

Editor's choice

Historical museum samples reveal signals of selection and drift in response to changing insecticide use in an agricultural pest moth

Elahe Parvizi¹, Andy Bachler^{2,3}, Andreas Zwick⁴, Tom K. Walsh³, Craig Moritz²,
Angela McGaughan^{1,2}

¹Department of Ecology, Biodiversity and Animal Behaviour, Te Aka Mātutua/School of Science, University of Waikato, Hamilton, New Zealand

²Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, ACT, Australia

³Commonwealth Scientific and Industrial Research Organisation, Land & Water, Black Mountain Laboratories, Canberra, ACT, Australia

⁴National Research Collections Australia, Commonwealth Scientific and Industrial Research Organisation, Black Mountain, Canberra, ACT, Australia

Handling editor: Xiang-Yi Li Richter, Associate editor: Neda Barghi

Corresponding author: Angela McGaughan, Te Aka Mātutua/School of Science, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand.

Email: amcgaugh@waikato.ac.nz

Abstract

In response to environmental and human-imposed selective pressures, agroecosystem pests frequently undergo rapid evolution, with some species having a remarkable capacity to rapidly develop pesticide resistance. Temporal sampling of genomic data can comprehensively capture such adaptive changes over time, for example, by elucidating allele frequency shifts in pesticide resistance loci in response to different pesticides. Here, we leveraged museum specimens spanning over a century of collections to generate temporal contrasts between pre- and post-insecticide populations of an agricultural pest moth, *Helicoverpa armigera*. We used targeted exon sequencing of 254 samples collected across Australia from the pre-1950s (prior to insecticide introduction) to the 1990s, encompassing decades of changing insecticide use. Our sequencing approach focused on genes that are known to be involved in insecticide resistance, environmental sensation, and stress tolerance. We found an overall lack of spatial and temporal population structure change across Australia. In some decades (e.g., 1960s and 1970s), we found a moderate reduction of genetic diversity, implying stochasticity in evolutionary trajectories due to genetic drift. Temporal genome scans showed extensive evidence of selection following insecticide use, although the majority of selected variants were low impact. Finally, alternating trajectories of allele frequency change were suggestive of potential antagonistic pleiotropy. Our results provide new insights into recent evolutionary responses in an agricultural pest and show how temporal contrasts using museum specimens can improve mechanistic understanding of rapid evolution.

Keywords: cotton bollworm, genetic drift, insecticide resistance, museum genomics, selection

Introduction

Some invasive and pest species show a remarkable ability to overcome novel environmental conditions that would otherwise constrain their survival in introduced ranges, with rapid post-introduction evolution directly contributing to invasion success (Reznick et al., 2019). Historical genomic data from museum and herbarium collections can provide a rich source of historical material to track adaptive evolutionary changes through time and shed light on the genetic architecture of adaptation to sudden environmental shifts (Benham & Bowie, 2023). Such data are especially valuable for understanding evolutionary responses of pest species within agroecosystems, where intense anthropogenic pressures like pest control practices often drive rapid evolutionary change (Cohen et al., 2022; Fritz et al., 2018). However, the extent to which genomic patterns of selection and drift in agricultural pests have been affected by the sequential use of insecticides with different mechanisms of action is unclear.

A major agricultural pest, the cotton bollworm (*Helicoverpa armigera* (Hübner, [1805])) is well known for repeated and rapid evolution of resistance globally across various pesticide

classes (Ahmad, 2007). Its high resistance capability can be linked to a wide geographic distribution (encompassing Africa, southern Europe, Asia, Australia, and New Zealand) and an ability to affect cotton crops, which have been extensively exposed to insecticides (Joußen et al., 2012). In Australia, insecticide resistance was first noted in *H. armigera* in the early 1970s, shortly after widespread intense nonspecific pesticide use. This included resistance to DDT (Dichloro-Diphenyl-Trichloroethane), parathion, endosulfan, endrin, and carbaryl (Wilson et al., 2018). Despite ongoing reports of resistance, pest control regimes remained almost exclusively reliant on such insecticides until the early 1980s, when integrated pest management approaches were adopted (Wilson et al., 2018). Genetically modified cotton was introduced in the late 1990s, however, pesticide use is still currently widespread in horticulture and grains (Bird, 2018; Walsh et al., 2022).

Insecticide resistance generally evolves by one of two main physiological mechanisms: (1) regulatory changes that enhance metabolism of the insecticide by increased activity of detoxifying enzymes, such as cytochrome P450s, glutathione-S-transferases,

Received October 19, 2023; revised April 9, 2024; accepted May 30, 2024

© The Author(s) 2024. Published by Oxford University Press on behalf of the European Society of Evolutionary Biology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

and carboxylesterases (e.g., [Schmidt et al., 2010](#)); and (2) mutation of the insecticide target protein, which makes it less sensitive to the actions of the insecticide (e.g., [Osta et al., 2012](#)). Although some single-gene regulatory and/or mutational pathways underlying insecticide resistance have been elucidated, whether these typically arise before or after the introduction of insecticides and/or whether they occur in tandem with other beneficial mutations (i.e., via polygenic adaptation) is uncertain. Additionally, the geographical and genetic complexity of resistance evolution can obscure understanding of fitness costs and trade-offs for resistance to different classes of insecticides.

Australian museums harbour a geographically extensive collection of *H. armigera* spanning >100 years. This includes specimens obtained before the application of insecticides (prior to the 1950s) and spans the use of various chemical classes through time as they have been introduced to the Australian market. Here, we used museum samples of Australian *H. armigera* collected between 1903 and 1995 to investigate patterns of evolutionary change through time. Leveraging the annotated reference genome of *H. armigera* ([Pearce et al., 2017](#)), we used a high-throughput targeted capture approach ([Jones & Good, 2016](#)) to extract a specific set of genes that are known to be involved in insecticide resistance, xenobiotic metabolism, environmental sensation, and tolerance ([ffrench-Constant, 2013](#); [Gao et al., 2022](#); [King & MacRae, 2015](#); [Vieira & Rozas, 2011](#)) (Table 1). Using these data, we assessed changes in genetic diversity, population structure, and allele frequency over time (i.e., before and after the introduction of insecticides) with a view towards examining shifting signatures of recent positive selection.

Materials and methods

Moth sample collection and temporal population definition

A total of 254 pinned specimens of *H. armigera* were obtained from several museums and government departments across Australia (Supplementary Table S1). After confirming a lack

Table 1. List of the genes used in the reference-based target capture sequencing of historical samples of *Helicoverpa armigera*, showing number per gene family.

Role	Gene family	n
Pesticide targets/ detoxification	ABC transporters (ABCs)	54
	Cadherins (CADs)	37
	Carboxyl/cholinesterases (CCEs)	103
	Glutathione-S-transferases (GSTs)	42
	Ion channels (ICs)	63
	Lipases (LIPs)	93
	Cytochrome P450s (P450s)	136
	Serine proteases (SERs)	230
	Uridine diphosphate glucuronosyltransferases (UGTs)	46
	Chemosensory receptors (CRPs)	424
Environmental sensation/tolerance	Heat shock proteins (HSPs)	41

of continent-wide population structure (see Results), a total of six “temporal populations” were defined as: pre1950s (1903–1945), 1950s (1950–1959), 1960s (1960–1969), 1970s (1970–1979), 1980s (1980–1989), and 1990s (1990–1995) which corresponded closely to the time before insecticides were used in Australia (pre1950s), and to the use of different classes of insecticides through time (Figure 1A).

DNA extraction and exon capture library preparation

A modified “salting-out” protocol ([Sunnucks & Hales, 1996](#)) was followed to extract genomic DNA. We used standard NGS library preparation steps but included several modifications due to the fragmented nature of the starting material (see Supplementary Methods for full details) and sequencing was carried out on an Illumina NextSeq500 (75 bp PE) at the Biomolecular Resource Facility (Australian National University). Baits were designed with target full exons of 1,269 genes (extracted from the *H. armigera* annotated genome, “Harm_1.0,” ~337 Mb; GenBank assembly accession: GCA_002156985.1; [Pearce et al., 2017](#)) in eleven gene families known to be involved in insecticide resistance and xenobiotic metabolism ([ffrench-Constant, 2013](#); [Gao et al., 2022](#)), and environmental sensation and tolerance ([King & MacRae, 2015](#); [Vieira & Rozas, 2011](#)) (Table 1). Because insecticide resistance can involve gene variation and/or regulatory changes, our capture design also encompassed regions upstream of the loci of interest to capture potential regulatory genomic features, such as promoters and enhancers or linked variants.

Bioinformatic processing

Quality control of raw reads was performed using FastQC v.0.10.1 ([Andrews, 2010](#)). Trimmomatic v.0.36 ([Bolger et al., 2014](#)) was then used to remove adapters, low quality bases and reads with lengths <36 bp. Trimmed reads were aligned to the Harm_1.0 *H. armigera* reference genome using the bwa v.0.7.5a mem algorithm ([Li & Durbin, 2009](#)) using read group information and default parameters. Duplicate reads were removed using picard v.2.10.6, and low quality and ambiguous alignments were removed with samtools v.1.5 ([Li et al., 2009](#)). Finally, ngsCAT v.0.1 ([López-Domingo et al., 2014](#)) was used to obtain various metrics of alignment quality associated with the targeted genomic regions.

Temporal population genomic analysis

To take genotype uncertainty into account, genomic analyses predominantly used Analysis of Next Generation Sequencing Data (ANGSD) software, v.0.931-11 ([Korneliussen et al., 2014](#)). We implemented standard population structure methods, including a principal component analysis (PCA) and admixture analysis and explored the impact of data quality on population structure using a PCA. Linkage disequilibrium (LD) was calculated (complete exons and flanking regions) from each chromosome using ngsLD v.1.1 ([Fox et al., 2019](#)). One-dimensional folded site-frequency spectra (SFSs) were inferred directly from genotype likelihoods for each temporal population using realSFS, and used to estimate nucleotide diversity [Watterson’s theta, θ_{ws} ([Watterson, 1975](#))] and Tajima’s estimator, θ_{π} , ([Tajima, 1989](#)) and Tajima’s D (T_D ; [Tajima, 1989](#)). We also generated allele frequency estimates for each temporal population and calculated pairwise F_{ST} for all possible temporal population pairs. Further details on all population genomic analyses are provided in Supplementary Methods.

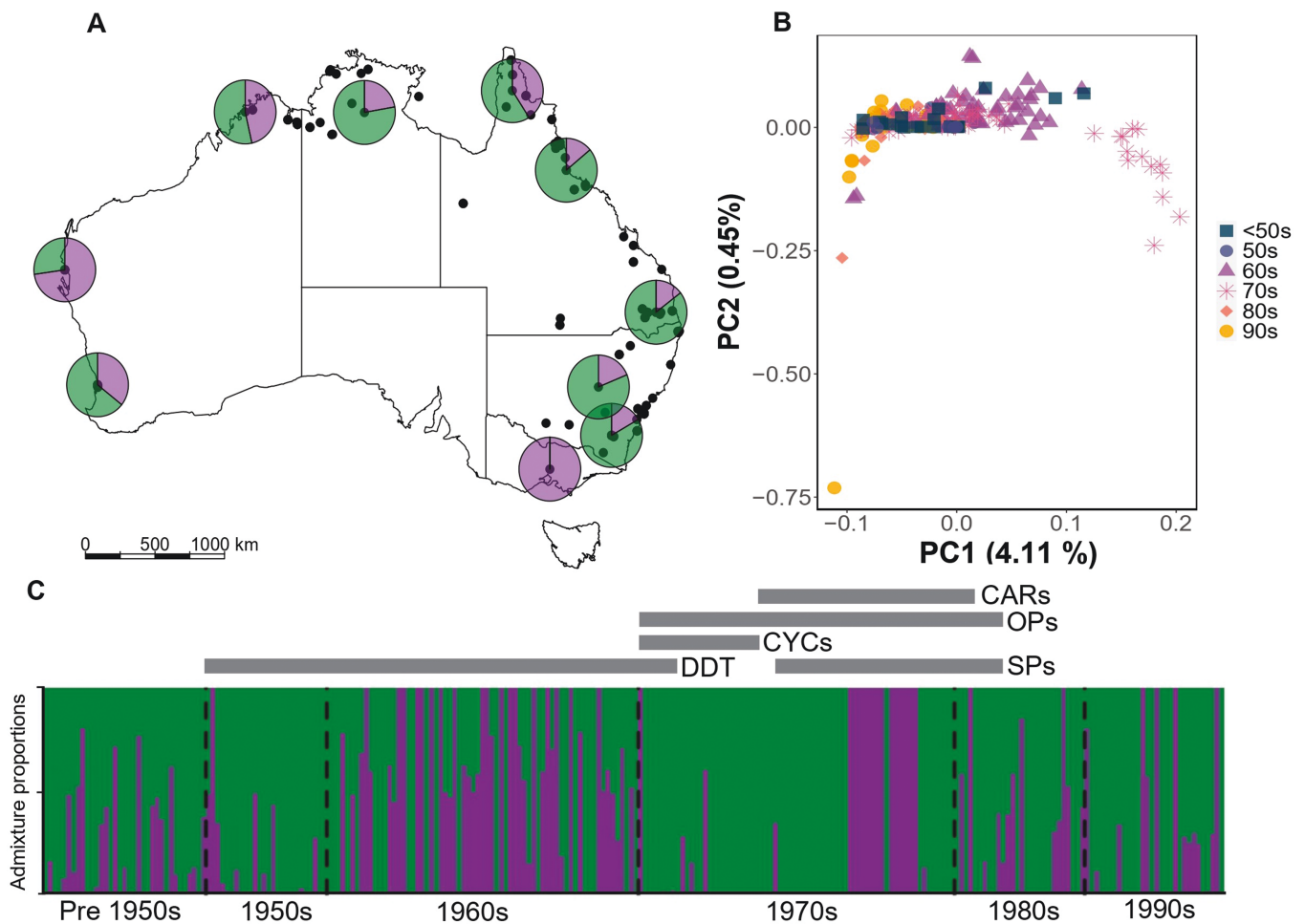


Figure 1. Temporal and spatial population genomic structure in *Helicoverpa armigera* in Australia. (A) Geographic distribution of historical samples used in this study. (B) Principal components analysis, with samples coloured by time according to the key at the right of the plot. Each data point in the PCA represents an individual specimen. The first and second two principal components (PCs) explain 4.11% and 0.45% of the total genetic variance, respectively. (C) Genetic clustering by individual across time based on admixture analyses at $K = 2$. In (C), each individual is partitioned into coloured segments that indicate cluster membership and the y-axis ranges from 0 to 1. Pie charts in (A) represent the sum of all individuals' membership in each cluster at each general locality on the map (proximate individual sample localities were pooled for clarity). Admixture results are plotted for the inferred best number of clusters ($K = 2$), with results for $K = 3$ provided in [Supplementary Figure S1](#). The grey horizontal bars at the top of the admixture plot in (C) indicate the first use (left hand side) and first noted resistance (right hand side) of/to five classes of insecticides (DDT = Dichlorodiphenyltrichloroethane; SPs = synthetic pyrethroids; CYCs = cyclodienes; OPs = organophosphates; CARs = carbamates). The number of individuals in each temporal population is as follows: pre1950s = 35; 1950s = 26; 1960s = 67; 1970s = 68; 1980s = 28; 1990s = 30.

Signatures of recent positive selection

We used two approaches to detect candidate SNPs involved in local adaptation. We first used a PCA-based approach in the R package PCAdapt v.4.1.0 (Luu et al., 2017) without any prior definition of populations. Using the resulting scree and score plots, we retained two PCs. We computed test statistics and p -values using a minimum minor allele frequency (MAF) of 0.05 and chose SNP outliers by applying a Bonferroni correction to the p -values, setting a false discovery rate of 5%. We then ran PCAdapt in a population-specific manner to identify outliers in each decade (see Supplementary Methods). Second, we identified outlier SNPs by computing F_{ST} between pre- and post-insecticide populations, defining F_{ST} outliers as those whose value was higher than three standard deviations above the mean for the given population comparison (Maiorano et al., 2018; Pintus et al., 2014). For both PCAdapt and F_{ST} methods, we counted the number of times outlier SNPs were present in each functional gene group and evaluated the degree of overlap in SNPs across decades.

Additionally, we annotated the SNP outliers that were common between PCAdapt and F_{ST} to provide a list of candidate genes potentially under selection in each decadal population.

Changes in allele frequency through time

Changes in allele frequency over time were examined using the PCAdapt outliers and two decadal comparisons: pre1950s vs. 1960s vs. 1980s; and pre1950s vs. 1980s vs. 1990s—the decades with the most complete data allowing comparison through time (since not all sites were variant across all decades likely due to stochastic variation in sequencing coverage and SNP calling) (Figure 1). We took the average allele frequency for each decade generated by realSFS, returning three values for each allele (e.g., a value for pre1950s, 1980s, and 1990s) and then the change across those three values was evaluated if it exceeded a 5% threshold (i.e., all changes <0.05 were considered nonsignificant). We then subset the allele frequency data into different allele frequency change classes: “monotonic increase,” “monotonic decrease,” “alternating” (i.e.,

allele frequency went up then down across time periods, or down then up), and “no change.”

Changes in MAF ratios in coding regions through time

To examine signatures of selection at the level of genes, we analyzed changes in non-synonymous/synonymous MAF ratios through time. We first converted per-decade genotype likelihoods to minor allele frequencies using ANGSD. We then converted these to variant calls using a custom Perl script (Supplementary Methods), and annotated them using SnpEff v.5.1d (Cingolani, Platts, et al., 2012) and the *H. armigera* reference genome. Gene information was extracted using SnpSift v.5.1d (Cingolani, Patel, et al., 2012) and analyzed in R. We then removed genes with low support (see Supplementary Methods) and calculated the mean MAF for non-synonymous and synonymous sites for all genes to estimate a base rate of MAF change. Finally, we calculated the ratio of non-synonymous to synonymous MAFs per gene for each decade to identify a change in temporal rate relative to the base rate that would be indicative of positive selection.

Results

Population genomic structure and admixture

PCA did not reveal significant overall clustering related to any Australian territorial states, with populations from different geographic regions clustering together. However, the analysis revealed certain levels of local clustering that may be related to decades. Notably, some of the individuals from the 1960s and 1970s collections, which coincides with the start of heavy nonspecific pesticide use, could be distinguished in the PCA plot (Figure 1B). The first PC explained 4.11% of total variance while the second PC explained only 0.45% (i.e., close to random).

Admixture analysis identified $K = 2$ as the optimal level of clustering and no geographic or temporal separation of the clusters. At $K = 2$, there was proportionally lower admixture within the 1970s collections ($n = 68$) compared to other decades (Figure 1A,C; see Supplementary Figure S1 for the $K = 3$ results).

Genomic characteristics

One-dimensional folded SFSs exhibited overall consistent patterns across different decades, with singleton alleles most prevalent and a general exponential decline for more frequent classes of alleles, and implied high genomic diversity in all studied populations (Supplementary Figure S2). However, population genomic statistics, including θ_w and θ_π , showed variable genomic diversity across the sampled loci through time, with lower values in the 1960s and 1970s (θ_w : 4.29–4.41; θ_π : 2.50–2.66) and higher values in the pre1950s, 1980s, and 1990s (θ_w : 6.27–6.90; θ_π : 3.60–3.98) (Table 2). Nucleotide diversity (θ_w) showed a significant difference across the temporal populations (See Supplementary Methods). Tajima's D (T_D) was negative for all populations (Table 2), indicating an excess of low-frequency variants suggestive of potential demographic events, such as sudden population expansion or selective sweeps.

Population differentiation

Genome-wide differentiation between temporal populations was low (Supplementary Table S2). The least differentiated

Table 2. Estimates of population genomic diversity indices across populations of *Helicoverpa armigera* collected in different decades. Numbers in parentheses indicate standard deviation.

Population	Diversity statistics		
	Watterson's theta (θ_w)	Tajima's estimator (θ_π)	Tajima's D (T_D)
Pre1950s	6.279 (11.793)	3.694 (6.344)	−1.268 (0.560)
1950s	6.906 (14.274)	3.609 (6.776)	−1.474 (0.595)
1960s	4.415 (8.445)	2.666 (5.012)	−1.047 (0.638)
1970s	4.296 (8.064)	2.502 (4.573)	−1.085 (0.631)
1980s	5.005 (9.059)	3.322 (5.797)	−1.067 (0.621)
1990s	6.444 (12.068)	3.987 (7.164)	−1.243 (0.584)

pairs of populations were the pre1950s vs. 1950s (mean $F_{ST} = 0.014$) and 1950s vs. 1970s (mean $F_{ST} = 0.014$), while the most differentiated pairs of populations were the 1960s vs. 1970s, and 1960s vs. 1980s (mean $F_{ST} = 0.022$ for both pairwise comparisons). Due to high levels of spatial genomic homogeneity across our study area, these pairwise temporal differences are unlikely to be associated with sampling location.

Signatures of recent positive selection

SNP outliers

PCAdapt identified the highest number of outliers in the 1960s ($n = 1,388$; including 483 unique) and 1980s ($n = 5,387$; 935 unique) (Table 3). The lowest number of outliers was detected for the pre1950s samples ($n = 16$ total and unique; Table 3). We found statistically significant variation in the number of total and unique outliers across consecutive decades based on chi-square tests (p -value < 0.05), except for the pre1950s and 1950s populations (Supplementary Table S3). Exploring the total number of PCAdapt outliers for each functional gene category showed that all gene categories had low numbers of outliers ($n = 0$ –5) in samples from the pre1950s and 1950s (Figure 2; Table 4). Samples from the 1960s had considerably more outliers per category ($n = 10$ –103), with the number dropping in the 1970s ($n = 2$ –30), increasing considerably in the 1980s ($n = 24$ –267) and dropping again in the 1990s ($n = 1$ –42) (Figure 2; Table 4).

F_{ST} outlier analysis identified a varying proportion of unique outliers across decades and functional gene groups in comparison to the pre1950s population (Table 5). The lowest proportions of unique outliers were found when comparing the pre1950s samples to 1950s (0.05), 1980s (0.07), and 1990s (0.05), with an increase in outliers in the 1960s and 1970s (0.20 and 0.19, respectively). Across comparisons, for all functional gene groups except ABCs (which remained high for all decades), the absolute number of F_{ST} outliers started off high ($n = 106$ out of 114 total for the pre1950s to 1950s comparisons), dropped below 50 for the 1960s and 1970s comparisons, then increased to 100 and 97 (0.88 and 0.85 of the total, respectively) in the pre1950s vs. 1980s and 1990s comparisons (Table 5).

When comparing the outliers derived from both methods, almost all of the PCAdapt outliers were represented in F_{ST} outlier analysis. The only exceptions were a single outlier (of 96 total) in the PCAdapt 1970s list (HaOG200560; an LIP gene), and six outliers (of 935 total) in the PCAdapt 1980s list

Table 3. The absolute number (and proportion in parentheses) of variant sites in the SNP dataset and the number of total and unique outliers identified overall and per decade, using PCAdapt and following Bonferroni correction. Variations in the number of outliers and number of unique outliers across consecutive decades were statistically significant based on chi-square tests, except for pre1950s and 1950s, with differences in the number of variant sites due to SNP calling being performed independently for each decade (i.e., individuals within decades share variant sites that may be absent from other decades). See [Supplementary Table S3](#) for the full details of the chi-square tests.

Metric	pre1950s	1950s	1960s	1970s	1980s	1990s
No. variant sites	101,901 (0.0002)	94,673 (0.0002)	1,00,062 (0.0048)	90,035 (0.0011)	1,01,337 (0.0092)	1,24,886 (0.0013)
No. outliers	16	19	1388	109	5387	316
No. unique outliers	16	17	483	96	935	165

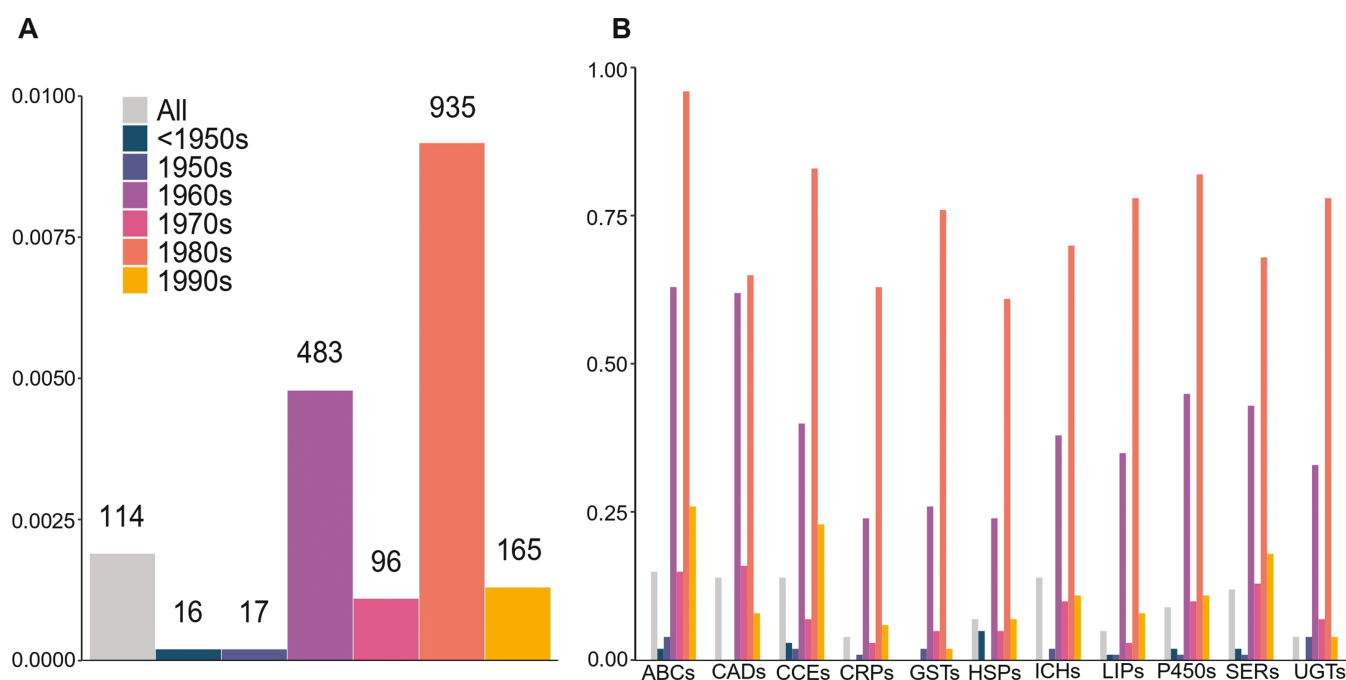


Figure 2. Proportion of exons containing PCAdapt SNP outliers. In each panel, numbers are plotted as the proportion of total sites that were identified as sitting in unique exons for each population and for the total dataset, as indicated by the provided colour key. (A) Overall results (with the total absolute number of unique exons containing outliers indicated above the bars); and (B) proportions in each of 11 gene family categories [ABCs: ATP-binding cassette transporters ($n = 54$), CADs: cadherin genes ($n = 37$), CCEs: carboxyl/cholinesterases ($n = 103$), CRPs: chemosensory receptor proteins ($n = 424$), GSTs: glutathione-S-transferases ($n = 42$), HSPs: heat shock proteins ($n = 41$), ICHs: ion channel-related genes ($n = 63$), LIPs: lipases ($n = 93$), P450s: cytochrome P450s ($n = 114$), SERs: serine-proteases ($n = 230$), UGTs: uridine diphosphate (UDP)-glucuronosyltransferases ($n = 46$); total number of exons: 1,247].

(HaOG200214 – CCE; HaOG200560 – LIP; HaOG200712, HaOG200818, HaOG200941 – CRPs; HaOG209424 – ICH). Both outlier detection methods identified at least one outlier in each of the studied genes (Table 1). Annotation of the outliers common between the two methods identified potentially adaptive genes (e.g., gustatory and odorant receptors, cytochrome P450, heat shock proteins, ATP-binding proteins, lipase, GST, and potassium channels; see [Supplementary Table S4](#)).

Allele frequency trajectories and changes in MAFs for coding regions through time

The majority of PCAdapt outlier allele frequencies changed over time in the two comparisons (pre1950s vs. 1960s vs. 1980s; pre1950s vs. 1980s vs. 1990s; see [Materials and Methods](#)), with around 40–50% of allele frequencies changing from one period to the next (Figure 3). The signal of temporal change was small for most genes except GSTs and HSPs.

The majority of these SNPs were low-impact variants (median number across functional gene families = 2,046),

while 372 were of moderate impact ([Supplementary Figure S3](#)). After filtering out low-support variants, a histogram of changes in non-synonymous/synonymous MAF ratios across decades indicated zero change compared to the base rate of change for the majority of these ratios ([Supplementary Figure S4](#)). However, significant variability in ratios over time (i.e., a change of ± 2 -fold deviation from the base rate change) occurred for 21 genes, encompassing eight gene families (Figure 4; [Supplementary Table S5](#)). CRP genes had the highest number of significant ratio changes through time ($n = 9$), followed by UGTs ($n = 3$), CCEs, ICHs, and SERs ($n = 2$), and GSTs, LIPs, and P450s ($n = 1$).

Discussion

We leveraged ~100 years of museum samples of *H. armigera* to show that pest populations experienced no major change in geographically driven population structure through time, but had a moderate reduction in genetic diversity in decades with intense applications of nonspecific insecticide use (1960s and

Table 4. The total number of outliers identified per gene family ($n = 1,247$) and the absolute number (and proportion per gene family in parentheses) of outliers identified overall and per decade for each gene family, using PCAdapt and following Bonferroni correction. No statistical test was possible in this case, since chi-square tests require a minimum of five observations per sample.

Gene family	Total in capture	All	pre1950s	1950s	1960s	1970s	1980s	1990s
ABCs	54	8 (0.15)	1 (0.02)	2 (0.04)	34 (0.63)	8 (0.15)	52 (0.96)	14 (0.26)
CADs	37	5 (0.14)	0 (0.00)	0 (0.00)	23 (0.62)	6 (0.16)	24 (0.65)	3 (0.08)
CCEs	103	14 (0.14)	3 (0.03)	2 (0.02)	41 (0.40)	7 (0.07)	85 (0.83)	24 (0.23)
CRPs	424	19 (0.04)	1 (0.00)	5 (0.01)	103 (0.24)	13 (0.03)	267 (0.63)	24 (0.06)
GSTs	42	0 (0.00)	0 (0.00)	1 (0.02)	11 (0.26)	2 (0.05)	32 (0.76)	1 (0.02)
HSPs	41	3 (0.07)	2 (0.05)	0 (0.00)	10 (0.24)	2 (0.05)	25 (0.61)	3 (0.07)
ICHs	63	9 (0.14)	0 (0.00)	1 (0.02)	24 (0.38)	6 (0.10)	44 (0.70)	7 (0.11)
LIPs	93	5 (0.05)	1 (0.01)	1 (0.01)	33 (0.35)	3 (0.03)	73 (0.78)	7 (0.08)
P450s	114	10 (0.09)	2 (0.02)	1 (0.01)	51 (0.45)	11 (0.10)	94 (0.82)	13 (0.11)
SERs	230	27 (0.12)	4 (0.02)	2 (0.01)	100 (0.43)	30 (0.13)	157 (0.68)	42 (0.18)
UGTs	46	2 (0.04)	0 (0.00)	2 (0.04)	15 (0.33)	3 (0.07)	36 (0.78)	2 (0.04)

Table 5. Results of the F_{ST} outlier analysis. The top row shows the number of unique outliers/number of total outliers, and the association proportion in parentheses for comparisons between populations collected in the pre1950s ("p50s") vs. the 1950s ("50s"), 1960s ("60s"), 1970s ("70s"), 1980s ("80s"), and 1990s ("90s"). Subsequent rows show the total number of outliers identified per gene family ($n = 1,247$), and the absolute number (and proportion per gene family in parentheses) of outliers identified overall and per decade for each gene family for those same population comparisons, following Bonferroni correction. Variations in the number of outliers and number of unique outliers across consecutive decades were statistically significant based on chi-square tests for some of the comparisons. See [Supplementary Table S6](#) for the full details of the chi-square tests.

Gene family	Unique outliers/Total outliers	p50s.50s	p50s.60s	p50s.70s	p50s.80s	p50s.90s
		1,099/23,021 (0.05)	421/2,105 (0.20)	447/2,328 (0.19)	983/13,707 (0.07)	1,039/20,748 (0.05)
Gene family	Total no. outliers in capture	p50s.50s	p50s.60s	p50s.70s	p50s.80s	p50s.90s
		p50s.50s	p50s.60s	p50s.70s	p50s.80s	p50s.90s
ABCs	54	53 (0.98)	48 (0.89)	49 (0.91)	50 (0.93)	52 (0.96)
CADs	37	25 (0.68)	20 (0.54)	19 (0.51)	30 (0.81)	27 (0.73)
CCEs	103	93 (0.90)	27 (0.26)	31 (0.30)	85 (0.83)	88 (0.85)
CRPs	424	339 (0.80)	73 (0.17)	83 (0.20)	283 (0.67)	308 (0.73)
GSTs	42	41 (0.98)	15 (0.36)	20 (0.48)	40 (0.95)	41 (0.98)
HSPs	41	35 (0.85)	10 (0.24)	13 (0.32)	30 (0.73)	30 (0.73)
ICHs	63	50 (0.79)	28 (0.44)	26 (0.41)	44 (0.70)	45 (0.71)
LIPs	93	84 (0.90)	42 (0.45)	37 (0.40)	82 (0.88)	83 (0.89)
P450s	114	106 (0.93)	52 (0.46)	51 (0.45)	100 (0.88)	97 (0.85)
SERs	230	182 (0.79)	69 (0.30)	77 (0.33)	167 (0.73)	175 (0.76)
UGTs	46	42 (0.91)	15 (0.33)	18 (0.39)	40 (0.87)	42 (0.91)

1970s). We further identified extensive signatures of selection following insecticide use and found evidence of alternating trajectories of allele frequency change over time.

Spatial and temporal patterns of population structure and diversity of *H. armigera* in Australia

Studies on the global population structure of *H. armigera* using SNPs obtained from contemporary samples have identified gene flow and population connectivity at inter-continental scales (Anderson et al., 2016; Jin et al., 2023). These studies, similar to previous microsatellite research (Endersby et al., 2007), also found an absence of discrete spatial population structure across mainland Australia (Anderson et al., 2016; Jin et al., 2023). Our targeted capture sequencing of historical samples confirmed these findings, showing that populations across mainland Australia were highly admixed, with no discernible geographic structure at genes likely to be relevant to insecticide resistance. Such notable population genetic

homogeneity can be attributed to the facultative migratory behaviour of *H. armigera*, its diverse host range (Jyothi et al., 2021), and its remarkable ability to disperse across high altitudes and long distances between different crop-growing areas (Jones et al., 2015, 2018).

We also found no signal for temporal changes in genetic diversity across most decades, but observed a moderate reduction following intense nonspecific insecticide use in the 1960s and 1970s. Previous microsatellite comparison of populations in Victoria across 3 years (1999, 2001, and 2004) with differing weather patterns also found no temporal genetic diversity changes, despite large observed differences in abundance of *H. armigera* during the study period (Endersby et al., 2007). Indeed, the lack of temporal diversity change observed here may be the result of a large effective population size of *H. armigera*, as found in previous mtDNA studies (e.g., Behere et al., 2007), and regional mixing—both of which could have hindered stochastic demographic impacts

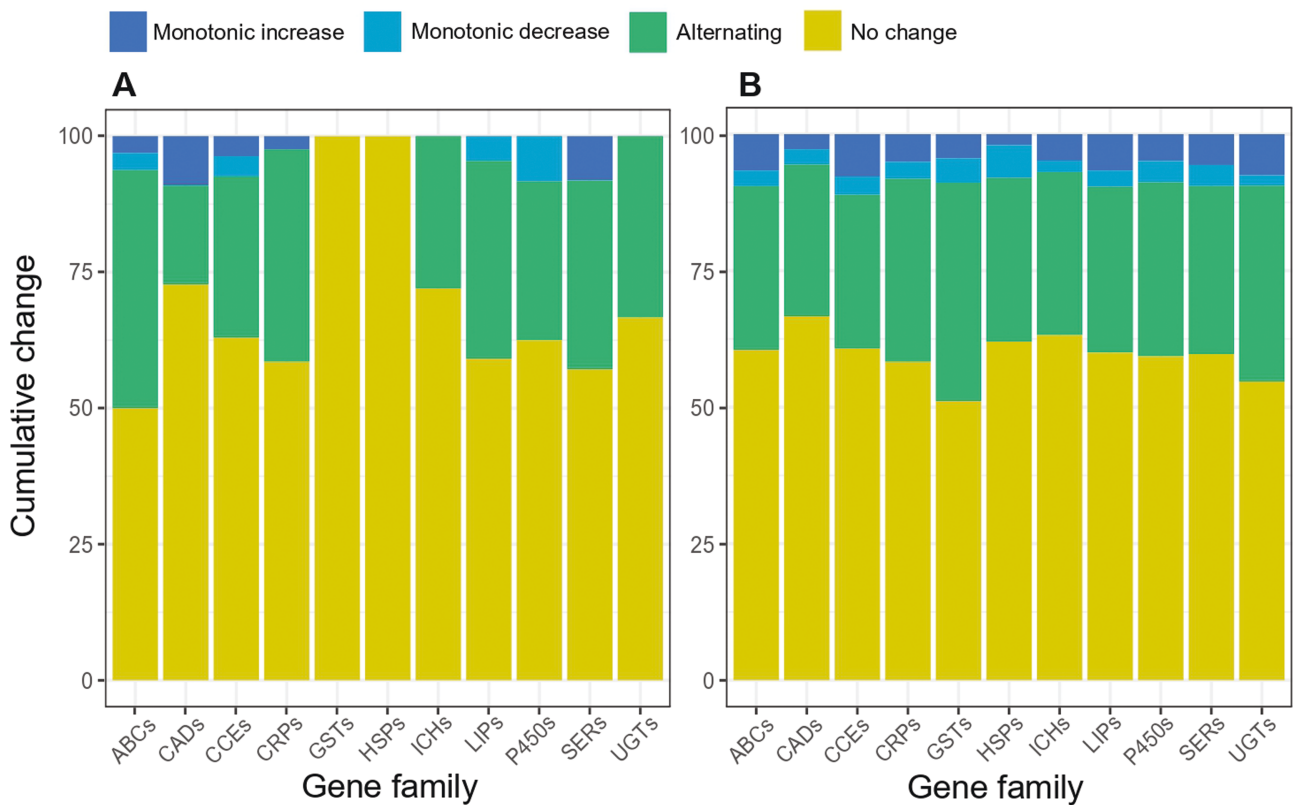


Figure 3. Temporal changes for PCAdept outliers >5% in allele frequency. Comparison of allele frequencies are shown between pre1950s and: (A) 1960s and 1980s; (B) 1980s and 1990s. See [Supplementary Figure S5](#) for a summarized overview of the “change vs. no change” patterns across different decades.

that might otherwise have driven shifts in population diversity over time (e.g., [Cousseau et al., 2016](#)).

Signatures of selection and drift through time

Evolved resistance in Australian *H. armigera* has been reported for all major classes of insecticides ([Daly, 1993](#); [Gunning et al., 1992, 1998](#)), with mechanisms including metabolic resistance (e.g., upregulation of detoxification enzymes in response to endosulfan and pyrethroids; [Daly, 1993](#); [Gunning & Easton, 1994](#)), and genetic changes that affect the sensitivity of target proteins to insecticides in response to pyrethroids, organophosphates and carbamates ([Daly, 1993](#); [Gunning et al., 1992, 1998](#)). Our temporal genome scans identified dynamic trends in the number of outlier loci across different decades. The pre1950s and 1950s samples had the fewest SNP outliers, possibly indicating a baseline condition in the absence of major insecticide pressure. However, a rise in genomic outliers during the 1960s was followed by a decline in the 1970s, a striking increase in the 1980s, and a subsequent drop in the 1990s. Two nonexclusive causes, including genetic drift associated with demographically driven initial reductions in genetic diversity and/or adaptive responses to heterogeneous selection pressures through time, could explain these trends and we elaborate on these below.

Demographic events typically associated with pest species that undergo major population contractions and subsequent expansions as selection pressures are imposed and insecticide resistance builds (i.e., bottlenecks or repeated gene flow), can drive patterns of allele frequency change ([Fritz, 2022](#); [Taylor et al., 2021](#)). In *H. armigera*, the slight divergence of the 1960s

and 1970s samples in the PCA plot, along with the identified reduction of genetic diversity in these two decades, could be explained by intense insecticide use in these two decades potentially contributing to an initial reduction in moth population sizes, with resulting increased genetic drift and stochasticity in the evolutionary trajectories of populations ([Saubin et al., 2023](#)).

Our temporal genome scans also identified varying numbers of outlier SNPs, indicative of positive selection throughout all decades. The majority of these variants had low impacts on genes and the majority of ratios of non-synonymous to synonymous MAFs for the functional gene families across decades were close to zero, indicating that purifying selection is most likely operating to preserve the amino-acid sequence ([Brookfield, 2000](#)). However, 21 genes from eight gene families (including CCE, UGT, GST, ICH, LIP, P450, SER, and CRP) showed >2-fold changes in non-synonymous/synonymous MAF ratios from the base rate of change, suggesting strong positive selection ([Dong et al., 2019](#); [Yang, 2004](#)) may be acting on these functionally important genes to facilitate rapid responses among moth populations to intense insecticide-driven pressures across decades. Alternatively, these allele frequency changes could have arisen due to environmental selective pressures unrelated to insecticides. Gene flow across a wide geographic scale in Australia has likely resulted in convergence patterns of allele frequency change in *H. armigera*.

The changes in numbers of outliers and alternating outlier allele frequencies over time are potentially suggestive of antagonistic pleiotropy—where alleles have opposing effects between components of fitness in the face of heterogeneous

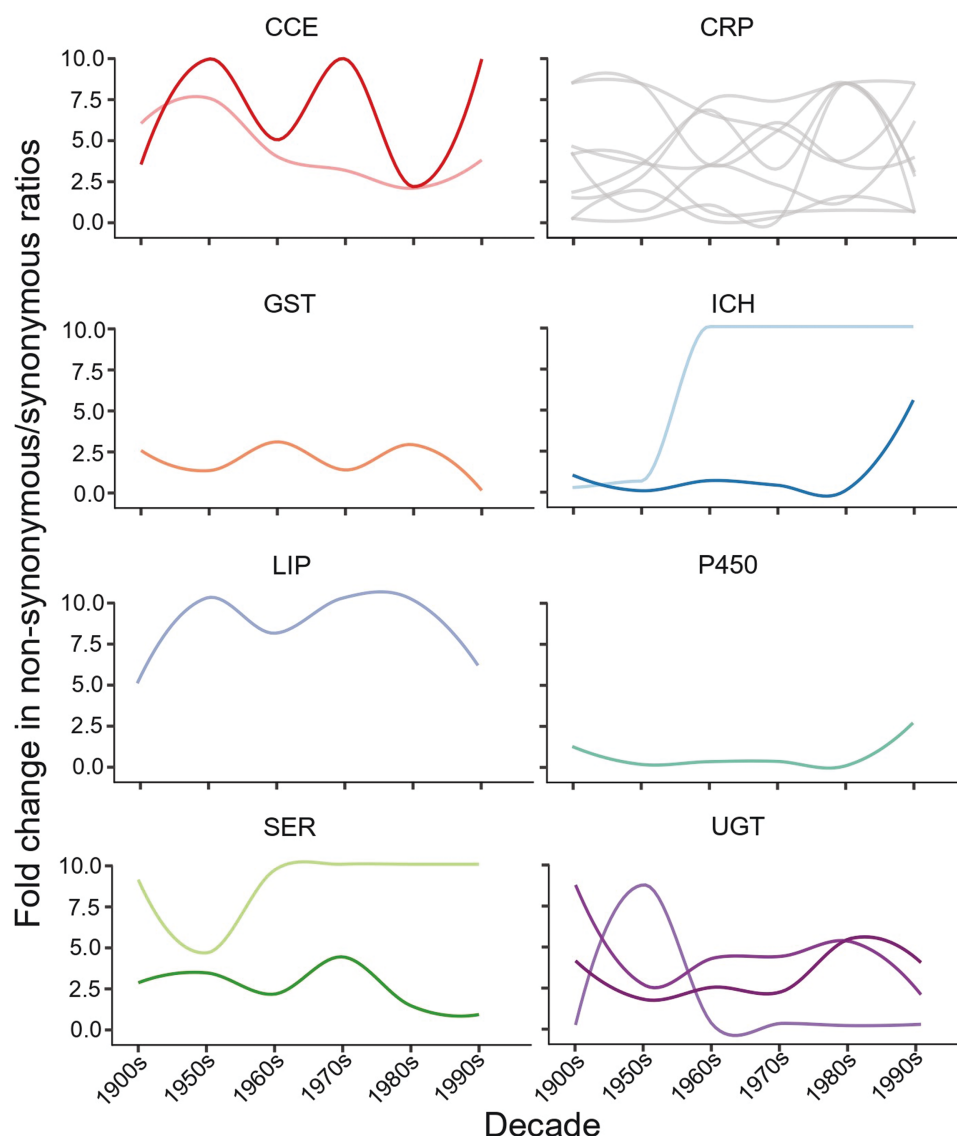


Figure 4. Highly variable non-synonymous/synonymous minor allele frequency (MAF) ratios across decades in different gene families. The changes in ratios for these genes fell outside the range of ± 2 -fold for at least one sequential decade. See [Supplementary Table S5](#) for the complete list of these genes, [Table 1](#) for gene family descriptors, and [Supplementary Methods](#) text for more details.

selection pressures (Chen & Zhang 2020; Connallon & Clark, 2012; Kawecki & Ebert 2004). In *H. armigera*, fitness benefits associated with insecticide resistance in the presence of a given insecticide may quickly incur costs when that insecticide was replaced with another that required adaptive alterations in a different gene or gene pathway. Trade-offs between the maintenance of insecticide resistance alleles and important life-history traits have been identified in several insect pests (Hawkins et al., 2019). For example, resistant strains of the codling moth, *Cydia pomonella*, showed lower fecundity and fertility, slower development, and shorter life-spans than their nonresistant counterparts (Boivin et al., 2001). In *Drosophila melanogaster*, seasonal fluctuation in susceptibility to insecticides is suggested to be associated with fitness costs of insecticide resistance factors (Miyo et al., 2000). Further, very high phytochemical similarity among different insecticides (which presumably require similar adaptive responses at the genetic level) has been shown to impede resistance adaptation via antagonistic pleiotropy in several insect pests (Crossley et al.,

2020). Alternatively, fluctuations in allele frequencies over time could be due to balancing selection and/or changes in directional selection in response to changing environmental selective pressures.

Explicit characterization of the relative roles of adaptive and demographic processes and their interaction through time would be a valuable future addition to this research, which would require whole genome resequencing data and in-depth demographic analyses that go beyond the scope of our study design. Even with such data, these analyses can prove challenging for museum datasets, where geographic specimen coverage and sample sizes may be poor, while teasing apart the effects of insecticide and other historical environmentally driven selective pressures is difficult. For *H. armigera*, we were fortunate to have representatives of multiple geographic regions in each decade, however further work investigating specific locations across Australia using higher replicates of temporal and geographic samples—especially for regions with a well-characterized history of insecticide use and lack

thereof—would be valuable. Coupled with broader genomic coverage, such data could also address gene regulatory evolution and the potential role of polygenic selection in rapid adaptation of *H. armigera*.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

Perl script to convert VCF to TSV is available in [Supplementary Methods](#). The genomic datasets used and/or analyzed in this study are available in [McGaughran \(2020\)](#) and were deposited in Genbank under BioProject ID PRJNA1097763.

Author contributions

Elahe Parvizi (Formal analysis [Supporting], Validation [Equal], Visualization [Equal], Writing—original draft [Lead], Writing—review & editing [Equal]), Andy Bachler (Formal analysis [Supporting], Methodology [Supporting], Software [Equal], Visualization [Equal], Writing—review & editing [Equal]), Andreas Zwick (Resources [Supporting], Writing—review & editing [Equal]), Tom Walsh (Validation [Supporting], Writing—review & editing [Equal]), Craig Moritz (Validation [Supporting], Writing—review & editing [Equal]), and Angela McGaughran (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Funding acquisition [Lead], Investigation [Lead], Methodology [Lead], Project administration [Lead], Resources [Lead], Validation [Lead], Writing—original draft [Equal], Writing—review & editing [Equal])

Funding

This work was funded by the Australian Research Council (Discovery Early Career Researcher Award DE160100685 to A.M.) and Commonwealth Scientific and Industrial Research Organisation (Sequencing lane to A.M.).

Acknowledgments

We wish to thank several museums and/or government departments across Australia for providing samples (including the Australian National Insect Collection, Canberra; the Department of Agriculture and Food, Western Australia; the Department of Agriculture and Fisheries, Queensland; the Agricultural Scientific Collections Trust, New South Wales; and Museum Victoria, Victoria).

Conflicts of interest

None declared.

References

Ahmad, M. (2007). Insecticide resistance mechanisms and their management in *Helicoverpa armigera* (Hübner)—A review. *Journal of Agricultural Research*, 45, 319–335.

Anderson, C. J., Tay, W. T., McGaughran, A., ... Walsh, T. K. (2016). Population structure and gene flow in the global pest,

Helicoverpa armigera. *Molecular Ecology*, 25(21), 5296–5311. <https://doi.org/10.1111/mec.13841>

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

Behere, G. T., Tay, W. T., Russell, D. A., ... Batterham, P. (2007). Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea*. *BMC Evolutionary Biology*, 7(1), 117. <https://doi.org/10.1186/1471-2148-7-117>

Benham, P. M., & Bowie, R. C. K. (2023). Natural history collections as a resource for conservation genomics: Understanding the past to preserve the future. *The Journal of Heredity*, 114(4), 367–384. <https://doi.org/10.1093/jhered/esac066>

Bird, L. J. (2018). Pyrethroid and carbamate resistance in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae) from 2008 to 2015: What has changed since the introduction of Bt cotton? *Bulletin of Entomological Research*, 108(6), 781–791. <https://doi.org/10.1017/S0007485317001316>

Boivin, T., D'Hières, C., Bouvier, J., ... Sauphanor, B. (2001). Pleiotropy of insecticide resistance in the codling moth, CYDIA POMONELLA. *Entomologia Experimentalis et Applicata*, 99, 381–386. <https://doi.org/10.1046/j.1570-7458.2001.00838.x>

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>

Brookfield, J. F. (2000). What determines the rate of sequence evolution? *Current Biology: CB*, 10(11), R410–R411. [https://doi.org/10.1016/s0960-9822\(00\)00506-6](https://doi.org/10.1016/s0960-9822(00)00506-6)

Chen, P., & Zhang, J. (2020). Antagonistic pleiotropy conceals molecular adaptations in changing environments. *Nature Ecology & Evolution*, 4(3), 461–469. <https://doi.org/10.1038/s41559-020-1107-8>

Cingolani, P., Patel, V. M., Coon, M., ... Lu, X. (2012). Using drosophila melanogaster as a model for genotoxic chemical mutational studies with a new program, SnpSift. *Frontiers in Genetics*, 3, 35. <https://doi.org/10.3389/fgene.2012.00035>

Cingolani, P., Platts, A., Wang, L. L., ... Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly*, 6(2), 80–92. <https://doi.org/10.4161/fly.19695>

Cohen, Z. P., François, O., & Schoville, S. D. (2022). Museum genomics of an agricultural super-pest, the Colorado potato beetle, *Leptinotarsa decemlineata* (Chrysomelidae), provides evidence of adaptation from standing variation. *Integrative and Comparative Biology*, 62(6), 1827–1837. <https://doi.org/10.1093/icb/icac137>

Connallon, T., & Clark, A. G. (2012). A general population genetic framework for antagonistic selection that accounts for demography and recurrent mutation. *Genetics*, 190(4), 1477–1489. <https://doi.org/10.1534/genetics.111.137117>

Cousseau, L., Husemann, M., Foppen, R., ... Lens, L. (2016). A longitudinal genetic survey identifies temporal shifts in the population structure of Dutch house sparrows. *Heredity*, 117(4), 259–267. <https://doi.org/10.1038/hdy.2016.38>

Crossley, M. S., Snyder, W. E., & Hardy, N. B. (2020). Insect–plant relationships predict the speed of insecticide adaptation. *Evolutionary Applications*, 14(2), 290–296. <https://doi.org/10.1111/eva.13089>

Daly, J. C. (1993). Ecology and genetics of insecticide resistance in *Helicoverpa armigera*: Interactions between selection and gene flow. *Genetica*, 90(2–3), 217–226. <https://doi.org/10.1007/bf01435041>

Dong, Y., Chen, S., Cheng, S., ... Xiang, Q. -Y. (2019). Natural selection and repeated patterns of molecular evolution following allopatric divergence. *ELife*, 8, e45199. <https://doi.org/10.7554/eLife.45199>

Endersby, N. M., Hoffmann, A. A., McKechnie, S. W., & Weeks, A. R. (2007). Is there genetic structure in populations of *Helicoverpa armigera* from Australia? *Entomologia Experimentalis et Applicata*, 122(3), 253–263. <https://doi.org/10.1111/j.1570-7458.2006.00515.x>

French-Constant, R. H. (2013). The molecular genetics of insecticide resistance. *Genetics*, 194(4), 807–815. <https://doi.org/10.1534/genetics.112.141895>

- Fox, E. A., Wright, A. E., Fumagalli, M., & Vieira, F. G. (2019). ngsLD: Evaluating linkage disequilibrium using genotype likelihoods. *Bioinformatics (Oxford, England)*, 35(19), 3855–3856. <https://doi.org/10.1093/bioinformatics/btz200>
- Fritz, M. L., DeYonke, A. M., Papanicolaou, A., ... Gould, F. (2018). Contemporary evolution of a Lepidopteran species, *Heliothis virescens*, in response to modern agricultural practices. *Molecular Ecology*, 27(1), 167–181. <https://doi.org/10.1111/mec.14430>
- Fritz, M. L. (2022). Utility and challenges of using whole-genome resequencing to detect emerging insect and mite resistance in agroecosystems. *Evolutionary Applications*, 15(10), 1505–1520. <https://doi.org/10.1111/eva.13484>
- Gao, L., Qiao, H., Wei, P., ... Wang, Y. (2022). Xenobiotic responses in insects. *Archives of Insect Biochemistry and Physiology*, 109(3), e21869. <https://doi.org/10.1002/arch.21869>
- Gunning, R. V., Balfe, M. E., & Easton, C. S. (1992). Carbamate resistance in *Helicoverpa armigera* (hübner) (lepidoptera: Noctuidae) in Australia. *Australian Journal of Entomology*, 31(2), 97–103. <https://doi.org/10.1111/j.1440-6055.1992.tb00464.x>
- Gunning, R. V., & Easton, C. S. (1994). Endosulfan resistance in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. *Australian Journal of Entomology*, 33(1), 9–12. <https://doi.org/10.1111/j.1440-6055.1994.tb00906.x>
- Gunning, R. V., Moores, G. D., & Devonshire, A. L. (1998). Insensitive acetylcholinesterase and resistance to organophosphates in Australian *Helicoverpa armigera*. *Pesticide Biochemistry and Physiology*, 62(3), 3.
- Hawkins, N. J., Bass, C., Dixon, A., & Neve, P. (2019). The evolutionary origins of pesticide resistance. *Biological Reviews of the Cambridge Philosophical Society*, 94(1), 135–155. <https://doi.org/10.1111/brv.12440>
- Jin, M., North, H. L., Peng, Y., ... Xiao, Y. (2023). Adaptive evolution to the natural and anthropogenic environment in a global invasive crop pest, the cotton bollworm. *Innovation (Cambridge (Mass.))*, 4(4), 100454. <https://doi.org/10.1016/j.xinn.2023.100454>
- Jones, C. M., Papanicolaou, A., Mironidis, G. K., ... Chapman, J. W. (2015). Genomewide transcriptional signatures of migratory flight activity in a globally invasive insect pest. *Molecular Ecology*, 24(19), 4901–4911. <https://doi.org/10.1111/mec.13362>
- Jones, C., Parry, H., Tay, W., ... Chapman, J. (2018). Movement ecology of pest *Helicoverpa*: Implications for ongoing spread. *Annual Review of Entomology*, 64, 1–19. <https://doi.org/10.1146/annurev-ento-011118-111959>
- Jones, M. R., & Good, J. M. (2016). Targeted capture in evolutionary and ecological genomics. *Molecular Ecology*, 25(1), 185–202. <https://doi.org/10.1111/mec.13304>
- Joußen, N., Agnolet, S., Lorenz, S., ... Heckel, D. G. (2012). Resistance of Australian *Helicoverpa armigera* to fenvalerate is due to the chimeric P450 enzyme CYP337B3. *Proceedings of the National Academy of Sciences*, 109(38), 15206–15211. <https://doi.org/10.1073/pnas.1202047109>
- Jyothi, P., Aralimarad, P., Wali, V., ... Sane, S. P. (2021). Evidence for facultative migratory flight behavior in *Helicoverpa armigera* (Noctuidae: Lepidoptera) in India. *PLoS One*, 16(1), e0245665. <https://doi.org/10.1371/journal.pone.0245665>
- King, A. M., & MacRae, T. H. (2015). Insect heat shock proteins during stress and diapause. *Annual Review of Entomology*, 60, 59–75. <https://doi.org/10.1146/annurev-ento-011613-162107>
- Kawecki, T. J., Ebert D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. doi: <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics*, 15(1), 356. <https://doi.org/10.1186/s12859-014-0356-4>
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, 25(14), 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Li, H., Handsaker, B., Wysoker, A., ... Durbin, R.; 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- López-Domingo, F. J., Florido, J. P., Rueda, A., ... Santoyo-Lopez, J. (2014). ngsCAT: A tool to assess the efficiency of targeted enrichment sequencing. *Bioinformatics (Oxford, England)*, 30(12), 1767–1768. <https://doi.org/10.1093/bioinformatics/btu108>
- Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, 17(1), 67–77. <https://doi.org/10.1111/1755-0998.12592>
- McGaughran, A. (2020). Effects of sample age on data quality from targeted sequencing of museum specimens: what are we capturing in time? *BMC Genomics*, 21(1), 1–10. <https://doi.org/10.1186/s12864-020-6594-0>
- Maiorano, A. M., Lourenco, D. L., Tsuruta, S., ... Silva, J. A. I. de V. (2018). Assessing genetic architecture and signatures of selection of dual purpose Gir cattle populations using genomic information. *PLoS One*, 13(8), e0200694. <https://doi.org/10.1371/journal.pone.0200694>
- Miyo, T., Akai, S., & Oguma, Y. (2000). Seasonal fluctuation in susceptibility to insecticides within natural populations of *Drosophila melanogaster*: Empirical observations of fitness costs of insecticide resistance. *Genes & Genetic Systems*, 75(2), 97–104. <https://doi.org/10.1266/ggs.75.97>
- Osta, M. A., Rizk, Z. J., Labbé, P., ... Knio, K. (2012). Insecticide resistance to organophosphates in *Culex pipiens* complex from Lebanon. *Parasites & Vectors*, 5(1), 132. <https://doi.org/10.1186/1756-3305-5-132>
- Pearce, S. L., Clarke, D. F., East, P. D., ... Wu, Y. D. (2017). Genomic innovations, transcriptional plasticity and gene loss underlying the evolution and divergence of two highly polyphagous and invasive *Helicoverpa* pest species. *BMC Biology*, 15(1), 63. <https://doi.org/10.1186/s12915-017-0402-6>
- Pintus, E., Sorbolini, S., Albera, A., ... Macciotta, N. P. P. (2014). Use of locally weighted scatterplot smoothing (LOWESS) regression to study selection signatures in Piedmontese and Italian Brown cattle breeds. *Animal Genetics*, 45(1), 1–11. <https://doi.org/10.1111/age.12076>
- Reznick, D. N., Losos, J., & Travis, J. (2019). From low to high gear: There has been a paradigm shift in our understanding of evolution. *Ecology Letters*, 22(2), 233–244. <https://doi.org/10.1111/ele.13189>
- Saubin, M., Louet, C., Bousset, L., ... Halkett, F. (2023). Improving sustainable crop protection using population genetics concepts. *Molecular Ecology*, 32(10), 2461–2471. <https://doi.org/10.1111/mec.16634>
- Schmidt, J. M., Good, R. T., Appleton, B., ... Robin, C. (2010). Copy number variation and transposable elements feature in recent, ongoing adaptation at the Cyp6g1 Locus. *PLoS Genetics*, 6(6), e1000998. <https://doi.org/10.1371/journal.pgen.1000998>
- Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase II in aphids of the genus Sitobion (Hemiptera: Aphididae). *Molecular Biology and Evolution*, 13(3), 510–524. <https://doi.org/10.1093/oxfordjournals.molbev.a025612>
- Tajima, F. (1989). The effect of change in population size on DNA polymorphism. *Genetics*, 123(3), 597–601. <https://doi.org/10.1093/genetics/123.3.597>
- Taylor, K. L., Hamby, K. A., DeYonke, A. M., ... Fritz, M. L. (2021). Genome evolution in an agricultural pest following adoption of transgenic crops. *Proceedings of the National Academy of Sciences of the United States of America*, 118(52), e2020853118. <https://doi.org/10.1073/pnas.2020853118>
- Vieira, F., & Rozas, J. (2011). Comparative genomics of the odorant-binding and chemosensory protein gene families across the arthropoda: Origin and evolutionary history of the chemosensory system. *Genome Biology and Evolution*, 3, 476–490. <https://doi.org/10.1093/gbe/evr033>
- Walsh, T. K., Heckel, D. G., Wu, Y., ... Oakeshott, J. (2022). Determinants of insecticide resistance evolution: Comparative analysis among heliothines. *Annual Review of Entomology*, 67(1), 387–406. <https://doi.org/10.1146/annurev-ento-080421-071655>

- Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology*, 7(2), 256–276. [https://doi.org/10.1016/0040-5809\(75\)90020-9](https://doi.org/10.1016/0040-5809(75)90020-9)
- Wilson, L., Whitehouse, M., & Herron, G. (2018). The management of insect pests in Australian cotton: An evolving story. *Annual Review of Entomology*, 63, 215–237. <https://doi.org/10.1146/annurev-ento-020117-043432>
- Yang, Z. (2004). Adaptive molecular evolution. In D. J. Balding, M. Bishop, & C. Cannings (Eds.), *Handbook of Statistical Genetics*. John Wiley & Sons, Ltd. <https://doi.org/10.1002/0470022620.bbc10>