Hope et al. 2008).

Consequently, a large international effort is required for devising new and sustainable strategies to manage resistance and prolonging the lifespan of the few available insecticides. Resistance management requires deep practice changes (Coleman and Hemingway 2007; Hollingworth and Dong 2008; Whalon et al. 2008), away from the common practice of using an insecticide until resistance becomes a limiting factor, which rapidly erodes the number of suitable insecticides (Penilla et al. 1998). A striking example is *An. gambiae* control: in the 50s-60s it became resistant to dieldrin (OC) in all Africa, leading to a shift to the mass use of

PYRs in the late 70s, in turn leading to wildspread PYR and DDT resistance (Chandre et al. 1999a).

Once insecticide resistance is established in a population, there is indeed a real danger of re-emergence of vector-borne diseases that had been presumed under control, even if in some instances resistance does not seem to compromise disease control on the short term (Brogdon and McAllister 1998; Asidi et al. 2005). Therefore, focusing on surveillance wherever possible is essential in order to react proactively once a regional population manifests shift in its susceptibility towards a class of insecticides used in public health (Nauen 2007). Resistance surveillance means i) providing baseline data for program planning and pesticide selection before the start of control operations; ii) detecting resistance at an early stage, and iii) continuously monitoring the effect of these resistance mechanisms on vector control strategies, so that timely management can be implemented (Brogdon and McAllister 1998).

a- Resistance management strategies

Resistance management strategies' goal is to prevent and delay the spread of resistance while maintaining a good control of vector populations (WHO 2006; Nauen 2007). The susceptibility of vectors and pests should be considered a valuable resource that must be preserved as long as possible. It is critical that the strategies of resistance management must be implemented preventively to preserve the efficacy of the few insecticides available for public health purposes (WHO 2006).

Different strategies can be implemented (reviews in Roberts and Andre 1994; Coleman and Hemingway 2007; Hollingworth and Dong 2008; Whalon et al. 2008; Hardstone and Scott 2009):

i) it is possible to vary the dose or frequency of pesticide applications, but increasing the dose of insecticides should be done with great caution, as it can increase the speed of resistance spread. However, in early stages, i.e. when resistance genes are mainly at the heterozygote state, it can possibly delay resistance spread if it is recessive. For example, high dose has been recommended for PYR-based *An. gambiae* control when *kdr* is at low frequency, because this gene is partially recessive (Corbel et al. 2004).

ii) insecticide application may also be narrowly focused on a small area or a short time period (e.g. only when endemic vector-borne diseases are present). For example, the onchocerciasis control program in West African began in 1975, with OP-based larviciding against the black fly (*Simulium damnosum* complex). To manage OP resistance, they are

only used at the beginning of rivers' high waters, to prevent the pic of black fly; other insecticides (PYRs and Bti) are used at other periods (Roberts and Andre 1994).

iii) such alternative use of various pesticides is probably the most efficient way of managing resistance (Nauen 2007). Several pesticides can be used in mixtures (all together), in mosaics (different insecticides in different sites), in sequences (different insecticides at different times) or in rotations (different insecticides both at different times and sites) (Roberts and Andre 1994; Hemingway and Ranson 2000). However, due to cross-resistance (Fig. 2), it requires using insecticides with different modes of action, rather than merely alternating members of one chemical class or different chemical classes that address the same target site (Nauen 2007). For example, for *An. gambiae* and *Cx. p. quinquefasciatus* control in highly PYR resistant populations of West and Equatorial Africa, successful trials have been implemented to use OPs or CXs as alternative to PYRs for ITN impregnation, in mixtures or mosaics (Kolaczinski et al. 2000; Hougard et al. 2003; Asidi et al. 2005; Sharp et al. 2007a; Oxborough et al. 2008).

All these resistance management strategies mainly rely on the fitness cost often associated with resistance alleles. Each resistance allele indeed corresponds to a recent adaptation, and is thus often associated with a diminution of the fitness, i.e. reduced reproductive ability in absence of insecticide (cost). This has been shown for example for *Cx. pipiens Ester* and *ace-1* OP resistance alleles (Lenormand et al. 1999; Berticat et al. 2002a; Berticat et al. 2004; Bourguet et al. 2004; Duron et al. 2006b), and for PYR resistance in *Ae. aegypti* (Kumar et al. 2009). Theoretical expectations are that costly resistance should rapidly disappear when insecticides cease to be used, natural selection favouring susceptible insects (Lenormand and Raymond 1998; Eritja and Chevillon 1999). However, as this adaptation evolves rapidly, new mutations that reduce or suppress the fitness cost have also been described (McKenzie 1996; Labbé et al. 2007a; ex: duplications of the ace-1 gene in the *Cx. pipiens* and *An. gambiae* complexes, Alout et al. 2009; Djogbénou et al. 2009; , or allele replacement and epistasy, Labbé et al. 2009), and may dramatically endanger resistance management and disease-vector control.

b- Examples

Resistance management is often planned using mathematical models, which can be very useful tools (e.g. Lenormand and Raymond 1998; Luz et al. 2009). In the field, however, proper detection and monitoring of resistance in vector populations are essential components of any resistance management program (Roberts and Andre 1994) and these field studies are often missing (Penilla et al. 1998; Hemingway and Ranson 2000). Some examples are

available, which show the benefits of evidence-based vector control with insecticide resistance management (Coleman and Hemingway 2007).

In Singapore, a sustainable control of dengue through *Ae. aegypti* control has been implemented successfully for 35 years (review in Ooi et al. 2006). It is based on entomological surveillance and reduction of *Aedes* larval habitat availability (no risk of resistance), mainly through environmental measures that require the implication of the population. This experience shows that public education and law, countinuous entomological surveillance (rather than emergency reactions) and a regional level collaboration between several countries are required for success.

In Vietnam, successful elimination of dengue and *Ae. aegypti* was achieved (review in Kay and Vu 2005). It relied on four key elements for its success: the continuous evaluation and quantification of *Ae. aegypti* larvae in the breeding sites, the use of local predatory copepods (cheap, no risk of resistance), the implication of the local community and the education of the population.

In Dar-es-salaam (Tanzania), a community level larviciding was established to control *An. gambiae*-vectored malaria, and *Aedes* and *Culex* biting nuisance. They used Bti and Bs, as alternative to PYRs for which resistance was present. It resulted in a 96% reduction of *An. gambiae* populations, 40% reduction of *P. falciparum* prevalence and 31% reduction of malaria cases, but was less successful with the other Culicidae (*Culex* and *Aedes*) nuisance. This strategy thus showed good potential, but was costly and evidenced the need for community implication and experienced actors (Fillinger et al. 2008).

Constant monitoring allows early detection of insecticide resistance and changes in policies. It prevented program failures in at least two initiatives: Bioko Island malaria control project and Lubombo spatial development initiative.
In Bioko Island (Equatorial Guinea), PYRs were used for *An. gambiae* control. The continuous monitoring kdr resistance showed an increase from 50% in 2003 to 85% in 2005. Consequently, the withdrawal of PYRs, replaced by CXs prevented *An. gambiae* and malaria control failure (Sharp et al. 2007a).
In Mozambic, DDT was used to control Anopheline vectors from 1946 until 1988 when it changed for PYRs. A systematic survey of *An. funestus* and *An. arabiensis* was engaged in 1999 by the Lubombo spatial development initiative (Mozambique, Swaziland and South Africa). It showed a high level of PYR resistance in these mosquitoes and led to a policy change: CXs replaced PYRs (no CX resistance in 2000). In 2003, high *ace-1*^{R} frequency was detected, so that DDT was reintroduced in 2006 to control CX resistance and for cost problems; in 2006, PYR resistance in *An. arabiensis* was no longer detectable (Casimiro et

al. 2006a; Casimiro et al. 2006b). Overall, resistance baseline data and monitoring allowed effective adaptation of policies keeping malaria under control (it decreased from 88% in 2000 to 33% in 2005, Sharp et al. 2007b)

Conclusion

The natural history of mosquito-borne diseases is complex, and the interplay of climate, ecology, vector biology, and many other factors defies simplistic analyses. The recent resurgence of many of these diseases is a major cause for concern. Its principal determinants are politics, economics and human activities (rather than climate change). In order to control these diseases and ameliorate the socio-economic burden they cause in developing countries, vector control remains a powerful and accessible tool. However, it is urgently required to change the treatment strategies to manage the development of insecticide resistance. This includes using alternative tools to insecticides for vector control, preserving the remaining insecticides by carefully planning their use to minimize resistance selection, and finally establishing continuous survey of resistance at a local scale by implicating the local population, a difficult but essential task to set goals and evaluate success. Several survey sites in different conditions are required for sentinel purposes, together with some baseline information, to rapidly detect resistance, identify the mechanisms and change the policies adequately (Kelly-Hope et al. 2008). In order to achieve this survey, basic tools like bioassays remains most powerful, and should always be a preliminary step before more complex and more costly analyses. Clearly, the greatest challenge for successful vector and disease control is the coordination of the different actors (chemical industries, researchers, politics, control agencies and local populations), which do not have the same agendas, motivations or economical interests.

Besides its implications in public health and development, insecticide resistance remains a powerful evolutionary biology model to study the contemporary adaptation of organisms to a changing environment. It indeed allows a complete and integrative study, from the molecular mechanisms to the fitness consequences at the individual level and their impacts on insect population dynamics and interactions with pathogens. Moreover, it is for once pleasant to constat that these rather fundamental approaches of evolutionary biology may have a direct impact in the society and help design new strategies for the successful control of some of the most threathening human diseases (Michalakis and Renaud 2009).

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Fig. 1: Mains classes of insecticides and their respective World scale market share (modified from Nauen 2006).

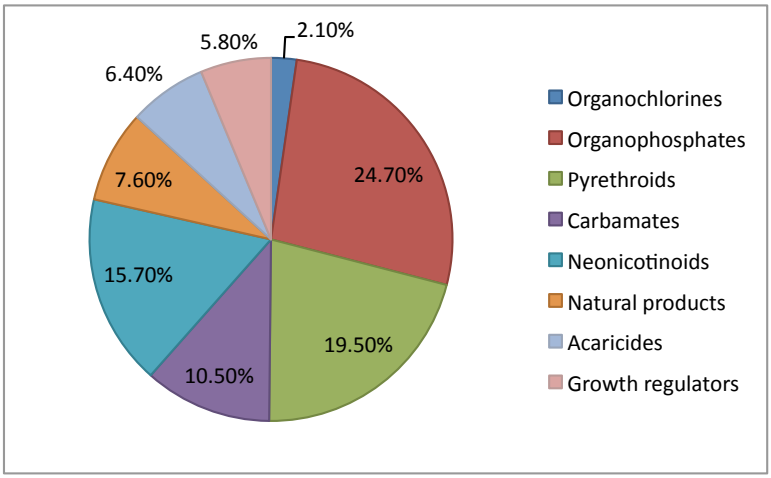

Fig. 2: Cross-resistance between main insecticide classes (modified from Brogdon and McAllister 1998). Metabolic resistances are indicated by continuous lines, while resistances by target site modification are represented by interrupted lines (*Nota*: no resistance has been yet indentified for fungi).

Resistance mechanisms (in italic): COE: carboxyl-esterases, MFO: multifunctionnal oxidases, GST: glutathione S-transferases, $AChE^R$: resistant acetylcholinesterase, kdr^R: knock-down resistance allele, Rdl^R: resistance-to-dieldrin allele.

Insecticide classes (in bold): CX: carbamates, OP: organophosphates, PYR: pyrethroids, OC: organochlorins, Nn: neonicotinoids, GR: growth regulators, CD: cyclodienes, Bti and Bs: bacterial toxins.

Note that, while target mutations confer cross-resistance to insecticides of different classes (interrupted lines), detoxifying enzymes give only "insecticide family cross-resistance" (i.e. a single gene does not confer cross resistance between classes of insecticides, but different genes of the same family can provide resistance to different insecticide classes; continuous lines).

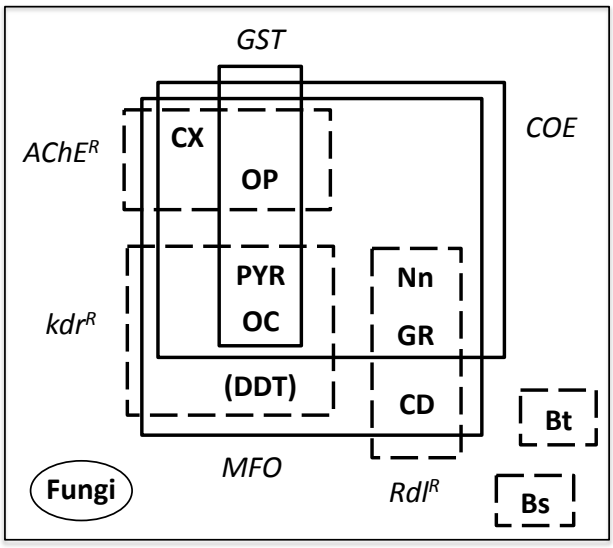

Table 1: Metabolic resistance mechanisms. Based on COE (carboxyl-esterases), MFO (multifunctionnal oxidases) and GST (glutathione S-transferases) for resistance to organophosphates (OP), organochlorids (OC), pyrethroids (PYR) and carbamates (CX) in various species.

Nota: *Cx. pipiens* is a species complex grouping *Cx. p. pipiens*, *Cx. p. quinquefasciatus* and *Cx. p. pallens*.

Gene family	Insecticides	Species
MFO	OP	*M. domestica* (Scott 1999), *D. melanogaster* (Daborn et al. 2007) *An. gambiae* (Muller et al. 2008), *Cx. pipiens* (Hardstone et al. 2007) *Ae. aegypti* (Marcombe et al. 2009)
	CX	*C. pipiens* (Shrivast et al. 1970)
	OC	*D. melanogaster, D. simulans* (Daborn et al. 2007)
	PYR	*An. stephensi*, *An. albopictus*, *An. gambiae* (Chandre et al. 1998; Hemingway and Ranson 2000), *Cx. p. quinquefasciatus* (Kasai et al. 1998), *Cx. p. pallens* (Shen et al. 2003), *M. domestica*, *B. germanica* (Scott 1999)
	Neonicotinoid	*D. melanogaster* (Daborn et al. 2002)
COE	OP	*Anopheles sp*., *M. domestica*, *L. cuprina* (Claudianos et al. 1999; Hemingway and Ranson 2000; Oakeshott et al. 2005; Perera et al. 2008), *Cx. pipiens* (Pasteur and Raymond 1996) *Cx. tarsalis* (Prabhaker et al. 1987), *Ae. aegypti* (Brengues et al. 2003)
	Malathion (OP)	*Anopheles sp., M. domestica, L. cuprina* (Claudianos et al. 1999; Hemingway and Ranson 2000; Oakeshott et al. 2005)*, Cx. tarsalis* (Hemingway et al. 2004)
	PYR	*Bo. microplus* (Hernandez et al. 2002), *Ae. aegypti* (Brengues et al. 2003)
GST	OC	*An. gambiae*, *An. arabiensis* (Hemingway and Ranson 2000; Hemingway et al. 2004), *An. dirus* (Enayati et al. 2005), *An. subpictus* (Perera et al. 2008), *An. albimanus* (Penilla et al. 2006), *Ae. aegypti* (Enayati et al. 2005)
	OP	*An. subpictus* (Perera et al. 2008), *M. domestica* (Scott 1999)
	PYR	*P. h. capitis* (Thomas et al. 2006), *Bl. germanica* (Scott 1999)

Table 2: Target-site modification resistance mechanisms. Mutations of insecticide targets are presented in various species.

Nota: *Cx. pipiens* is a species complex grouping *Cx. p. pipiens*, *Cx. p. quinquefasciatus* and *Cx. p. pallens*.

Target	Mutation		Species
GABA receptor	*Rdl*^{R}	A302S	*D. melanogaster* (ffrench-Constant et al. 1993), *D. simulans, M. domestica*, *Bl. germanica*, *L. cuprina*, *Ae. aegypt*i (ffrench-Constant et al. 2000), *An. gambiae* (Davidson 1956) *An. stephensi*, *An. arabiensis* (Du et al. 2005), *Cx. p. quinquefasciatus* and *Ae. albopictus* (Tantely et al. 2010)
		A302G	*D. simulans* (ffrench-Constant et al. 2000), *An. gambiae* (Du et al. 2005)
Na-channels	*kdr*	L1014F	*M. domestica* (Williamson et al. 1996), *Bl. germanica* (Hollingworth and Dong 2008), *An. gambiae* (Martinez-Torres et al. 1998), *An. stephensi* (Enayati et al. 2003), *An. arabiensis* (Diabate et al. 2004), *An. sacharovi* (Luleyap et al. 2002), *An. subpictus* (Perera et al. 2008), *An. culicifacies* (Singh et al. 2009), *An. arabiensis* (Stump et al. 2004), *Cx. pipiens* (Martinez-Torres et al. 1999; Xu et al. 2006), *H. irritans* (Soderlund and Knipple 2003)
		L1014S	*Cx. pipiens* (Martinez-Torres et al. 1999), *An. arabiensis* (Ranson et al. 2000), *An. sacharovi* (Luleyap et al. 2002)
		others	*P. h. capititis* (Thomas et al. 2006), *D. melanogaster, Bo. microplus* (Soderlund and Knipple 2003), *Ae. aegypti* (Brengues et al. 2003; Saavedra-Rodriguez et al. 2007)
	super-*kdr*	M918T	*M. domestica* (Williamson et al. 1996), *H. irritans* (Soderlund and Knipple 2003)
		others	*Bl. germanica* (Soderlund and Knipple 2003)
	?		*Bo. microplus* (Hernandez et al. 2002)
AChE	*ace-1*^{R}	G119S	*An. albimanus, An. nigerimus*, *An. atroparvus* (Hemingway et al. 2004), *An. subpictus* (Perera et al. 2008), *An. gambiae*, *Cx. pipiens* (Weill et al. 2003), *Cx. vishnui* (Alout et al. 2007)
		F331W	*Cx. tritaeniorhynchus* (Nabeshima et al. 2004; Alout et al. 2007)
		F290V	*Cx. pipiens* (Alout et al. 2007; Alout et al. 2009)
	ace-1^D	G119S	*Cx. pipiens* (Lenormand et al. 1998a; Labbé et al. 2007a), *An. gambiae* (Djogbénou et al. 2009)
		F290V	*Cx. pipiens* (Alout et al. 2009)
	ace-2		*D. melanogater, M. domestica* (Fournier and Mutéro 1994)