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Carbamate resistance in a UK population of the halophilic mosquito *Ochlerotatus detritus* implicates selection by agricultural usage of insecticide

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Abstract

The salt marsh mosquito *Ochlerotatus detritus* Haliday, 1833 (Diptera: Culicidae) is locally abundant in some areas of the UK, can be a pernicious summer nuisance biter and is one of eleven British mosquitoes considered as a potential bridge vector of West Nile Virus. WHO bioassays of insecticide susceptibility were performed on *O. detritus* from Parkgate Marshes, Little Neston, Wirral, UK using three insecticides, the pyrethroid permethrin, the carbamate bendiocarb and the organophosphate fenitrothion. *O. detritus* were fully susceptible to permethrin and fenitrothion but exhibited strong resistance to bendiocarb (66% of females alive following 1hr exposure; LT_{50} of 116 minutes). Sequencing of the known resistance-causing mutations in the acetylcholinesterase target site of carbamate/organophosphates, and of pyrethroids, the voltage-gated sodium channel, revealed no known target-site mutations indicating the carbamate resistance is not target-site mediated. Pre-exposure to the synergist piperonyl butoxide recovered full bendiocarb susceptibility further implicating metabolic resistance. We show that UK mosquitoes have the potential to develop resistance to insecticides and suggest that the carbamate resistance detected could be a result of exposure to agricultural insecticides.

Keywords:

Metabolic insecticide resistance, Ace-1, synergist, PBO, agriculture

Introduction

The UK has 34 species of mosquitoes including six species of *Anopheles*, three species of *Aedes*, four species of *Culex* and 11 species of *Ochlerotatus* (Mackenzie-Impoinvil et al. 2015). The salt marsh mosquito *Ochlerotatus detritus* (= *Aedes detritus*) Haliday, 1833 (Diptera: Culicidae) is locally abundant in coastal regions of the UK but found also in a variety of inland sites (Burke 1946; Marshall 1933; Ramsdale and Snow 1995; Service 1973). In addition, it is found throughout European coastal sites and inland saline water-bodies in Europe and North Africa (Cranston et al. 1987). It is one of thirteen British species of mosquitoes thought to be a possible bridge vector of mosquito-borne viruses (Medlock et al. 2005) and has been demonstrated to be a competent vector of west Nile virus and Japanese encephalitis virus (Blagrove et al. 2016; Mackenzie-Impoinvil et al. 2015). As *O. detritus* feeds on birds, humans and livestock and is the most common human biting mosquito of the UK, this species has the potential to become a significant vector of disease (Blagrove et al. 2016; Clarkson and Setzkorn 2011).

In some areas of the UK this species can be a pernicious biting nuisance in the summer months, particularly outdoors (Snow 1990). Areas with particular levels of nuisance biting complaints include close to the Dee estuary salt-marsh of south-west Wirral, and at Sandwich in Kent (Kampen et al. 2015; Medlock et al. 2012; Ramsdale and Snow 1995). In south-west Wirral, complaints of nuisance biting have resulted in council-led programmes of environmental management on the Dee estuary marshes (cutting of draining channels) and treatment with *Bacillus thuringiensis israeliensis* (Davies 1995; Clarkson and Setzkorn 2011). However, as a Site of Special Scientific Interest, no chemical insecticides are directly applied to the site.

The rise in insecticide resistance particularly in the main mosquito vectors (*Aedes, Anopheles* and *Culex*) is a major public-health concern, particularly across Africa, Asia and South America (Ranson and Lissenden 2016; Moyes et al. 2017). Whilst insecticide resistance can be driven through direct selection pressure from insecticide use in vector control programmes, the use of agrochemicals has been identified as an additional driving mechanism for the development of insecticide resistance in mosquitoes (Reid and McKenzie 2016). Whilst the use of insecticides in UK agricultural and horticultural practices is seen as detrimental from a biodiversity perspective (Lentola et al., 2017; Shardlow, 2017) its role in the development of insecticide resistance in UK mosquitoes is unexplored.

This study investigated the prevalence of insecticide resistance in *O. detritus* from North West England using bioassays to three classes of insecticides: pyrethroids (permethrin), carbamates (bendiocarb) and organophosphates (fenitrothion) and the partial nucleotide and protein sequences is reported of the *O. detritus Vgsc* and *Ace-1* genes in order to investigate possible resistance mechanisms.

2. Materials and methods

2.1 Study area and mosquito collection

O. detritus is a multivoltine species that appears most abundantly from March to November with eggs that can survive over a year (Blagrove et al. 2016). *O. detritus* larvae were collected during October 2016 and August 2017 by dipping from the salt marshes of Little Neston (53.280°N 3.057°W), located on the Wirral Peninsula, Cheshire, England. Larvae were sampled from a range of pools and returned to the laboratory for emergence and testing of adults. Larvae were maintained in field collected water, fed on crushed cat biscuits and pupae separated before eclosion in cages (Bugdorm, Taiwan). Adults were provided with access to 10% sugar water. Samples of the insecticide susceptible *Aedes aegypti* New Orleans and Liverpool strains were raised from eggs and tested concurrently. Adult *O. detritus* were identified morphologically (following Snow, 1990).

2.2 Insecticide bioassays

Insecticide bioassays were carried out to WHO specifications using WHO insecticide susceptibility kits and insecticide treated papers (WHO 2016) on 3-5 day old unfed, adult male and female mosquitoes. Three insecticides were tested: 0.75% permethrin, 0.1% bendiocarb and 1% fenitrothion with papers used up to six times as per WHO protocol. Approximately 20-25 mosquitoes (both male and female; mean 23.01 95% C.I. 21.91-24.11) were exposed to insecticide papers with control exposures (papers without insecticide) conducted concurrently. Following exposure, mosquitoes were returned to the resting tube, provided with 10% sugar water (*ad libitum*) and mortality recorded 24 hours later. Following initial exposures for 60 minutes mosquitoes were subsequently exposed to a range of exposure times from 2.5 to 120 minutes in order to calculate the LT_{50} (as per Bagi et al., 2015). Male and female time response curves were created and medium lethal time 50 (LT_{50}) calculated using a custom R-script. A sample of surviving and dead mosquitoes was retained in ethanol for molecular analysis.

Synergist bioassays were conducted following a 1h pre-exposure to 4% piperonyl butoxide papers. Control mosquitoes were also pre-exposed to PBO papers in synergist assays before testing with control papers.

2.3 Amplification and sequencing of partial *Ace-1* and *voltage-gated sodium channel* sequences

DNA from a sample of surviving and dead mosquitoes following bendiocarb and permethrin exposure (twelve for bendiocarb and eight for permethrin) was purified using the GeneJet Genomic DNA Purification Kit (Thermofisher, UK).

Mosquitoes possess two forms of acetylcholinesterase, AChE1 and AChE2, encoded by *Ace-1* and *Ace-2* genes. So far, only mutations in AChE1 have been implicated in resistance to organophosphates and carbamates (Liu 2015). A single amino acid substitution of a glycine to serine at position 119 of the *Ace-1* gene results in a high level of AChE1 insensitivity in multiple mosquito species (Weill et al. 2004b). PCR primer pairs to amplify exon 3 of *Ace-1* in *O. detritus* were designed from alignments of the *A. aegypti* sequence (Weill et al. 2004b) and *Cx. quinquefasciatus* sequence (accession number KF680946) biasing primer design to *Aedes*. A 559-bp genomic DNA fragment of the *Ace-1* gene encompassing codon 119 was then PCR amplified using the primers Ace_Och_F (5'-CGAATTGTAGATGCCGAATTAG-3') and Ace_Och_R (5'-CGATACTGCAGTGAAACTACG-3').

Target site resistance (knock-down resistance or *kdr*) to pyrethroids and DDT, occurs due to changes in the VGSC as a result of non-synonymous point mutations. In *A. aegypti* two point mutations at amino acid position 1016 have been identified, resulting in either a valine to glycine (V1016G) or valine to isoleucine (V1016I) substitution (Moyes et al. 2017). *Ae. aegypti* can also have an isoleucine to methionine substitution at amino acid position 1011 that results in pyrethroid and DDT cross-resistance (Lima et al. 2011). Additionally, a mutation at codon 1534 has been seen to be resistance associated (Harris et al. 2010).

The VGSC sequences in the region of exons 20 and 21 from *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* (from Davies et al. 2007) were used with designed primers biased to *Aedes*. Primers Kdr_Och_F (5'-GTGTTCCGGGTATTGTGCG-3') and Kdr_Och_R (5'-GGACGCAATCTGGCTTGTTA-3') amplify a 371-bp genomic DNA fragment of the voltage-gated sodium channel encompassing the regions of potential resistance mutations at codons 1011, 1014 and 1016.

PCR conditions for both fragments were an initial denaturation step of 3 minutes at 95˚C, followed by 35 cycles of 95˚C for 30 seconds, 55˚C for 30 seconds, 72˚C for 1 minute with a final extension at 72˚C for 5 minutes. Successful amplicons were purified using the GeneJet PCR Purification Kit and sequenced (GATC Biotech, Constanz, Germany). Sequences were manually edited and aligned using CodonCode Aligner (CodonCode Corportation).

2.4 HPLC analysis of residual bendiocarb on WHO papers

Concentration of bendiocarb on filter papers was analysed using high-pressure liquid chromatography (HPLC). Bendiocarb was extracted from 15.24 cm^2 of treated filter papers using 5 ml of acetone spiked with 100 µg/ml of dicyclohexyl phthalate (DCP) internal standard. Samples were sonicated for 15 mins

at 21˚C and 1 ml of extract was removed and acetone evaporated. Analyte residue was resuspended in 1 ml acetonitrile, centrifuged at 13,000 rpm for 20 mins at 21˚C and 10 µl of supernatant injected onto a Hypersil Gold C18 Reverse-Phase column at 21°C on an Agilent 1100 series HPLC. Analytes were measured using an isocratic mobile phase of 70% acetonitrile and 30% water with a flow-rate of 1 ml/min at a monitoring absorbance wavelength of 232 nm. At these conditions, the retention times of bendiocarb and DCP are 4 mins and 18.5 mins, respectively. Concentration of bendiocarb on the papers was quantified from a standard curve produced using known concentrations of authenticated bendiocarb standards.

3. Results

3.1 Insecticide bioassays

O. detritus were fully susceptible to permethrin following exposures from 2.5-60 minutes. Small numbers of mosquito were paralysed but twitching following up to 30 min exposure and 24 hr recovery (Table 1) but following WHO guidelines (WHO 2016) were classified as dead.

Very low mortality rates to 1hr bendiocarb exposure was observed in *O. detritus* for both female (33.7%) and male (67.2%) mosquitoes suggesting resistance (Table 1; Fig. 1) with surviving *O. detritus* in all of the bendiocarb bioassays. According to the WHO (2016) criteria, <90% mortality 24 hours post-exposure indicates resistance and the mortality of female *O. detritus* to bendiocarb at 34% by this criterion indicates resistance to carbamates. The longest period of exposure to bendiocarb was 120 minutes and even for this exposure time high numbers of female mosquitoes (53.2%) survived, giving an LT_{50} value of 115.6 minutes (Fig. 1). Resistance to bendiocarb differed between sexes with 33.7% female mortality and 67.2% male mortality (χ^2=17.61 $P < 0.0001$). When *O. detritus* were pre-exposed (for 1hr) to the synergist piperonyl butoxide (4%) mosquitoes recovered near full susceptibility to carbamates (98% mortality in females, 100% mortality in males – Table 1).

Because we identified resistance to bendiocarb in *O. detritus* we examined the efficacy of the bendiocarb papers through concurrent insecticide bioassays on *Aedes aegypti* New Orleans and Liverpool strains. Surprisingly, these strains did not exhibit full susceptibility to carbamates (91.2% and 86.1% female mortality respectively).

O. detritus exhibited susceptibility to fenitrothion with 100% mortality following 60 minutes exposures (Table 1).

3.2 *Ace-1* gene

Sequence analysis of exon 3 of the *Ace-1* gene from both resistant and susceptible mosquitoes showed very little variation in the nucleotide sequence and none in the protein sequence (Fig. 2). From twelve sequenced individuals (four resistant to bendiocarb (60-120 minutes), 2 susceptible to bendiocarb, 6 susceptible to fenitrothion), there were four single nucleotide polymorphisms (SNPs), two of which were in the non-coding intron and those in the coding region were synonymous. These polymorphisms were very common between the mosquito specimens regardless of whether they were resistant or susceptible. The G119S substitution associated with resistance to organophosphate and carbamate insecticides was not evident in *O. detritus*. Comparison of the *Ace-1* homolog gene protein coding sequence to *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* showed only a small amount of

variation demonstrating high conservation of the amino acid sequence with that of other mosquitoes (Fig. 2).

3.3 Voltage-gated sodium channel gene

Sequencing of *O. detritus* exon 20 and 21 which contain three of the possible amino acid residues known to confer resistance in the closely related *Ae. aegypti* revealed two synonymous SNPs in codons 1045 (T/C), 1047 (G/A) and in one mosquito two nucleotide changes at codon 1062 of a G/A and T/C (Fig. 3) from eight studied individuals (twitching following 5-30 minute exposures but dead following 24hr recovery time). The two nucleotide changes in succession at position 1062 result in an amino acid change from serine (AGT) to asparagine (AAC). This mutation occurred only in one female mosquito that was twitching 24hr after 5 minutes of exposure to permethrin. The known mutations in *Anopheles* (L1014F and L1014S) and *Ae. aegypti* (V1016G and I1011M) were not present in *O. detritus*. Comparison of the VGSC channel exon 20 and 21 protein coding sequence to *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* showed only a small amount of variation demonstrating high conservation of the amino acid sequence with that of other mosquitoes. At codon 1062 *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* have an asparagine amino acid whilst *O. detritus* exhibits either a serine or asparagine as described above (Fig. 3).

3.4 HPLC analysis of residual bendiocarb on WHO papers

Levels of bendiocarb were analysed in four used (4-6 times) papers. New papers are supplied as 0.1%. After usage, levels varied from 1.8-2.25µg bendiocarb/cm^2 (≡0.05-0.06%).

4. Discussion

In comparison to countries where vector-borne diseases are prevalent and a major concern to public health, chemical insecticides are not used widely in the UK domestic market. Insecticide resistance is not therefore expected in UK mosquito populations. This study shows that UK *O. detritus* were, as expected, susceptible to pyrethroids and organophosphates. However, resistance to carbamates was found in the population investigated. Whilst *O. detritus* has been a nuisance species to the public around Neston for a number of years (Medlock et al. 2012) chemical insecticides are not used to control mosquitoes in their larval habitat as this area of Cheshire is a UK site of Special Scientific Interest (SSSI) and an EU Special Area of Conservation, which imposes restrictions on potential control methods due to its conservation status. The Local Authority had regularly sprayed a small area of the marsh with the biological control agent *Bacillus thuringiensis israelensis* (Davies 1995; Clarkson and

Setzkorn 2011) however, this does not explain the development of carbamate resistance. Whilst it is possible that *ad hoc* usage of domestic insecticide products, and potentially on domestic garden plants (e.g. Lentola et al. 2017) where mosquitoes may rest or feed on emergence, has selected for resistance in adults, carbamate insecticides are not a typical component of these products which are largely pyrethroid or neonicitinoid-based. However, carbamate insecticides are used widely across the UK where agriculture is a major industry and pest control important (Vale and Lotti 2015). The most recent UK pesticide usage survey by the Food and Environment Research Agency in Northern England shows that more carbamate insecticides were used in comparison to pyrethroids and organophosphates (Garthwaite et al*.* 2015). Carbamates were used across 63,671 ha with 18,574 kg applied, compared to pyrethroids across 680,778ha and 9,209 kg (Garthwaite et al*.* 2015). Organophosphates were used at an even lower frequency across 3,805 ha with a total applied weight of 2,806 kg (Garthwaite et al*.* 2015). Higher agricultural use of carbamates in the North of England could explain the resistance to bendiocarb observed in this study. Carbamates applied to crops can pollute streams running off farms and this could be the indirect route of how these insecticides may be present in the salt marshes which are fed by local streams and flooded by the River Dee at spring tides. Recent analyses of UK river samples have shown that neonicitinoid insecticides can be found at detectable levels in river water (Shardlow 2017) and this may be true also for other insecticide classes. Indeed, the amount of agricultural pesticides that can be detected in the upper reaches of the River Dee, which feeds into the estuarine environment at Little Neston, has risen (Anon. 2017) although it is not clear from which classes these compounds are from. This exposure route would however act upon larval stages and require that the resistance mechanism selected be retained into the adult stage. Future work is clearly needed to specifically test the waters at Little Neston for carbamate levels.

The development of insecticide resistance as a consequence of exposure to insecticides used in agricultural practices has been seen in a number of studies (Reid and McKenzie, 2016), most noticeably in Benin, West Africa. Yadouleton et al*.* (2009) found that the increasing development of urban agriculture and farming practices in Benin has led to the high usage of insecticides as vegetable pest control. This increase in insecticide use was correlated with the emergence of insecticide resistance in *An. gambiae* (Yadouleton et al*.* 2009). This is a growing issue in control and management of malaria vectors. Corbel *et al.* (2007) report similar results and concluded that the increase in agricultural practices and intense use of insecticides are a main factor in selecting for resistance in both *An. gambiae* and *Cx. quinquefasciatus*.

We note that post-usage our bendiocarb papers (official in-date, WHO supplied) levels of bendiocarb were at 50-60% of the indicated supplied level (from 0.1% down to 0.5-0.6%) and lower levels of insecticide in testing equipment may result in inflated resistance levels. Indeed the <100% mortality

of the susceptible strains (we note that no data exists for carbamate mortality in these strains – their use as susceptible controls is based upon their lack of resistance to pyrethroids and DDT which are the main insecticides used for control of this species) of *Aedes aegypti* (approx. 90%) led us to measure the residual insecticide in the papers. However, even if bendiocarb levels in these papers had been lower on supply, the resistance levels in *O. detritus* are significantly higher than for *Aedes* (66.3% female survival to 1hr exposure versus 6.8% and 13.9% in the New Orleans and Liverpool strains respectively) and are removed by pre-exposure to the P450 synergist piperonyl butoxide indicating that there is genuine metabolic resistance in these samples.

The two main and most studied mechanisms of insecticide resistance in mosquitoes are metabolic resistance and target site insensitivity. These are due to either heightened detoxification activities or physiological changes of the insecticide's protein target (Nkya et al. 2013). Insecticides are classified into organochlorines, organophosphates, carbamates and pyrethroids based on their chemical structure (WHO 2006). The target site of pyrethroids and organochlorines are voltage-gated sodium channels (VGSC) to which they bind, preventing the switch from an activated (ion-conducting) to an inactivated (non-conducting) state (Davies et al. 2007). The target site for organophosphates and carbamates is the enzyme acetylcholinesterase (AChE) (Russell et al. 2004) which terminates nerve impulses by catalysing the hydrolysis of acetylcholine from its cholinergic nerve receptors. Organophosphates inhibit AChE by covalently phosphorylating an active site serine residue whereas carbamates carbamylate this. Analysis of exon 3 of the *Ace-1* gene revealed very little variation among the *O. detritus* population and only synonymous mutations. The G119S substitution due to a GGC to AGC SNP in the *Ace-1* gene found in *An. gambiae* and *Cx. pipiens* known to cause high levels of AChE1 insensitivity and associated with both carbamate and organophosphate resistance (Weill et al. 2004b) was not present in *O. detritus* (Weill et al. 2004a) and was not seen here. Weill et al. (2004a) found that the absence of the G119S mutation in *Ae. aegypti* is likely due to differences in the sequence of the 119 codon. In *Ae. aegypti*, glycine at 119 of AChE1 is encoded by GGA, whilst in other mosquitoes is encoded by GGC (Weill et al. 2004a). Serine can be encoded by AGY or TCN, therefore a substitution of glycine to serine requires one mutation when glycine is encoded by GGY, but two mutations when encoded by GGR as seen in *Ae. aegypti* and *O. detritus* (Weill et al. 2004a). Sequence analysis showed that the glycine at codon 119 of *O. detritus* in this study was also coded by GGA and this would cause a heavy constraint on its ability to spontaneously mutate, similar to *Ae. aegypti* (Weill et al. 2004a). Two mutations make it unlikely to occur and the sequencing of resistant individuals indicate that this has not happened here, therefore is not the cause of carbamate resistance found in the mosquito population of this study. Other mutations have been seen in mosquitoes e.g. F455W in *Culex tritaeniorhynchus* (Liu, 2015) and we studied only the region containing the G119S mutation. However,

O. detritus was susceptible to fenitrothion which shares the AChE1 target site of carbamates and therefore, it is unlikely that target-site resistance is responsible for the carbamate insensitivity observed in this study.

Carbamate resistance observed in *O. detritus* could also be due to metabolic resistance. Metabolic resistance is caused most commonly by elevated levels and activities of enzymes that result in an insecticide being detoxified or sequestered before reaching its target site (Li et al. 2007). Enzyme groups associated with carbamate resistance include cytochrome P450 monooxygenases (P450s) and esterases, however glutathione-S-transferases (GSTs) can mediate resistance to organophosphates, organochlorines, and pyrethroids (Li et al. 2007). Here, P450s are implicated by the synergist bioassay results. P450s have a broad substrate specificity, genetic diversity and catalytic versatility, consequently they have been associated to all classes of insecticides in a number of insects (Li et al. 2007). The most common P450s involved with carbamate resistance belong to the CYP6 subfamily. Using microarray techniques Edi et al. (2014) found that in *An. gambiae* there was the overexpression of multiple CYP6 P450 genes including *CYP6P3* and *CYP6M2* with *CYP6P3* being able to metabolise bendiocarb *in vitro* (Edi et al. 2014). In *An. funestus* Ibrahim et al. (2016) demonstrated the involvement of CYP6Z1 in carbamate resistance showing that this CYP6 enzyme could metabolise bendiocarb *in vitro*. Esterases that cleave carboxylester and phosphodiester bonds are also important in resistance to carbamates (Edi et al. 2014; Polson et al. 2011). Polson et al. (2011) found that bendiocarb resistance in *Ae. aegypti* corresponded to elevated activity levels of α- and β- esterases. This is similar in *Cx. quinquefasciatus* where the main cause of metabolic resistance is the co-amplification of *estα2* and *estβ2* (Hemingway 2000; Hemingway et al. 2004). This esterase genotype was observed in over 90% of the insecticide resistant *Cx. quinquefasciatus* (Hemingway et al. 2004). Therefore, the mechanism of carbamate resistance in these *O. detritus* could be metabolic and not target-site based.

Although the *O. detritus* population exhibited no resistance to pyrethroids, we note that as per the majority of studies conducted on *Aedes* (Moyes *et al*., 2017), we used testing papers with 0.75% permethrin rather than the *Aedes* diagnostic dose of 0.25%. The presence of twitching, but paralysed mosquitoes post-exposure suggested that mosquitoes may have survived an exposure to 0.25%. We therefore studied sequence variation of the VGSC gene. Analysis of exon 20 and 21 showed little variation. Common *kdr* point mutations in *Anopheles* (L1014F and L1014S) and *Aedes aegypti* (V1016G and I1011M) (Moyes et al. 2017) were not present in *O. detritus* and this was expected due to their susceptibility to permethrin. This *O. detritus* population did exhibit a non-synonymous mutation at codon 1062 resulting in an amino acid change from serine (AGT) to asparagine (AAC). However, when the amino acid sequence of exon 20 and 21 of the VGSC gene were compared between *O. detritus*,

An. gambiae, *Ae. aegypti* and *Cx. quinquefasciatus*, all of the mosquitoes display an asparagine at codon 1062 apart from *O. detritus*. However, since this change is in the Pre-S1 domain III linker, it seems unlikely to have consequences for the resistance phenotype.

The ability of *O. detritus* to vector disease and the potential of incipient resistance to bendiocarb in this population could raise issues in the future. Recent studies have also found that changes in mosquito distributions are driven by climatic changes (Medlock and Leach 2015). For example, the vector *Aedes albopictus* is historically native to tropical and subtropical Southeast Asia (Benedict et al. 2007). However, it has now been reported in 25 European countries and in 2014 was involved in the transmission of dengue and chikungunya in France (Medlock and Leach 2015) and resistance to insecticide is now being detected in European populations (Pichler et al. 2018). The ability of mosquitoes to evolve resistance mechanisms to important insecticides could pose a potential threat to control and management in the UK in the future. This study provides an insight into the status of insecticide resistance in *O. detritus* in the UK and poses the question of whether other species of UK mosquitoes show insensitivity to the classes of insecticides used in agriculture, an issue of importance given the northward spread of disease vector mosquitoes (Kampen et al. 2015). The mechanism of carbamate resistance in this population is still not fully characterised and further research is needed into the mechanism of the resistance detected.

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Table 1. Time-mortality data for *Ochlerotatus detritus* and *Aedes aegypti* New Orleans and Liverpool strains exposed to the carbamate insecticide bendiocarb, bendiocarb + the synergist piperonyl butoxide (PBO), the organochlorine fenitrothion and the pyrethroid permethrin. Female and male data are displayed separately. No mortality was seen in control exposures.

			Females		Males	
Species	Insecticide	Time	Number tested	Mortality	Number tested	Mortality
O. detritus	Bendiocarb	15	54	5.6	23	4.3
		30	68	7.4	25	12.0
		60	101	33.7	64	67.2
		120	62	46.8	27	100.0
O. detritus	PBO + bendiocarb	60	87	97.7	27	100.0
O. detritus	Fenitrothion	15	30	90.0	26	100.0
		30	43	95.3	9	100.0
		60	64	100.0	70	100.0
		120	30	100.0	17	100.0
O. detritus	Permethrin	2.5	43	100.0	10	100.0
		5	41	100.0	9	100.0
		7.5	33	100.0	13	100.0
		15	44	100.0	36	100.0
		20	19	100.0	6	100.0
		30	35	100.0	37	100.0
		60	11	100.0	36	100.0
A. aegypti NO	Bendiocarb	60	88	93.2	37	91.9
A. aegypti LIV	Bendiocarb	60	158	86.1	92	90.2

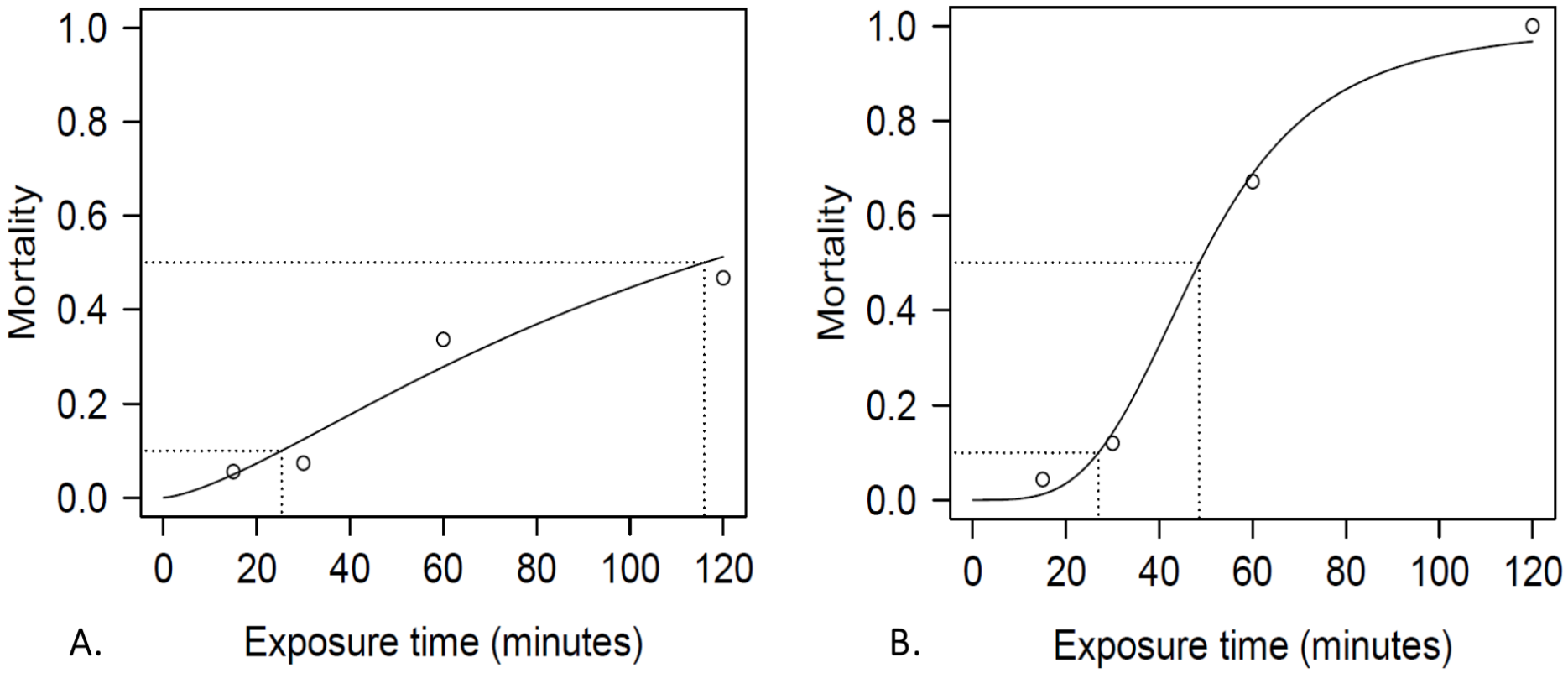

Figure 1. Time-mortality data measured for 2-5 day-old adult female (A) and male (B) *Ochlerotatus detritus* (female *N*= 285; male *N*=139) exposed to 0.1% bendiocarb treated papers. Dotted lines show the LT_{10} and LT_{50} for the population.

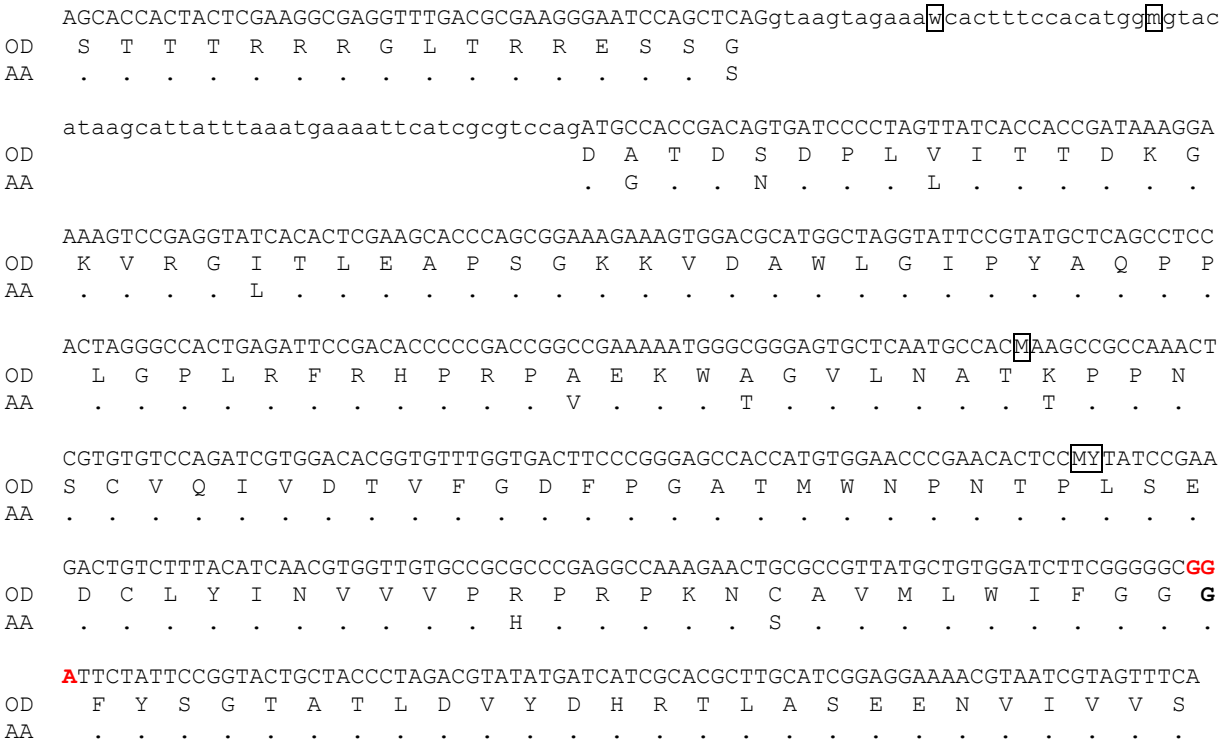

Figure 2. Consensus DNA sequence of *Ochlerotatus detritus* partial *Ace-1* sequence. Exon sequence is in upper case and intron in lower case. *O. detritus* amino sequence (OD) is depicted below the DNA sequence with *Aedes aegypti* (AA) aligned with identical amino acids depicted as (.). The 119G codon is emboldened. Variable bases are boxed and given as the relevant IUPAC ambiguity codon.

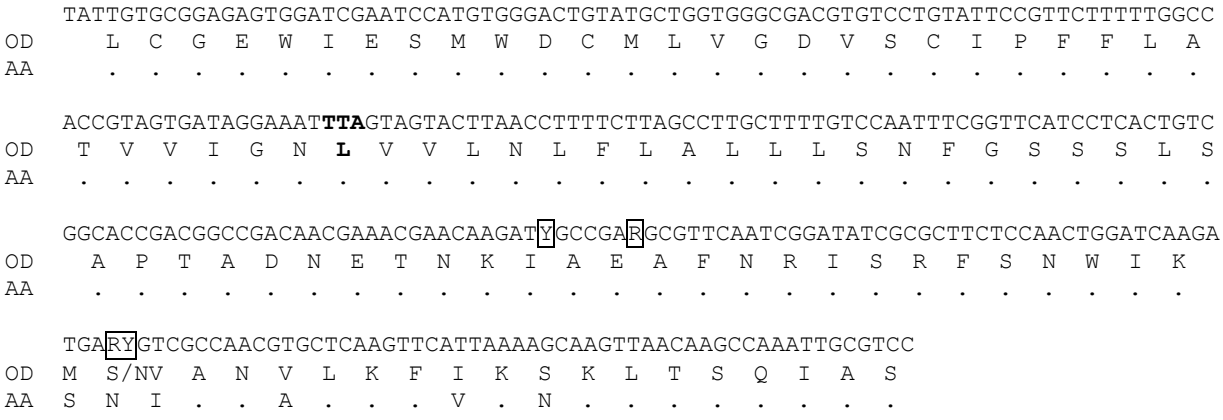

Figure 3. Consensus DNA sequence of *Ochlerotatus detritus* partial *Vgsc* sequence. *O. detritus* amino sequence (OD) is depicted below the DNA sequence with *Aedes aegypti* (AA) aligned with identical amino acids depicted as (.). The 1014L codon is emboldened. Variable bases are boxed and given as the relevant IUPAC ambiguity codon.