# Natural and experimental admixture of invasive blowflies in New Zealand and Australia

A thesis

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**Master of Science (Research) in Molecular and Cellular Biology**

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THE UNIVERSITY OF
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# Abstract

Across the globe, native species are being outcompeted and often reach extinction due to introduced species becoming invasive. Previously confined to their native areas due to geographical, ecological, or environmental barriers that have prevented them from expanding, human impacts have resulted in a significant increase in the number of introduced species. Such species become invasive when they begin to expand their range demographically and typically cause negative impacts in the new environment. There is no current model that allows us to predict and prevent future biological invasions, though next generation sequencing, population genomics analysis, and experimental laboratory manipulations are helping to fill critical gaps in our understanding of the invasion process.

My first analysis (**Chapter 2**) explored the ability of population genetics analyses of single nucleotide polymorphism (SNP) data to identify hybridisation levels and the rate of admixture occurring in wild populations of *Calliphora hilli* and *Calliphora stygia* – two invasive blowflies found in New Zealand and originally from Australia. I analysed samples from various locations and found patterns of population genetic connectivity and structure that supported Australia as the source of the New Zealand invasion for both species. This research provided highly valuable new insights into the population structure of these two species, with hybridisation and gene flow playing a key role in their respective biological invasions.

My second analysis (**Chapter 3**) first explored the population structure of the highly invasive blowfly species, *Callipohra vicina*, using SNP data to analyse population genomic patterns, such as genetic diversity and admixture. Following this, low genetic diversity colonies were generated from isofemale lines to simulate an invasive population that had undergone a genetic bottleneck. These low diversity lines were compared to relatively high

diversity lines for a number of traits, including fecundity, body size, developmental rate, and lifespan to determine the effects of genetic diversity on population fitness. We found genetic differentiation between North and South Island New Zealand populations in the wild, while high diversity lines outcompeted low diversity lines for all measured traits in the laboratory. These results demonstrated the importance of genetic bottlenecks on invasion scenarios and suggested interesting new ideas for follow-up research.

Predicting and preventing future invasions is a significant current gap in invasion biology. Population genomic and ecological assays can together help to fill this gap to help us identify the mechanisms underlying invasive success.

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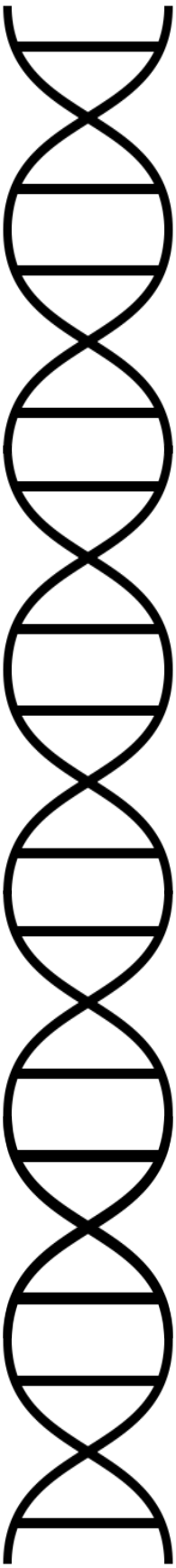

# Chapter 1.

## General information

## 1.1 Introduction

Native species can be defined as those that reside in a given region or ecosystem as a result of local natural evolution (Simberloff, 2013). They are usually constrained to their native ranges due to geographical, ecological, or environmental barriers that prevent them from expanding. In contrast, introduced species are non-native species that become resident in new ecosystems due to anthropogenic transport (McCarthy et al, 2019). They go on to become invasive when they demographically expand, causing negative impacts on the ecosystem and surrounding biodiversity (Matheson, 2022; Tobin, 2018).

Negative impacts of invasive species affect native biodiversity, human health, agricultural/primary industries, and the economy – all on a global scale (Sagoof, 2005; Doherty et al, 2016; Mazza and Tricarico, 2018; Keller, 2010). For each invasive species colonisation, there is a 16.6% decrease in native species richness (Tobin et al, 2018) and it is estimated that an additional one million species will become extinct in the next 50 years due to competition with invasives (Lieurance, 2022). Many costs associated with the control of invasive species are not monitored, however their global economic impact is estimated to be upwards of 100 billion dollars per year, with this only expected to rise due to global trade and climate change (Jardine and Sanchirico, 2016; Diagne et al, 2021). New Zealand is no exception, with large economic costs associated with invasive species. For example, economic losses from unproductive sheep grazing due to invasive rabbits costs an estimated $50 million annually, with an average of 16 rabbits consuming as much pasture as a single sheep (Lantham *et al*, 2019).

## 1.2 Stages of invasion

Invasive species entering new environments undergo four stages, known as: (1) transport; (2) introduction; (3) establishment; and (4) spread (Fig. 1.1).

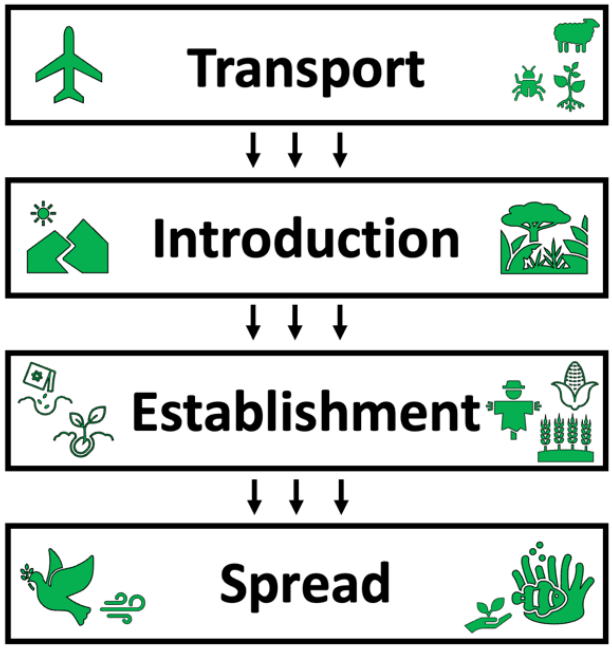


**Figure 1.1**. Simplified model showing the stages of invasion (adapted from Lieurance e*t al,* 2022).

Transport occurs when a species (eggs, seeds, larvae, or adults) is transported beyond the boundaries of its native habitat (Matheson, 2023; Lieurance, 2022). Humans have contributed to the transport of invasive species since the Middle Ages (Hulme, 2009). Human-mediated transport has previously caused introductions that are both accidental and intentional, with some intentional examples including the introduction of invertebrates used as a food source for animals (due to their high protein content), the biocontrol of other pest species, and the establishment of global livestock populations and food crops (an estimated 99% of these are introduced species) (Kumschick et al, 2016; Pimentel et al, 2007). Accidental introductions can occur due to unintentional releases and stowaway species in vehicles, shipping, and air cargo (Hulme, 2009). Roughly 10% of introduced species go on to reach the establishment stage (Tobin, 2018).

Following establishment, a small subset (~1%) of species ultimately spread to become invasive in the new environment (Matheson, 2022; Tobin, 2018). This often requires the

ability to tolerate or respond to new environmental conditions and successful invasive species often have traits, such as heat and environmental tolerance, that assist with this (Rilov and Crooks, 2004). The environmental spread of an established invasive species also requires reproductive success and dispersal of offspring in the invaded range (Lieurance *et al*, 2022).

Successful invasive species have the ability to colonise new environments due to a combination of pre- and post-adaptations. The pre-adaptation hypothesis states that a species has the ability to become invasive if the characteristics that promote invasiveness are already present in the native range (Elst et al, 2015). This may include phenotypic characteristics, such as faster reproductive rates, rapid dispersal, the ability to outcompete native species, and plasticity in traits like behavior and heat tolerance (Forman and Kesseli, 2003; Flores-Moreno *et al*, 2014; Allendorf and Lundquist, 2003; Davidson et al, 2011). In contrast, post-adaptation results from rapid evolution in the invasive range, with genetic diversity playing an important role during invasion by impacting the species' adaptive potential (i.e., capacity to rapidly evolve in response to new selective pressures; Seaborn *et al*, 2021). Finally, demographic factors, such as propagule pressure, are important in determining the success of invasive species (e.g., a greater number of founding individuals improves the likelihood of establishment; Simberloff *et al*, 2009). In practice, these various factors are considerably difficult to separate, and we know much less about the role of genetic factors in invasion because most of the focus to date has been trait-based.

## 1.3 Genetic processes in invasion

Next generation sequencing (NGS) has allowed genomic analyses of more DNA sequences than have previously been available. For example, the DNA of model organisms has been disproportionately examined, but model organisms may not be representative of closely related species or may not be informative for questions pertinent to invasion (Rius et al,

2015). NGS has advanced our ability to explore genomic questions with any species, including invasives. Identifying genetic contributions to adaptation during invasion is a key focus, allowing insights into processes like hybridisation and bridgehead events (reproduction between two genetically different populations/species, and the introduction of an invasive species to a new area via another introduced population, respectively, Abbott *et al*, 2013; Valetin et al, 2017), as well as specific genes (e.g., for pesticide resistance) (Makino and Kawata, 2019).

Population genomics – analyses of genomes and specific genes and loci in order to better understand the role of genetic diversity in evolution (Luikart et al, 2018) – is a key area of research that is increasingly applied to the understanding of genetic processes in invasive species (Sakai *et al*, 2001). For example, species that establish via a large number of individuals or via multiple introductions are significantly more likely to have invasive success due to having higher genetic diversity (Gatto-Almeida, 2022). Yet, during most invasion events, the invading population will undergo a significant demographic bottleneck, causing a decrease in genomic diversity (Rius and Darling, 2014) (Fig. 1.2). Genetic processes like hybridisation and gene flow can work to increase genetic diversity, for example through the addition of new genes into the existing gene pool in a process known as genetic admixture (when individuals from two or more previously isolated populations interbreed; Rius and Darling, 2014; Moran and Alexander, 2014), and these processes can be identified using population genomic analyses.

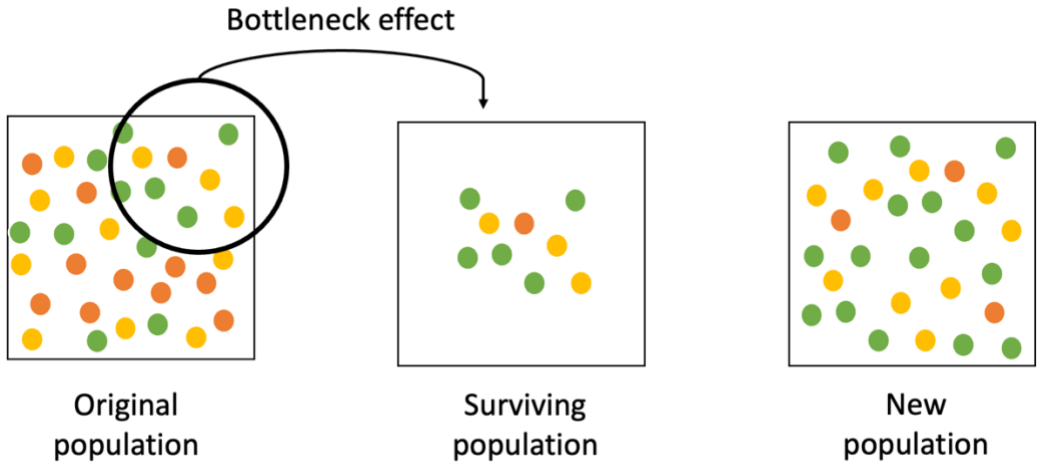


**Figure 1.2.** Simplified model showing the changes undergone by a species before, during, and after a genetic bottleneck.

Genetic data can also be used to examine population structure, which can inform understanding of transport pathways and connectivity (i.e., gene flow) among invasive populations. In particular, methods such as principal component analysis (PCA) can be used to group genetically similar individuals together in order to investigate genetic ancestry and/or the correlation between genetic and geographical variation (Price *et al*, 2010; Patterson *et al,* 2006). Admixture analysis can also be performed to cluster the genome into related components to understand how ancestral diversity is distributed among individuals (Edea *et al,* 2015), while hybrid analyses can be used to determine whether hybridisation is occurring (i.e., whether an individual has ancestry derived from more than one species) (Baiakhmetov *et al*, 2021).

### 1.4 Blowflies as a model organism

Blowflies have beneficial environmental impacts, including their ability to pollinate, their use in forensic entomology, and their role as carrion feeders (Cook *et al,* 2020; Fremdt et al,

2014; Norris, 1965). However, they can also cause detrimental effects to the agricultural sector. For example, flystrike is caused when *Calliphora* blowflies – typically *Lucilia cuprina* and *Lucilia sericata* – lay eggs on a sheep, with the resulting larvae going on to hatch and feed on the living sheep tissue (Tellam and Bowles, 1997; Heath and Bishop, 2006). Flystrike has an estimated annual cost of $30-40 million to the wool industry which, combined with resistance to relevant insecticides and chemical residues being left in the wool, make them a significant pest to farmers (Bishop *et al,* 1996: Tellam and Bowles, 1997).

Despite their detrimental effects, little is known about the invasive history of *Calliphora*, especially in a New Zealand context. New Zealand is home to several *Calliphora* species, of which one (*Calliphora quadrimaculata*) is endemic (Dear, 1986). Among the invasives are three species targeted in this research – *Calliphora hilli* and *Calliphora stygia* invaded from Australia and were first recorded in New Zealand in 1841, while *Calliphora vicina* was likely introduced from Europe via Australia in the mid-1800s (Dear, 1986). *Calliphora hilli* and *C. stygia* are morphologically similar golden blowflies with an Australasian distribution, that dominate warmer environments in Australia throughout both summer and winter months, while *C. vicina* has a global distribution and prefers cooler temperatures (Dear, 1986).

*Calliphora* blowflies are a highly advantageous model system for the examination of questions relating to invasion success due to their invasive properties, significant ease of collection (due to baited net trapping), fast developmental rate, and ease of phenotyping.

### 1.5 Thesis structure

My thesis uses genomic and ecological tools to understand the evolutionary mechanisms embedded within invasive blowfly species in New Zealand and Australia that have likely contributed to their invasive success. Following this introductory chapter, **Chapter 2**

investigates the independent invasions of *C. hilli* and *C. stygia* from Australia to examine the extent of intra- and interspecific hybridisation and admixture that is occurring in the wild for these two invasive species. **Chapter 3** explores the impacts of high and low genetic diversity on population success of the highly invasive *C. vicina* to understand the impact of genetic bottlenecks on evolutionary fitness. Finally, **Chapter 4** provides an overall discussion of the broader implications of my research and identifies caveats and future steps.

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# Chapter 2.

## Independent invasions of two Calliphora blowfly species provide evidence for post-invasion intra- and interspecific hybridisation in the wild

Image by: Nathan Butterworth, Monash University

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Contributions: NB assisted with blowfly identification; CF extracted the DNA; LC and PM performed the analyses, with assistance AM as required; LC wrote the manuscript draft. All authors provided feedback on the final manuscript.

## 2.1 Abstract

When a species invades a new geographical area, it can become invasive and have negative effects on native biodiversity. Among other genetic processes, hybridisation has been shown to facilitate invasion by producing new combinations of genetic variation that increase adaptive potential and associated population fitness. Yet the role of hybridisation (and resulting gene flow) in biological invasion for invertebrate species is under-studied.

Blowflies are important agricultural pests and two *Calliphora* species (*C. hilli* and *C. stygia*), separately invaded New Zealand from Australia *c.* 1779-1841. Here, we used genome-wide single nucleotide polymorphism (SNP) data from samples collected from various locations across New Zealand and Australia to understand the potential roles of hybridisation and gene flow within/between wild populations of these two species.

We found patterns of population genetic connectivity and structure that supported Australia as the source of the New Zealand invasion for both species. Genomic data indicated that *C. hilli* and *C. stygia* are hybridising in the wild, with admixture also occurring within species at appreciable rates. Collectively, our study provides new insights into the population structure of these two invasive invertebrates, and of the roles of hybridisation and gene flow following their respective biological invasions.

## 2.2 Introduction

Species range limits can be constrained by a variety of factors, including geographic and abiotic conditions, dispersal limitations among taxa, and ecological elements such as interspecific competition (Case and Taper, 2000; Sexton *et al*, 2009). Climate change and other anthropogenic impacts are also re-defining species ranges across the globe, especially as rates of biological invasion increase and temperatures warm (Parvizi *et al,* 2022; Waters *et al*, 2013; Legault, 2020; Spence and Tingley, 2020).

An invasive species is often defined as a population that is self-sustaining while having evidence of spread from the original geographic location (Hobbs, 2000). In new environments, invasive species can often cause detrimental impacts on native biodiversity, ecosystem function, and agricultural productivity (Keller, 2010). Invasive success can be determined by phenotypic characteristics (including faster reproductive rates, rapid responses to new environments, and the ability to out-compete native species; Forman and Kesseli, 2003; Flores-Moreno *et al*, 2014; Allendorf and Lundquist, 2003) that assist the species to disperse, establish, and densely populate the new area. However, it is often also due in part to the ability of invasive species to respond at the genetic level to natural selection (Lee, 2002), whether by rapid adaptation or the expression of phenotypic plasticity in the invasive range (Davidson *et al*, 2011). Among genetic processes, hybridisation (reproduction between individuals from genetically differentiated populations – which may include different species – resulting in offspring with mixed genetic characteristics; Abbott *et al*, 2013) and the resulting gene flow have been shown to facilitate invasion (e.g., Lee, 2002; Zbawicka *et al*, 2019; Pfeilsticker *et al*, 2023). However, their roles in the invasive success of invertebrates have historically received little attention (Kirk *et al*, 2013).

During invasions, populations often undergo bottlenecks and/or changes in selection pressures that can constrain establishment and spread. Hybridisation and gene flow can provide the required counteraction by increasing adaptive potential and/or associated population fitness (Rius and Darling, 2014) in three major ways: (1) genetic admixture (the incorporation of new alleles into existing lineages) can result in novel mixing of parental genes in the hybrid, upon which selection may act to promote hybrid vigour – the enhanced fitness of the hybrid relative to either parent (Ackermann, 2011; Qiao, 2019; Keller, 2010; Rius and Darling, 2014); (2) transfer of beneficial genes from locally adapted species to the invasive species can confer a fitness advantage to the invader; and (3) transfer of beneficial

genes from the invader to locally adapted species can enhance fitness of the local species (Abbott *et al,* 2013).

Many plant invasions are associated with hybrid vigour. For example, the willow tree species *Salicaceae alba* and *Salicaceae fragilis* produce hybrid offspring in numbers as abundant as the respective parental species, while *Tamaricaceae ramosissima* and *Tamaricaceae chinensis* hybrids account for up to 87% of the *Tamaricaceae* population in Asia (Gaskin, 2017). Continuous gene flow causing admixture in previously diverged populations in the early stages of invasion has also assisted wild sunflowers to rapidly establish and adapt when entering new environments globally (Mondon *et al,* 2017; Hübner *et al*, 2022). An example in which interspecific gene exchange facilitates invasion is the escape of beneficial transgenes from crop plants to invasive relatives – this has been documented in sunflower crops and assessed as likely to have occurred in some species of insects (Stewart *et al,* 2003; Whitehouse *et al,* 2007). Meanwhile, hybridisation between the invasive pest moth, *Helicoverpa armigera*, and local *H. zea* in Brazil resulted in the transfer of genes in the other direction, conferring rapid fenvalerate resistance in *H. zea* (Valencia-Montoya *et al*, 2020).

Calliphorid blowflies include many species that have both beneficial and detrimental environmental impacts (Norris, 1965). For example, blowflies can have positive effects in their role as carrion feeders, with larvae assisting in the breakdown of carcasses in rural areas (Norris, 1965). They can also provide valuable ecosystem services, such as pollination (Cook *et al,* 2020). In New Zealand, invasive blowflies can detrimentally cause flystrike – when female blowflies deposit eggs onto sheep and the resulting larvae hatch and feed on the living sheep tissue (Heath and Bishop, 2006). With an estimated annual cost of 30-40 million dollars to the farming industry (Bishop *et al,* 1996), blowflies are important agricultural pests, yet we understand little about their invasion pathways into New Zealand or the ways in

which genetic processes, such as hybridisation and gene flow, operate during or after invasion. Such questions are particularly interesting for blowflies, given that they are highly vagile, excellent dispersers (e.g., Tsuda *et al*, 2009; Butterworth *et al*, 2023) that may be expected to maintain high rates of population connectivity post-invasion.

There are several invasive blowflies in New Zealand, with two golden blowflies – *Calliphora hilli* and *Calliphora stygia* – first recorded in 1841 during specimen collections in the Bay of Islands (Dear, 1986). *C. hilli* and *C. stygia* are thought to have been introduced to New Zealand from Australia somewhere between 1779 and 1841 (Dear, 1986). The two species are very similar morphologically and fulfil the same ecological niche (Muller, 1939; Dear, 1986). Despite being some of the most common flies in Australia and New Zealand, little is currently known about the population structure of these two species, or the role of hybridisation in their evolutionary histories. However, *C. stygia* has been shown to produce hybrids with *C. albifrontalis* in Australia under laboratory conditions and *C. hilli* produces hybrids with *C. valiforons* – suggesting that hybridisation within the species complex may be occurring in the wild (Wallman and Adams, 1997; Monzu 1977).

Here, we use genome-wide SNP data from samples collected from various locations across New Zealand and Australia to investigate population structure and examine the potential roles of hybridisation and gene flow – within and between wild *C. hilli* and *C. stygia* populations and species – following biological invasion.

## 2.3 Methods

*Sample collection and identification*

Sampling kits and set-up instructions were sent to friends and colleagues to use in their backyards in various locations across New Zealand. Sampling traps consisted of a modified bottle trap (Hwang and Turner, 2005) made from two plastic bottles, with meat bait placed in

the lower bottle, which was covered in black tape to block out light. Flies could enter the trap through slots in the side of the lower bottle and were funneled towards the light in the upper bottle, where they were trapped until collection. Traps were left outside for 3-4 days and checked and emptied daily. Emptying traps involved placing the upper bottle in the freezer to euthanise the flies, which were then placed into a 50 mL falcon tube containing 69% ethanol for postage back to the University of Waikato.

Alongside the New Zealand backyard sites, we obtained 43 samples from nine locations in Australia. Together, this resulted in a total of 81 samples from 20 sites for *C. hilli* and 100 samples from 26 sites for *C. stygia* (Fig. 2.1, Tables 2.1, A2.1). All specimens were identified to species level using the taxonomic key of Dear (1986).

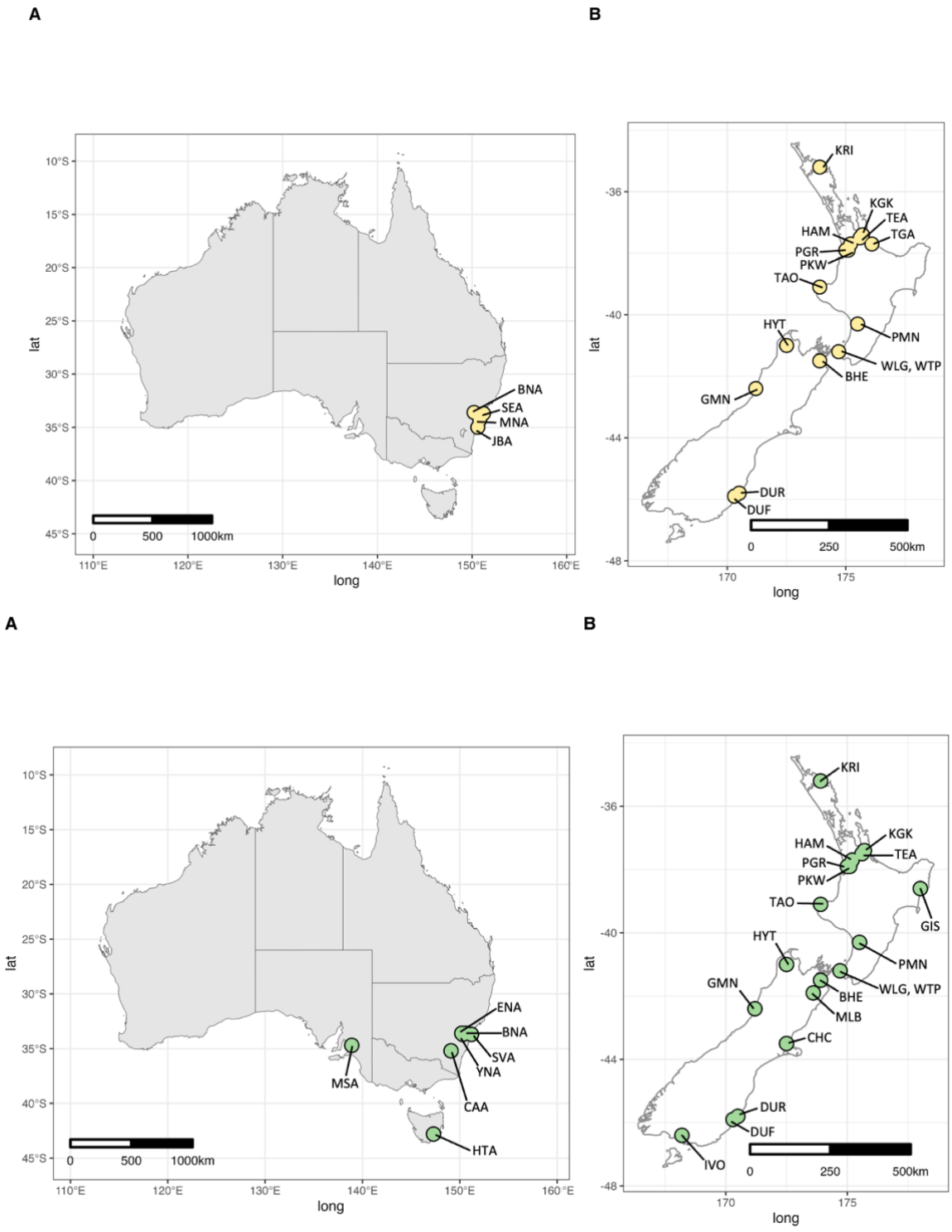


**Figure 2.1**. Geographical maps showing sampling sites where *Calliphora hilli* (yellow dots) and *Calliphora stygia* (green dots) were collected.

*DNA extraction and sequencing*

DNA was extracted for 181 samples using a DNeasy Blood and Tissue kit (Qiagen) and associated protocol, quantified using a Qubit fluorometer (Thermo Fisher Scientific), and sent to AgResearch Ltd for genotyping-by-sequencing (GBS).

A single GBS library was constructed according to the methods outlined in Elshire *et al*. (2011), with modifications as outlined in Dodds *et al*. (2015). The GBS library was prepared using a PstI-MspI double-digest and included negative control samples (no DNA). Libraries underwent a Pippin Prep (SAGE Science, Beverly, Massachusetts, United States) to select fragments in the size range of 220-340 bp (genomic sequence plus 148 bp of adapters). Single-end sequencing (1x101bp) was performed on a NovaSeq6000 utilising v1.5 chemistry.

*SNP filtering*

Raw fastq files were quality checked using FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). IPYRAD v0.7.28 (Eaton and Overcast, 2020) was used to filter and remove low quality data, identify homology among reads through *de novo* assembly, make single nucleotide polymorphism (SNP) calls, and format output files for each species dataset. Reads were processed with the following non-default parameter settings: filter_adapters (2, where adapters were removed), filter_min_trim_len (60), and trim_reads (10, -140, 0, 0); and SNPs were exported in variant call format (VCF).

The VCF file was filtered using VCFTOOLS v0.1.13 (Danecek *et al*., 2011), with --missing-indv, --max-missing-count and --maf parameters applied to filter data with >98% missing data, 20% missing genotypes across all individuals, and a minor allele frequency cut-off of 5%. This resulted in datasets of 16,144 and 12,494 SNPs, for *C. hilli* and *C. stygia*, respectively.

Because of the morphological similarities between *C. stygia* and *C. hilli,* a combined dataset for both species was also run through the IPYRAD and VCFTOOLS pipeline outlined above, and an initial Principal Component Analysis (PCA; see below) of this combined dataset (16,333 SNPs) was performed. Any potential hybrids (i.e. samples that overlapped in the PC space) were then removed from the individual species datasets to create 'pure' VCF files for population analysis for each species (see Results; Table A2.1).

*Population analyses*

The following analyses were conducted in R v4.3.0 (R Core Team, 2020). Geographic maps were first created to visualise the geographic distribution of samples using the function map_data within the ggplot2 package v3.3.6; (Wickham, 2016). Population genetic diversity (heterozygosity) and differentiation ($F_{ST}$) were determined for each population and species using the hierfstat package v0.5-11 (Goudet, 2005).

PCAs were conducted using the glPCA function implemented in the adegenet package v2.1.10 (Jombart, 2008), and plotted using ggplot2. Admixture analyses were conducted by first converting the VCF file into geno format using the R package, LEA v3.6.0 (Frichot and Francois, 2015). The optimal K value was determined using a cross entropy plot produced by using the snmf function in LEA on the geno file. The function qmatrix from the tess3r package v1.1.0 (Caye and Francois, 2016), along with ggplot, were used to produce an admixture bar plot for each species. Using the meta function within the terra package (Hijmans R, 2023), a new VCF file containing only neutral SNPs was created, and both the PCA and admixture analyses were repeated on these neutral datasets. The neutral and non-neutral datasets produced predominantly consistent results; thus, we present the neutral plots in the main text and provide the non-neutral plots in the Supplementary Information.

Finally, we investigated potential hybrids between *C. stygia* and *C. hilli* in several ways: (i) we examined the PCA for the combined species dataset (see above); (ii) we

performed admixture analysis for K = 2-5 as outlined above on the combined dataset; and (iii) we performed a specific hybrid analysis using the 'gl.nhybrids' function from the DartR package v2 (Gruber *et al*, 2018; Mijangos *et al*, 2022). This latter hybrid analysis produced probability proportions for identification of individuals as 'pure' species, hybrids, or backcrosses, and we plotted these proportions as a bar chart, as per Baiakhmetov (2021).

## 2.4 Results

*Calliphora hilli*

Observed heterozygosity (Ho) was low across all sampled populations, and significantly lower than expected heterozygosity (He) (mean Ho = 0.100, range = 0.010-0.200, and mean He = 0.242, range = 0.140-0.270; $T_{37} = -11.295$; $P < 0.001$) (Table A2.1). Comparing New Zealand and Australian populations, there was no significant difference in mean Ho (mean Ho = 0.107 and 0.073 for New Zealand and Australia, respectively; $T_{19} = 1.581$; $P = 0.065$). Pairwise population $F_{ST}$ ranged from 0.000 to 0.287 (Table 2.2), indicating generally low genetic differentiation overall. Wellington Te Papa had the lowest mean $F_{ST}$ (0.025) while the highest mean $F_{ST}$ was found for Mount Keira (New South Wales, NSW) Australia (0.1219). Among all pairwise comparisons, the highest $F_{ST}$ (0.287) was seen for Pirongia (North Island, New Zealand) versus Mt Keira (NSW, Australia). Within the islands of New Zealand, mean $F_{ST}$ was 0.025 (North Island) and 0.012 (South Island), while mean $F_{ST}$ between all populations in the North Island versus all populations in the South Island was 0.080. Mean $F_{ST}$ between New Zealand and Australia populations was four-fold higher (0.124) and ranged from 0-0.264 within Australian populations (Table 2.2).

The PCA was consistent with the $F_{ST}$ results, with individuals from Australia forming two main clusters distinct from New Zealand and no clear separation between the North and South islands (Figs. 2.2, A2.1). The Australian samples in the upper cluster corresponded to

Mt Kiera and Jervis bay, while the lower cluster of individuals corresponded to NSW (Blackheath and Jervis Bay), and VIC (Seaford) (Fig. A2.2).

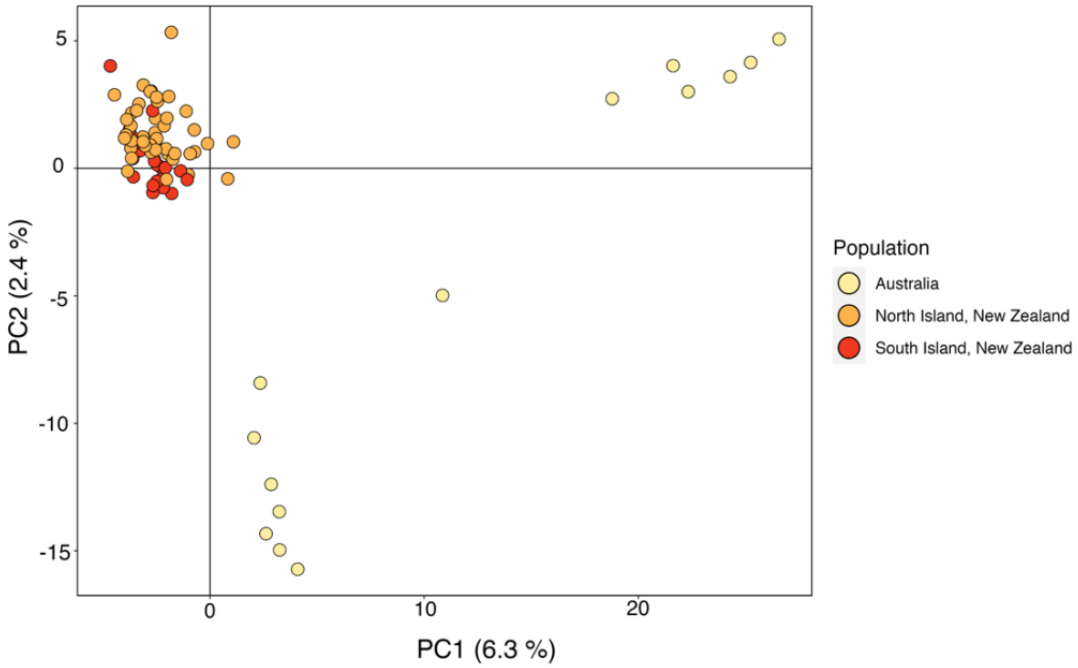


**Figure 2.2**. PCA plot showing *Calliphora hilli* samples (neutral dataset: 16,636 SNPs), with samples coloured by geographic location, as indicated by the key.

Admixture analysis confirmed the genetic differentiation between New Zealand and Australian populations of *C. hilli*: for the optimal K-value (K = 2), Australian populations mainly consisted of one genetic group (light blue colour in top panel of Figs. 2.3; A2.3) while New Zealand populations showed two genetic ancestries and included admixture with the Australian group. New Zealand individuals showed no clear difference in admixture patterns between North and South islands. The admixture results for K = 3, 4, and 5 were consistent with K = 2 in that the New Zealand populations were discrete from the Australian ones and showed generally higher rates of admixture (Figs. 2.3; A2.3).

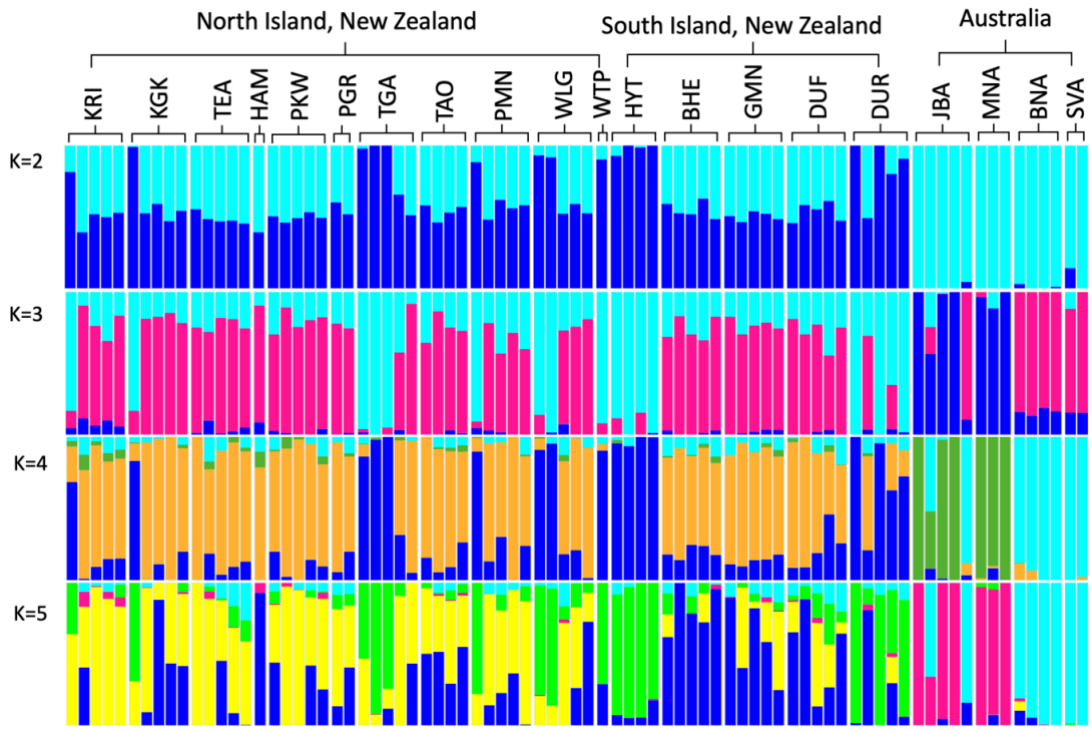


**Figure 2.3**. Admixture plots for *Calliphora hilli* (neutral dataset: 16,636 SNPs), where the optimal K value was determined to be K = 2. Admixture proportions showing K = 2-5 are presented, with populations in order from left to right corresponding to the top of the North Island, through to the bottom of the South Island of New Zealand, followed by Australia.

*Calliphora stygia*

Similar to *C. hilli*, Ho was low within all sampled populations, and was significantly lower than He (mean Ho = 0.118, range = 0.030-0.200, and mean He = 0.277, range = 0.160-0.310; $T_{48}$ = -20.053; $P < 0.001$), and there was no significant difference in mean Ho between New Zealand and Australian populations (mean Ho = 0.121 and 0.110, $T_{25}$ = 0.877; $P$ = 0.195) (Table 2.1).

Population specific $F_{ST}$ was low/moderate among *C. stygia* populations, ranging from 0.000 to 0.314 (Table 2.3). Hamilton again had the lowest mean $F_{ST}$ (0.002), while the highest mean $F_{ST}$ was found for Yarramundi (NSW, Australia; 0.219). Among all pairwise comparisons, the highest $F_{ST}$ (0.214) was found for Heapthy track (South Island, New

Zealand) versus Yarramundi (NSW, Australia). Within New Zealand's North and South islands, mean $F_{ST}$ was very low (North: 0.005, South: 0.050), while mean $F_{ST}$ between all populations in the North Island versus all populations in the South Island was 0.027. Australia and New Zealand showed approximately six times higher genetic variation, with a mean $F_{ST}$ of 0.075, and $F_{ST}$ among Australian populations was relatively high (> 0.204) for all comparisons that included Yarramundi (Table 2.3).

Supporting the $F_{ST}$ results, there was no clear signal of geographic structure within New Zealand's North and South islands for *C. stygia* in the PCA, though there were five outlier individuals from locations in the North and South islands in the bottom right PC space (Figs. 2.4, A2.4). Compared to *C. hilli*, there was less separation between New Zealand and Australian samples, though four outlier Australian individuals from Yarramundi were present in the upper left PC space (Fig. A2.5).

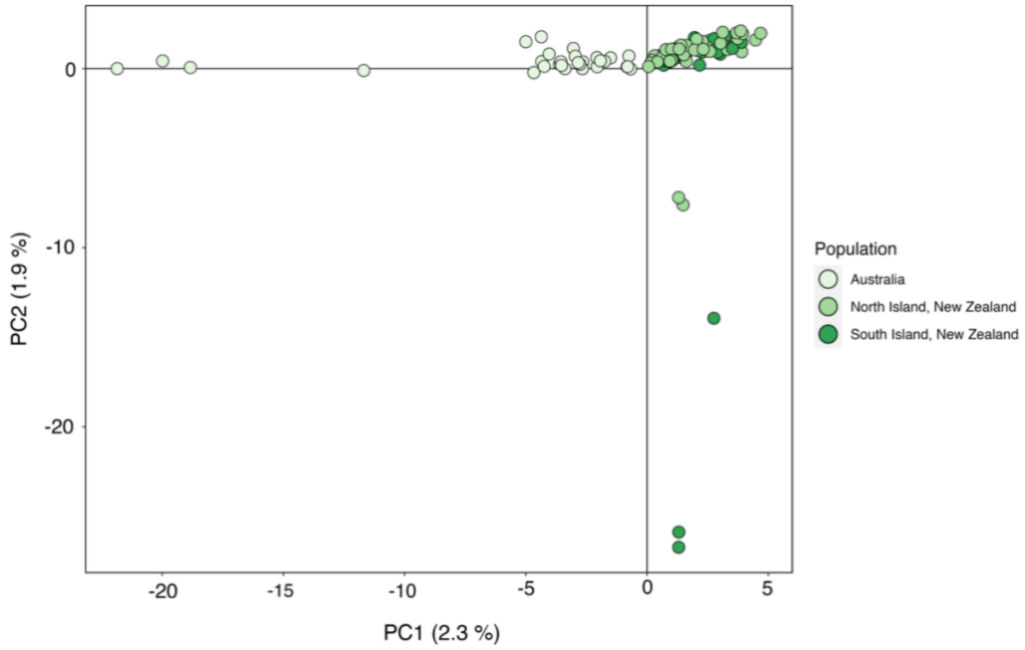


**Figure 2.4**. PCA plot showing *Calliphora stygia* samples from the neutral data set (12,841 SNPs), with samples coloured by geographic location, as indicated by the key.

The optimal K-value for the admixture analysis of *C. stygia* was K = 3. This showed a distinct Australian population from Yarramundi (NSW) – consistent with the $F_{ST}$ results – that was made up almost entirely from one genetic cluster (pink colour for K = 3 in Figs. 2.5; A2.6), which was present in lower proportions (< 0.40) for the rest of the Australian sampling sites. New Zealand and the remaining Australian populations showed signals of admixture, with all individuals sharing various degrees of ancestry from three genetic groups and a greater dominance of the dark blue genetic group in New Zealand versus Australian samples (with the latter showing greater dominance of the pink genetic group). As for *C. hilli*, there was no clear difference between patterns among North and South Island populations. Similar patterns can be observed in the K = 2, 4, and 5 plots, where Yarramundi was genetically distinct compared to the rest of Australia and New Zealand (Figs. 2.5; A2.6).

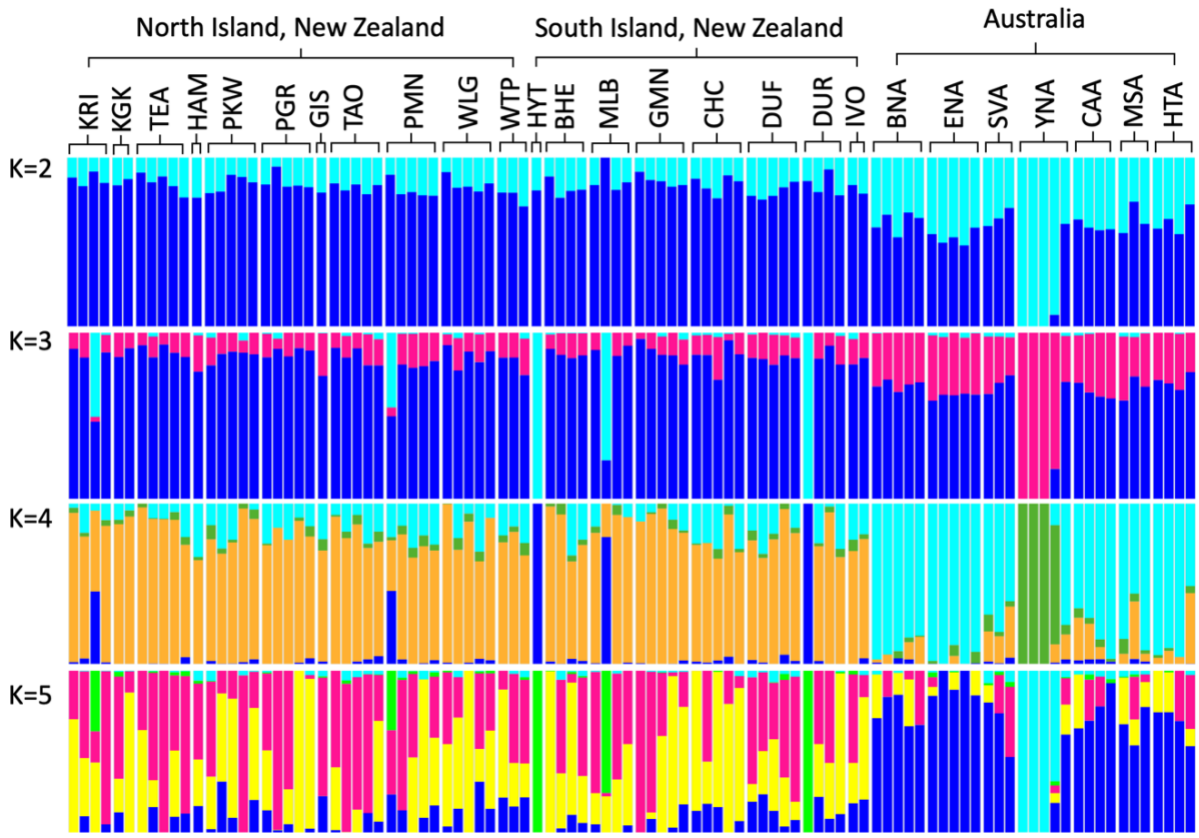


**Figure 2.5**. Admixture plots for *Calliphora stygia* were produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the neutral dataset containing 12,841 SNPs, where the optimal K value was determined to be K = 3. Admixture proportions showing K = 2 to K = 5 are presented, with populations in order from left to right corresponding to the top of the North Island, through to the bottom of the South Island of New Zealand, followed by Australia.

*Hybrid analyses*

The combined PCA for *C. hilli* and *C. stygia* showed an overall pattern of individuals generally clustering together within their 'pure' species group, however, several individuals from both species can be observed together in the centre of the plot. (Figs. 2.6, A2.7).

Similarly, the combined species admixture plot for K = 2 showed two distinct genetic groups corresponding to the two species, with small amounts of admixture highlighting

putative hybrids (Figs. 2.7, A2.8). Neither species showed clear differentiation between New Zealand and Australian samples at K = 2, however higher values of K resulted in differentiation of Australian and New Zealand samples for *C. hilli*.

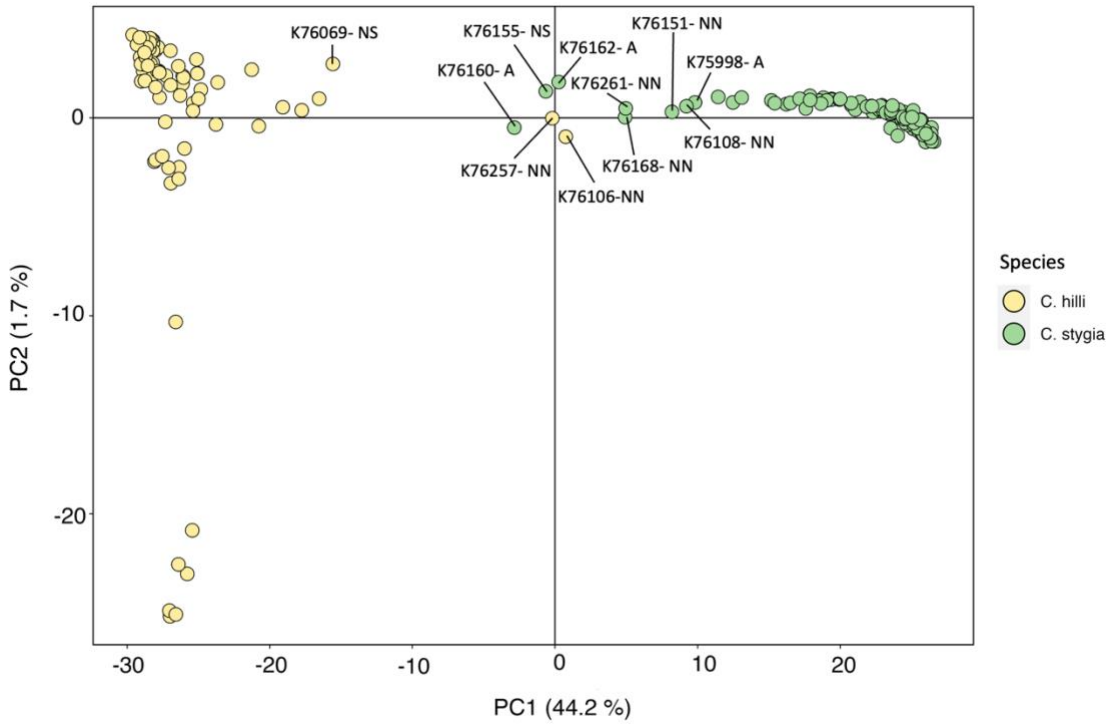


**Figure 2.6**. PCA plot showing *Calliphora hilli* and *Calliphora stygia* samples from the combined species neutral dataset (12,155 SNPs), including potential hybrids labeled by individual codes. Individuals are coloured by taxonomically identified species as per the key. '-A' in the individual code indicates Australian samples, '-NN' indicates New Zealand North Island, and '-NS' indicates New Zealand South Island samples.

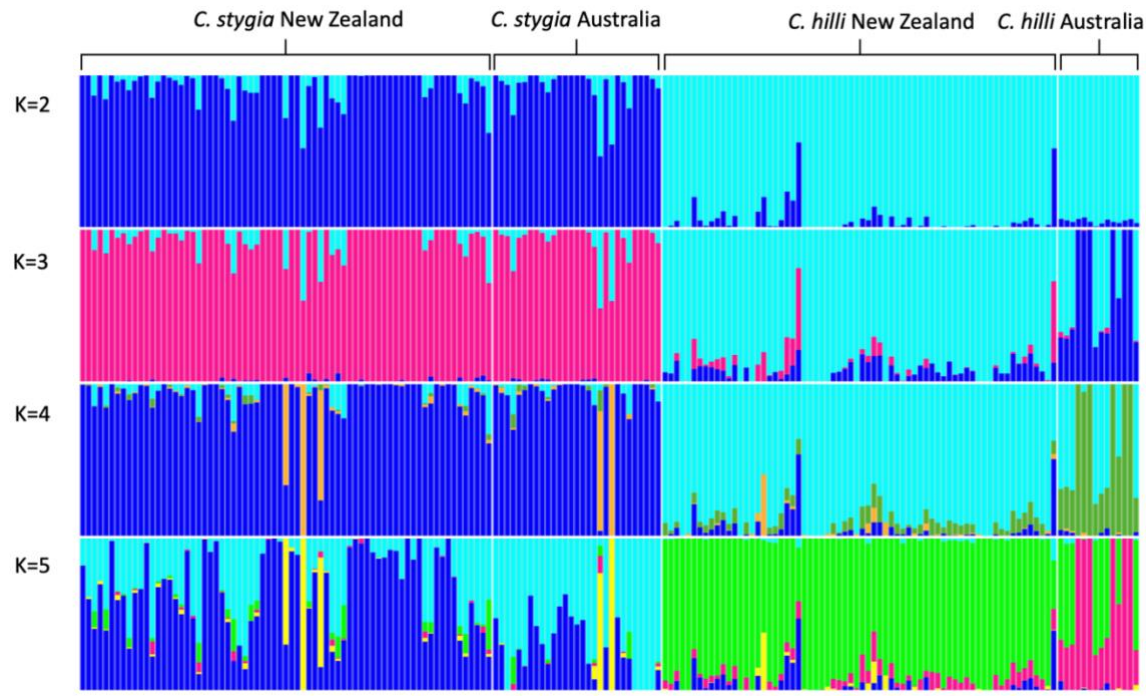


**Figure 2.7**. Admixture plot for the combined *Calliphora stygia* and *Calliphora hilli* dataset, produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the neutral dataset (12,155 SNPs). We examine K = 2 here, and show the results for other K-values in Figure A2.8.

Hybrid analysis using the 'gl.nhybrids' function from DartR identified 11 hybrid and backcrossed individuals for *C. hilli* and *C. stygia* samples (Figs. 2.8, A2.9), including three Australian individuals. In the plot, the first two columns provide examples of 'pure' *C. hilli* and *C. stygia* individuals, and K75998, K76108, and K76261 (taxonomically identified as *C. stygia*) showed > 0.99% ancestry to the noted species. However, the *C. hilli* individual K76257 (from Wellington, Te Papa) showed a hybrid genetic pattern, with 78% and 21% genetic contributions from *C. hilli* and *C. stygia*, respectively. Individual K76160 (Mt Crawford, South Australia) showed a hybrid/backcrossed genetic pattern, individual K76106 (Hamilton) was identified as an F2 hybrid, and the remaining individuals K76155, K76162,

K76168, and K76151 (all taxonomically identified as *C. stygia*) were classified as backcrossed (Figs. 2.8, A2.8).

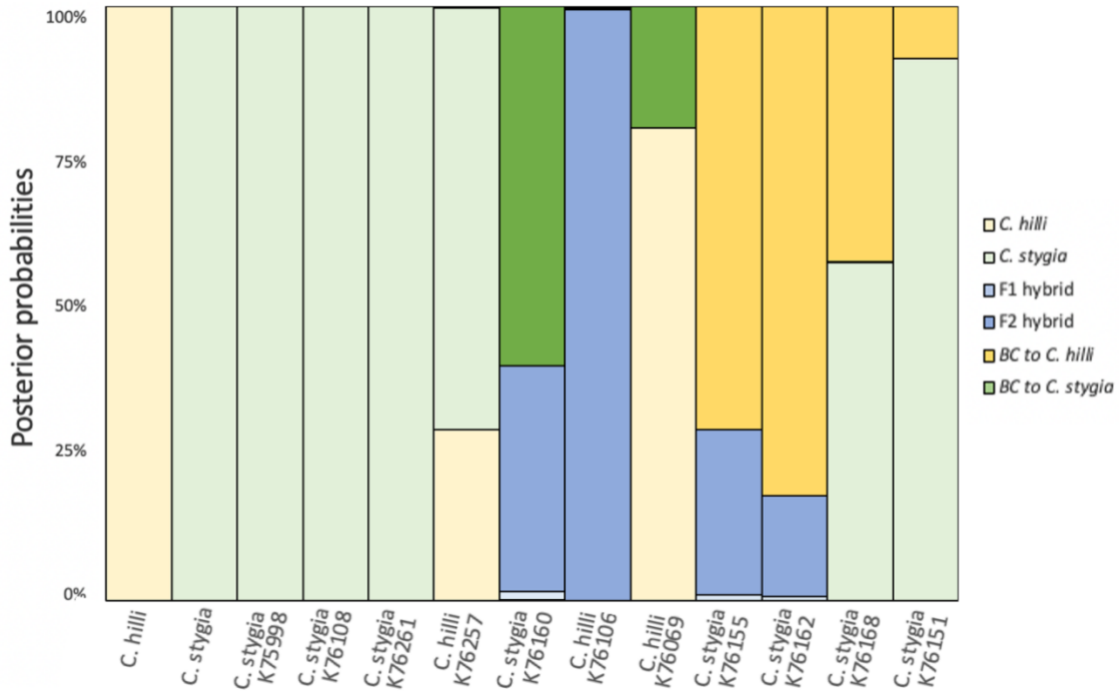


**Figure 2.8**. Hybrid analyses for the combined *Calliphora stygia* and *Calliphora hilli* neutral dataset (12,155 SNPs), indicating 'pure', hybrid, or backcrossed status of 13 individuals. F1 and F2 hybrids represent offspring from first- and second-generation crosses between *C. stygia* and *C. hilli,* respectively. BC to *C. hilli* and BC to *C. stygia* indicates first generation back-crossed individuals to the respective species. Each individual is labeled according to it's taxonomic identification, with individual codes corresponding to Table A2.1.

**Table 2.1**. Sampling information for *Calliphora hilli* and *Calliphora stygia*, including population names, population codes, GPS coordinates, population sample numbers and observed (Ho) and expected (He) heterozygosity. New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island. See Table A2.1 for further sampling details.

| **Population name** | **Population code** | **GPS Coordinates** | **No. samples** | | **Heterozygosity** | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ***C. hilli*** | ***C. stygia*** | ***C. hilli* – Ho** | ***C. hilli* – He** | ***C. stygia* – Ho** | ***C. stygia* – He** |
| Kerikeri | KRI | -35.2089, 173.9619 | 5 | 4 | 0.11 | 0.26 | 0.12 | 0.29 |
| Karangahake | KGK | -37.4343, 175.7255 | 5 | 2 | 0.10 | 0.27 | 0.12 | 0.29 |
| Te Aroha | TEA | -37.5386, 175.6932 | 5 | 5 | 0.08 | 0.25 | 0.11 | 0.31 |
| Hamilton | HAM | -37.7977, 175.2729 | 1 | 1 | 0.01 | -- | 0.20 | - |
| Kaniwhaniwha | PKW | -37.9339, 175.0777 | 5 | 5 | 0.08 | 0.27 | 0.11 | 0.29 |
| Pirongia | PGR | -37.9683, 175.1504 | 2 | 5 | 0.10 | 0.26 | 0.11 | 0.30 |
| Tauranga | TGA | -37.7327, 176.1799 | 5 | - | 0.13 | 0.26 | - | - |
| Gisborne | GIS | -38.6595, 178.0039 | - | 1 | - | - | 0.16 | - |
| Taranaki Oakura | TAO | -39.1157, 173.9522 | 4 | 5 | 0.09 | 0.26 | 0.11 | 0.29 |
| Palmerston North | PMN | -40.3785, 175.5866 | 5 | 5 | 0.11 | 0.26 | 0.13 | 0.28 |
| Wellington | WLG | -41.2950, 174.7989 | 5 | 5 | 0.12 | 0.26 | 0.12 | 0.28 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Wellington Te Papa | WTP | -41.2904, 174.7820 | 1 | 3 | 0.20 | - | 0.13 | 0.28 |
| Heaphy track | HYT | -41.0984, 172.5351 | 4 | 1 | 0.16 | 0.26 | 0.03 | - |
| Blenheim | BHE | -41.5075, 173.9299 | 5 | 4 | 0.09 | 0.26 | 0.12 | 0.29 |
| Marlborough | MLB | -41.9805, 173.6659 | - | 3 | - | - | 0.12 | 0.28 |
| Greymouth | GMN | -42.4646, 171.2029 | 5 | 5 | 0.09 | 0.25 | 0.11 | 0.29 |
| Christchurch | CHC | -43.5317, 172.5794 | - | 5 | - | - | 0.12 | 0.29 |
| Dunedin Fairfield | DUF | -45.9000, 170.3823 | 5 | 5 | 0.09 | 0.25 | 0.12 | 0.28 |
| Dunedin Ravensbourne | DUR | -45.8640, 170.5494 | 5 | 4 | 0.15 | 0.26 | 0.11 | 0.28 |
| Invercargill | IVO | -46.4361, 168.2832 | - | 2 | - | - | 0.14 | 0.28 |
| Jervis Bay, NSW Australia | JBA | -35.0925, 150.6187 | 5 | - | 0.06 | 0.18 | - | - |
| MtKeira, NSW Australia | MNA | -34.3970, 150.8534 | 3 | - | 0.06 | 0.14 | - | - |
| Blackheath, NSW Australia | BNA | -33.6144, 150.2683 | 4 | 5 | 0.07 | 0.21 | 0.11 | 0.27 |
| Echo Point, NSW Australia | ENA | -33.7296, 150.3116 | - | 5 | - | - | 0.10 | 0.27 |
| Seaford, NSW Australia | SVA | -33.7928, 151.2409 | 2 | 3 | 0.10 | 0.19 | 0.12 | 0.26 |
| Yarramundi, NSW Australia | YNA | -33.6563, 150.6617 | - | 5 | - | - | 0.10 | 0.16 |
| Canberra, Australia | CAA | -35.2803, 149.1310 | - | 4 | - | - | 0.11 | 0.25 |
| Mt Crawford, SA Australia | MSA | -34.7185, 138.9579 | - | 4 | - | - | 0.12 | 0.27 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Hobart, TAS Australia | HTA | -42.8829, 147.3264 | - | 4 | - | - | 0.11 | 0.28 |

**Table 2.2**. *Calliphora hilli* $F_{ST}$ values presented by population (population codes as referred to in Table 2.1); values in bold correspond to the mean $F_{ST}$ for each population. New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island.

| | KRI | KGK | TEA | HAM | PKW | PGR | TGA | TAO | PMN | WLG | WTP | HYT | BHE | GMN | DUF | DUR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KRI | **0.044** | | | | | | | | | | | | | | | |
| KGK | 0.004 | **0.029** | | | | | | | | | | | | | | |
| TEA | 0.015 | 0.008 | **0.054** | | | | | | | | | | | | | |
| HAM | 0.105 | 0.103 | 0.129 | **0.123** | | | | | | | | | | | | |
| PKW | 0.000 | 0.000 | 0.016 | 0.106 | **0.037** | | | | | | | | | | | |
| PGR | 0.000 | 0.000 | 0.015 | 0.145 | 0.000 | **0.054** | | | | | | | | | | |
| TGA | 0.012 | 0.002 | 0.006 | 0.097 | 0.013 | 0.031 | **0.053** | | | | | | | | | |
| TAO | 0.004 | 0.000 | 0.025 | 0.108 | 0.002 | 0.013 | 0.016 | **0.046** | | | | | | | | |
| PMN | 0.025 | 0.006 | 0.020 | 0.112 | 0.000 | 0.039 | 0.002 | 0.024 | **0.053** | | | | | | | |
| WLG | 0.024 | 0.000 | 0.025 | 0.113 | 0.000 | 0.008 | 0.000 | 0.008 | 0.000 | **0.048** | | | | | | |
| WTP | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | **0.025** | | | | | |
| HYT | 0.011 | 0.000 | 0.016 | 0.119 | 0.000 | 0.021 | 0.000 | 0.001 | 0.008 | 0.000 | 0.000 | **0.045** | | | | |
| BHE | 0.007 | 0.014 | 0.033 | 0.134 | 0.000 | 0.031 | 0.028 | 0.001 | 0.018 | 0.007 | 0.000 | 0.000 | **0.048** | | | |
| GMN | 0.045 | 0.039 | 0.014 | 0.128 | 0.015 | 0.001 | 0.056 | 0.026 | 0.032 | 0.031 | 0.000 | 0.004 | 0.018 | **0.058** | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DUF | 0.027 | 0.002 | 0.029 | 0.111 | 0.026 | 0.038 | 0.046 | 0.014 | 0.039 | 0.035 | 0.000 | 0.006 | 0.000 | 0.039 | **0.068** | |
| DUR | 0.027 | 0.014 | 0.054 | 0.091 | 0.004 | 0.048 | 0.037 | 0.019 | 0.027 | 0.015 | 0.000 | 0.009 | 0.024 | 0.021 | 0.220 | **0.071** |
| JBA | 0.168 | 0.190 | 0.199 | 0.139 | 0.194 | 0.191 | 0.213 | 0.201 | 0.215 | 0.208 | 0.091 | 0.200 | 0.188 | 0.209 | 0.207 | 0.225 |
| MNA | 0.211 | 0.000 | 0.233 | 0.272 | 0.204 | 0.287 | 0.230 | 0.221 | 0.231 | 0.240 | 0.285 | 0.248 | 0.245 | 0.254 | 0.243 | 0.256 |
| BNA | 0.101 | 0.097 | 0.104 | 0.151 | 0.092 | 0.091 | 0.117 | 0.114 | 0.122 | 0.107 | 0.058 | 0.115 | 0.096 | 0.111 | 0.124 | 0.136 |
| SVA | 0.059 | 0.069 | 0.089 | 0.178 | 0.039 | 0.069 | 0.094 | 0.080 | 0.082 | 0.093 | 0.043 | 0.101 | 0.072 | 0.068 | 0.079 | 0.114 |

| | JBA | MNA | BNA | SVA |
|---|---|---|---|---|
| KRI | | | | |
| KGK | | | | |
| TEA | | | | |
| HAM | | | | |
| PKW | | | | |
| PGR | | | | |
| TGA | | | | |
| TAO | | | | |
| PMN | | | | |
| WLG | | | | |
| WTP | | | | |
| HYT | | | | |
| BHE | | | | |
| GMN | | | | |

| | | | | |
|---|---|---|---|---|
| DUF | | | | |
| DUR | | | | |
| JBA | 0.172 | | | |
| MNA | 0.000 | 0.219 | | |
| BNA | 0.135 | 0.245 | 0.112 | |
| SVA | 0.101 | 0.264 | 0.003 | 0.089 |

**Table 2.3**. *Calliphora stygia* $F_{ST}$ values presented by population (population codes as referred to in Table 2.1); values in bold correspond to the mean $F_{ST}$ for each population. New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island.

| | KRI | KGK | TEA | HAM | PKW | PGR | GIS | TAO | PMN | WLG | WTP | HYT | BHE | MLB | GMN | CHC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KRI | **0.031** | | | | | | | | | | | | | | | |
| KGK | 0.000 | **0.037** | | | | | | | | | | | | | | |
| TEA | 0.000 | 0.002 | **0.028** | | | | | | | | | | | | | |
| HAM | 0.000 | 0.000 | 0.000 | **0.002** | | | | | | | | | | | | |
| PKW | 0.000 | 0.007 | 0.000 | 0.000 | **0.034** | | | | | | | | | | | |
| PGR | 0.004 | 0.009 | 0.000 | 0.000 | 0.018 | **0.032** | | | | | | | | | | |
| GIS | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | **0.010** | | | | | | | | | |
| TAO | 0.000 | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 | 0.019 | **0.034** | | | | | | | | |
| PMN | 0.000 | 0.009 | 0.000 | 0.000 | 0.005 | 0.002 | 0.000 | 0.023 | **0.032** | | | | | | | |
| WLG | 0.000 | 0.004 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | **0.037** | | | | | | |
| WTP | 0.038 | 0.026 | 0.009 | 0.000 | 0.000 | 0.007 | 0.002 | 0.009 | 0.027 | 0.013 | **0.039** | | | | | |
| HYT | 0.166 | 0.231 | 0.186 | 0.000 | 0.188 | 0.190 | 0.000 | 0.195 | 0.170 | 0.211 | 0.275 | **0.186** | | | | |
| BHE | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.013 | 0.222 | **0.029** | | | |
| MLB | 0.000 | 0.000 | 0.014 | 0.001 | 0.000 | 0.003 | 0.069 | 0.000 | 0.000 | 0.008 | 0.022 | 0.153 | 0.000 | **0.033** | | |
| GMN | 0.004 | 0.022 | 0.006 | 0.000 | 0.000 | 0.009 | 0.000 | 0.017 | 0.024 | 0.027 | 0.003 | 0.199 | 0.000 | 0.016 | **0.036** | |
| CHC | 0.000 | 0.019 | 0.000 | 0.000 | 0.015 | 0.003 | 0.000 | 0.001 | 0.004 | 0.015 | 0.017 | 0.200 | 0.000 | 0.026 | 0.005 | **0.036** |
| DUF | 0.020 | 0.025 | 0.002 | 0.005 | 0.000 | 0.012 | 0.004 | 0.017 | 0.022 | 0.028 | 0.024 | 0.199 | 0.000 | 0.000 | 0.000 | 0.014 |
| DUR | 0.012 | 0.000 | 0.006 | 0.000 | 0.004 | 0.022 | 0.000 | 0.013 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 | 0.033 | 0.026 |
| IVO | 0.010 | 0.002 | 0.002 | 0.000 | 0.000 | 0.000 | 0.017 | 0.000 | 0.016 | 0.004 | 0.002 | 0.273 | 0.000 | 0.002 | 0.000 | 0.000 |
| BNA | 0.059 | 0.049 | 0.057 | 0.000 | 0.042 | 0.060 | 0.000 | 0.067 | 0.042 | 0.046 | 0.039 | 0.213 | 0.023 | 0.038 | 0.067 | 0.055 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ENA | 0.080 | 0.071 | 0.079 | 0.000 | 0.076 | 0.079 | 0.013 | 0.082 | 0.066 | 0.082 | 0.058 | 0.201 | 0.063 | 0.069 | 0.092 | 0.090 |
| SVA | 0.031 | 0.034 | 0.036 | 0.000 | 0.030 | 0.024 | 0.019 | 0.028 | 0.030 | 0.057 | 0.043 | 0.243 | 0.039 | 0.019 | 0.032 | 0.032 |
| YNA | 0.237 | 0.267 | 0.210 | 0.032 | 0.203 | 0.202 | 0.084 | 0.222 | 0.254 | 0.252 | 0.226 | 0.314 | 0.246 | 0.244 | 0.238 | 0.245 |
| CAA | 0.063 | 0.065 | 0.000 | 0.000 | 0.057 | 0.061 | 0.010 | 0.071 | 0.046 | 0.074 | 0.067 | 0.245 | 0.055 | 0.062 | 0.056 | 0.063 |
| MSA | 0.011 | 0.025 | 0.041 | 0.012 | 0.023 | 0.037 | 0.000 | 0.039 | 0.019 | 0.045 | 0.032 | 0.153 | 0.017 | 0.038 | 0.033 | 0.045 |
| HTA | 0.043 | 0.042 | 0.049 | 0.000 | 0.188 | 0.047 | 0.013 | 0.049 | 0.037 | 0.054 | 0.031 | 0.208 | 0.036 | 0.042 | 0.013 | 0.034 |

| | DUF | DUR | IVO | BNA | ENA | SVA | YNA | CAA | MSA | HTA |
|---|---|---|---|---|---|---|---|---|---|---|
| KRI | | | | | | | | | | |
| KGK | | | | | | | | | | |
| TEA | | | | | | | | | | |
| HAM | | | | | | | | | | |
| PKW | | | | | | | | | | |
| PGR | | | | | | | | | | |
| GIS | | | | | | | | | | |
| TAO | | | | | | | | | | |
| PMN | | | | | | | | | | |
| WLG | | | | | | | | | | |
| WTP | | | | | | | | | | |
| HYT | | | | | | | | | | |
| BHE | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MLB | | | | | | | | | | |
| GMN | | | | | | | | | | |
| CHC | | | | | | | | | | |
| DUF | **0.037** | | | | | | | | | |
| DUR | 0.020 | **0.026** | | | | | | | | |
| IVO | 0.004 | 0.000 | **0.025** | | | | | | | |
| BNA | 0.017 | 0.060 | 0.015 | **0.049** | | | | | | |
| ENA | 0.086 | 0.096 | 0.019 | 0.036 | **0.070** | | | | | |
| SVA | 0.048 | 0.000 | 0.032 | 0.027 | 0.033 | **0.044** | | | | |
| YNA | 0.244 | 0.265 | 0.181 | 0.204 | 0.200 | 0.251 | **0.219** | | | |
| CAA | 0.050 | 0.050 | 0.048 | 0.003 | 0.041 | 0.003 | 0.230 | **0.067** | | |
| MSA | 0.043 | 0.018 | 0.007 | 0.000 | 0.018 | 0.012 | 0.208 | 0.004 | **0.037** | |
| HTA | 0.051 | 0.015 | 0.000 | 0.016 | 0.030 | 0.000 | 0.217 | 0.245 | 0.042 | **0.060** |

## 2.5 Discussion

Using genome-wide SNP data and a variety of population genomic analyses, we have shown that population genetic structure and connectivity differs among New Zealand and Australian populations of *C. hilli* and C. *stygia*, and that hybridisation is occurring at detectable rates among wild populations in the invasive (New Zealand) and native (Australia) range.

The timing of invasion is an important factor in determining how an invasive species may impact native biodiversity. For example, there is often a lag time that occurs immediately after invasion in which the invasive species has little impact before a burst in population growth (Sakai *et al*, 2001). *C. hilli* and *C. stygia* are thought to have invaded New Zealand in the late 1700s / early 1800s, however, their morphological similarity casts some doubt on whether they invaded simultaneously or consecutively (Dear, 1986). Our analysis supports *C. hilli* as entering New Zealand prior to *C. stygia*, with Australian and New Zealand *C. hilli* more genetically distinct (e.g., higher $F_{ST}$ and higher percent variation on the PCA) from each other than their *C. stygia* counterparts, suggesting a more ancient separation. Sample size was not uniform among Australian sites in our study, with *C. hilli* represented by 14 samples from four Australian sites, and *C. stygia* represented by 28 samples and seven sites. Nevertheless, *C. hilli* showed a stronger genetic separation between the North and South islands of New Zealand, while geographical structure within New Zealand was more limited for *C. stygia*; this further supports an earlier arrival of *C. hilli* populations, with the pairwise $F_{ST}$ and PCA analyses for both species consistent with this hypothesis.

Another factor important in driving invasion success is the genetic process of hybridisation and the resulting gene flow. We found that New Zealand populations of *C. hilli* had higher admixture levels when compared to the Australian populations, while the dominant genetic group represented a greater proportion of the individuals' genetic ancestry for New Zealand versus Australian populations of *C. stygia*. This is consistent with *C. hilli*

and *C. stygia* having invaded New Zealand from Australia (and with *C. hilli* arriving earlier and/or experiencing greater post-invasion admixture) and hints that intraspecific hybridisation – following either a single or multiple incursions for each species – may have played a role in facilitating invasion for these species.

Australia and New Zealand have significantly different climates, hence post-invasion hybridisation leading to admixture within populations may have provided new combinations of genetic variants that promoted adaptive processes in the introduced range. Previous work has supported the role of admixture in driving success of colonising populations (Barker et al, 2018; Blumenfeld *et al*, 2021; Bras *et al*, 2022), including when selection favors locally adapted genotypes (Rius and Darling, 2014). For example, the salt marsh grass *Spartina alterniflora* spread rapidly into China from the southern Atlantic coast and the Gulf of Mexico, with hybrids in the invasive populations having enhanced fecundity, plant height, and shoot regeneration (Qiao *et al,* 2019). Similarly, population admixture led to superior reproductive ability, and hunger and cold tolerance under laboratory conditions in the predatory ladybird *Cryptolaemus montrouzieri* (Li *et al*, 2018). Future work using full genome resequencing data should investigate the potential impacts of post-invasion intraspecific admixture in these two blowflies to provide greater understanding of this phenomenon, while broader sampling of the Australian meta-population will help to determine the number of incursion events to New Zealand.

We also found evidence – in the form of overlapping samples assigned to opposite species in the PC space for our PCA analysis, admixture in the combined species admixture analysis, and identification of hybrid and backcrossed individuals in our hybrid analysis – that interspecific hybridisation is occurring in the wild between *C. stygia* and *C. hilli*. Reproduction between species has been shown to facilitate invasive success in a number of studies (e.g. Rius and Darling, 2014; Yamaguchi *et al,* 2018). For example, hybrid lineages of

the apple snails *Pomacea canaliculate* and *Pomacea maculata* in the invasive range in Malaysia acquired traits that significantly enhanced invasiveness via improved desiccation and cold tolerance (Kannan *et al*, 2021). Meanwhile, hybridisation between the sunflowers *Helianthus annuus* and *Helianthus debilis* (forming the natural hybrid *H.annuus ssp. texanus*) resulted in hybrid fitness exceeding that of control lines by up to 51% within seven generations in a common garden experiment (Mitchell *et al,* 2019). Of course, our hybrid analyses may have been affected by sample contamination during DNA extraction and/or sequencing. However, combined with previous knowledge about the propensity of both species to readily hybridise under laboratory conditions (Wallman and Adams, 1997; Monzu 1977), as well as their phylogenetic and ecological closeness (Dear, 1986), our findings provide compelling support for interspecific hybridisation. The future work using full genome resequencing data suggested above would also provide useful data for a broader comparative genomics study that could look more closely at the key genetic traits resulting from hybridisation that may aid invasion in invertebrates.

Overall, our study has provided new insights into the population structure of two invasive blowflies, as well as the role of hybridisation in their respective evolutionary histories. In future, similar studies on other invasive invertebrates – especially those that differ in their degree of invasiveness and time since invasion – will enable broader advances in our understanding of how hybridisation acts in the wild to facilitate invasion. At the laboratory scale, the selective use of crosses and their associated fitness effects would help to further elucidate the adaptive impacts of hybridisation more generally.

## 2.6 Literature cited

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# Chapter 3.

## Population genetic diversity following bottlenecks in introduced species.

Image by: Nathan Butterworth, Monash University

To be submitted as: Croft, L., Butterworth, N., & McGaughran, A. (202x). Title and Journal to be decided.

Contributions: Genomics: NB assisted with blowfly identification, Chloe Flemming extracted the DNA, LC performed analyses and wrote the manuscript sections; Diversity lines: LC set up the reproductive crosses, measured the phenotypes, analysed the data, and wrote the manuscript. All: AM and NB provided assistance where required and helped edit the manuscript.

## 3.1 Abstract

Genetic variation is important for invasive species due to the potential increase in adaptive potential it can provide to facilitate population and species persistence. However, it is not uncommon for introduced populations to undergo demographic bottlenecks that cause a decrease in genetic diversity and associated reductions in population fitness. Investigating the effects of genetic diversity on population fitness for invasive species is therefore key to understanding how they may survive genetic bottlenecks in new environments.

We used Calliphorid blowflies to examine the effects of genetic diversity on population fitness in an invasive context. *Calliphora vicina* (Robineau-Desvoidy 1830) invaded New Zealand in 1889, where it is now widespread. We collected samples from across its distribution range and used 21,159 SNPs to investigate population genomic patterns, such as diversity, population structure, and admixture. We also explored the impacts of repeated bottlenecks on population fitness by producing high and low diversity lines in the laboratory and then measuring a variety of fitness traits.

We found low overall genetic diversity, patterns of genetic admixture, and some genetic differentiation between North and South Island New Zealand populations, with strong genetic links between the South Island and Australia. In our simulated laboratory bottlenecks, we found significant impacts of genetic diversity on fitness, with high diversity lines outcompeting low diversity lines for all measured traits.

Our research indicated that genetic bottlenecks reduce population fitness as expected, but provided intriguing insights into compensatory methods that may assist invasive species in overcoming this issue – collectively demonstrating the value of a tractable model system for investigating processes that may facilitate or hamper biological invasion.

## 3.2 Introduction

Invasive species are on the move across the globe, due to changing land and sea use, direct exploitation of organisms, climate change, pollution, and declines of native species (IPBES, 2023). These factors also interact, with climate change affecting the quality and quantity of ideal environments (e.g., increasing carbon dioxide and temperature levels, which can be more effectively utilised by invasive plants and pests, respectively; Bellard et al, 2018) – further facilitating significant increases in the number of species moving outside their native range (Finch *et al*, 2021).

As well as climatic factors promoting invasions, invasive species may come equipped with, or rapidly evolve, phenotypic traits that facilitate adaptive responses to new environments. For example, higher reproduction rates in invasive species can result in them rapidly outcompeting natives (Forman and Kesseli, 2003; Flores-Moreno *et al*, 2014; Allendorf and Lundquist, 2003). Genetic factors are also important, with genomic variation facilitating pre-invasion adaptation as species establish and spread in new environments (e.g., Tepolt *et al*, 2022; Battlay *et al,* 2023) and/or post-introduction adaptation in the form of beneficial *de novo* mutations that arise in the introduced range (e.g, Exposito-Alonso *et al*, 2018). Meanwhile, genetic processes, such as hybridisation and introgression, can promote invasion via the mixing of divergent lineages resulting in new sources of adaptive diversity (Popovic et al. 2021).

Genetic diversity in particular plays an important role in invasion by underscoring evolutionary potential to increase the likelihood of population and species persistence (Kardos et al., 2021; Ørsted et al., 2019). However, introduced populations often undergo demographic bottlenecks that decrease genetic diversity (Kanuch, 2020, Schrieber, 2016) and result in small population sizes that would often cause native species to reach extinction (Estoup, 2016). Bottleneck effects can lead to inbreeding depression, genetic drift, and

reduced responses to selection pressures (Schrieber, 2016), resulting in declines in population fitness as key alleles are lost (Markert, 2010). However, this 'genetic paradox of invasion', where invasive species flourish despite undergoing invasion bottlenecks, has been questioned as many invasive species do retain high diversity when measured at appropriate genetic markers (Estoup, 2016), while others thrive due to alternative aspects, such as propagule pressure, phenotypic plasticity, asexual reproduction, and hybridisation (Li, 2022). Investigating the effects of genetic diversity on population fitness for invasive species is key to understanding how such species thrive, yet such work requires the development of model-like systems where populations can be reared and genetically crossed under differing conditions, and a variety of fitness traits measured.

We generally expect both increasing inbreeding and reduced diversity after bottlenecks to impair fitness outcomes – e.g., by reinforcing deleterious mutations and/or leading to inbreeding depression, etc (Willoughby *et al*, 2017). However, in many invasive species and invertebrates (e.g., *Drosophila*), studies show that there can be benefits associated with inbreeding (Kokko and Ots, 2007), and low genetic diversity can still produce high phenotypic diversity and plasticity (David *et al*, 2005). Yet, studies explicitly comparing high and low diversity lineages of the same species, and the consequences of genetic bottlenecks on population fitness, remain rare (Markert *et al*, 2010).

Calliphorid blowflies are an ideal model for investigating invasive species due to their high biotic potential, excellent dispersal ability, and rapid adaptation to different environmental conditions (Oliveira and Vasconcelos, 2020). Alongside this, they are easily collected and reared in the laboratory (Norris, 1965). In New Zealand there are 54 species of Calliphoridae across seven genera, including *Calliphora, Lucilia, Hemipyrellia, Pollenia, Ptilonesia, Xenocalliphora,* and *Chrysomya.* Among these, *Calliphora vicina* (Robineau-Desvoidy 1830) is an invasive species that originates from Europe and was introduced to

New Zealand in 1889 (Dear, 1986). It is globally widespread and can be found on all continents except Antarctica, though it has reached the sub-Antarctic (Daly, 2023; Williams and Villet, 2006). Previous work has shown that *C. vicina* distributes geographically in response to temperature changes, indicating a strong role of climate change in its evolution and invasion success (Fuentes-Lopez, 2020; Henning, 2005). However, the main focus of research so far has been the fly's development and general biology – particularly with respect to its use in forensic entomology (Marchenko 2001; Defilippo and Bonilauri, 2013).

Here, we used the New Zealand invasive population of *C. vicina* to investigate population genomic patterns, including diversity, population structure, and admixture, from 21,159 SNPs. In addition, we used *C. vicina* as a model to explore the impacts of repeated genetic bottlenecks on population fitness by creating high and low diversity lineages and comparing their fitness across various phenotypic measures.

## 3.3 Methods

Population genetic analysis

*Sample collection, sequencing, and analyses*

Samples were collected by friends and colleagues who had been sent sampling kits and set-up instructions to use in their backyards in various locations across New Zealand. Sampling traps consisted of a modified bottle trap as per Hwang and Turner (2005). Traps were emptied daily and were left outside for 3-4 days. Emptying traps involved placing the upper bottle in the freezer to euthanize the flies, which were then placed into a 50 mL falcon tube containing 69% ethanol for postage back to the University of Waikato.

Alongside the New Zealand backyard sites, we obtained four samples from one location in Australia, resulting in a total of 59 samples from 16 sites (Fig. 3.1; Tables 3.1, A3.1). All specimens were identified to species level using the taxonomic key of Dear (1986).

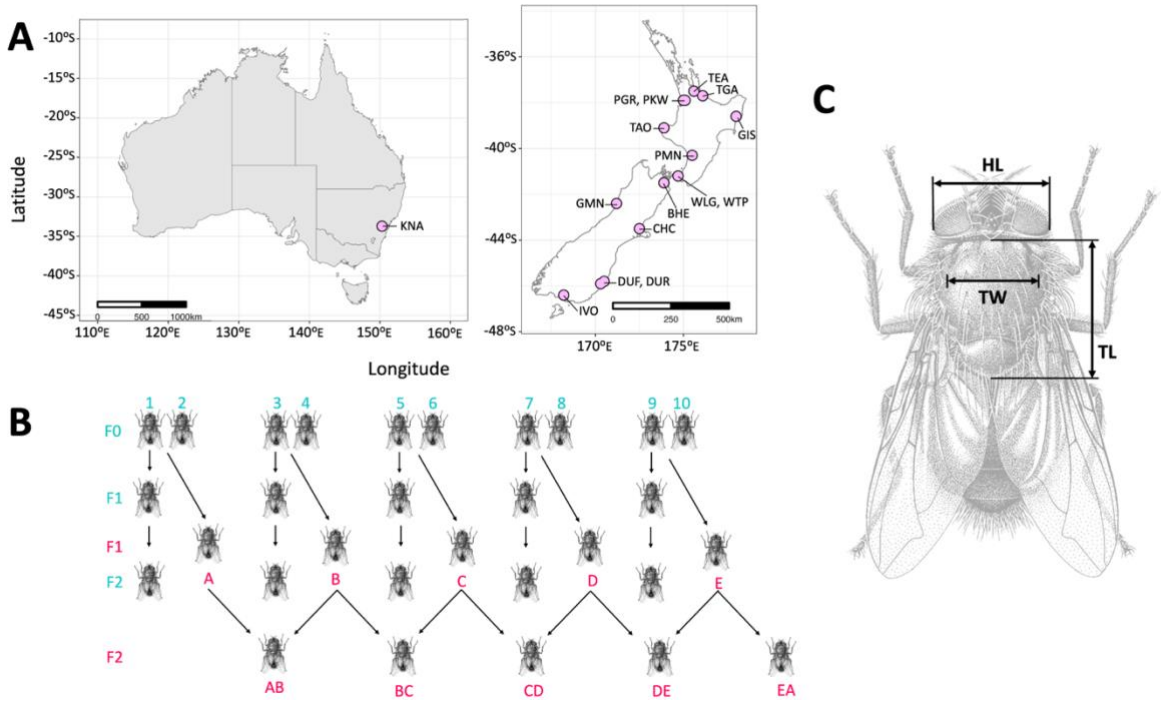



**Figure 3.1**. (A) Geographical maps showing sampling sites where *Calliphora vicina* was collected; (B) Schematic showing the use of reproductive crosses to generate high and low diversity lines to the F2 generation. Colours represent the subsequent diversity lines produced (C) Visualisation of body size traits measured: HL = head length, TL = thorax length, TW = thorax width. Blowfly image modified from Dear (1985).

DNA was extracted for 59 samples using a DNeasy Blood & Tissue Kit (Qiagen) and associated protocol, quantified using a Qubit fluorometer (Thermo Fisher Scientific), and sent to AgResearch Ltd (Christchurch, New Zealand) for genotyping-by-sequencing (GBS).

A single GBS library was constructed according to the methods outlined in Elshire *et al*. (2011), with modifications as outlined in Dodds *et al*. (2015). The GBS library was prepared using a PstI-MspI double-digest and included negative control samples (no DNA).

Libraries underwent a Pippin Prep (SAGE Science, Beverly, Massachusetts, United States) to select fragments in the size range of 220-340 bp (genomic sequence plus 148 bp of adapters). Single-end sequencing (1x101bp) was performed on a NovaSeq6000 using v1.5 chemistry.

Raw fastq files were quality checked using FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). IPYRAD v0.7.28 (Eaton and Overcast, 2020) was used to filter and remove low quality data, identify homology among reads through *de novo* assembly, make single nucleotide polymorphism (SNP) calls, and format output files for each species dataset. Reads were processed with the following non-default parameter settings: filter_adapters (2, where adapters were removed), filter_min_trim_len (60), and trim_reads (10, -140, 0, 0); and SNPs were exported in variant call format (VCF).

The VCF file was filtered using VCFTOOLS v0.1.13 (Danecek *et al*., 2011), with --missing-indv, --max-missing-count and --maf parameters applied to filter data with >98% missing data, 20% missing genotypes across all individuals, and a minor allele frequency cut-off of 5%. This resulted in a dataset of 21,159 SNPs.

Maps were created to visualise the geographic distribution of samples using the function map_data within the ggplot2 package v3.3.6; (Wickham, 2016) in R v4.3.0 (R Core Team, 2020). Population genetic diversity (heterozygosity) and differentiation ($F_{ST}$) were determined for each population and species using the hierfstat package v0.5-11 (Goudet, 2005) in R.

PCAs were conducted using the glPCA function implemented in the adegenet package v2.1.10 (Jombart, 2008) in R, and plotted using ggplot2. Admixture analyses were conducted by first converting the VCF file into geno format using the R package, LEA v3.6.0 (Frichot and Francois, 2015). The geno file was then input to the snmf function in LEA to produce a cross entropy plot to discover the optimal K value. The function qmatrix from the tess3r

package v1.1.0 (Caye, 2016) in R, along with ggplot, were used to produce an admixture barplot for each species. Using the meta function within the terra package (Hijmans R, 2023) in R, a new VCF file containing only neutral SNPs was created and both the PCA and admixture analyses were repeated on the neutral dataset. The neutral and non-neutral datasets produced consistent results; thus, we present the neutral plots in the main text and provide the non-neutral plots in Appendix A3.

**Table 3.1**. Sampling information for *Calliphora vicina*, including population names, codes, and GPS coordinates, and per-population sample numbers and heterozygosity. New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island. See Table A3.1 for further sampling details.

| **Population name** | **Population code** | **GPS coordinates** | **No. samples** | **Heterozygosity** | |
|---|---|---|---|---|---|
| | | | | **Ho** | **He** |
| Te Aroha | TEA | -37.5386, 175.6932 | 2 | 0.10 | 0.27 |
| Pirongia | PGR | -37.9683, 175.1504 | 4 | 0.08 | 0.25 |
| Kaniwhaniwha | PKW | -37.9339, 175.0777 | 5 | 0.08 | 0.25 |
| Tauranga | TGA | -37.7327, 176.1799 | 5 | 0.08 | 0.28 |
| Gisborne | GIS | -38.6595, 178.0039 | 3 | 0.09 | 0.25 |
| Taranaki Oakura | TAO | -39.1157, 173.9522 | 5 | 0.08 | 0.27 |
| Palmerston North | PMN | -40.3785, 175.5866 | 1 | 0.10 | - |
| Wellington | WLG | -41.2950, 174.7989 | 5 | 0.08 | 0.28 |
| Wellington Te Papa | WTP | -41.2904, 174.7820 | 3 | 0.11 | 0.25 |
| Blenheim | BHE | -41.5075, 173.9299 | 5 | 0.09 | 0.27 |
| Greymouth | GMN | -42.4646, 171.2029 | 4 | 0.09 | 0.26 |
| Christchurch | CHC | -43.5317, 172.5794 | 5 | 0.07 | 0.26 |
| Dunedin Fairfield | DUF | -45.9000, 170.3823 | 3 | 0.09 | 0.26 |

| | | | | | |
|---|---|---|---|---|---|
| Dunedin Ravensbourne | DUR | -45.8640, 170.5494 | 3 | 0.10 | 0.24 |
| Invercargill | IVO | -46.4361, 168.2832 | 1 | 0.11 | - |
| Katoomba,NSW Australia | KNA | -33.7118, 150.3118 | 4 | 0.08 | 0.22 |

Laboratory crossing experiments.

*Sample collection/F0 blowflies*

Wild *C. vicina* were caught in late 2022 at two sites approximately 36 km apart (PGR and PKW; Fig. 3.1A, Table 3.1). Trapping involved preparation of a bait, where beef mince was left outside to attract Calliphorid blowflies; after the flies laid eggs, larvae were left to feed on the meat for three days, then the container was tightly sealed and stored in the fridge before being taken to the field. At the field site, the bait was placed on grass and attracted flies were trapped using nets. A total of 16 female trapped individuals were caught, eight from each site; these are hereafter referred to as 'F0'.

Each singular female F0 individual was placed along with an F0 male in a breeding cage (494L x 322W x 258H mm; 27 L total volume), with access to 50 g of raw beef mince, raw sugar, and water in a room with 12:12hr light:dark cycling at 22 ℃. Once eggs were laid, an excess of meat was transferred to a container (750 mL) with a mesh lid, containing chaff as a pupariation material. The larvae had access to meat until they burrowed into the chaff to pupate. Once F1 flies began to emerge, cages were checked at least once every 24 h and individuals were separated by sex and placed into separate cages to prevent uncontrolled premature mating and maintain virgin status of males and females, as males and females do not reach maturity until approximately 2-3 days after eclosion (Butterworth et al. 2020). Male and female cages always had access to non-limiting amounts of sugar and water.

*Generating high and low diversity lines*

Five low genetic diversity lines were generated using repeated population bottlenecks, with mating among male and female siblings. Fifteen virgin F1 males and 15 virgin F1 females that had reached sexual maturity were placed in a single cage under the same environmental conditions described above to produce the F2 population; these lines were labeled '1', '2', '3', '4', and '5' (Fig. 3.1B).

Five high diversity lines were produced by crossing 15 F1 males and 15 F1 females that had reached sexual maturity, were unrelated, and were from different collection locations. Groups of 30 flies were placed together under the same environmental conditions as described above. These high diversity F1 flies were labeled 'A', 'B', 'C', 'D', and 'E' (Fig. 3.1B). To produce the F2 high diversity generation, 15 males and 15 females were crossed from the separate F1 lines to produce lines 'AB', 'BC', 'CD', 'DE', and 'EA' (Fig. 3.1B).

*Phenotyping*

Two weeks after the first F2 fly emerged from each high and low diversity line, 15 sibling males and 15 females were put into a cage together with constant access to beef mince as an oviposition medium. The meat was replaced every second day. For all lines, back-up F2 cages of separate males and females from the same line were maintained and used to replace dead individuals in the relevant main cage to maintain population density at n = 30 until the experiment's end. All phenotyping was performed for these main cages. *Fecundity*: Laid F3 eggs were counted daily under a microscope to measure the fecundity of the F2 flies. *Lifespan*: All cages were checked daily and the date each fly died was recorded, with overall lifespan recorded as the number of days from when the egg was laid until the adult died. *Developmental rate*: Progression through the life cycle (from the date eggs were laid, to the average dates of pupation and average emergence for each individual line) was measured in number of days. *Body size*: Three body size-related traits were measured for the first 30 F2 (15 males and 15 females) flies for each line, including head length (HL), thorax length (TL),

and thorax width (TW) (Fig. 3.1C). The three measures were then multiplied to produce an overall body size measurement for each fly. To ensure that measuring the first 15 males and females for each cage was representative of the entire pool (i.e., since individuals were replaced to maintain density; see above), we measured all flies (first 30 plus all replacements) for one high and low diversity line each, and examined the distribution of values for all flies versus the first 30 individuals. We found no significant difference between each dataset ($T_{3,26}$= 1.237, P = 0.108), hence proceeded with measuring body size for just the first 30 flies for all other lines. All body size measurements were performed using a binocular microscope with an ocular micrometer, and the resulting images were analysed using ImageJ v1.53t (Schneider *et al*, 2012).

*Data analysis*

Boxplots were created using ggplot2 package v3.3.6 (Wickham, 2016) in R v4.3.0 (R Core Team, 2020). To understand how life history traits were impacted by diversity, time, and/or sex, linear models were run on the various traits (fecundity, lifespan, body size, and developmental rate), with time (measured in days) or sex included as predictor variables, using the lm function in the base R stats package v3.6.2.

## 3.3 Results

Population genetic analysis

Observed heterozygosity (Ho) was low across all of the populations (range 0.07-0.11), and considerably lower than expected heterozygosity (He) (range 0.22-0.28). There was no significant difference in Ho or He between the New Zealand populations and the single Australian population (where n = 4).

Pairwise population $F_{ST}$ showed a range of genetic differentiation values (range 0-0.124, Table 3.2). Te Aroha had the lowest mean $F_{ST}$ (0.014), while Katoomba, Australia had

the highest (mean $F_{ST}$ = 0.092). The highest pairwise $F_{ST}$ among New Zealand populations was 0.109 (between Kaniwhaniwha in the North Island and Christchurch in the South Island), while the lowest was between Tauranga (North Island) and Invercargill (South Island) ($F_{ST}$ = 0.002). Within the New Zealand islands, mean $F_{ST}$ was 0.025 (North Island) and 0.020 (South Island), while mean $F_{ST}$ between all populations in the North Island versus all populations in the South Island was 0.035.

PCA analysis showed genetic differentiation between New Zealand's North and South islands, while the four Australian individuals clustered together with the South Island populations (Figs. 3.2A, A3.1A). Within New Zealand, there was clear separation between the North and South islands, with individuals from Wellington, Kaniwhaniwha, and Taranaki slightly distant from the main North Island cluster (Figs. 3.2B, A3.1B).

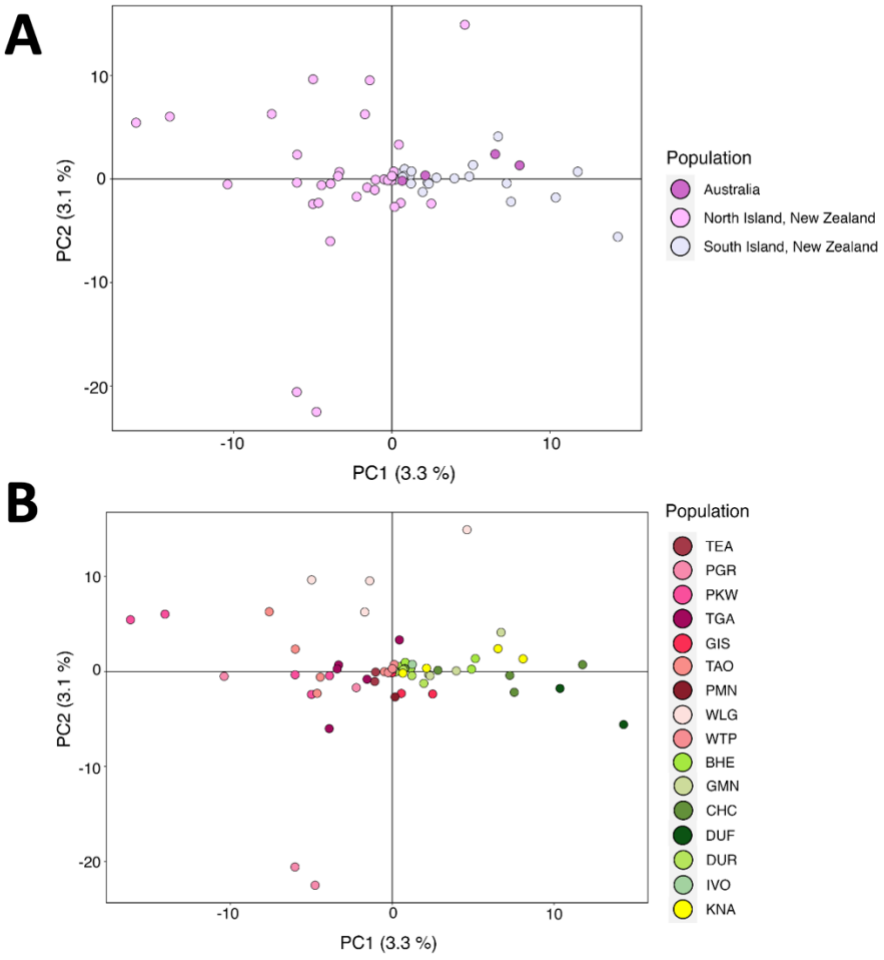



**Figure 3.2**. PCA plots showing *Calliphora vicina* samples (neutral data set: 21,159 SNPs) from: (A) broad regions (Australia, and North and South islands of New Zealand); and (B) populations.

Consistent with the $F_{ST}$ and PCA results, admixture plots showed differences in admixture between the North and South islands of New Zealand. Although the optimal K-value was one genetic cluster, results for K = 2 to K = 5 showed admixture among individuals, with differences between the North and South islands and the Australian population indistinguishable from the South Island (Figs. 3.3, A3.2).

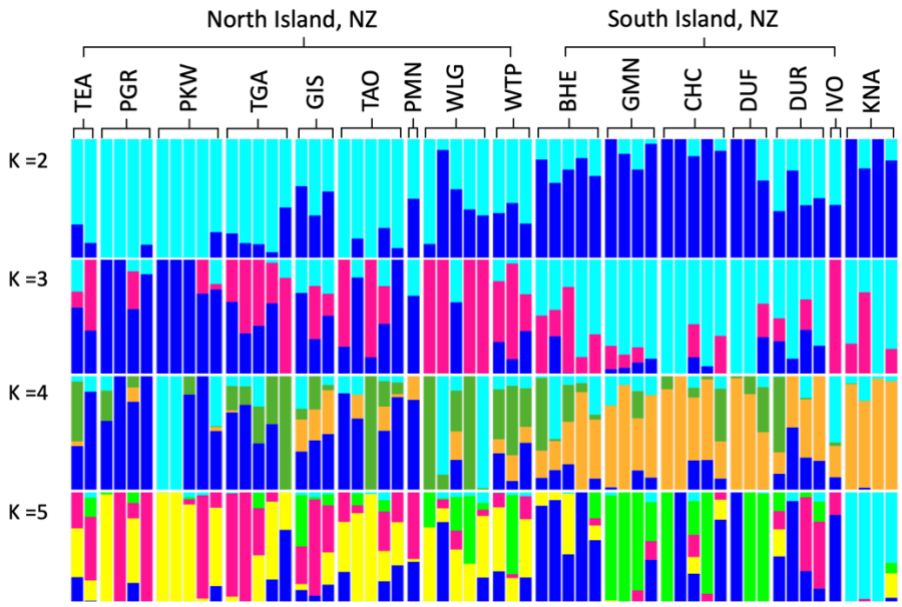



**Figure 3.3**. Admixture plots for *Calliphora vicina* were produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the neutral dataset containing 21,159 SNPs, where the optimal K value was determined to be K = 1. Admixture proportions showing K = 2 to K = 5 are presented, with populations in order from left to right corresponding to the top of the North Island, through to the bottom of the South Island of New Zealand, followed by Australia.

**Table 3.2**. *Calliphora vicina* $F_{ST}$ values presented by population (see Table 3.1 for population codes); values in bold correspond to the mean $F_{ST}$ for each population. New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island.

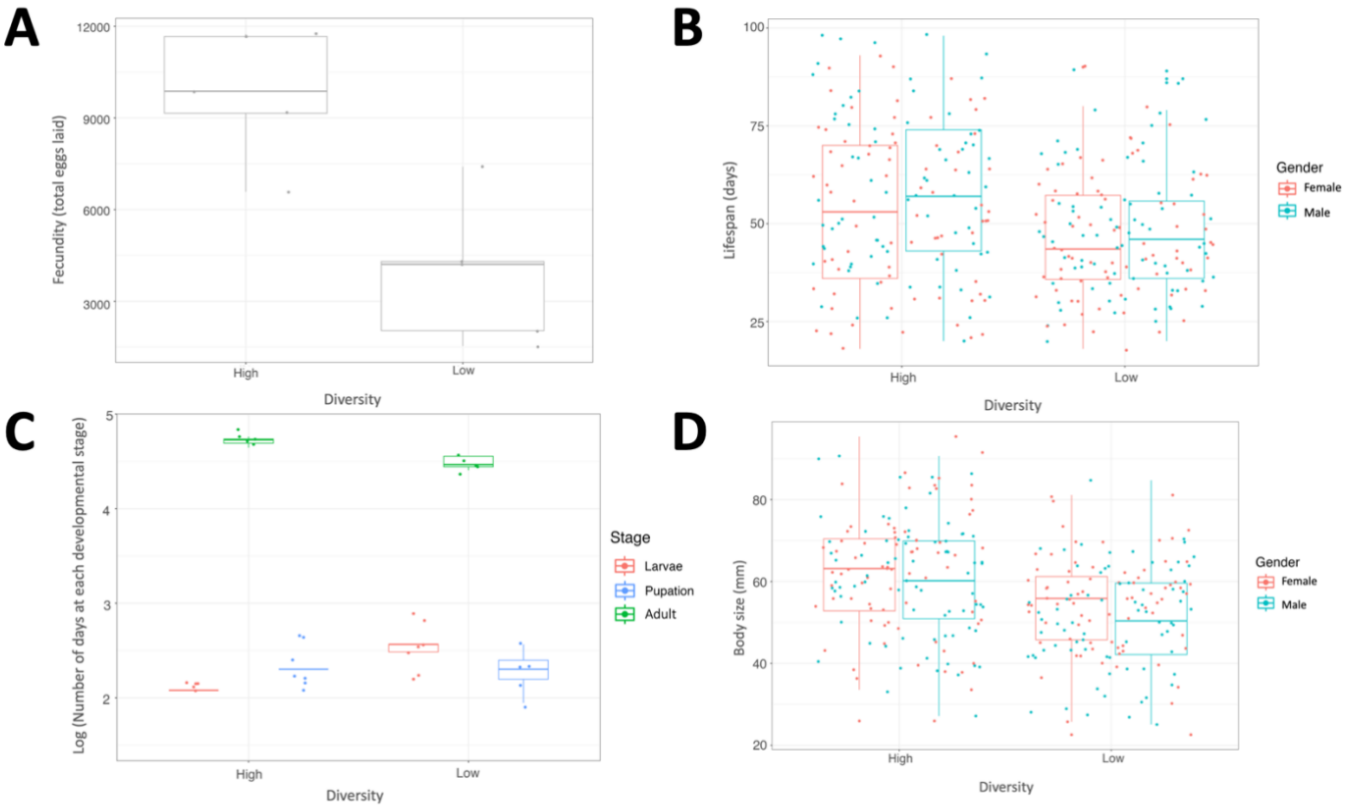
| | **TEA** | **PKW** | **PGR** | **TGA** | **GIS** | **TAO** | **PMN** | **WLG** | **WTP** | **BHE** | **GMN** | **CHC** | **DUF** | **DUR** | **IVO** | **KNA** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TEA** | **0.014** | | | | | | | | | | | | | | | |
| **PKW** | 0.015 | **0.063** | | | | | | | | | | | | | | |
| **PGR** | 0.015 | 0.063 | **0.052** | | | | | | | | | | | | | |
| **TGA** | 0.000 | 0.055 | 0.046 | **0.032** | | | | | | | | | | | | |
| **GIS** | 0.000 | 0.070 | 0.070 | 0.022 | **0.038** | | | | | | | | | | | |
| **TAO** | 0.000 | 0.055 | 0.048 | 0.027 | 0.033 | **0.033** | | | | | | | | | | |
| **PMN** | 0.014 | 0.014 | 0.033 | 0.009 | 0.013 | 0.014 | **0.025** | | | | | | | | | |
| **WLG** | 0.009 | 0.070 | 0.059 | 0.018 | 0.041 | 0.004 | 0.005 | **0.029** | | | | | | | | |
| **WTP** | 0.024 | 0.009 | 0.019 | 0.000 | 0.000 | 0.005 | 0.031 | 0.000 | **0.019** | | | | | | | |
| **BHE** | 0.003 | 0.081 | 0.051 | 0.015 | 0.037 | 0.026 | 0.023 | 0.015 | 0.006 | **0.029** | | | | | | |
| **GMN** | 0.013 | 0.101 | 0.060 | 0.045 | 0.032 | 0.045 | 0.049 | 0.016 | 0.012 | 0.024 | **0.039** | | | | | |
| **CHC** | 0.013 | 0.109 | 0.076 | 0.049 | 0.045 | 0.061 | 0.016 | 0.058 | 0.025 | 0.010 | 0.008 | **0.041** | | | | |
| **DUF** | 0.008 | 0.102 | 0.075 | 0.055 | 0.044 | 0.051 | 0.018 | 0.039 | 0.020 | 0.017 | 0.046 | 0.016 | **0.041** | | | |
| **DUR** | 0.014 | 0.040 | 0.027 | 0.037 | 0.015 | 0.027 | 0.039 | 0.018 | 0.034 | 0.024 | 0.012 | 0.017 | 0.000 | **0.027** | | |
| **IVO** | 0.013 | 0.047 | 0.030 | 0.002 | 0.017 | 0.004 | 0.000 | 0.000 | 0.025 | 0.023 | 0.032 | 0.022 | 0.034 | 0.022 | **0.024** | |
| **KNA** | 0.069 | 0.120 | 0.111 | 0.097 | 0.124 | 0.089 | 0.103 | 0.079 | 0.070 | 0.085 | 0.089 | 0.093 | 0.088 | 0.076 | 0.089 | **0.092** |

Laboratory crossing experiments.

*Fecundity*

Mean total fecundity was higher for the high (n = 9,801 eggs laid) versus low (n = 3,896 eggs) diversity lines (Fig. 3.4A). The fitted regression model was significant ($R^2 = 0.725$, $F_{3,80} = 70.23$; $P < 0.001$), with line diversity ($\beta = -218.722$; $P < 0.001$) and day ($\beta = -6.309$; $P < 0.001$) significantly predicting total fecundity, but their interaction non-significant ($\beta = 1.559$; $P = 0.080$) (Table 3.3). For the low diversity lines, > 1,000 eggs were laid on the first day of exposure to meat, and this was followed by stochastic smaller peaks for the first ~25 days, and a stabilistion in number of eggs laid per day thereafter (Fig. A3.3). In contrast, the high diversity lines showed a steady declining slope from an initial peak of around 500 eggs laid on Day 1, and a slight peak at Day ~47 that may correspond to a last effort to reproduce (mean lifespan of the high diversity females was ~56.2 days) (Fig. A3.3).

**Figure 3.4**. Differences among high and low diversity lines in various phenotypes: (A) Fecundity (total eggs laid by each line); (B) Lifespan (total time alive, from day eggs were laid to day of adult death) for males and females; (C) Log-transformed development rate (average number of days spent as larvae, pupae, and adults for each line); and (D) Overall body size (i.e., thorax width x thorax length x head length) in males and females.



*Lifespan*

Mean lifespan was higher for the high (56 days) versus low (47 days) diversity lines (Fig. 3.4B). The fitted regression model was weakly significant ($R^2 = 0.027$, $F_{3,296} = 2.703$; $P = 0.046$), but none of the individual predictors were significant (Table 3.3). Variability in lifespan was also higher for the high diversity lines (ranging from 120-136 days and from 104-121 days for high and low diversity lines, respectively; Fig. 3.4B). Mean male lifespan (58.6 and 48.4 days, for high and low diversity lines, respectively) was slightly higher than mean female lifespan (53.7 and 45.9 days, for high and low diversity lines, respectively) for both lines.

*Developmental rate*

The low diversity lines spent an average of 13 days (range 9-18) in the larval stage compared to the high diversity lines, which each spent exactly eight days (Fig. 3.4C). Both high and low diversity lines underwent pupation for an average of 10 days, while the high diversity lines had a significantly longer lifespan than the low diversity flies ($F_{1,2,26} = 5.039$; $P = 0.034$)

*Body size*

Mean overall body size was larger for high diversity (61.5 mm) compared to the low diversity lines (52.6 mm) (Fig. 3.4D). These differences were significant in the fitted regression model ($R^2 = 0.105$, $F_{3,296} = 11.63$; $P < 0.001$), with line diversity ($\beta = -7.297$; $P = 0.001$), but not sex, or the interaction between diversity and sex, significantly predicting body size (Table 3.3). For both lines, the interquartile ranges of the males and females overlapped, with males slightly smaller in both and no significant sex effects overall.

**Table 3.3**. Linear model results, showing model equations, coefficients, and ANOVA statistics, for each of the modeled traits.

| | **Coefficients** | | | | |
|---|---|---|---|---|---|
| **Trait** | **Response** | **Estimate** | **Standard error** | **T value** | **P value** |
| Fecundity | Equation | 509.1 – 218.7 (Diversity) – 6.309 (Day) + 1.559 (Diversity:Day) | | | |
| | Intercept | 509.1 | 29.15 | 17.465 | **< 2e-16** |
| | Diversity | -218.7 | 42.84 | -5.105 | **2.19e-06** |
| | Day | -6.309 | 0.552 | -11.43 | **< 2e-16** |
| | Diversity:Day | 1.559 | 0.879 | 1.774 | 0.079 |
| Lifespan | Equation | 72.13 – 3.278 (Diversity) + 4.947 (Sex) – 2.991 (Diversity:Sex) | | | |
| | Intercept | 72.13 | 2.138 | 33.74 | **<2e-16** |
| | Diversity | -3.278 | 3.013 | -1.088 | 0.278 |
| | Sex | 4.947 | 3.023 | 1.636 | 0.103 |
| | Diversity:Sex | -2.991 | 4.276 | -0.700 | 0.485 |
| Body size | Equation | 62.10 – 7.297 (Diversity) – 1.706 (Sex) – 2.431 (Diversity:Sex) | | | |
| | Intercept | 62.102 | 1.533 | 40.50 | **< 2e-16** |
| | Diversity | -7.297 | 2.161 | -3.376 | **0.001** |
| | Sex | -1.706 | 2.168 | -0.787 | 0.432 |
| | Diversity:Sex | -2.431 | 3.067 | -0.793 | 0.428 |

| | **ANOVA** | | | | | |
|---|---|---|---|---|---|---|
| **Trait** | **Response** | **Df** | **Sum of Squares** | **Mean Sum of Squares** | **F value** | **P value** |
| Fecundity | Diversity | 1 | 2,934 | 2,934 | 31.74 | **2.5e-07** |
| | Day | 1 | 1,625 | 1,625 | 175.7 | **< 2.2e-16** |
| | Diversity:Day | 1 | 2,908 | 2,908 | 3.145 | 0.079 |
| | Residuals | 80 | 7,396 | 9,245 | | |
| | Diversity | 1 | 1,718 | 1,718 | 5.013 | **0.025** |
| | Sex | 1 | 893 | 893 | 2.606 | 0.107 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lifespan | Diversity:sex | 1 | 168 | 167 | 0.489 | 0.484 |
| | Residuals | 296 | 1,014 | 167.7 | | |
| Body size | Diversity | 1 | 5,399 | 5,399 | 30.62 | **6.899e-08** |
| | Sex | 1 | 640 | 3.629 | 3.629 | **0.057** |
| | Diversity:sex | 1 | 111 | 110.8 | 0.628 | **0.428** |
| | Residuals | 296 | 5,218 | 176.3 | | |
| Developmental rate | Diversity | 1 | 270 | 270 | 5.039 | 0.033 |
| | Stage | 2 | 5,370 | 2,685 | 501.1 | **<2e-16** |
| | Residuals | 26 | 1,393 | 54 | | |

## 3.5 Discussion

This study provided new insights into the population structure and response to controlled population bottlenecks in wild-caught populations of *C. vicina* in New Zealand.

Using genome-wide SNP data and a range of population genomic analyses, we found some genetic differentiation between North and South Island populations of *C. vicina*, indicating a period of time in isolation post-invasion and/or the introduction(s) of individuals from multiple source populations to the different islands. The small number of Australian samples (n = 4) clustered together with the South Island populations, suggesting close genetic linkages between these populations that may be indicative of limited post-invasion genomic change in the South Island. However, the source of the New Zealand incursion cannot be determined in the current study, since we lack comparative samples from other areas across the full distribution range of *C. vicina.*

Previous studies have concluded that global spatial and demographic expansions of *C. vicina* were incredibly fast and likely assisted by human movements and the international livestock trade, making it likely that multiple invasions into some environments have facilitated its invasion success (Fuentes-Lopez, 2020). However, only mitochondrial diversity has been examined to date, revealing a large number of haplotypes with no geographic

structuring (Fuentes-Lopez, 2020). Here, we found low levels of observed heterozygosity. Similarly low genetic diversity in wild blowfly populations was observed in the blowfly *Chrysomya latifrons* over a wide expanse of New South Wales rainforests, though it is unknown whether this is tied to its adaptive capacity, as *C. latifrons* occurs in high abundance throughout its range (Butterworth *et al*, 2022). Low genomic diversity has also been observed in the Queensland fruit fly (*Bactrocera tryoni*) yet this species remains an incredibly invasive pest within Australian horticulture (Popa-Baez et al, 2020).

Investigating the direct impacts of genetic diversity on population fitness requires a tractable system. Here, we used laboratory crosses to establish high and low diversity lines of *C. vicina* to investigate the potential effects of genetic bottlenecks on fitness in the context of invasion. We found that high diversity lines outperformed low diversity lines for all measured traits, suggesting that pre-existing levels of genetic diversity may play an important role in invasion for this species by directly affecting fitness. This is consistent with a similar study done on the estuarine crustacean (*Americsmysis bahia*), where low and high genomic diversity lines were experimentally manipulated and lower genetic diversity was heavily associated with lower population fitness in both permissive and stressful environments (Markert *et al*, 2010). Durkee *et al*, 2023 performed a similar study exploring the effect of admixture in response to climate change in the flour beetle (*Tribolium castaneum*). Four populations with varying degrees of admixture were experimentally manipulated, ranging from completely inbred to high admixture (no inbreeding), and populations with higher admixture and genomic diversity had increased fitness in the form of surviving offspring (Durkee *et al*, 2023).

The size of adult *C. vicina* blowflies has previously been linked to the availability of nutrients from the meat source when in the larval stage, where gross overcrowding among the larvae can lead to reduced adult body size (Saunders et al, 1999). Here, the high diversity

replicate lines produced almost three times the number of adult flies compared to the low diversity lines, thus would be expected to have reduced body size as a result of significantly more larvae competing for the same amount of meat. However, surprisingly, the average overall body size of high diversity blowflies was almost 10 mm larger than that of average low diversity individuals. Thus, the effects of low diversity appear to have trumped potential resource limitation. Indeed, the effects of an ecosystem's biodiversity on its function can vary across time and space (Symstad and Tilman, 2003).

The excellent dispersal ability and broad environmental tolerance of blowflies likely facilitates the maintenance of genetic diversity following invasion, particularly if multiple incursions from divergent genetic sources are common. The implications of higher fitness associated with more diverse lines are considerable. For example, our developmental rate data indicated that high diversity replicates were in the larval stage for shorter periods (eight days, compared to 9-18 days in the low diversity lines). This pattern was also found for the adult stage, with high diversity adults living for an average of three weeks longer than their low diversity counterparts. Progression through development likely has major impacts on population turnover, affecting the time taken to reach sexual maturity and produce eggs and/or the number of generations that can be progressed through in a single season (Roff, 2000). Indeed, the high diversity lines here were able to produce over two times the total number of eggs when compared to the low diversity lines. However, despite their lower overall fecundity, low diversity lines had laid roughly a third of their total egg capacity on the first day of being exposed to a meat source, while the high diversity females had a slow decline in the number of eggs laid per day over their entire lifespan. This suggests there may be some trade-off between development time and fecundity, where laying many eggs initially may counteract the additional time spent in development (Roff, 2000). In fact, despite the expected costs of inbreeding and bottlenecks, the low diversity populations still performed

reasonably well here (e.g., relative to the high diversity lines, their overall average lifespan was just nine days less, and they produced more offspring in the initial few days). Thus, though bottlenecks clearly have a negative impact on fitness, invasive species such as *C. vicina* may be so successful because they perform relatively well even in the face of bottlenecks. Testing the fitness impacts of genetic diversity under different challenges (e.g., varying spatial and temporal resources, and different temperature or other abiotic regimes), will allow researchers to tease apart this question further.

In sum, we investigated population genomic patterns, and the effects of genomic diversity on fitness, in the invasive blowfly *C. vicina*. We found genetic differentiation between New Zealand North and South Island populations and close genetic links between the South Island and Australian populations. The high genetic diversity lines we generated significantly outcompeted the low genomic diversity lines for all measured traits, consistent with expectations of invasion biology research. Our experimental manipulation of genomic diversity within invasive species demonstrates the value of a tractable study system in testing theoretical aspects of invasion success. Replications of these methods with additional invasive invertebrates is a key priority for future research to investigate how the impacts of genetic diversity on fitness may change with degree of invasiveness, age of invasion, and other factors of interest.

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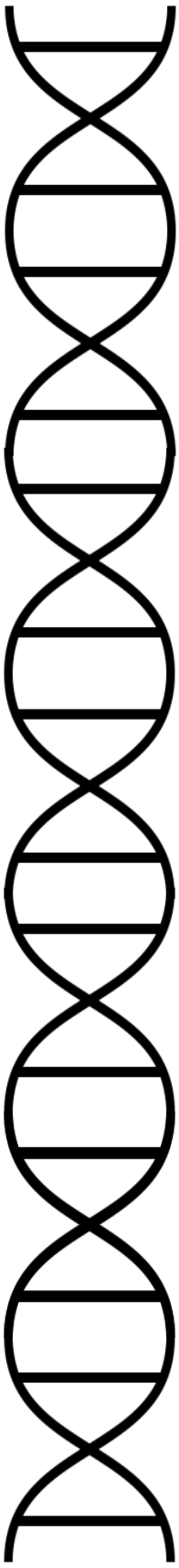

# Chapter 4.

## Discussion

## 4.1 General overview

My thesis aimed to provide insights into the population genomics and phenotypic responses at play within invasive Calliphorid blowflies from New Zealand and Australia.

In **Chapter 2**, I highlighted the role of hybridisation and gene flow in the evolutionary history and biological invasion to New Zealand from Australia of two invasive golden blowflies, *C. hilli* and *C. stygia*. These results were achieved using genome-wide single nucleotide polymorphism (SNP) data to perform a variety of population genetic analyses. From them, I have not only discovered that these two species have the ability to produce viable hybrid offspring in the wild but have also provided new insights into whether the invasions of these two species were simultaneous or consecutive.

In **Chapter 3**, I advanced knowledge into the effects of genetic diversity on invasion processes, using a specific example (*C. vicina*) to demonstrate how fitness traits are impacted in response to bottleneck effects commonly experienced when small populations invade new areas. I used a combination of population genomic analyses on genome-wide SNP data to demonstrate genetic differentiation between New Zealand populations of *C. vicina*, and laboratory crosses to replicate continuous bottleneck effects that are typically experienced by wild invasive species. These results provided new knowledge about the specific traits that are affected by decreases in diversity, along with potential mechanisms undertaken by the *C. vicina* to offset or combat these disadvantages.

## 4.2 Considerations and caveats

Invasion events are highly complex and not entirely understood. Thus, significantly more research needs to be done in this space to assist in the preparation and prevention of future invasion events that are inevitable in this current day. Evolutionary genetics can provide highly valuable data that allows significant insights into the invasion success of many highly

invasive species and their ability to respond to natural selection through changes in factors like genetic architecture, selection, and adaptation (Lee, 2002). Current research is heavily focused on the exploration of invasive species using population genetic analyses, but the accuracy and resolution of these studies could be significantly improved in certain cases by using whole genome sequencing (North *et al*, 2021). Future approaches will also benefit from developments in 'big data' analysis. For example, machine learning has emergent applications in evolutionary data analysis and can be used to recognise patterns of variation in the wild (Schrider and Kern, 2018).

Global access to previously studied genomic data through open access data sharing is significantly steering the progression of evolutionary and invasion genomics in the right direction. Open access data sharing provides collaboration that would otherwise not occur. For example, during the 2013 Ebola outbreak in Guinea researchers made three viral genomes public, which assisted significantly in the control of the outbreak and saved many lives due to the progression in treatments available (Yozwiak *et al,* 2015). Large genome sequencing consortium efforts are also expanding the taxonomic scope of publicly available data. For example, the Earth BioGenome project aims to sequence all eukaryotic genomes by 2030 (Lewin *et al*, 2022). Currently in GenBank (a well-known DNA data depository), there are over 4,000 animal genome assemblies available spanning 24 phyla. This availability in data is a significant breakthrough for the study of invasive species however, currently there is a domination of sequenced vertebrates compared to invertebrates (Hotaling *et al,* 2021). Open access data sharing also often does not take indigenous values and beliefs into account. Addressing the indigenous values associated with genomic data is a particularly challenging area for invasive species, as many communities want to keep control over their environment, animals, and plants, but invasives span multiple environments where crossover with indigenous cultures and values may need to be considered (Hudson et al, 2020).

Invasive species are a highly pertinent issue in New Zealand, with >30 mammals, 34 birds, 2,000 invertebrates, and 2,200 plants invading from other continents (Norton, 2009). New Zealand has successfully eradicated invasive predators from 10% of offshore islands, and has set a highly ambitious goal of having the entire country predator free by 2050 (Russell *et al*, 2015). Understanding the mechanisms that drive or hinder invasion success, using both ecological and genomic approaches as demonstrated here, will provide important information to assist in management initiatives such as this.

## 4.3 Recommendations

A major finding from **Chapter 2** was that the *C. hilli* and *C. stygia* have the ability to hybridise in the wild, ultimately causing more admixture within the species and likely providing them with the mechanisms to become highly successful invaders due to new combinations of successful genes. Hybridisation has been a driver of success in many invasive populations, such as the salt marsh grass and the ladybird (Qiao *et al,* 2019; Li *et al*, 2018). In Chapter 2, the two species under study were identified with a microscope following taxonomic keys, and this process could have been improved using genetic barcoding. Genetic barcoding is a method of taxonomic identification using DNA sequence data; however it is estimated that only ~15% of animal species have barcoded sequences available to the public (deWaard et al, 2019). Genetic barcoding is reasonably challenging when being used for invertebrates, due to their vast numbers and high degree of genomic diversity (Evans and paulay, 2012). In future, development of genomic resources, including both barcodes and whole genome sequencing data alongside open access data sharing, will assist in future studies — especially those on morphologically similar species.

Hybridisation and interspecific admixture can be advantageous to a potentially invasive species when entering a new area, and whole genome sequencing could also be used

in the future to explore the impacts of this phenomenon in other blowfly species. In this study, I concluded that both *C. hilli* and *C. stygia* had come to New Zealand from Australia however, we lacked uniform sample sizes from the Australian populations. Thus, future studies should aim to examine hybridisation and admixture processes using more samples from the native range to increase overall accuracy and confidence in the results of the invasive route undertaken by these blowflies.

My findings from **Chapter 3** provided valuable understanding of the population structure and genomic and phenotypic responses to bottlenecks that are often experienced by small populations moving to new geographical areas. Sequencing of samples at the beginning and end of the experiment to confirm diversity changes and potentially identify other genomic signals associated with genetic bottlenecks (e.g., loss of specific alleles) was outside the scope of the current study but would be very interesting for future work. My research supported the idea that bottleneck effects negatively impact low diversity populations due to genetic drift and the associated loss of specific genetic variants. However, although the low diversity lines had a significantly longer development time, resulting in less time to reproduce, they laid a significant proportion of their eggs immediately upon exposure to a meat source. This is an exciting finding that may indicate tradeoffs between developmental rate and fecundity that should be investigated further. In addition, it would be interesting to compare the responses of high and low genetic diversity lines to other phenotypic traits, such as heat tolerance and desiccation resistance, given that invasive species often have broad tolerance to a range of abiotic factors and/or may be expected to evolve these in response to climate change (Silva et al, 2021).

This study was the first experimental laboratory colony of high and low diversity lines to be set up for *C. vicina* in the Invasomics Lab (University of Waikato) and because of this, improvements were identified during/after the process. For example, during the

fecundity tests for each line, dead flies were replaced with back-ups from the same line in order to keep the fecundity cage at a constant population density. An alternative would have been to not replace dead flies, measuring fecundity only for the original 30 flies, which would have allowed an estimation of extinction rates. Future work should investigate extinction among high and low diversity lines.

Finally, this study benefited from samples being collected by friends and colleagues, who undertook sampling in their backyards. This let me skip the time and expense associated with travelling, as well as letting work proceed when travel restrictions were in place due to COVID-19. Due to the incredibly fast spread of invasive species, future work should involve more citizen scientists, who can help to increase the rate of biosecurity responses (Matheson, 2023), as well as engage directly with conservation outcomes.

## 4.4 Conclusion

My thesis showed how DNA sequencing and experimental manipulation of the invasion process can together provide new insights relevant to the prediction and prevention of future biological invasions. Population genomics analyses are an incredibly useful aspect of invasive species research, and their use and value will continue to grow as genomic resources and invasion rates each escalate. Using genomic data and population genomic analyses, I provided evidence that *C. hilli* and *C. stygia* are undergoing hybridisation in the wild, that there are high levels of admixture occurring within invasive populations, and that the New Zealand populations most likely invaded from Australia. Furthermore, I showed that genetic diversity in *C. vicina* has major consequences on population fitness that are likely important as species invade new habitats globally. This work has firmly established Calliphorid blowflies as a new model system for the Invasomics Lab, laying the foundation for future efforts to tease apart the ecological and genomic drivers of invasion success.

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# Appendix A2

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**Figure A2.1**. PCA of *Calliphora hilli* individuals plotted using the non-neutral dataset (18,324 SNPs) and coloured by island, according to the key. This plot is identical to the PCA for the neutral dataset (Fig. 2.2).

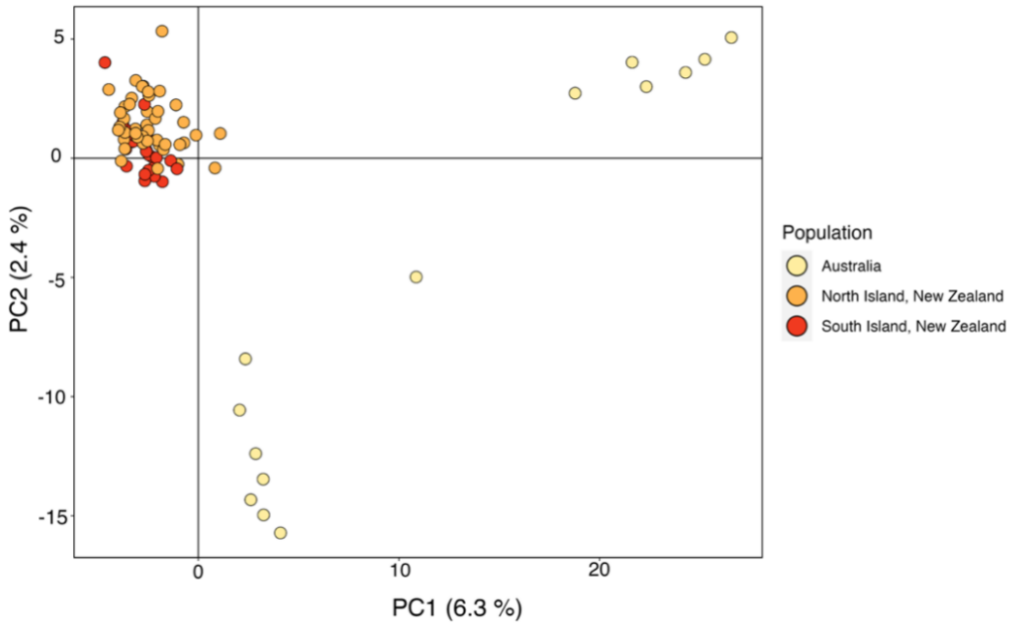

**Figure A2.2**. PCA of *Calliphora hilli* individuals plotted using the non-neutral dataset (18,324 SNPs) and coloured by population, according to the key.

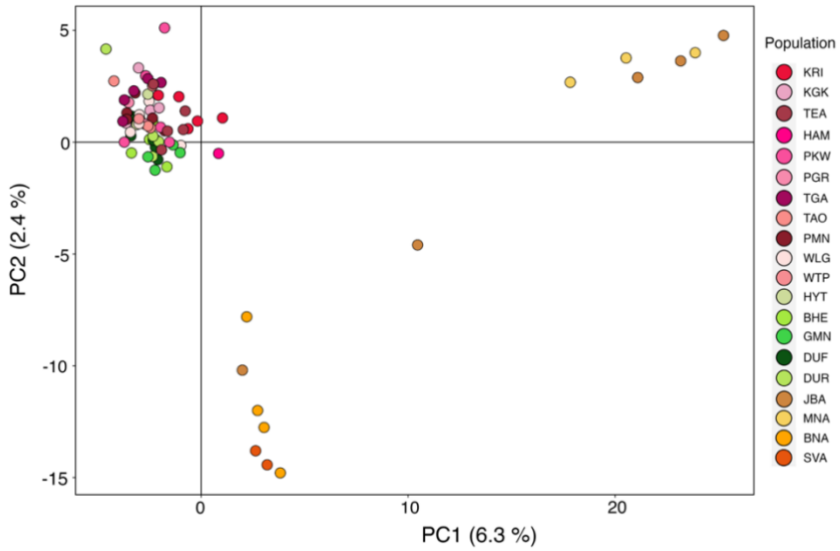

**Figure A2.3**. Admixture plots for *Calliphora hilli*, produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the non-neutral dataset (16,144 SNPs), where the optimal K value was determined to be K = 2. Admixture proportions are presented for K = 2 to K = 5. Results are consistent with the neutral dataset (Fig. 2.3), though dark blue admixture proportions here are higher at K = 2.

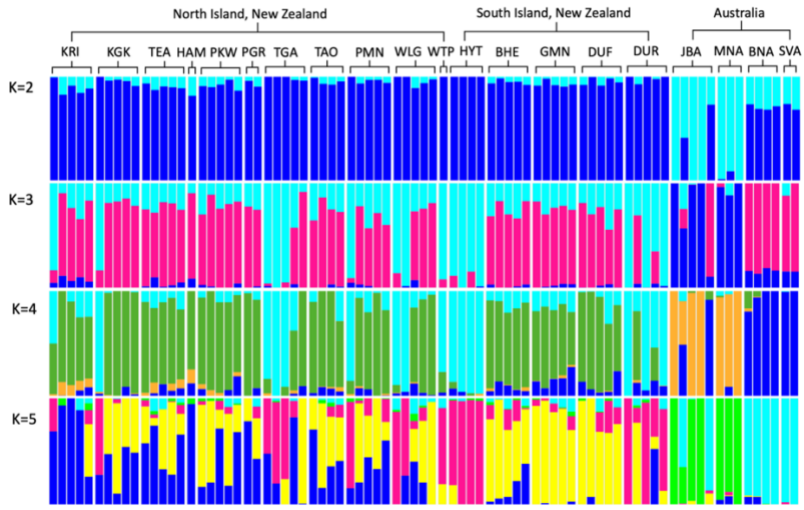


**Figure A2.4**. PCA of *Calliphora stygia* individuals plotted using the non-neutral dataset (16,115 SNPs) and coloured by geographic location, according to the key. Results are highly consistent with the neutral dataset (Fig. 2.4).

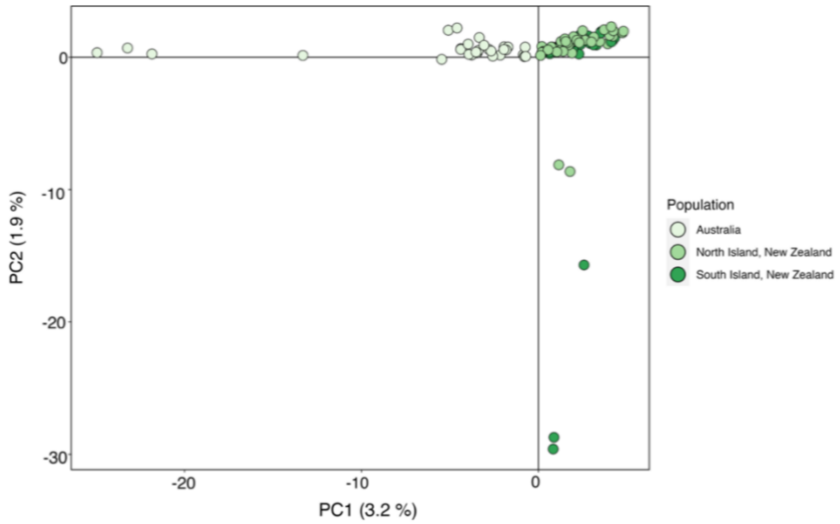

**Figure A2.5**. PCA of *Calliphora stygia* individuals plotted using the non-neutral dataset (16,115 SNPs) and coloured by population, according to the key.

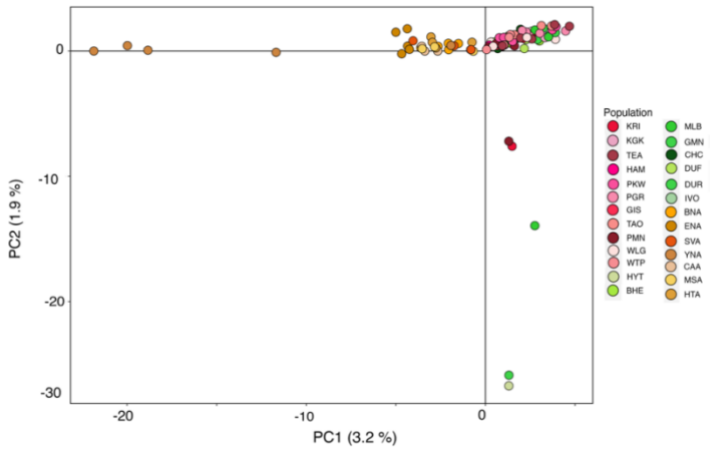


**Figure A2.6**. Admixture plots for *Calliphora stygia,* produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the non-neutral dataset (16,115 SNPs), where the optimal K value was determined to be K = 3. Admixture proportions are shown for K = 2 to K = 5. Results are highly concordant with the neutral dataset (Fig. 2.5).

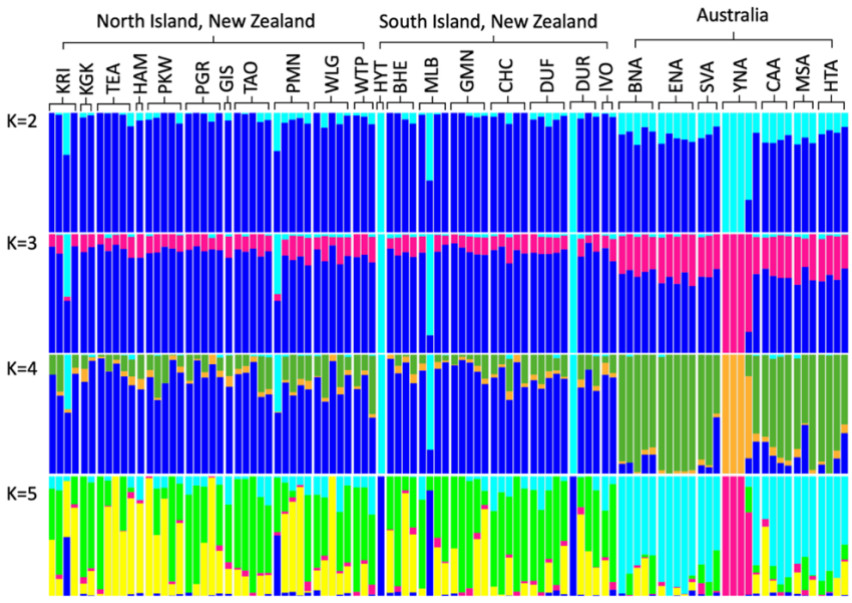

**Figure A2.7**. PCA plot showing *Calliphora hilli* and *Calliphora stygia* samples from the non-neutral dataset (16,333 SNPs), including potential hybrids labelled by individual codes. Individuals are coloured by taxonomically identified species as per the key. Results are highly consistent with the neutral dataset (Fig. 2.6).

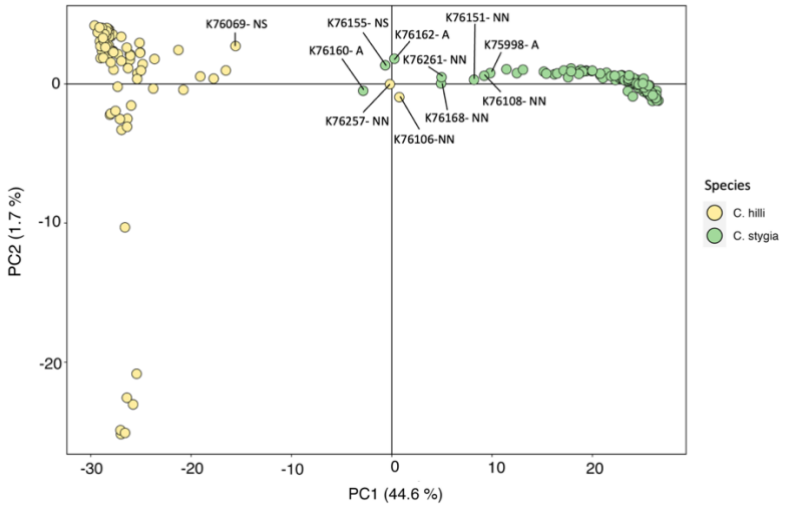


**Figure A2.8**. Admixture plot for the combined *Calliphora stygia* and *Calliphora hilli* dataset for K-values of 2-5, produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the non-neutral dataset (16,333 SNPs).

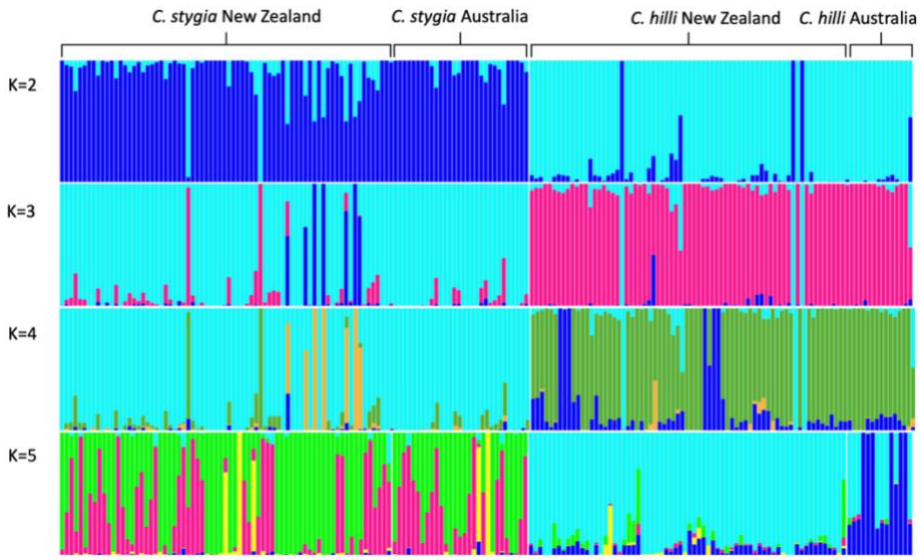

**Figure A2.9** Hybrid analyses for the combined *Calliphora stygia* and *Calliphora hilli* non-neutral dataset (16,333 SNPs), indicating 'pure', hybrid, or backcrossed status of 13 individuals. F1 and F2 hybrids represent offspring from first- and second-generation crosses between *C. stygia* and *C. hilli*, respectively. BC to *C. hilli* and BC to *C. stygia* indicates first generation back-crossed individuals to the respective species. Results are consistent with the neutral dataset (Fig. 2.8).

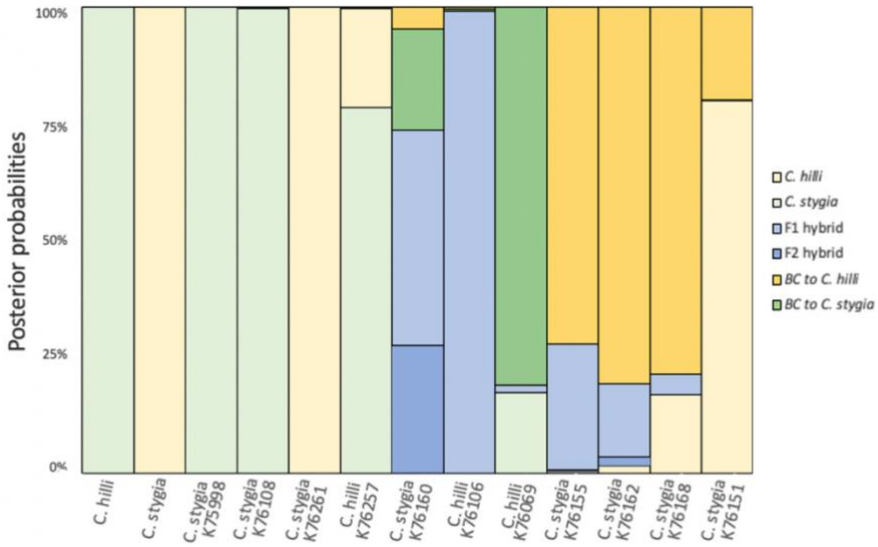

**Table A2.1.** Individual sampling information for *Calliphora hilli* and *Calliphora stygia*, including population names, specimen identification codes, and dates of collection (unknown dates are left blank or indicated with 'x'). New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island.

| Location | Sample ID | Sample collection date | Species |
|---|---|---|---|
| KRI | K76149 | xx.11.20 | *C. stygia* |
| KRI | K76150 | xx.11.20 | *C. stygia* |
| KRI | K76151 | xx.11.20 | *C. stygia* |
| KRI | K76152 | xx.11.20 | *C. stygia* |
| KRI | K76144 | xx.11.20 | *C. hilli* |
| KRI | K76145 | xx.11.20 | *C. hilli* |
| KRI | K76146 | xx.11.20 | *C. hilli* |
| KRI | K76147 | xx.11.20 | *C. hilli* |
| KRI | K76148 | xx.11.20 | *C. hilli* |
| KGK | K76137 | 18.12.21 | *C. stygia* |
| KGK | K76139 | 18.12.21 | *C. stygia* |
| KGK | K76132 | 18.12.21 | *C. hilli* |
| KGK | K76133 | 18.12.21 | *C. hilli* |
| KGK | K76134 | 18.12.21 | *C. hilli* |
| KGK | K76135 | 18.12.21 | *C. hilli* |
| KGK | K76136 | 18.12.21 | *C. hilli* |
| TEA | K76240 | 29.01.22 | *C. stygia* |
| TEA | K76241 | 29.01.22 | *C. stygia* |
| TEA | K76242 | 29.01.22 | *C. stygia* |
| TEA | K76243 | 29.01.22 | *C. stygia* |
| TEA | K76244 | 29.01.22 | *C. stygia* |
| TEA | K76233 | 29.01.22 | *C. hilli* |
| TEA | K76234 | 29.01.22 | *C. hilli* |
| TEA | K76235 | 29.01.22 | *C. hilli* |
| TEA | K76236 | 29.01.22 | *C. hilli* |
| TEA | K76237 | 29.01.22 | *C. hilli* |
| HAM | K76108 | 22.11.21 | *C. stygia* |
| HAM | K76106 | 22.11.21 | *C. hilli* |
| PKW | K76195 | 10.12.21 | *C. stygia* |
| PKW | K76196 | 10.12.21 | *C. stygia* |
| PKW | K76197 | 10.12.21 | *C. stygia* |
| PKW | K76198 | 10.12.21 | *C. stygia* |
| PKW | K76199 | 10.12.21 | *C. stygia* |
| PKW | K76190 | 10.12.21 | *C. hilli* |
| PKW | K76191 | 10.12.21 | *C. hilli* |
| PKW | K76192 | 10.12.21 | *C. hilli* |
| PKW | K76193 | 10.12.21 | *C. hilli* |
| PKW | K76194 | 10.12.21 | *C. hilli* |
| PGR | K76183 | 18.01.22 | *C. stygia* |

| | | | |
|---|---|---|---|
| PGR | K76184 | 18.01.22 | *C. stygia* |
| PGR | K76185 | 18.01.22 | *C. stygia* |
| PGR | K76186 | 18.01.22 | *C. stygia* |
| PGR | K76187 | 18.01.22 | *C. stygia* |
| PGR | K76175 | 18.01.22 | *C. hilli* |
| PGR | K76177 | 18.01.22 | *C. hilli* |
| TGA | K76223 | 01.09.20 | *C. hilli* |
| TGA | K76224 | 01.09.20 | *C. hilli* |
| TGA | K76225 | 01.09.20 | *C. hilli* |
| TGA | K76226 | 01.09.20 | *C. hilli* |
| TGA | K76227 | 01.09.20 | *C. hilli* |
| GIS | K76083 | 25.10.20 | *C. stygia* |
| TAO | K76213 | | *C. stygia* |
| TAO | K76214 | | *C. stygia* |
| TAO | K76215 | | *C. stygia* |
| TAO | K76216 | | *C. stygia* |
| TAO | K76217 | | *C. stygia* |
| TAO | K76208 | | *C. hilli* |
| TAO | K76209 | | *C. hilli* |
| TAO | K76211 | | *C. hilli* |
| TAO | K76212 | | *C. hilli* |
| PMN | K76168 | 09.11.21 | *C. stygia* |
| PMN | K76169 | 09.11.21 | *C. stygia* |
| PMN | K76170 | 09.11.21 | *C. stygia* |
| PMN | K76171 | 09.11.21 | *C. stygia* |
| PMN | K76172 | 09.11.21 | *C. stygia* |
| PMN | K76163 | 09.11.21 | *C. hilli* |
| PMN | K76164 | 09.11.21 | *C. hilli* |
| PMN | K76165 | 09.11.21 | *C. hilli* |
| PMN | K76166 | 09.11.21 | *C. hilli* |
| PMN | K76167 | 09.11.21 | *C. hilli* |
| WLG | K76019 | 09.02.21 | *C. stygia* |
| WLG | K76020 | 09.02.21 | *C. stygia* |
| WLG | K76021 | 09.02.21 | *C. stygia* |
| WLG | K76249 | 09.02.21 | *C. stygia* |
| WLG | K76250 | 09.02.21 | *C. stygia* |
| WLG | K76016 | 09.02.21 | *C. hilli* |
| WLG | K76017 | 09.02.21 | *C. hilli* |
| WLG | K76018 | 09.02.21 | *C. hilli* |
| WLG | K76247 | 09.02.21 | *C. hilli* |
| WLG | K76248 | 09.02.21 | *C. hilli* |
| WTP | K76259 | 10.02.18 | *C. stygia* |
| WTP | K76260 | 10.02.18 | *C. stygia* |
| WTP | K76261 | 14.11.12 | *C. stygia* |
| WTP | K76256 | 20.11.20-10.12.20 | *C. hilli* |
| HYT | K76119 | 26.01.21 | *C. stygia* |
| HYT | K76114 | 26.01.21 | *C. hilli* |
| HYT | K76115 | 26.01.21 | *C. hilli* |

| | | | |
|---|---|---|---|
| HYT | K76116 | 26.01.21 | *C. hilli* |
| HYT | K76117 | 26.01.21 | *C. hilli* |
| BHE | K76033 | 12.01.21 | *C. stygia* |
| BHE | K76034 | 12.01.21 | *C. stygia* |
| BHE | K76035 | 12.01.21 | *C. stygia* |
| BHE | K76036 | 12.01.21 | *C. stygia* |
| BHE | K76027 | 12.01.21 | *C. hilli* |
| BHE | K76028 | 12.01.21 | *C. hilli* |
| BHE | K76029 | 12.01.21 | *C. hilli* |
| BHE | K76030 | 12.01.21 | *C. hilli* |
| BHE | K76031 | 12.01.21 | *C. hilli* |
| MLB | K76155 | 12.01.21 | *C. stygia* |
| MLB | K76156 | 12.01.21 | *C. stygia* |
| MLB | K76157 | 12.01.21 | *C. stygia* |
| GMN | K76097 | 16.06.21 | *C. stygia* |
| GMN | K76098 | 16.06.21 | *C. stygia* |
| GMN | K76099 | 16.06.21 | *C. stygia* |
| GMN | K76100 | 16.06.21 | *C. stygia* |
| GMN | K76101 | 16.06.21 | *C. stygia* |
| GMN | K76087 | 16.06.21 | *C. hilli* |
| GMN | K76088 | 16.06.21 | *C. hilli* |
| GMN | K76089 | 16.06.21 | *C. hilli* |
| GMN | K76090 | 16.06.21 | *C. hilli* |
| GMN | K76091 | 16.06.21 | *C. hilli* |
| CHC | K76044 | 13.11.20 | *C. stygia* |
| CHC | K76045 | 13.11.20 | *C. stygia* |
| CHC | K76046 | 13.11.20 | *C. stygia* |
| CHC | K76047 | 13.11.20 | *C. stygia* |
| CHC | K76048 | 13.11.20 | *C. stygia* |
| DUF | K76004 | 08.06.21 | *C. stygia* |
| DUF | K76005 | 08.06.21 | *C. stygia* |
| DUF | K76006 | 08.06.21 | *C. stygia* |
| DUF | K76058 | 08.06.21 | *C. stygia* |
| DUF | K76059 | 08.06.21 | *C. stygia* |
| DUF | K76001 | 08.06.21 | *C. hilli* |
| DUF | K76002 | 08.06.21 | *C. hilli* |
| DUF | K76003 | 08.06.21 | *C. hilli* |
| DUF | K76054 | 08.06.21 | *C. hilli* |
| DUF | K76055 | 08.06.21 | *C. hilli* |
| DUR | K76070 | 24.05.21 | *C. stygia* |
| DUR | K76071 | 24.05.21 | *C. stygia* |
| DUR | K76072 | 24.05.21 | *C. stygia* |
| DUR | K76073 | 24.05.21 | *C. stygia* |
| DUR | K76065 | 24.05.21 | *C. hilli* |
| DUR | K76066 | 24.05.21 | *C. hilli* |
| DUR | K76067 | 24.05.21 | *C. hilli* |
| DUR | K76068 | 24.05.21 | *C. hilli* |
| DUR | K76069 | 24.05.21 | *C. hilli* |

| | | | |
|---|---|---|---|
| IVO | K76124 | 09.11.21 | *C. stygia* |
| IVO | K76125 | 09.11.21 | *C. stygia* |
| JBA | K76127 | | *C. hilli* |
| JBA | K76128 | | *C. hilli* |
| JBA | K76129 | | *C. hilli* |
| JBA | K76130 | | *C. hilli* |
| JBA | K76131 | | *C. hilli* |
| MNA | K76010 | xx.02.05 | *C. hilli* |
| MNA | K76011 | xx.02.05 | *C. hilli* |
| MNA | K76012 | xx.02.05 | *C. hilli* |
| BNA | K75995 | 24.04.21 | *C. stygia* |
| BNA | K75996 | 24.04.21 | *C. stygia* |
| BNA | K75997 | 24.04.21 | *C. stygia* |
| BNA | K76025 | 24.04.21 | *C. stygia* |
| BNA | K76026 | 24.04.21 | *C. stygia* |
| BNA | K75992 | 24.04.21 | *C. hilli* |
| BNA | K75993 | 24.04.21 | *C. hilli* |
| BNA | K75994 | 24.04.21 | *C. hilli* |
| BNA | K76024 | 24.04.21 | *C. hilli* |
| ENA | K76078 | 03.06.08 | *C. stygia* |
| ENA | K76079 | 03.06.08 | *C. stygia* |
| ENA | K76080 | 03.06.08 | *C. stygia* |
| ENA | K76081 | 03.06.08 | *C. stygia* |
| ENA | K76082 | 03.06.08 | *C. stygia* |
| SVA | K76205 | 10.05.01 | *C. stygia* |
| SVA | K76206 | 10.05.01 | *C. stygia* |
| SVA | K76207 | 10.05.01 | *C. stygia* |
| SVA | K76013 | 10.05.01 | *C. hilli* |
| SVA | K76015 | 10.05.01 | *C. hilli* |
| YNA | K76265 | xx.10.20 | *C. stygia* |
| YNA | K76266 | xx.06.19 | *C. stygia* |
| YNA | K76267 | xx.06.19 | *C. stygia* |
| YNA | K76268 | xx.xx.20 | *C. stygia* |
| YNA | K76269 | xx.06.19 | *C. stygia* |
| CAA | K75998 | 13.10.04 | *C. stygia* |
| CAA | K75999 | 13.10.05 | *C. stygia* |
| CAA | K76000 | 13.10.04 | *C. stygia* |
| CAA | K76043 | 13.10.04 | *C. stygia* |
| MSA | K76158 | | *C. stygia* |
| MSA | K76159 | | *C. stygia* |
| MSA | K76161 | | *C. stygia* |
| MSA | K76162 | | *C. stygia* |
| HTA | K76008 | 30.09.03 | *C. stygia* |
| HTA | K76009 | 30.09.03 | *C. stygia* |

# Appendix A3

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**Figure A3.1**. PCA plots showing *Calliphora vicina* samples (non-neutral data set 21,354 SNPs) from: (A) broad regions (Australia, and North and South islands of New Zealand); and (B) populations.

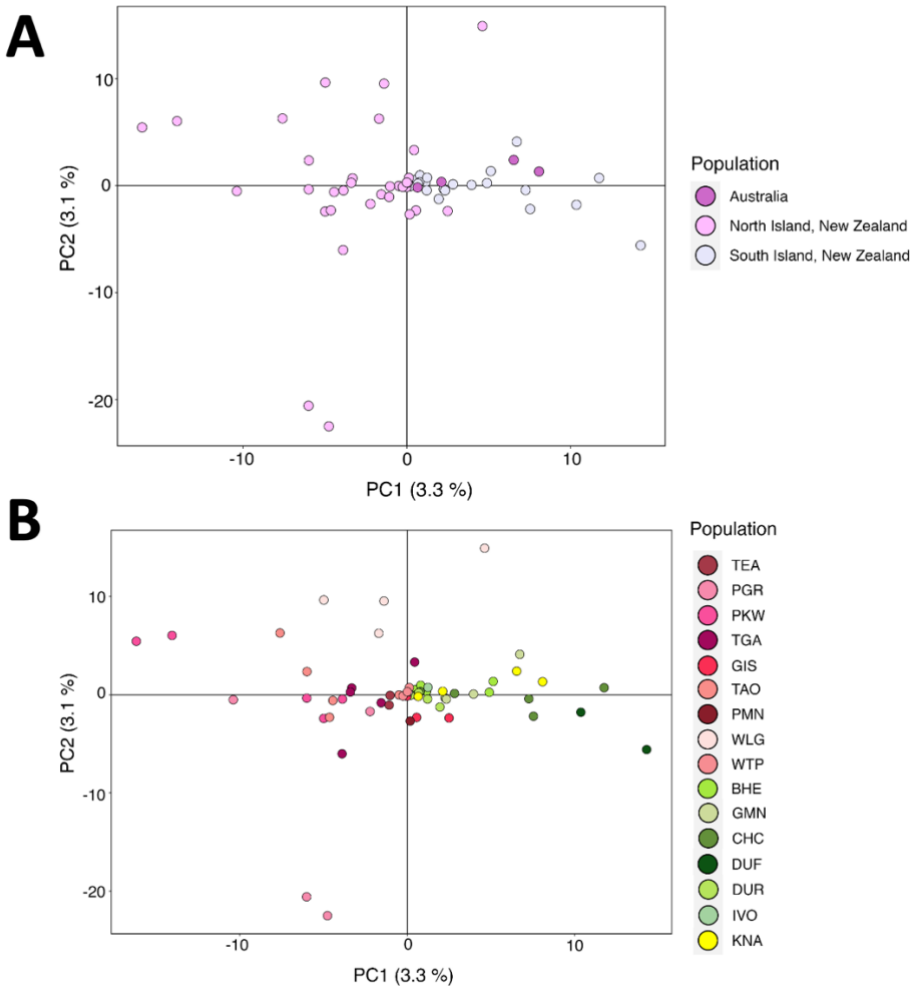

**Figure A3.2**. Admixture plots for *Calliphora vicina* were produced using Sparse Non-Negative Matrix Factorisation (sNMF) analysis of the non-neutral dataset containing 21,354 SNPs, where the optimal K value was determined to be K = 1. Admixture proportions showing K = 2 to K = 5 are presented, with populations in order from left to right corresponding to the top of the North Island, through to the bottom of the South Island of New Zealand, followed by Australia.

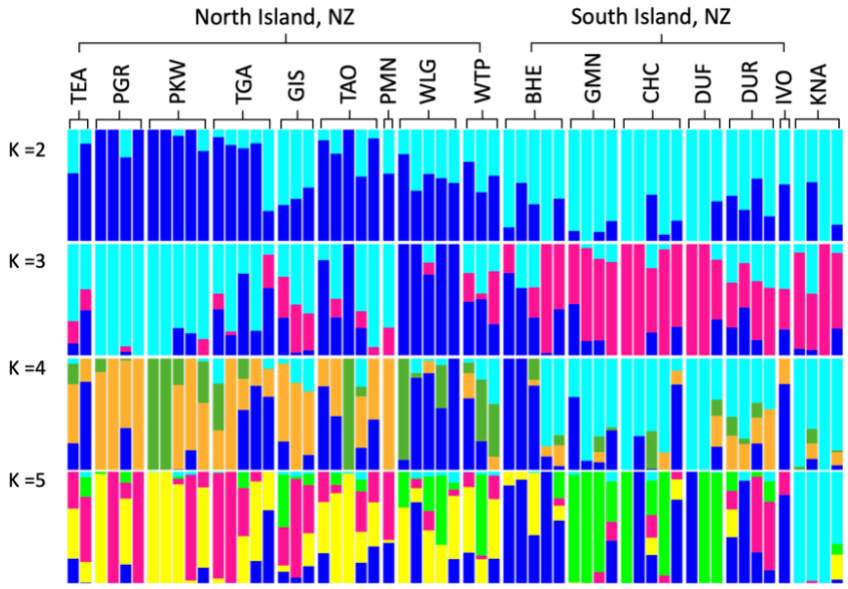

**Figure A3.3.** Fecundity plotted as the log-transformed total eggs laid per day for both high (left) and low (right) diversity lines. The grey shading represents standard error.

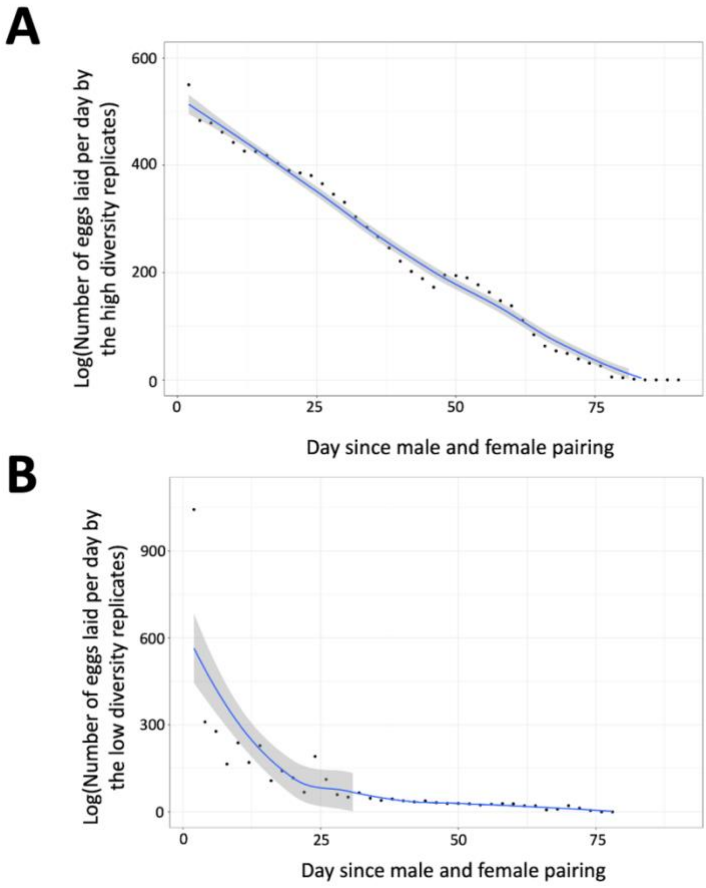

**Figure A3.4**. Boxplots showing the overall body size measurement for the first 15 males/females vs all individuals in a single cage (following replacement to maintain n = 30 density throughout) for one high and one low representative line: (A) Overall body size (i.e., thorax width x thorax length x head length) for 30 males and females for one replicate low diversity line; (B) Overall body size of all flies within the low diversity replicate; (C) Overall body size in the 30 males and females for one replicate high diversity line; and (B) Overall body size of all flies within the high diversity replicate.

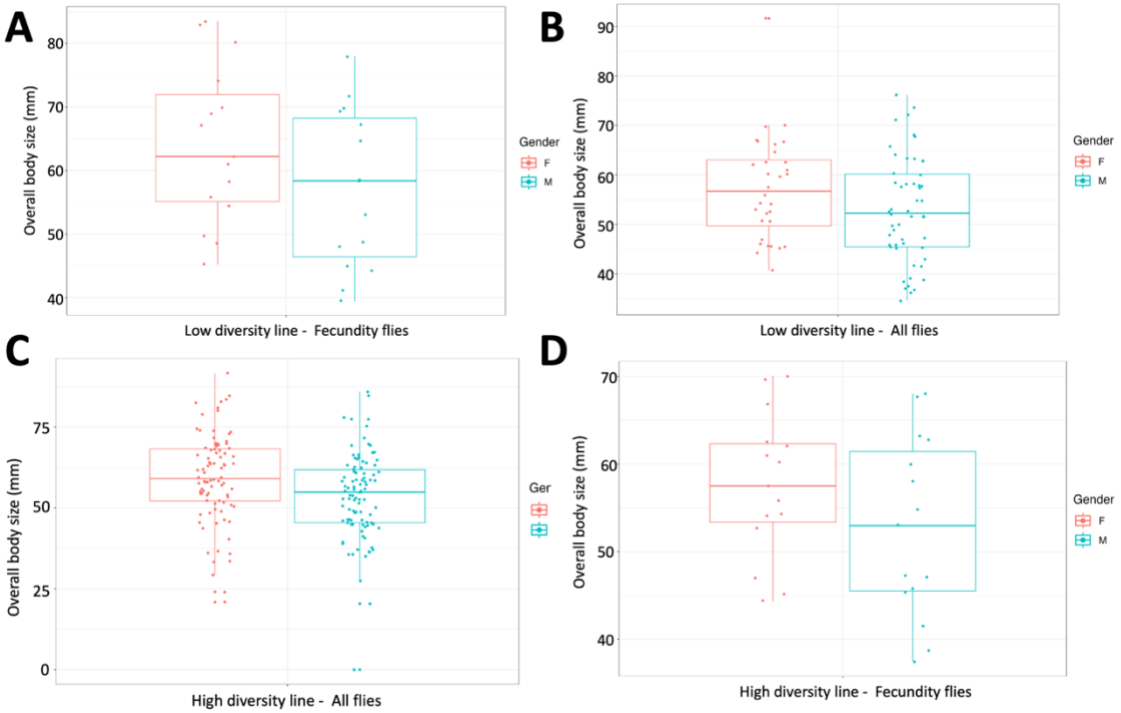

**Figure A3.5**. Body size boxplots for all all individuals for each of the three separate body size measures (head length, thorax length, thorax width): (A) Head length measured as per Fig. 3.1 for both high and low diversity lines; (B) Thorax width for high and low diversity; and (C) Thorax length for high and low diversity.

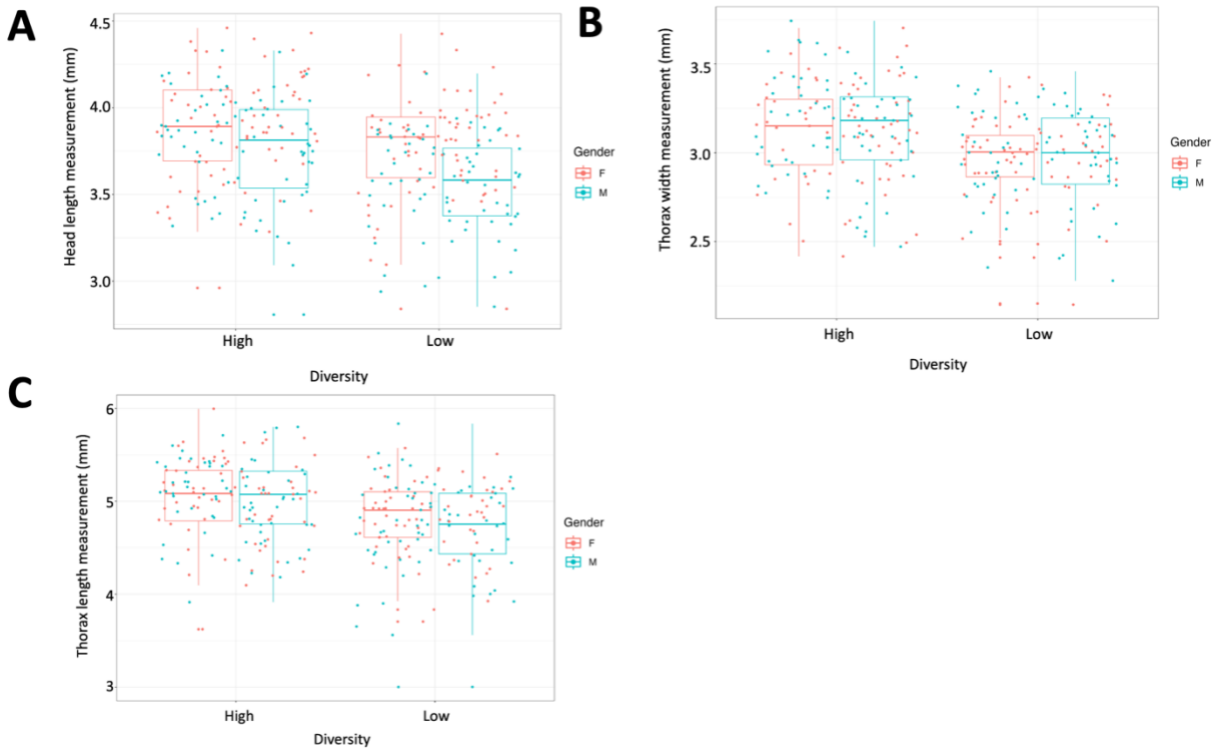

**Table A3.1**. Individual sampling information for *Calliphora vicina*, including population names, specimen identification codes, and dates of collection (unknown dates are left blank). New Zealand populations are listed in rough geographical order from the top of the North Island to the bottom of the South Island.

| Sample ID | Location | Sample collection date |
|---|---|---|
| K76245 | TEA | 29.01.22 |
| K76246 | TEA | 29.01.22 |
| K76022 | PGR | 18.01.22 |
| K76023 | PGR | 18.01.23 |
| K76188 | PGR | 18.01.22 |
| K76189 | PGR | 18.01.22 |
| K76200 | PKW | 15.12.21 |
| K76201 | PKW | 15.12.21 |
| K76202 | PKW | 15.12.21 |
| K76203 | PKW | 15.12.21 |
| K76204 | PKW | 15.12.21 |
| K76228 | TGA | 01.09.20 |
| K76229 | TGA | 01.09.20 |
| K76230 | TGA | 01.09.20 |
| K76231 | TGA | 01.09.20 |
| K76232 | TGA | 01.09.20 |
| K76084 | GIS | 25.10.20 |
| K76085 | GIS | 25.10.20 |
| K76086 | GIS | 25.10.20 |
| K76218 | TAO | |
| K76219 | TAO | |
| K76220 | TAO | |
| K76221 | TAO | |
| K76222 | TAO | |
| K76173 | PMN | 09.11.21 |
| K76251 | WLG | 09.02.21 |
| K76252 | WLG | 09.02.21 |
| K76253 | WLG | 09.02.21 |
| K76254 | WLG | 09.02.21 |
| K76255 | WLG | 09.02.21 |

| | | |
|---|---|---|
| K76262 | WTP | xx.xx.19 |
| K76263 | WTP | xx.xx.19 |
| K76264 | WTP | xx.xx.19 |
| K76037 | BHE | 12.01.21 |
| K76038 | BHE | 12.01.21 |
| K76039 | BHE | 12.01.21 |
| K76040 | BHE | 12.01.21 |
| K76041 | BHE | 12.01.21 |
| K76102 | GMN | 16.06.21 |
| K76103 | GMN | 16.06.21 |
| K76104 | GMN | 16.06.21 |
| K76105 | GMN | 16.06.21 |
| K76049 | CHC | 13.11.20 |
| K76050 | CHC | 13.11.20 |
| K76051 | CHC | 13.11.20 |
| K76052 | CHC | 13.11.20 |
| K76053 | CHC | 13.11.20 |
| K76060 | DUF | 08.06.21 |
| K76061 | DUF | 08.06.21 |
| K76062 | DUF | 08.06.21 |
| K76074 | DUR | 24.05.21 |
| K76075 | DUR | 24.05.21 |
| K76076 | DUR | 24.05.21 |
| K76077 | DUR | 24.05.21 |
| K76126 | IVO | 09.11.21 |
| K76140 | KNA | 24.04.21 |
| K76141 | KNA | 24.04.21 |
| K76142 | KNA | 24.04.21 |
| K76143 | KNA | 24.04.21 |