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Fitness consequences of population bottlenecks in an invasive blowfly

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Abstract

Invasive species often undergo demographic bottlenecks that cause a decrease in genetic diversity and associated reductions in population fitness. Despite this, they manage to thrive in novel environments. Investigating the effects of inbreeding and genetic bottlenecks on population fitness for invasive species is, therefore, key to understanding how they may survive in new environments. We used the blowfly *Calliphora vicina* (*Sciences, Mathématiques et Physique*, 1830, 2, 1), which is native to Europe and was introduced to Australia and New Zealand, to examine the effects of genetic diversity on population fitness. We first collected 59 samples from 15 populations across New Zealand and one in Australia, and used 20,501 biallelic SNPs to investigate population genomic diversity, structure and admixture. We then explored the impacts of repeated experimental bottlenecks on population fitness by creating inbred and outbred lines of *C. vicina* and measuring a variety of fitness traits. In wild-caught samples, we found low overall genetic diversity, signals of genetic admixture and limited (<3%) genetic differentiation between North and South Island populations, with genetic links between the South Island and Australia. Following experimental bottlenecks, we found significant reductions in fitness for inbred lines. However, fitness effects were not felt equally across all phenotypic traits. Moreover, they were not enough to cause population collapse in any experimental line, suggesting that *C. vicina* (when under relaxed selection, as in laboratory settings) may be able to compensate for population bottlenecks even when highly inbred. Our results demonstrate the value of a tractable experimental system for investigating processes that may facilitate or hamper biological invasion.

KEYWORDS
biological invasion, blowflies, fitness, genetic diversity, inbreeding, population genomics

Nathan J. Butterworth and Angela McGaughran are joint senior authors.

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1 | INTRODUCTION

Invasive species are shifting and/or expanding their ranges globally, due to changing land and sea use, climate change, pollution and declines of competing native species (Finch et al., 2021; IPBES, 2023). Alongside environmental factors promoting invasions, invasive species may come equipped with, or rapidly evolve, intrinsic phenotypic and/or genetic characteristics that aid their establishment and spread. For example, higher reproductive rates in invasive species can result in them rapidly out-competing natives (Allendorf & Lundquist, 2003; Flores-Moreno et al., 2015; Forman & Kesseli, 2003), while genetic variation can facilitate adaptive responses to novel selective pressures in new environments (e.g. Battlay et al., 2023; Exposito-Alonso et al., 2018; Tepolt et al., 2022). In the latter case, demographic bottlenecks typically associated with population foundation can decrease genetic diversity (Kaňuch et al., 2021; Schrieber & Lachmuth, 2017), potentially limiting adaptive potential (but see Estoup et al., 2016).

Theoretical expectations are that both increased inbreeding and reduced diversity following demographic bottlenecks should decrease fitness – for example by reinforcing deleterious mutations and/or leading to inbreeding depression (Frankham, 1995; Willoughby et al., 2017). While some studies have found support for this (e.g. Harrisson et al., 2019; Reed & Frankham, 2003), others have identified potential evolutionary benefits associated with inbreeding (Kokko & Ots, 2006; Verhoeven et al., 2011; Szulkin et al., 2013), or shown that invasive species with low genetic diversity can still undergo rapid adaptation (e.g. Hjort et al., 2023), produce high phenotypic diversity (e.g. Castillo et al., 2018) and/or maintain fitness (Yates et al., 2019). Further, many invasive species, in fact, retain high diversity when measured at appropriate genetic markers (Estoup et al., 2016), while others avoid genetic bottlenecks (e.g. via high propagule pressure during introduction or asexual reproduction) or recover from them with rapid adaptive responses (e.g. genetic hybridization among divergent lineages) (Li et al., 2022).

The relationship between neutral genetic diversity, adaptive capacity and population fitness has also been called into question (Teixeira & Huber, 2021) and subsequently debated (DeWoody et al., 2021). One possibility is that invasive species may benefit from compensatory mechanisms which mitigate the consequences of inbreeding and low genetic diversity in small populations (Estoup et al., 2016; Schrieber & Lachmuth, 2017). Examples include genetic purging and drift (Benazzo et al., 2017; Facon et al., 2011; Grossen et al., 2020; Hedrick & Garcia-Dorado, 2016; Khan et al., 2021; Larsen et al., 2011; Pérez-Pereira et al., 2021; Toczydlowski & Waller, 2023), inbreeding × environment interactions (Schrieber & Lachmuth, 2017), high mutation rates (Kaňuch et al., 2021; Talla, Johansson et al., 2019; Talla, Soler et al., 2019), phenotypic plasticity (Davidson et al., 2011; Gioria et al., 2023; Richards et al., 2006) and polyandry (with multiple paternity reducing the extent of inbreeding by generating half-siblings in the same offspring generation; Cornell & Tregenza, 2007; Kaňuch et al., 2021; Pekkala et al., 2014). However, the direct impacts of inbreeding (and subsequent loss of genetic diversity) on population fitness are difficult to study empirically due to the requirement to genotype or cross individuals to generate experimental lines with differing levels of inbreeding that can then be reared and assayed for a variety of fitness-related traits. This often limits the scope of such research to model systems (e.g. *Drosophila*; Charlesworth, 2015; Flatt, 2020; Frankham, 1995). Further research – including in non-model and wild-caught species – into the effects of inbreeding and genetic diversity on population fitness is necessary to provide broader insights into the evolutionary dynamics of invasive species.

Blowflies are an ideal model for investigating biological invasion due to their high biotic potential (Fenton et al., 2008; French et al., 1995; Wall et al.,1995, 2000), excellent dispersal ability (Coutinho et al., 2012), capacity to adapt to varying environmental conditions (Johnson et al., 2022; Oliveira & Vasconcelos, 2020) and ease of rearing under laboratory conditions (e.g. Butterworth et al., 2020). Among the 54 species (seven genera) of Calliphoridae found in New Zealand, *Calliphora vicina* (Robineau-Desvoidy, 1830) originates from Europe and was first identified in New Zealand in 1889 (Dear, 1986). Geographically widespread, *C. vicina* can be found on all continents except Antarctica (though it has reached the sub-Antarctic; Daly, Gerlich et al., 2023; Williams & Villet, 2006), with changing temperatures facilitating its global spread (Fuentes-López et al., 2020; Henning et al., 2005).

Here, we used 16 invasive populations (15 from New Zealand and one from Australia) of *C. vicina* to first investigate the extent of population genomic diversity, structure and admixture in wild-caught populations, analysing 20,501 biallelic genome-wide single nucleotide polymorphisms (SNPs). Next, we explored the impacts of repeated genetic bottlenecks on population fitness in *C. vicina* by creating inbred and outbred lineages in the laboratory and measuring their fitness across various phenotypic traits.

2 | MATERIALS AND METHODS

2.1 | Population genetic analysis

2.1.1 | Sample collection, sequencing and analysis

Individual flies 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 kits consisted of a modified bottle trap as per Hwang and Turner (2005). Traps were left outside for 3–4 days and were emptied daily by placing the upper part of the bottle trap into 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 (Figure 1, Table 1 and Table S1). All specimens were identified to species level using the taxonomic key of Dear (1986).

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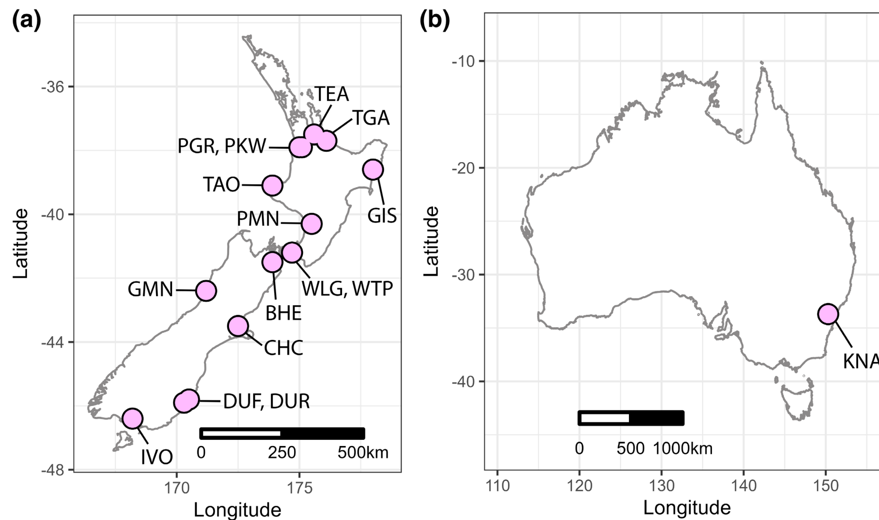

FIGURE 1 Geographical map showing sampling sites where specimens of *Calliphora vicina* (Diptera: Calliphoridae) were collected for population genomic analyses. (a) New Zealand field sites; and (b) the single Australian field site.

TABLE 1 Sampling information for *Calliphora vicina*, including population names, codes and GPS coordinates, and per-population sample numbers and heterozygosity (for populations where $n \geq 3$; more conservative, Ho_1, He_1, and less conservative, Ho_2, He_2 datasets).

				Heterozygosity			
Population name	**Population code**	**GPS coordinates**	**No. samples**	**Ho_1**	**He_1**	**Ho_2**	**He_2**
Te Aroha	TEA	−37.5386, 175.6932	2	–	–	–	–
Pirongia	PGR	−37.9683, 175.1504	4	0.08	0.25	0.10	0.26
Kaniwhaniwha	PKW	−37.9339, 175.0777	5	0.08	0.25	0.07	0.26
Tauranga	TGA	−37.7327, 176.1799	5	0.08	0.28	0.07	0.26
Gisborne	GIS	−38.6595, 178.0039	3	0.09	0.25	0.08	0.26
Taranaki Oakura	TAO	−39.1157, 173.9522	5	0.08	0.27	0.08	0.28
Palmerston North	PMN	−40.3785, 175.5866	1	–	–	–	–
Wellington	WLG	−41.2950, 174.7989	5	0.08	0.28	0.07	0.29
Wellington Te Papa	WTP	−41.2904, 174.7820	3	0.11	0.25	0.11	0.25
Blenheim	BHE	−41.5075, 173.9299	5	0.09	0.27	0.07	0.29
Greymouth	GMN	−42.4646, 171.2029	4	0.09	0.26	0.07	0.27
Christchurch	CHC	−43.5317, 172.5794	5	0.07	0.26	0.07	0.25
Dunedin Fairfield	DUF	−45.9000, 170.3823	3	0.09	0.26	0.09	0.32
Dunedin Ravensbourne	DUR	−45.8640, 170.5494	3	0.10	0.24	0.10	0.25
Invercargill	IVO	−46.4361, 168.2832	1	–	–	–	–
Katoomba, NSW Australia	KNA	−33.7118, 150.3118	4	0.08	0.22	0.07	0.22

Note: 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 S1 for further sampling details.

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 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) to select fragments in the size range of 220–340 bp (genomic sequence plus 148 bp of adapters). Single-end sequencing (1 × 101 bp) 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 & Overcast, 2020) was used to filter and remove low-quality data (e.g. $Q < 20$, phred$Q < 33$), 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 (72,481 SNPs) was filtered using VCFTOOLS v0.1.13 (Danecek et al., 2011), with --missing-indv, --max-missing-count and --maf parameters explored to create 'more conservative' and 'less conservative' datasets. The --missing-indv filter indicated that 0 individuals had >98% missing data, while one individual had >95% and six individuals had >90% missing data – to retain as many individuals in the dataset as possible, we ultimately decided not to apply this filter. However, the more conservative dataset applied filters to remove sites with >20% missing genotypes across all individuals, and an overall minor allele frequency cut-off of 5%; resulting in 20,501 biallelic SNPs. The less conservative dataset applied filters to remove sites with >10% missing genotypes across all individuals, and an overall minor allele frequency cut-off of 5%; resulting in 41,219 biallelic SNPs. The two datasets showed similar results (e.g. similar genetic clustering in PCA plots; see Figure 2a,b vs. Figure S2C,D), with clearer delineations in the more conservative dataset. Thus, we present the results for the more conservative dataset in the main text, and for the less conservative in the Appendix.

Maps were created to visualize 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, 2024). Population genetic diversity (heterozygosity, calculated as Ho = 1 − ∑_k ∑_i Pkii/*np*, where Pkii represents the proportion of homozygotes *i* in sample *k* and np the number of samples) was determined for each population for which $n \geq 3$ using the hierfstat package v0.5–11 (Goudet, 2005) in R. Pairwise F_{ST} was calculated between all populations with $n \geq 3$ using the gl.fst.pop function from the dartR package (Gruber et al., 2017), with significance tested using 1000 bootstrap replications. 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 & François, 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 & Francois, 2016) in R and ggplot2 were used to produce an admixture barplot for each species. We next removed SNPs under selection using an F_{ST} outlier approach. After using snmf to estimate the optimal *K* value (above), we computed F_{ST} statistics on the best snmf run and calculated the GIF (inflation factor) for that run. Adjusted *p*-values were then computed from the combined z-scores and a histogram of *p*-values was plotted against different lambda/inflation values. A false discovery rate threshold of 0.05 was then used to identify candidate F_{ST} outliers, which were removed from the dataset to create new VCF file containing only neutral SNPs (20,815 and 41,477 SNPs for the more and less conservative datasets respectively). Both the PCA and admixture analyses were repeated on the neutral dataset. The neutral and non-neutral datasets produced consistent results for both the more and less conservative datasets; thus, we present the neutral plots in the main text and the non-neutral plots in the Appendix (Figures S1 and S2).

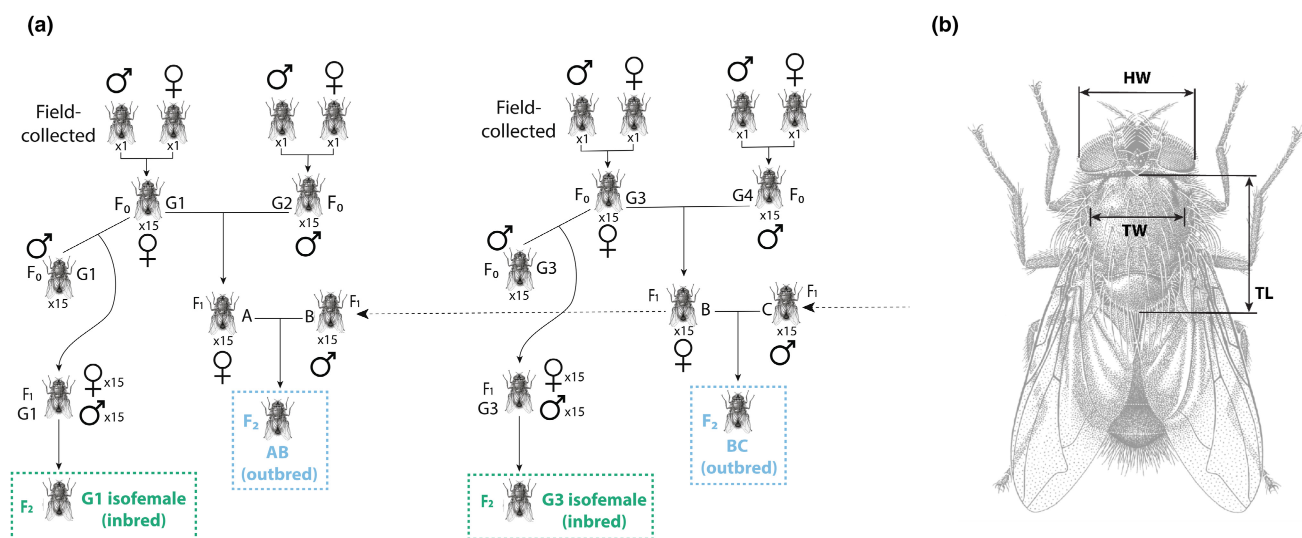

FIGURE 2 (a) Schematic showing the use of reproductive crosses to generate inbred and outbred lines to the F2 generation (see 2. Materials and Methods; *Generating inbred and outbred lines*). Number of flies used in each cross is presented beneath each fly. Methods are presented for generating only the 'AB' and 'BC' lines, but were the same for the 'CD', 'DE' and 'EA' lines – originating from G5 to G10 lines (also not shown). Green and blue dashed boxes represent the generations that were phenotyped for all fitness traits (fecundity, lifespan, development, body size and population performance); (b) Visualization of body size traits measured: HL = head length, TL = thorax length, TW = thorax width. Blowfly illustration from Dear (1986); The pictured species is *Calliphora quadrimaculata* (not *C. vicina*), artist D.W. Helmore.

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2.2 | Laboratory crossing experiments

2.2.1 | Sample collection/F0 blowflies

Wild *C. vicina* were caught in late 2022 at two sites approximately 36 km apart (Pirongia, PGR and Kaniwhaniwha, PKW; Figure 1 and Table 1). Trapping involved preparation of a bait, where beef mince was left outside to attract blowflies; after the flies laid eggs, larvae were left to feed on the meat for 3 days, and then the container was tightly sealed and stored in the fridge before being taken to the field. The subsequent mortality of actively feeding blowfly larvae, and their anaerobic decomposition (in combination with the decomposing beef mince) results in the production of specific volatiles that are highly attractive to a wide diversity of blowfly species (Butterworth et al., 2023). The bait presumably exploits the preference of primary and secondary colonizing blowflies for cues associated with carrion that is in the active and mid-stages of decomposition (Dawson et al., 2021). At the field site, the bait container was placed on grass and attracted 'F0' flies were trapped using hand nets. Live flies were identified following the key of Dear (1986). Ten individual F0 females were placed into separate breeding cages (494 L × 322 W × 258 H mm; 27 L total volume), each with a single F0 male, and provided with access to 50 g of raw beef mince, raw sugar and water in a room with 12:12-h light:dark cycling at 22°C. Though it is possible that females had mated prior to collection in the field, providing females with a single male prior to egg-laying ensures last sperm precedence (e.g. Laturney et al., 2018), reducing the chance of multiply-sired offspring. These field-collected females and males are referred to as 'field-collected' (Figure 2a). Once eggs were laid by field-collected individuals (referred to as G1–G10 and the F0 generation), 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 flies began to emerge, cages were checked at least once every 24 h and individuals were separated by sex into different cages to prevent uncontrolled premature mating and to maintain the virgin status of males and females, as *Calliphora* sp. do not generally reach maturity until at least 3–7 days after eclosion (Butterworth et al., 2020). Male and female cages always had access to non-limiting amounts of sugar and water. Laboratory temperature was checked regularly via several thermometers placed around the room. Cages were shuffled randomly to account for microenvironmental effects.

2.2.2 | Generating inbred and outbred lines

Five 'inbred' lines were generated using repeated population bottlenecks of five of the 10 F0 lines, with mating within lines only occurring among male and female siblings. Each new generation was started with 15 sibling virgin males and 15 sibling virgin females (i.e. both males and females all from the same mother) that had reached sexual maturity and were placed in a single cage under the same environmental conditions described above (Figure 2a).

Five 'outbred' lines were produced by crossing 15 non-sibling F1 males and 15 non-sibling F1 females (i.e. all females from one F0 mother, all males from another) that had reached sexual maturity. These outbred F1 flies were labelled 'A', 'B', 'C', 'D' and 'E' (Figure 2a). To produce the F2 outbred generation, 15 males and 15 females from separate F1 lines, respectively, were crossed to produce lines 'AB', 'BC', 'CD', 'DE' and 'EA' (Figure 2a).

2.2.3 | Phenotyping

For phenotyping, 15 male and female F2 flies from each inbred and outbred line were put into a cage together with constant access to beef mince as an oviposition medium. The meat was replaced every second day to ensure it never became a limiting resource. For each line, single back-up F2 cages of separate males and females from that line were maintained and used to replace dead individuals in the relevant main cage to maintain population density at $n = 30$ until the experiments' end; total density varied across back-up cages but had no detectable effect on fitness outcomes (Table S2). All phenotyping targeted traits of the F2 flies from the main cages. *Fecundity*: Laid eggs (i.e. the F3 generation) 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 defined 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 earliest dates of pupation and emergence for each individual line) was measured in number of days. *Body size*: Three body size-related traits were measured for the first 30 flies for each line (15 males and 15 females), including head length (HL), thorax length (TL) and thorax width (TW) (Figure 2b). 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 inbred and one outbred line and examined the distribution of values for all flies versus the first 30 individuals in each line. We found no significant difference between the full and $n = 30$ datasets ($T_{3,26} = 1.237$, $p = 0.108$), 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 micrometre, and the resulting images were analysed using ImageJ v1.53t (Schneider et al., 2012). *Population performance*: To estimate the consequences of inbreeding for the longer term performance of populations of *C. vicina*, we calculated two measures of population performance that account for both survival through the life stages and subsequent reproduction – thus reflecting general measures of population fitness. We use equations from Ross et al., 2019 (our Equation 1) and Facon

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et al., 2011 (our Equation 2) but with slightly modified variables. In our equations, F is the total fecundity of each population, S is the proportion of pupae surviving to adulthood for each population and D is the minimum larval duration in days for each population.

$$\text{Index of performance} = \ln(FS) / D \tag{1}$$

$$\text{Lifetime performance} = FS \tag{2}$$

2.2.4 | Data analysis

All analyses were run in R v. 4.2.2 (R Core Team, 2024). To understand how life-history traits were impacted by inbreeding, linear models were run on line-averaged values for fecundity, developmental rate, emergence success and population performance. A linear mixed-effects model (using the 'lme4' v. 1.1 package; Bates et al., 2015) was run on lifespan with inbred/outbred status and sex as predictor variables and line as a random effect, and a linear-mixed effect model was run on body size with inbred/outbred status and sex as predictor variables and line as a random effect. Type III analysis of variance (ANOVA) was run on the outputs of all models to assess statistically significant differences between treatments. The 'emmeans' v. 1.8.3 package (Lenth et al., 2018) was then used to predict means and standard errors from the respective models and these values were plotted with ggplot2 v. 3.4.4 (Wickham, 2016).

3 | RESULTS

3.1 | Population genetic analysis

Observed heterozygosity (Ho) in wild-caught *C. vicina* populations for which $n \geq 3$ ranged from 0.07 to 0.11 and was always lower than expected heterozygosity (He) (range 0.22–0.28). Levels of Ho and He were similar between the New Zealand populations and the single Australian population (where $n=4$) (Table 1). Pairwise population F_{ST} showed a range of genetic differentiation values (range 0.000–0.171 for populations with $n \geq 3$ for the neutral dataset; Figure S3A). Wellington Te Papa/WTP had the lowest mean F_{ST} (0.035), while Katoomba, Australia/KNA had the highest (mean $F_{ST}=0.124$). The highest pairwise F_{ST} among New Zealand populations was 0.128 (between Kaniwhaniwha/PKW in the North Island and Fairfield, Dunedin/DUF in the South Island), while there were several instances of 0.000 F_{ST} values among populations from the same New Zealand Island. Within islands, mean F_{ST} was 0.041 (North Island) and 0.053 (South Island), while mean F_{ST} between all populations in the North Island versus all populations in the South Island was 0.067 (Figure S3A).

PCA analysis showed minimal clustering (<3.3% differentiation) of New Zealand's North and South Island populations, while the four Australian individuals clustered together with the South Island populations (Figure 3 and Figure S1).

Consistent with the F_{ST} and PCA results, admixture plots showed differences between the North and South Islands of New Zealand and similarity in the admixture pattern between the South Island and Australia. Although the optimal K-value was one genetic cluster, we present the results for $K=2$ to $K=5$ in Figure 4 and Figure S2.

3.2 | Laboratory crossing experiments

3.2.1 | Fecundity

Mean total fecundity differed for the outbred lines ($n=9801$ eggs laid) versus inbred ($n=3896$ eggs) lines, with inbred/outbred status significantly predicting total fecundity in the linear model (Figure 5a and Table 2). Exploring this further, the total number of eggs laid per day differed, such that outbred lines experienced a steady decline while inbred lines had a rapid drop-off from a similar high initial number of ~500 eggs laid on Day 1 (Figure 5b). Using a linear mixed effects model, we found that line inbred/outbred status significantly affected rates of egg laying (Figure 5c). However, these differences were driven by a major time effect, with no significant differences in fecundity between inbred and outbred lines detected over the first 5 days of measurement (Figure 5d).

3.2.2 | Developmental rate

The mean minimum larval duration (averaged for each of the five inbred and outbred lines) was reduced in outbred lines, with an average of 13 days (range 9–18) for the inbred lines and exactly 8 days for the outbred lines. There was no difference in minimum time spent in the pupal stage, as both lines spent an average minimum of 10 days as pupa (Figure 6a).

Using the developmental rate data, we calculated the proportion of adults that survived to adulthood (i.e. those that emerged from pupa divided by the total number of pupae for each line). Here, we found that outbred lines had a significantly higher chance of emergence – 25% greater than the inbred lines (Table 2 and Figure 6b).

3.2.3 | Body size

Mean overall body size was significantly different between males and females, and was significantly larger in outbred lines for both sexes compared to inbred lines (Figure 6c and Table 2). We next explored whether body size correlated with higher fecundity and better survival (potentially suggesting a mechanistic link between genetic diversity and fitness). We found that line-averaged female body size was predictive of the total fecundity of both inbred and outbred lines (Figure S4 and Table S3).

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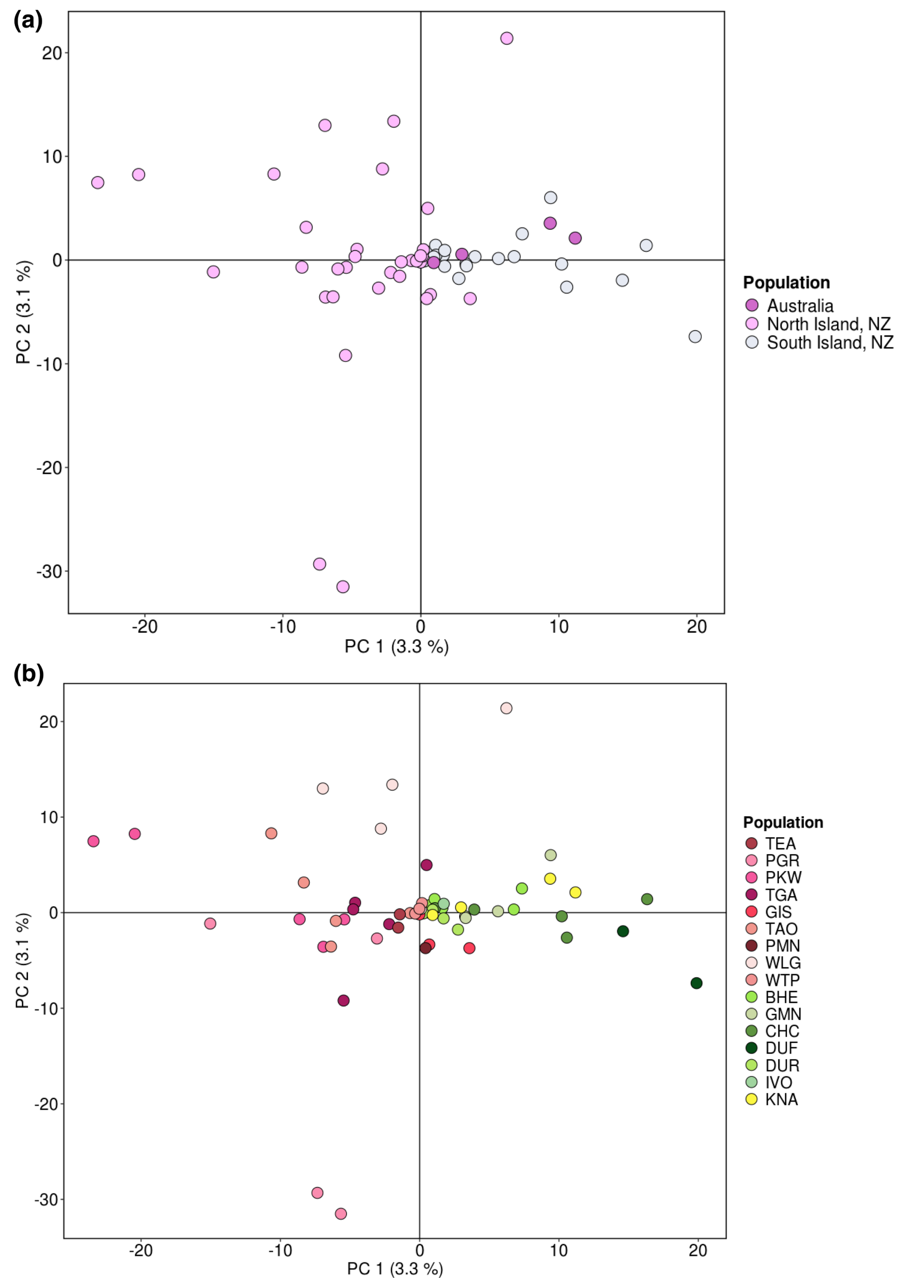

FIGURE 3 PCA plots showing *Calliphora vicina* samples (20,501 SNPs) from: (a) broad regions (Australia and North and South Islands of New Zealand); and (b) populations (see Table 1 for population codes).

3.2.4 | Lifespan

Mean lifespan was slightly reduced in inbred lines for both sexes, but this was not significantly different based on the linear mixed effects model (Figure 6d and Table 2). There were also no significant differences in lifespan between males and females (Figure 6d and Table 2).

3.2.5 | Population performance

Population performance was found to be significantly reduced in inbred lines, which spend longer durations as larvae, had lower survival to adulthood and had lower lifetime fecundity (Figure 6a,b and Table 2).

4 | DISCUSSION

We characterized the population structure of invasive New Zealand populations of a cosmopolitan blowfly and measured the fitness effects resulting from controlled population bottlenecks in wild-caught populations. We found that wild-caught populations were genetically depauperate with limited population structure. Our experiments revealed that fitness was significantly reduced following laboratory-induced population bottlenecks, but the effects were not felt equally across all phenotypic traits. Moreover, although estimates of long-term population fitness were reduced following inbreeding, they were not sufficient for any single line to completely crash, suggesting that *C. vicina* populations exposed to low-stress and permissive environments (such as laboratory conditions, where

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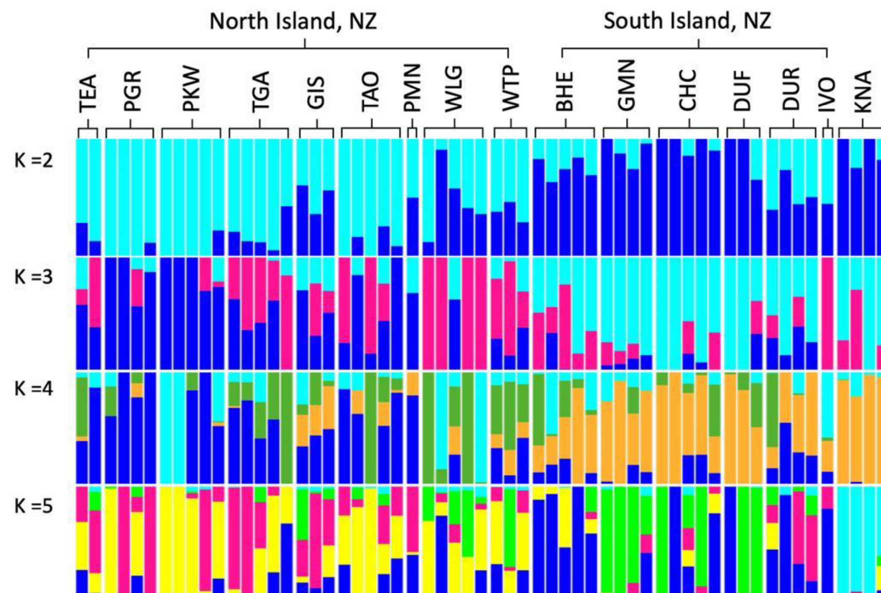

FIGURE 4 Admixture plots for *Calliphora vicina* were produced using Sparse Non-Negative Matrix Factorization (snmf) analysis of the neutral dataset containing 20,501 SNPs, where the optimal K value was determined to be $K=1$. Admixture proportions showing $K=2$ to $K=5$ are presented, with populations 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 (KNA). See Table 1 for population codes.

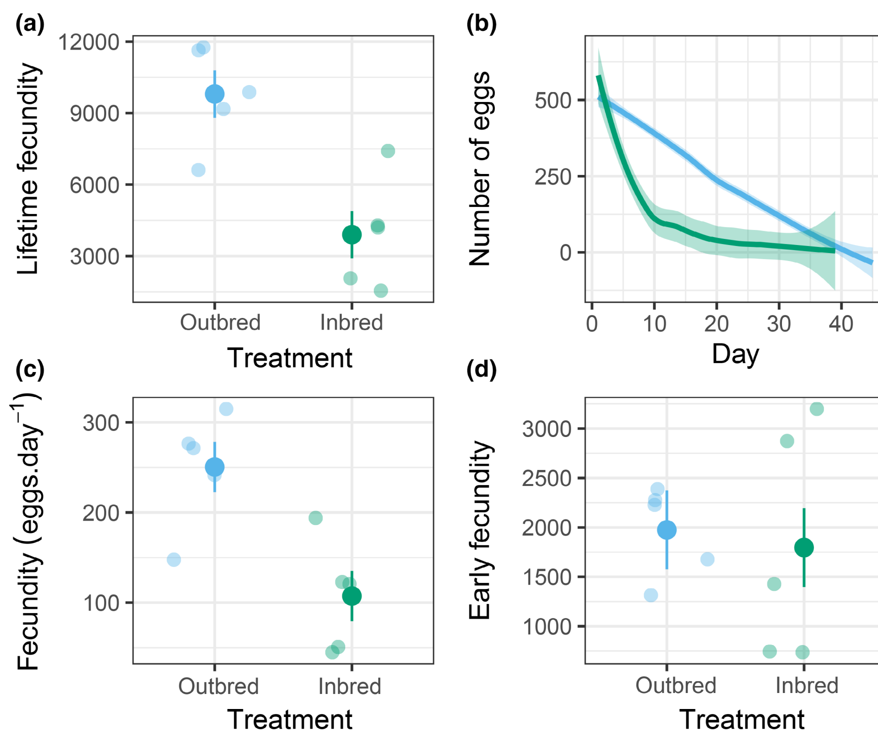

FIGURE 5 Differences in several measures of fecundity among inbred and outbred lines. (a) Lifetime fecundity (total number of eggs produced per replicate line); (b) Number of eggs laid per day (calculated for each line as: Lifetime fecundity/number of egg-laying days; lines fit with geom_smooth, method = 'loess'; blue = outbred; green = inbred); (c) Fecundity rate (number of eggs laid per day); (d) Early fecundity, measured as the total number of eggs laid in the first 5 days. Darker points in panels (a, c, d) represent means for inbred and outbred lines, and lighter points represent the individual values of each of the five inbred or outbred lines.

resources are non-limiting) may be able to compensate for population bottlenecks even when inbreeding is high.

Only mitochondrial diversity had been examined in *C. vicina* prior to our study, and this revealed a large number of haplotypes with no geographic structuring among populations in Spain and Portugal (Fuentes-López et al., 2020), and in Germany and England (Limsopatham et al., 2018). We found a similar lack of localized population structure using genome-wide SNP data. The small number of Australian samples ($n=4$) in our study clustered together with the South Island populations, suggesting close genetic linkages that may be indicative of source-to-sink relationships. However, the invasion pathway into New Zealand cannot be accurately determined yet, as we lack comparative samples from other areas across the full distribution range of *C. vicina*.

As well as a lack of genetic structure, we found low levels of genetic diversity (low observed heterozygosity values comparable

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TABLE 2 Results of the analysis of variance (type III) for the effects of inbred/outbred status (two levels: Inbred or outbred), sex (two levels: Male or female) and their interactions on lifetime fecundity, egg-laying rate, early fecundity, minimum larval duration, minimum pupal duration, emergence success, body size, lifespan and population performance.

Trait	Variable		
Fecundity (lifetime) (Figure 5a)	Inbred/outbred status	*F*	17.619
		df	1,8
		p	**.003**
Egg-laying rate (fecundity) (Figure 5c)	Inbred/outbred status	*F*	13.155
		df	1,8
		p	**.007**
Fecundity (early) (Figure 5d)	Inbred/outbred status	*F*	0.103
		df	1,8
		p	.759
Minimum duration (larvae) (Figure 6a)	Inbred/outbred status	*F*	11.905
		df	1,8
		p	**.009**
Minimum duration (pupae) (Figure 6a)	Inbred/outbred status	*F*	0.082
		df	1,8
		p	.782
Emergence success (Figure 6b)	Inbred/outbred status	*F*	51.23
		df	1,8
		p	**<.001**
Body size (Figure 6c)	Inbred/outbred status	$\chi 2$	5.735
		df	1
		p	**.017**
	Sex	$\chi 2$	4.088
		df	1
		p	**.043**
	Inbred/outbred status × Sex	$\chi 2$	0.715
		df	1
		p	.398
Lifespan (Figure 6d)	Inbred/outbred status	$\chi 2$	1.488
		df	1
		p	.223
	Sex	$\chi 2$	2.716
		df	1
		p	.099
	Inbred/outbred status × Sex	$\chi 2$	0.5
		df	1
		p	.479
Index of performance (Figure 7a)	Inbred/outbred status	*F*	29.222
		df	1,8
		p	**<.001**
Lifetime performance (Figure 7b)	Inbred/outbred status	*F*	27.526
		df	1,8
		p	**<.001**

Note: Bold values indicate significant values.

with other invasive populations; e.g. Hjort et al., 2023; Matheson et al., 2023) in wild populations. Similarly low genetic diversity has been observed in wild populations of other flies, including *Chrysomya latifrons* over a wide expanse of New South Wales (Australia) rain forests (Butterworth et al., 2023), the North American *Phormia regina* (Picard & Wells, 2009) and the Queensland fruit fly *Bactrocera tryoni* (an incredibly invasive pest of Australian horticulture; Popa-Báez et al., 2020). Thus, several widely successful dipteran species appear to remain ecologically successful despite their low genetic diversity.

Exploring the direct impacts of inbreeding and relative genetic diversity on population fitness in *C. vicina*, we found that outbred lines outperformed inbred lines in all measured traits (i.e. lifetime and daily fecundity, body size, minimum larval duration and emergence success) except lifespan. This was generally expected, as traits tied closely to fitness (i.e. fecundity, development and body size) will be most heavily impacted by inbreeding depression – though not all traits are expected to show equivalent responses (e.g. DeRose & Roff, 1999; Roff, 1998). Regarding fecundity, our results are consistent with those across many diverse taxa, where inbreeding has been shown to reduce this measure of fitness (Pedersen et al., 2005; Roff, 1998; Ross et al., 2019). For example, research on the estuarine crustacean *Americamysis bahia* found that lower genetic diversity was associated with lower population fitness in both permissive and stressful environments (Markert et al., 2010). Similarly manipulated populations of the flour beetle *Tribolium castaneum* – ranging from completely inbred to highly admixed – showed increased fitness (in the form of surviving offspring) at higher levels of genetic diversity (Durkee et al., 2023). Interestingly, we found no effect of inbreeding on lifespan in either sex – which may be because lifespan is not closely tied to fitness in these populations of *C. vicina*, as the majority of offspring are laid in the first 2 weeks of adult emergence, after which fecundity has been shown in other blowflies to drop significantly (Readshaw & van Gerwen, 1983). Other studies, however, have found contrasting effects of inbreeding on lifespan, with higher levels of inbreeding either increasing (Bilde et al., 2009) or decreasing lifespan (Vermeulen & Bijlsma, 2004).

The implications of higher fitness associated with more diverse lines are considerable. For example, our developmental rate data indicated that outbred lines spent shorter minimum periods in the larval stage (8 days, compared to 13 days for the inbred lines). Outbred lines also experienced a 25% greater likelihood of emergence as adults and a longer overall lifespan. Survival and progression through development have 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 outbred lines here were able to produce over two times the total number of eggs when compared to the inbred lines, and scaling up trait-specific patterns into metrics of population performance further supported the notion that population fitness is substantially higher in outbred lines. However, despite their lower overall fecundity and a rapid drop in egg production, there was no difference in the number of eggs laid by inbred lines

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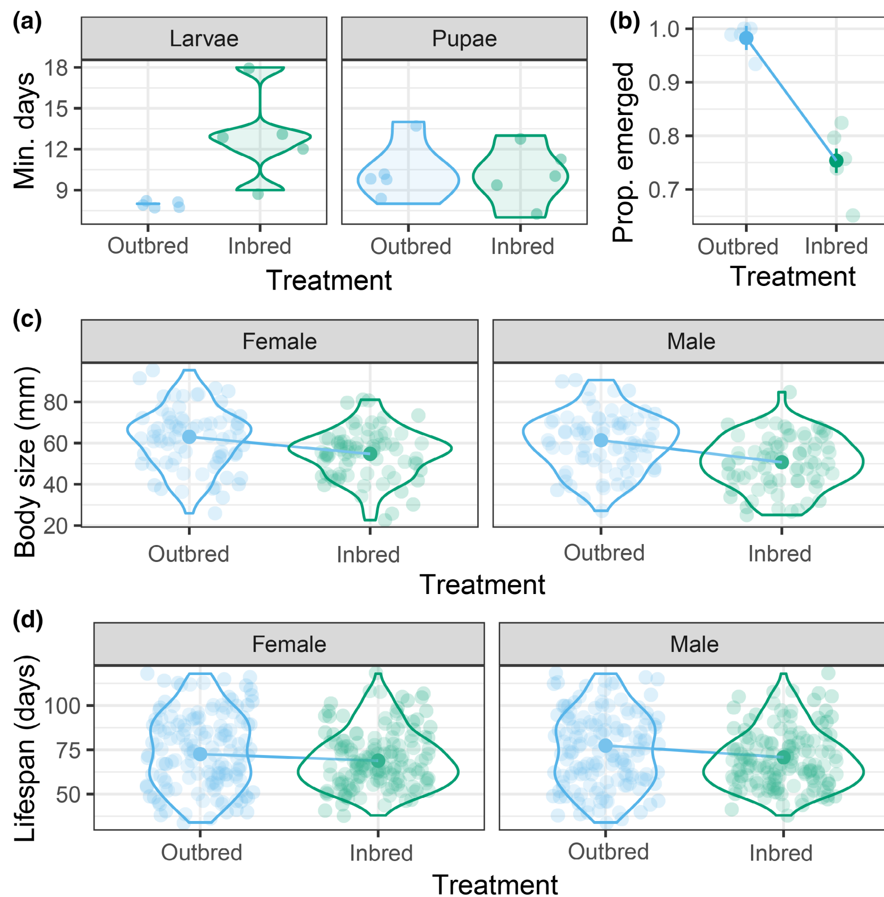

FIGURE 6 Life-history traits (a) Developmental rate (average minimum time spent in each life stage [averaged across all five populations per line]); (b) Predicted mean emergence proportions (number of adult flies/number of pupae) (i.e. survival to the adult stage) per line for the F2 generation based on a linear model (emergence proportion ~ inbred/outbred status); (c) Overall body size (i.e. thorax width × thorax length × head length) in males and females, plotted for both inbred and outbred lines; (d) Lifespan (total number of days alive, from the day eggs were laid to the day of adult death) for inbred and outbred lines. There were no significant differences found between male and female lifespan.

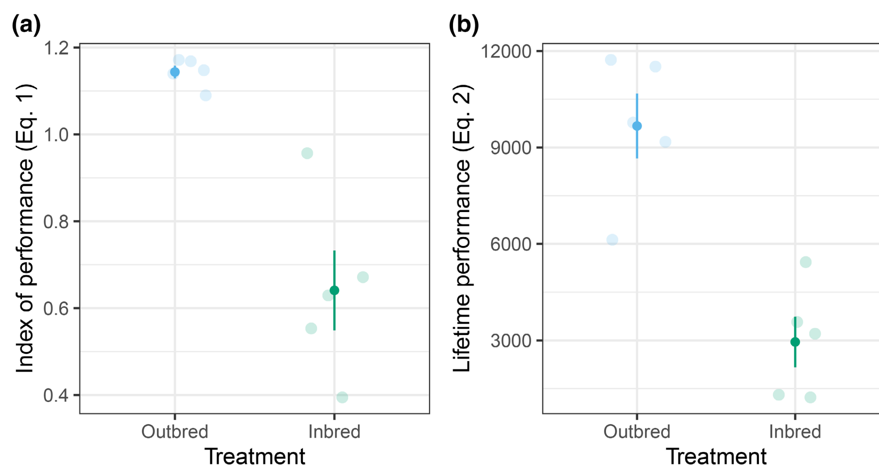

FIGURE 7 Metrics of population performance for inbred and outbred *C. vicina*. (a) Index of performance from Ross et al. (2019) (Equation 1); and (b) Lifetime performance from Facon et al. (2011) (Equation 2) Dark-coloured circles represent treatment means, lighter circles represent line-specific measures and bars represent ±SE.

over the first 5 days. Thus, there may be some compensation in the inbred lines, such that laying many eggs initially may to some extent counteract the negative impacts of genetic bottlenecks on fitness.

While the extent of inbreeding was relatively severe here, none of the inbred lines became extinct. Notably, however, this was following only two generations of inbreeding, and the outcomes of longer term inbreeding in *C. vicina* remain unclear (although healthy laboratory stocks of *C. vicina* can be reared for at least 70 generations with no new introduction of genetic diversity; pers. obs.). Other fly species seem to be similarly resistant to the effects of laboratory inbreeding. For example, inbred lines of the house fly *Musca domestica* experienced similar reductions in fitness but were able to persist for as many as 18 generations of inbreeding (Day et al., 2003). Conversely, research in *Aedes aegypti* mosquitoes showed that

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80% of inbred lines went extinct after 12 generations of inbreeding. Larsen et al. (2011) provides a unique case in the guppy *Poecilia reticulata*, where some genotypes were able to purge deleterious mutations and recover to pre-inbreeding levels of fitness after 10 generations of inbreeding, while other genotypes (presumably those with higher genetic loads) became extinct. Thus, the likelihood of extirpation following heavy inbreeding may depend on the initial genetic background. Whether invasive species are more likely to have genetic backgrounds that facilitate purging remains an open question that rearing of *C. vicina* populations over longer terms may help to address.

Other (potentially idiosyncratic) factors may also ultimately drive tolerance of inbreeding effects, and low genetic diversity does not necessarily preclude successful populations, particularly those that adopt previously underutilized niches where competitors are scarce (Benazzo et al., 2017; Daly, Chabrerie et al., 2023; Daly, Gerlich et al., 2023). As a synanthropic species, *C. vicina* has spread widely by capitalizing on urban environments (Hwang & Turner, 2009) and may have been one of the first urban-exploiting blowflies to arrive in New Zealand. Multiple ongoing introductions via trade are likely and subsequent genetic and demographic rescue and heterosis are likely in the event of initial incursions and small founder populations. The species may also benefit from genetic purging, like other small populations (Benazzo et al., 2017; Khan et al., 2021). Finally, a polyandrous mating system may help to minimize the effects of inbreeding on genetic diversity – polyandry is frequently observed among blowflies (Butterworth et al., 2019; Jones et al., 2017) and females can likely store sperm from multiple males (Jones et al., 2015; Smith et al., 1988), which would enable half-siblings to be generated in small populations and may mitigate effects of inbreeding depression (Cornell & Tregenza, 2007).

An important caveat is that our results are from laboratory populations, which generally experience vastly different selective pressures compared to wild populations (Calisi & Bentley, 2009). Laboratory populations of *C. vicina* in the present study were afforded non-limiting beef mince as a resource and experienced reduced competition, a stable environment, the absence of predators and very different community compositions compared to wild populations. Such differences in both soft selection (selection on fitness relative to that of conspecifics) and hard selection (selection on absolute fitness) are essential to consider in understanding the effects of inbreeding depression (Bell et al., 2021; Benazzo et al., 2017). Estimating and measuring the population growth rates (i.e. as per Emiljanowicz et al., 2014) of inbred and outbred lines across a range of environments and selective pressures to assess inbreeding × environment interactions (i.e. Schrieber & Lachmuth, 2017) is a logical next step that would enable further exploration of the effects of relative genetic diversity/inbreeding on rates of invasive population persistence in the real world.

Mechanistic explanations for our finding that inbreeding significantly reduces population-level fecundity and has varying effects on different phenotypic traits will similarly require additional work. For example, explanations related to inbreeding depression and the exposure of deleterious variants related to growth, development and fecundity in inbred lines (e.g. Frankham, 1995; Hewett et al., 2024; Willoughby et al., 2017) will require DNA sequencing. Alternative explanations could include the effects of kin recognition in larvae (Khodaei & Long, 2019) or inbreeding avoidance by adult siblings (Szulkin et al., 2013). For example, adults in the inbred lines may be less inclined to mate, which could explain their reduction in total fecundity. In regards to developmental traits, the extent of relatedness in a group has been shown to alter phenotypic trait expression (Dudley et al., 2013; Khodaei & Long, 2019) – including in the context of traits related to invasion (Kubisch et al., 2013; Van Petegem et al., 2018). In this study, it is possible that increased relatedness among individuals in the inbred lines resulted in slower larval development and lower proportions of emergence.

The influence of inbreeding depression and reduced genetic diversity on the fitness of invasive populations is a long-standing question of eco-evolutionary importance. Improving our understanding may have relevance to better mitigating invasive species and in targeted conservation of endangered and restricted small populations of native species through genetic and demographic rescue (Hufbauer et al., 2015; Keller & Waller, 2002). Moving forward, replication of our study methods on other invasive (and non-invasive) taxa is a key priority for further investigation, as is analysis of how the impacts of genetic diversity/inbreeding on fitness may change with a species' degree of invasiveness, age of invasion and other factors of interest, such as the extent of environmental perturbation.

AUTHOR CONTRIBUTIONS

AM designed and supervised the research, contributed all resources and co-wrote the manuscript; NB co-designed the research, performed statistical analyses and co-wrote the manuscript; LC performed the lab experiments, analysed the genetic data, performed preliminary statistical analyses and wrote the first manuscript draft with assistance from AM and NB as required; PM analysed the genetic data and co-wrote parts of the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The raw sequence data are deposited in the NCBI Sequence Read Archive (SRA) database, Accession no. PRJNA1097288; associated metadata are provided in Table S1. All phenotype data and R code

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used to create manuscript figures and tables are available at: https://github.com/invasomics/inbreeding.

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data, as described above.

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