The Vulnerability of Aotearoa to Introduced Mosquito Species

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1. Focus species

Endemic: *Culex pervigilans*

- Endemic to and wide spread within New Zealand
- Bird-biters(1)
- Low vector competence(1)

Exotic: *Culex quinquefasciatus*

- Most widespread species in the world
- Introduced in 1830's but only recently established in the South Island(2)
- Mammal-biters(2)
- High vector competence(2,3,4)

3. Methods

Data was pooled from 12 district health boards around New Zealand spanning the last 13 years. ANOVA and multiple regression analysis will be performed using SAS statistical software and Excel.

Genetic analysis has been done with 5 microsatellite primers developed in previous studies (6,7). PCR protocols were trialled and altered before beginning to successfully test 3 samples of each of the 2 species over 3 time periods (2007-08, 2013-14 & 2017-18). Gel electrophoresis helped to indicate suitable samples to be sent to Massey University Genome Services and Geneious software was used for visualising results. Genalex software will be used for microsatellite interpretation.

For the larval morphometric analysis, a minimum of 7 individuals all in the last stage of development (instar 4) of each of the 2 species from a minimum of 3 localities were measured. Photo's were obtained using a compound microscope with a Leica ICC50W camera attachment and LASEZ computer program.

4. Preliminary Results

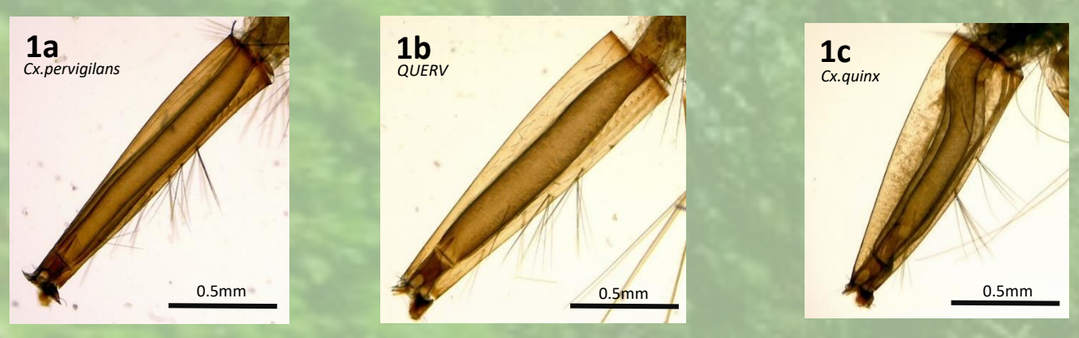

Figure 1: a) *Cx.pervigilans* long, narrow siphon b) Variation Culex (QUERV) long, bulging siphon c) *Cx.quinx* short, bulging siphon.

Larval populations showed variation between species with both intermediate characteristics and also mispairing of species features (5).

Some samples had long, thin siphons (*Fig. 1*) like the native Culex but also had few, pointed teeth on the mental plate (*Fig. 2*) which is suggestive of the exotic. These 'QUERV'(5) characteristics were also observed in some adult samples which had contradicting scale patterns.

Most interestingly was a sample collected in Northland in 2018 which showed a half-and-half profile of both *Cx. pervigilans* and *Cx. quinx!*

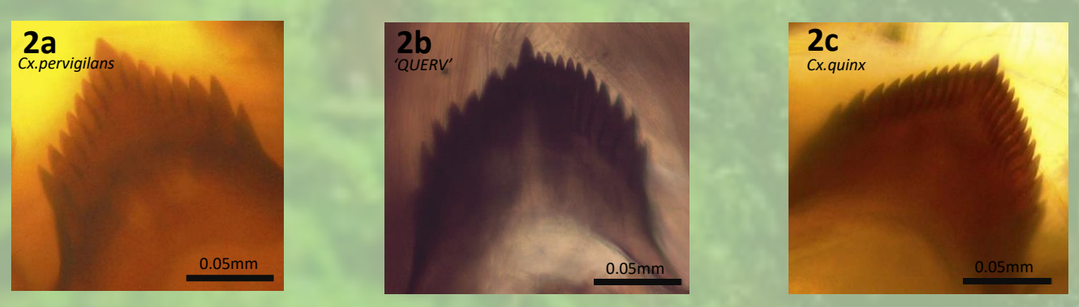

Figure 2: Mental plates a) *Cx.pervigilans* large, few teeth b) 'QUERV' half-and-half profile of wide, few teeth and narrow, many teeth c) *Cx.quinx* narrow, many teeth.

2. Background

Cx. quinquefasciatus (*Cx.quinx*) is known to transmit harmful human diseases such as Ross River Virus, West Nile Virus and Filariasis. It is also a known vector of avian malaria and multiple parasitic worms which could threaten our bird and lizard species(2). Such diseases have yet to become established in New Zealand however it is clear that if an outbreak were to occur, establishment is highly likely (3,4).

With increased international travel, trade and climate changes, New Zealand's border biosecurity is more important now than ever (2,3,4). Our current mosquito surveillance doesn't target established species like *Cx.quinx* which is dangerous as it means we cannot quantify how many *Cx.quinx* interceptions had originated in NZ or from overseas (5).

Our aim is to assess the population changes of both species over the past 13 years looking particularly at larval morphological differences between species and genetic mixing of samples from different regions. Our big questions include:

1. Is there a statistical relationship between exotic and native Culex population numbers over the last 13 years?
2. Is there preliminary evidence of interbreeding or hybridisation between the two Culex species?
3. Is there genetic evidence of unnoticed, international introductions of *Cx.quinx* over the past 10 years?

4. Preliminary Results

The microsatellite results have indicated a widening genetic pool across the last 10 years however this will not be conclusive until a larger data set has been analysed.

For most primers, *Cx.quinx has* larger pool of allele frequencies particularly than *Cx.pervigilans*. There was also significant overlap of alleles between species which indicates relatability(8,9).

	CxqA115	CxqA118	CxqATG9	CxqGA12	CxqGT14
Total pop.	21	9	28	13	3
Cx.pervigilans	10	7	15	6	1
% Alleles present	47.6	77.8	53.6	46.2	33.3
Cx.quinx	16	5	19	7	2
% Alleles present	76.2	55.6	67.9	53.8	66.7

Figure 3: The above table shows the results of the 79 analysed microsatellites (we predict 216 will be needed for accurate final analysis). Percentage of alleles refers to the number of alleles found in each species relative to the total number of alleles observed per primer.

5. Discussion & Further…

Preliminary results suggest that there is a diverse group of alleles present particularly in *Cx. quinx* populations however more samples are required to draw any further conclusions regarding locational differences.

Significant overlap of alleles and mixing of physical characteristics is suggestive of interbreeding and possible hybridisation. The continuation of the project will be able to isolate key movements and introductions hopefully showing the flow and transfer of alleles throughout New Zealand (5).

The Hardy Weinberg equilibrium will be used to determine the extent of mixing between populations and will also show a relative amount of international introductions which have gone unnoticed. We hope that further results from the project will confirm doubts regarding New Zealand's biosecurity efforts, promoting reform and improvement for the continued well-being of Aotearoa.

6. References

1) Vector profile: *Culex pervigilans* (2008). *SMS-NZB* Retrieved: www.smsl.co.nz. ***2)*** Vector profile: *Culex quinquefasciatus* (2008). *SMS-NZB* Retrieved: www.smsl.co.nz ***3)*** P. Holder et al. (1999). The mosquitoes of New Zealand and their animal disease significance. *Surveillance,* 26,12-15. ***4)*** E.B. Spurr, & G.R. Sandlant (2004). Risk Assessment for the Establishment of West Nile Virus in New Zealand. *Manaaki Whenua Press*. Series No. 25. ***5)*** NZ BioSecure (pers. coms) 2019. ***6)*** F. E. Edillo et al. (2007). A set of broadly applicable microsatellite markers for analysing the structure of *Culex pipiens* populations. *Department of Medical Entomology* 44: 145-149. ***7)*** J. L. Smith et al. (2005). Cross-species comparison of microsatellite loci in the *Culex pipiens* complex and beyond. *Molecular Ecology Notes*, 5: 697-700. ***8)*** H. Ellegren (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, 5:435-445. 9) A. B. B. Wilke et al. (2014) Population genetics of neotropical *Culex quinquefasciatus* (Diptera: *Culicidae). Parasites and Vectors*, 7:468.