Check for updates

Received: 8 June 2023 | Revised: 3 December 2023 | Accepted: 19 February 2024

DOI: 10.1111/mec.17326

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Patterns of genetic variation and local adaptation of a native herbivore to a lethal invasive plant

Nitin Ravikanthachari[1,2] | Rachel A. Steward[1,2,3] | Carol L. Boggs[1,2,4]

[1]Department of Biological Sciences, University of South Carolina, Columbia, South Carolina, USA

[2]Rocky Mountain Biological Laboratory, Crested Butte, Colorado, USA

[3]Department of Biology, Lund University, Lund, Sweden

[4]School of Earth, Ocean & Environment, University of South Carolina, Columbia, South Carolina, USA

Correspondence
Nitin Ravikanthachari, Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208, USA.
Email: ravikanth.nitin@gmail.com

Present address
Nitin Ravikanthachari, Division of Biological Sciences, University of Montana, Missoula, Montana, USA

Funding information
Rocky Mountain Biological Laboratory Graduate Fellowship; University of South Carolina College of Arts & Sciences; NSF Graduate Research Fellowship Program

Handling Editor: Samridhi Chaturvedi

Abstract

Understanding the evolutionary processes that influence fitness is critical to predicting species' responses to selection. Interactions among evolutionary processes including gene flow, drift and the strength of selection can lead to either local adaptation or maladaptation, especially in heterogenous landscapes. Populations experiencing novel environments or resources are ideal for understanding the mechanisms underlying adaptation or maladaptation, specifically in locally co-evolved interactions. We used the interaction between a native herbivore that oviposits on a patchily distributed introduced plant that in turn causes significant mortality to the larvae to test for signatures of local adaptation in areas where the two co-occurred. We used whole-genome sequencing to explore population structure, patterns of gene flow and signatures of local adaptation. We found signatures of local adaptation in response to the introduced plant in the absence of strong population structure with no genetic differentiation and low genetic variation. Additionally, we found localized allele frequency differences within a single population between habitats with and without the lethal plant, highlighting the effects of strong selection. Finally, we identified that selection was acting on larval ability to feed on the plant rather than on females' ability to avoid oviposition, thus uncovering the specific ontogenetic target of selection. Our work highlights the potential for adaptation to occur in a fine-grained landscape in the presence of gene flow and low genetic variation.

KEYWORDS
brassicaceae, evolutionary traps, gene flow-selection balance, maladaptation, novel resources, *Pieris*

1 | INTRODUCTION

Strong spatially heterogenous selection can lead to locally adapted populations. The ability of populations to adapt to local conditions depends on substantial genetic variation, large effective population sizes and the changes in their habitat (Franch-Gras et al., 2018; Hedrick, 2011; Lande, 1976; Lenormand, 2002; Perrier et al., 2020; Simons, 2011). Additionally, local adaptation is maintained through the balance of strong selection and limited gene flow between divergently adapted populations (Blanquart et al., 2013; Hereford, 2009; Savolainen et al., 2013; Whitlock, 2015). Across a heterogeneous landscape, the interplay between gene flow and selection can lead to a geographic mosaic with varying levels of adapted and maladapted sub-populations (Gomulkiewicz et al., 2000; Thompson, 1999a, 2005).

Novel environments can derail adaptative evolution by decreasing the fitness of formerly locally adapted individuals (Brady, Bolnick, Angert, et al., 2019; Reed et al., 2003; Robertson & Hutto, 2006; Schlaepfer et al., 2002, 2005). The outcome of interactions between native populations and novel environments depends strongly on population connectivity among divergently adapted populations as well as the population's potential to adapt

 © 2024 John Wiley & Sons Ltd.

to novel conditions (Bolnick & Nosil, 2007; Farkas et al., 2015, 2016; Nosil et al., 2019). This often results in persistent mismatches with or maladaptation to the new resources or conditions (Brady, Bolnick, Angert, et al., 2019; Chew, 1977b; Cotto & Ronce, 2014; Singer & Parmesan, 2018; Steward et al., 2022; Steward & Boggs, 2020). Such cases provide unique opportunities for understanding the process of local adaptation because the character, scale and timeline of the environmental change are often well known (Chew, 1977b; Singer & Parmesan, 2018, 2019; Steward et al., 2022; Steward & Boggs, 2020). Identifying and understanding these interactions are critical to understanding species responses to novel environments, especially in the face of anthropogenic disturbance and climate change.

Herbivorous insects often have a narrow range of plants that they recognize as hosts and that support their development (Ehrlich & Raven, 1964; Hardy & Otto, 2014; Jaenike, 1990). While dietary specialization can arise from neutral evolutionary processes (i.e. drift; Forister & Jenkins, 2017; Hardy et al., 2016), most specialization in herbivorous insects has been attributed to local adaptation or even antagonistic coevolution with their host plants (Ehrlich & Raven, 1964; Hardy & Otto, 2014; Jaenike, 1990; Joshi & Thompson, 1995; Thompson, 1999b). Over time, specialist insects have evolved the behavioural, sensory and physiological mechanisms to recognize suitable hosts and mitigate plant defences. While specialists often exhibit some degree of sloppiness in host choice, overall, they tend to be well adapted to the plant communities with which they interact (Gomulkiewicz et al., 2000; Thompson, 2005). The introduction of non-native or invasive species may interact with this sloppiness, leading to new host associations (Agosta, 2006). However, in many cases, invasive species break down long-standing locally adapted or coevolved interactions and lead to maladaptation in either or both of the interacting partners, either by outcompeting and replacing native species or through eco-evolutionary traps (LaForgia et al., 2020; Richard et al., 2019; Saul & Jeschke, 2015; Schlaepfer et al., 2005; Schweiger et al., 2010). Eco-evolutionary traps arise when previously adaptive traits become maladaptive in the face of novel resources or environments, such that the organisms interact with the low fitness-value novel resource over the high fitness-value native resources (Robertson et al., 2013; Robertson & Chalfoun, 2016; Robertson & Hutto, 2006; Schlaepfer et al., 2002).

Eco-evolutionary traps resulting from maladaptive oviposition choice have been documented in many insects (Horváth et al., 2007, 2010; Singer & Parmesan, 2018). Lepidoptera, specifically butterflies, are susceptible to laying eggs on low-quality novel host plants (Nakajima & Boggs, 2015; Singer & Parmesan, 2010, 2019; Steward et al., 2022; Steward & Boggs, 2020; Yoon & Read, 2016). Hence, butterflies serve as an ideal system to test the impacts of novel resources on local adaptation in species interactions. We here use a landscape genetics approach to identify genomic signatures of local adaptation in the specialist herbivore *Pieris macdunnoughii* Remington, 1954 (Pieridae; formerly *P. napi macdunnoughii*) (Chew & Watt, 2006) in the presence of the toxic invasive plant *Thlaspi arvense* (Brassicaceae). *Pieris macdunnoughii* females recognize *T. arvense* as a potential host plant due to chemical cue similarity with the native brassicaceous hosts and lay eggs on it (Chew, 1977a, 1977b, 1980; Nakajima, 2014; Steward et al., 2019, 2022). However, larvae from eggs laid on *T. arvense* have significant mortality due to the toxicity of the plant (Chew, 1977b; Steward et al., 2019). We sampled *P. macdunnoughii* in the East River Valley, Gunnison County, Colorado, where the butterfly oviposits on both native host plants and *T. arvense*.

Using genome-wide data from whole-genome resequencing of individuals from areas where both the plants occur and from areas where *T. arvense* is absent, we identified candidate single nucleotide polymorphisms (SNPs) that are under selection in relation to the presence of *T. arvense* in the habitat. We specifically quantify: (i) population structure of *P. macdunnoughii* in the East River Valley, (ii) gene flow patterns of *P. macdunnoughii* between areas with and without *T. arvense* and (iii) underlying signatures of selection in *P. macdunnoughii* in response to the presence of *T. arvense* in the landscape. Our work builds on decades of existing research on this system to address the genetic basis underlying maladaptive behaviour in the face of novel resources.

2 | MATERIALS AND METHODS

2.1 | Study organisms

Pieris macdunnoughii is a montane butterfly distributed across the southern Rocky Mountains in North America. *Pieris macdunnoughii* is a specialist herbivore, and females oviposit on and the larvae feed on several native Brassicaceae (mustard) plants including *Boechera* spp., *Cardamine cordifolia*, *Draba aurea* and *Descurainia incana* (Chew, 1977a, 1977b, 1980). Females of *P. macdunnoughii* visit multiple host plants in the habitat during oviposition and lay 1–2 eggs per plant on the underside of apical leaves followed by nectaring and/or basking on nearby foliage and lay between 10 and 400 (Chew, 1977b). Early instar larvae feed on the hostplant after hatching and rarely move to other hostplants. However, late instar larvae are mobile and frequently wander in search of hostplants and/or substrates for pupation. Pierine larvae have evolved resistance to the toxicity of glucosinolates (secondary metabolites) found in the Brassicaceae by modifying the glucosinolate hydrolysis pathway to form thiocyanates and epithionitrilies that are less toxic, instead of forming isothiocyanates (Edger et al., 2015; Wheat et al., 2007). Although pierine butterflies are resistant to glucosinolate toxicity, they are susceptible to species whose glucosinolate concentration or composition differs from the ones with which they have coevolved locally (Keeler & Chew, 2008).

Thlaspi arvense (L.) (Brassicaceae) is native to Eurasia and was introduced to the Gunnison basin, Colorado in the 1880s (Chew, 1977b). It is an early successional plant and is found in heavily disturbed areas. It has colonized elevations up to 2900 m (Best & McIntyre, 1975; Warwick et al., 2011). *Pieris macdunnoughii*

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

females recognize and lay eggs on *T. arvense* as a potential host plant in areas where they both co-occur due to similarity of glucosinolates, and other cues shared among the native and non-native host plants. Previous studies have demonstrated that oviposition is nonrandom with regard to habitat preference and female experience (Nakajima et al., 2013; Steward & Boggs, 2020). Additionally, evidence also suggests that overall preference between *T. arvense* and *C. cordifolia* (a native host) is mildly heritable when tested on whole plants and cut stems, but not on methanol extracts, suggesting that differences in glucosinolates between the hostplants were not the primary driver of oviposition preference (Steward et al., 2022). Although females exhibit variation in the number of eggs laid on *T. arvense* in the laboratory (with some females ovipositing exclusively on *T. arvense* compared to native hosts, while some completely avoid *T. arvense*), we did not find any differences in gene expression in females preferring different host plants. (Ravikanthachari, 2024). These results highlight that female oviposition preference is not driven by a few genes/alleles of major effect involved in differentiating chemical signatures between the two hostplants. Early instar larvae reared on *T. arvense* in the laboratory experience high mortality, thus leading to an evolutionary trap (Nakajima, 2014; Steward & Boggs, 2020). However, 4th and 5th instar larvae can successfully feed on *T. arvense* and larvae can be rescued from death if they are provided with suitable native host plants (Chew, 1977b). Previous research has indicated that the chemical profile of *T. arvense* is comprised mostly of the aliphatic glucosinolate sinigrin (2-propenyl), whereas native mustards (e.g. *C. cordifolia*) that contain both aliphatic and aromatic glucosinolates (Rodman & Chew, 1980). Additionally, recent research has indicated that sinigrin acts as a pre-ingestive deterrent and early instar larvae exhibit delayed feeding on *T. arvense* compared to *C. cordifolia* (Steward et al., 2019).

2.2 | Population sampling and study area

The study area is situated in the East River valley, Gunnison County, Colorado spanning an altitude from ca. 2800 to 3400 m a.s.l. (Figure 1). We sampled 100 female adult butterflies during June–August 2016 from five locations and 235 adult butterflies of both sexes during June–August 2019 from seven locations. Our final sample size consisted of 335 individuals from 11 locations. The distance between sampling sites ranged from 1 km to 28 km. All butterflies were caught using an aerial net. We collected whole-body specimens of females and stored them in 98% ethanol in 2016. In 2019, 2 mm × 2 mm square of hindwings and a pair of midlegs were clipped and stored in 98% ethanol, after which the butterflies were marked and released. We also surveyed the presence/absence of *T. arvense* in all our sampling locations. We considered *T. arvense* present at a site if the site contained at least 20 plants at the flowering stage. We recorded *T. arvense* as present in two sites in 2016 (of 5 sites sampled) and 2019 (of 7 sites sampled). Our final sampling list consisted of three sites where *T. arvense* was present (of 11 sites sampled).

2.3 | DNA extraction and WGS library preparation

We extracted genomic DNA from thorax (2016 samples) and from wing clips and a pair of midlegs (2019 samples) using the DNeasy Blood & Tissue Kit (Qiagen, USA). We followed the manufacturer's protocol, with the following modifications: We increased the Proteinase K incubation step to 16 h, used ice-cold ethanol to precipitate DNA, and heated the elution buffer to 57°C before DNA elution. We quantified the DNA concentration using a NanoDrop™ 2000/2000c. We used an in-house Tn5 tagmentation protocol following Andolfatto et al. (2011) for our library preparation. Genomic DNA of 2–10 ng/ul was tagmented with 1:4 diluted Tn5 transposase and 40 uM pre-annealed oligonucleotides. The tagmentation was carried out in a final volume of 20uL containing 4 uL of 5x TAPS buffer. Samples were incubated at 55°C for 7 min followed by rapidly lowering the holding temperature to 10°C. The Tn5 transposase was deactivated using 2.5 uL 0.2% SDS at 55°C for 7 min with a heated lid. PCR-based barcoding and enrichment were carried out in a reaction mixture of 20 uL consisting of 2 uL of the tagmentation reaction product, 10 uL of 2x OneTaq Hot Start DNA polymerase, 4 uL of nuclease-free water and 2 uL (10 uM) each of i7 and i5 Illumina primer. The thermocycling conditions included initial denaturation at 72°C for 3 min followed by denaturation at 94°C, followed by 10 cycles with 10 s at 94°C, 15 s at 62°C, 30 s at 68°C and final extension for 5 min at 68°C. We constructed five replicate libraries for each sample, then pooled all the replicates and samples (1920 libraries). Size selection of 300–500 bp was carried out using 0.8x volume and 0.6x volume AmpureXP beads. All the libraries were sequenced at MedGenome on an Illumina Novaseq S4 platform, generating 150 bp paired end reads.

2.4 | SNP (single-nucleotide polymorphisms) calling

Figure 2 provides an overview of the methods detailed here. Demultiplexed raw Illumina reads and adapter sequences were trimmed using fastp (Chen et al., 2018) and mapped against the reference genome (which consisted of 25 chromosomes and 17 unplaced scaffolds) (Steward et al., 2021) using bwa mem with default parameters (Li, 2013). The resulting SAM files were converted to BAM format, sorted and indexed using samtools (Li et al., 2009). Duplicates were marked and identifier groups were added using PICARD TOOLS with default parameters (Picard toolkit., 2019). The genome dataset had a coverage of 6.84x ± 2.42x (mean ± s.d) and was quantified using genozip (Lan et al., 2021). Single nucleotide polymorphisms (SNPs) were called across all samples using GATK HaplotypeCaller to generate individual intermediate gVCF files that were then imported using GATK GenomicsDBImport and were finally genotyped using GATK GenotypeGVCFs. We used GATK VariantFilteration to further hard filter SNPs and Indels separately using the best recommended workflow practices (Van der Auwera et al., 2013), and finally used GATK SelectVariants to include only those variants that met the criteria: QD>2, base

1365294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

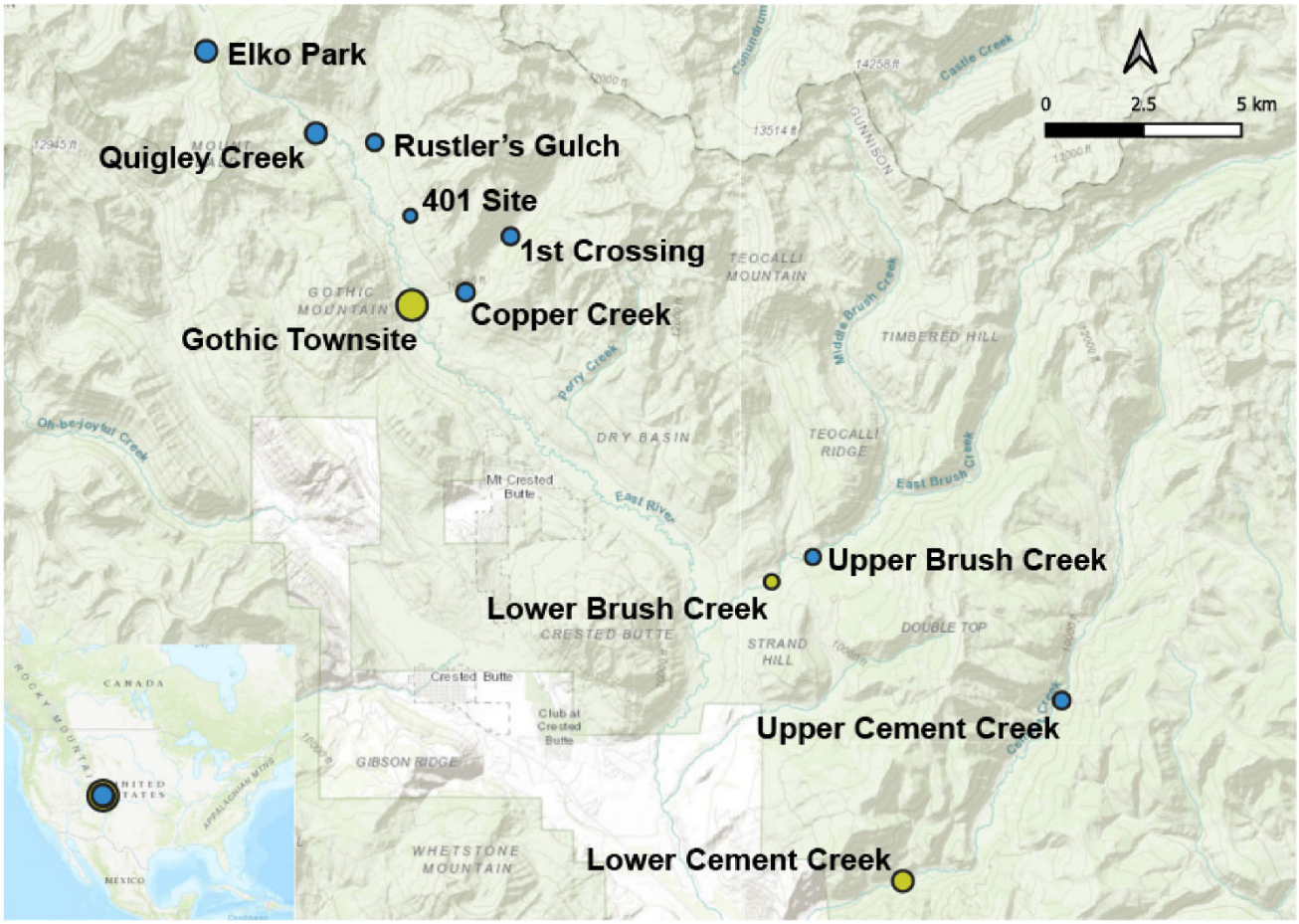


FIGURE 1 Map of sites where butterflies were collected in the East River valley. Sites in blue represent areas where *Thlaspi arvense* does not occur and sites in yellow represent areas where *T. arvense* is present. The size of the circle represents the magnitude of sample sizes from each location.

quality >30, SOR <3.0, FS < 60, MQ > 40, MQ Rank Sum >12.5, Read Pos Rank Sum >8.0. We then used VCFtools to retain only high-quality bi-allelic variants using the following parameters: minor allele count = 4, max missing = 0.20, min q = 30, min-mean DP = 6, max mean DP = 60, minDP = 6, max DP = 60. We further used PopLDdecay (Zhang et al., 2019) to estimate the extent of LD decay (Figure S1) in our SNPs and used PLINK (Purcell et al., 2007) to prune those loci that were at LD with the following parameters: $r^2 > .2$ in a window of 50 bp. Our final sample size consisted of 335 individuals and 768,339 SNPs.

2.5 | Population structure

We used a subset of 735,000 putatively neutral SNPs that were obtained after removing outlier loci (see below) to discern the population structure. Principal component analysis (PCA) clustering was carried out using PLINK (Purcell et al., 2007). We complemented our PCA analysis with archetypal analysis following Gimbernat-Mayol et al. (2022), to test biases in the PCA analysis due to irregular sample sizes and to identify latent factors. We removed multi-allelic SNPs prior to running archetypal analysis and performed the analysis with k ranging from 2 to 4. We picked the k whose PC1 and PC2 axes together accounted for the most variance. We additionally used ADMIXTURE to corroborate the results from PC and archetypal analysis and to assess population structure and ancestry (Alexander et al., 2009). We performed ADMIXTURE analysis for ancestral clusters K ranging from 1 to 6 and selected the K value with the lowest cross-entropy as the best estimate of population admixture.

2.6 | Nucleotide summary statistics

We used geoVar (Biddanda et al., 2020) to assess if alleles were shared among habitats/sites or if they were localized to each habitat/site. We estimated allele frequency distribution (site frequency spectrum) in (a) all 11 sites in the East River Valley and (b) in areas with and without *T. arvense*. We converted our VCF into a frequency table using geoVar ($n = 768,339$ SNPs), and then calculated the cumulative fraction of variants that contributed to the allele frequency pool for each site/habitat. We used the allele frequency distribution in geoVar in place of widely used SFS

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

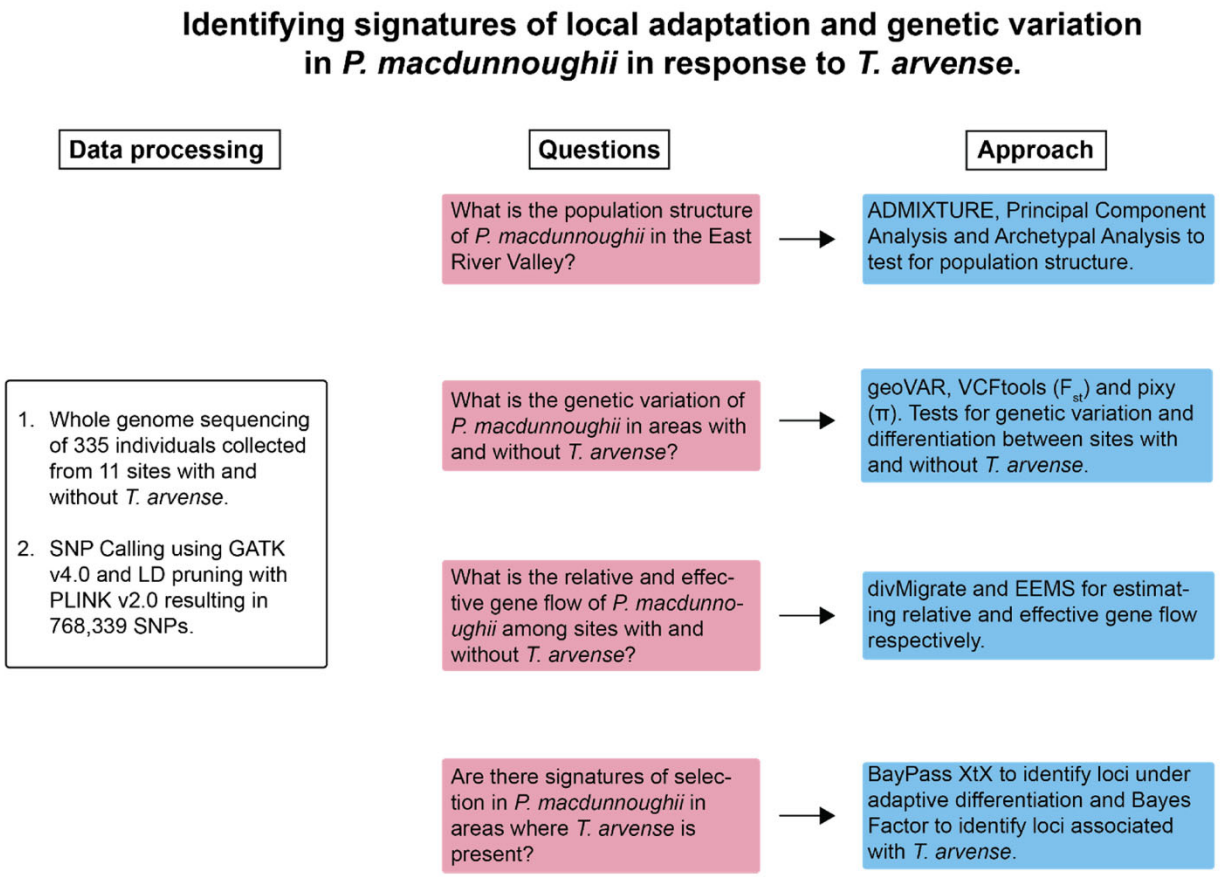


FIGURE 2 Outline of bioinformatic pipelines employed in the analysis of whole-genome sequences of *Pieris macdunnoughii* to dissect signatures of selection in response to *Thlaspi arvense*.

(site frequency spectrum) methods (Gutenkunst et al., 2009), since geoVar permits simultaneous comparisons of SFS for more than two populations. Furthermore, geoVar allows us to classify minor alleles into common (>5% frequency among all samples), low (1%–5% frequency), rare (<1% frequency) and unobserved (allele not present). geoVar specifically uses minor allele frequency to classify variants into the bins mentioned above as rare/low-frequency alleles are typically the result of recent mutations that are localized geographically, thus providing an accurate estimation of fine-scale differences among populations that arise due to selection, drift or other evolutionary processes.

Using VCFtools (Danecek et al., 2011) on our SNP dataset (consisting only of variant sites, $n = 768{,}339$ sites), we calculated expected and observed heterozygosity of butterflies for sites where *T. arvense* is present and sites where *T. arvense* is absent. We used pixy (Korunes & Samuk, 2021) to estimate genome-wide nucleotide (π) diversity. For the input for pixy, we specifically used both the invariant and variant sites in our input VCF file ($n = 69{,}339{,}609$ sites) as recommended by the authors to prevent bias in our estimates. We used a 10 kb sliding window with a 50 bp step to calculate π estimates. We used Bartlett's test to test differences in heterozygosity and nucleotide diversity between the habitats. We used VCFtools on our SNP dataset (consisting only of variant sites, $n = 768{,}339$ sites) to calculate pairwise F_{st} for all combinations of sites in the East River Valley. We used a sliding window approach with an interval window of 1 kb with a 50 bp step for estimating pairwise F_{st}.

2.7 | Migration surface/gene flow

We used divMigrate from the diveRsity package (Keenan et al., 2013) in R (R Core Team, 2020) to estimate directional relative migration rates among sampling sites and PGDSpider (Lischer & Excoffier, 2012) to process the input for divMigrate. The relative migration network was scaled to the largest estimated magnitude based on N_m as a measure of genetic distance. We assessed the significance of the migration network by running 1000 bootstrap iterations.

We used EEMS (Petkova et al., 2015) to examine spatial variation in migration among populations (demes) and genetic diversity within populations. EEMS estimates genetic differentiation using an isolation-by-distance model of geo-referenced samples to visualize patterns of potential barriers and corridors of gene flow. We used PLINK to convert the VCF file to BED files and calculated a genetic dissimilarity matrix using bed2diff in EEMS. We ran EEMS using 400 and 800 demes and a MCMC run with 1.5 million burn-in iterations followed by 15 million sampling iterations. We

13652394x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ran multiple iterations and adjusted the proposal variance rates after each run until runs converged (Figure S2). The parameters used for the final run are provided in the (Table S2). We then combined the final output of all the demes to produce a composite migration and diversity landscape. Migration and diversity rates were illustrated on a log10 scale relative to the overall migration and diversity across the entire landscape, such that a rate of one indicated tenfold higher migration and diversity rates relative to the average.

2.8 | Genotype-environment association (GEA) analysis

Genome-wide scans for outlier loci were carried out in BayPass (Gautier, 2015). We converted our VCF file into an allele count matrix ($n = 768{,}339$ SNPs) using an in-house python script for BayPass input. We first used the standard covariate model with the importance sampling (IS) approximation (−covis) in BayPass to obtain the following parameters: (a) the population scaled covariance matrix and (b) the XtX (SNP-specific F_{st} that corrects for observed population covariance) score of overall differentiation among sites. We then used the auxiliary covariate model using the MCMC approximation along with the Bayesian auxiliary variable to identify loci that were associated with the presence/absence of *T. arvense*.

To differentiate between neutral loci and those under selection in our XtX analysis, we estimated the posterior XtX distribution under the null model by generating pseudo observed datasets (POD) using the function simulate.baypass in the BayPass package. We randomly generated 100,000 loci from the core inference model (−covis) with the underlying population covariance parameters fixed to the posterior means obtained from the analysis of original data. We then analysed the POD dataset using the same MCMC parameters as stated above for our original data. The resulting XtX values from each simulated loci were combined to produce an empirical distribution, and quantiles of this distribution were used to calibrate the observed XtX for each locus in the original dataset. We used the top 1% significance threshold from the POD dataset (XtX >26) as a cut-off to distinguish between neutral and selected SNPs.

For environmental association, we used the Bayes Factor (BF) metric in deciban units (dB) as a measure of support for association with *T. arvense*. We used a cut-off of db >20 (decisive evidence) in favour of association based on Jefferey's rule (Jeffreys, 1961). Loci associated with *T. arvense* as well as those under adaptive differentiation were used as input in SNPEff (Cingolani et al., 2012) and BlastX (Altschul et al., 1990) to identify the underlying genes and their effects on protein structure.

To determine if the observed outlier loci from BayPass analysis were inflated due to other underlying biases in the dataset such as SNP density, allele frequency distributions, population origin among others, we conducted 20 BayPass permutation analyses (Chaturvedi et al., 2022). We randomized the environmental covariate (presence/absence of *T. arvense*) before implementing BayPass. We reran BayPass analysis on these 20 permuted datasets as described above for the observed dataset. We then compared the resulting number of outlier loci based on the criteria outlined above from the permuted datasets with our observed dataset to determine the validity of the outliers detected by BayPass.

3 | RESULTS

3.1 | Population structure and admixture

Our PCA and archetypal analysis using neutral loci failed to separate individuals into distinct clusters and hence did not reveal any population structure among all sites in the East River Valley (PCA: PC1 = 6.56% and PC2 = 5.65%; Figure 3a; archetypal analysis: PC1 = 52.92%, PC2 = 47.08%; Figure 3b). Our ADMIXTURE analysis corroborated the PCA and archetypal analysis. The lowest cross validation (CV) indicated K = 1 (Table S1). Together, these results indicate that *P. macdunnoughii* sampled from sites throughout the East River valley comprise a single population.

3.2 | Genetic variation and differentiation

We quantified the relative abundance of private and shared alleles in (a) between all sites where *T. arvense* is absent or present (i.e. between habitats) and (b) pairwise comparison among individual sites. The allele frequency distribution for *P. macdunnoughii* in habitats with and without *T. arvense* was mostly low-frequency alleles that were localized to each habitat (Figure 4a; 26% of cumulative fraction of variants), followed by 22% of shared and common alleles. The rest of the variants were either rare or low-frequency alleles that were localized to each habitat (Figure 4a). In contrast to the habitat-based allele frequency distribution, pairwise comparison of allele frequencies among sites consisted of alleles that were shared/common among all the sites (Figure 4b; 14% of the cumulative fraction of variants).

Overall, we found higher genetic variation among *P. macdunnoughii* in areas without *T. arvense* (heterozygosity: 0.140 ± 0.024; mean ± s.d) compared to areas with *T. arvense* (heterozygosity: 0.119 ± 0.030; mean ± s.d) (Figure 5a; Bartlett's $K^2 = 6.95$; $df = 1$; $p = .008$). The observed heterozygosity (0.139 ± 0.024; mean ± s.d) was less than expected (0.152 ± 0.015; mean ± s.d) in areas without *T. arvense* (Figure 5a; Bartlett's $K^2 = 39.63$; $df = 1$; $p < .001$) and the observed (0.107 ± 0.028; mean ± s.d) versus expected (0.118 ± 0.026; mean ± s.d) heterozygosity was similar in areas with *T. arvense*. Our genome-wide average estimates of nucleotide diversity (π) indicated a significant increase in π in sites without *T. arvense* (0.019 ± 0.008; mean) compared to sites with *T. arvense* (0.018 ± 0.007; mean ± s.d) (Figure 5b; Bartlett's $K^2 = 9.611$; $df = 1$; $p = .001$).

Our estimate of genome-wide mean pairwise F_{st} among all combinations of sites was less than <0.001 (Figure S3), indicating low genetic differentiation and high relatedness among sites. A

13652394x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

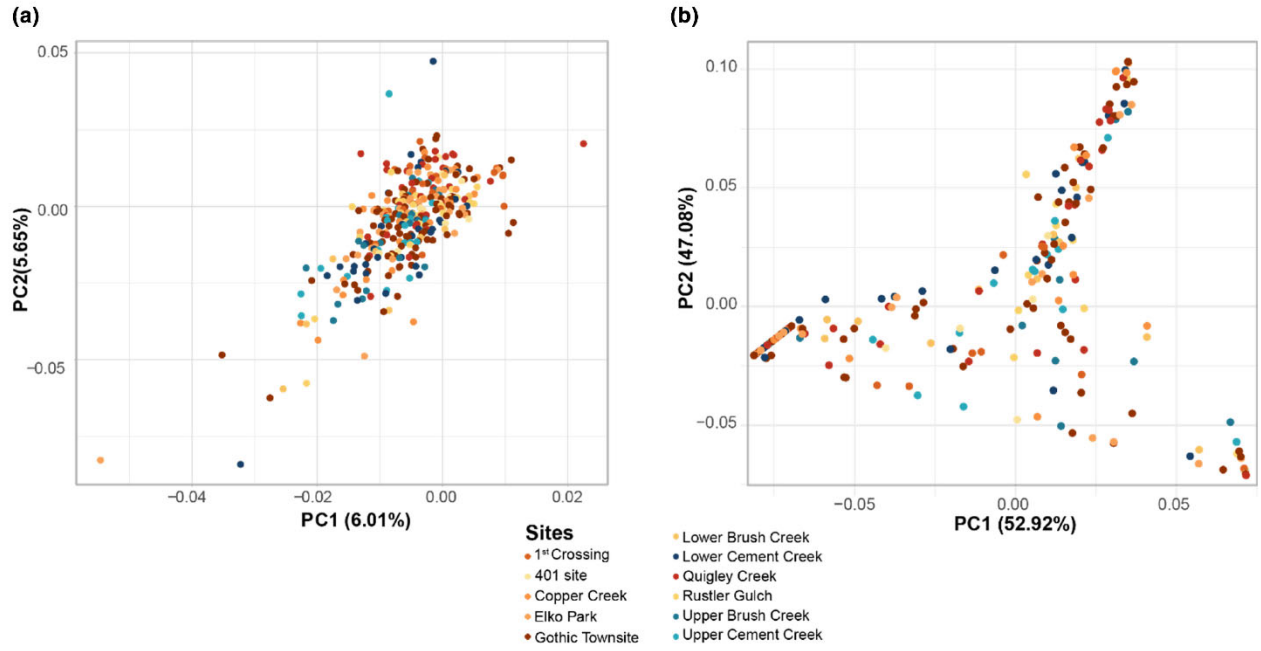


FIGURE 3 (a) Genome-wide principal component analysis (axes 1 and 2) using neutral loci indicating a mixed population without distinct clustering by site. (b) Archetypal analysis (axes 1 and 2) with k = 3 again showing a mixed population without site-specific clusters.

correlation map based on the population co-variance matrix indicated that all sites were weakly positively correlated. Accordingly, the Mantel test did not reveal any signs of isolation by distance ($p = .14$, $r = .05$).

3.3 | Migration

We observed significant variation in the magnitude and direction of relative migration of *P. macdunnoughii* among all sites (Figure 6, Table S4). The estimated relative migration rates ranged from 0 to 1 with an average of 0.56. We observed unidirectional and bidirectional migration from areas with *T. arvense* to areas without *T. arvense* and vice versa. The highest rates of gene flow (Nm >0.85) were observed from Gothic Townsite to Elko Park (Nm = 0.89) and vice versa (Nm = 1), and from Quigley Creek to Gothic Townsite (Nm = 0.96). Additionally, we observed high rates of relative gene flow to and from Gothic Townsite compared to other sites (Figure 6, Table S4).

Estimated effective migration surface (EEMS) contours revealed the low relative effective gene flow of *P. macdunnoughii* in the East River Valley (Figure 7a). Most of the potential barriers also showed high posterior probabilities in the Bayesian estimation of migration parameters (Figure S4). Posterior probabilities of migration parameters for sites in the Upper East River valley (Copper Creek, Copper Creek 1st Crossing, Gothic Townsite, Rustler's Gulch, Quigley Creek and Elko Park) were higher (>0.95) compared to the sites in the Lower East River valley (Lower and Upper Brush Creek and Lower and Upper Cement Creek), which had lower posterior probabilities of <.90.

We also estimated the relative effective genetic diversity of *P. macdunnoughii* in the East River valley. Our analysis highlighted low relative effective genetic diversity of *P. macdunnoughii* in all sampled sites (Figure 7b). All sites showed a posterior probability of >.90 (Figure S5).

Several areas that were not sampled for *P. macdunnoughii* showed higher than average effective migration rates and diversity rates (Figure 7a,b). However, none of these areas had high posterior probability values, suggesting these estimates were driven by the prior used in the MCMC iterations and hence have been excluded from our interpretation of migration and diversity patterns.

3.4 | Genotype-environment association (GEA) analysis

The BayPass core model (−covis) allowed us to estimate the scaled covariance matrix of population allele frequencies that quantify genetic relationship among pairs of sites. The results of Ω estimates agreed with our F_{st} estimates, indicating that all sites are genetically similar (Figure S3).

To identify outlier loci, the XtX (SNP-specific F_{st}) estimates were calibrated by analysing a POD (pseudo-observed dataset) of 100,000 SNPs. At the 1% threshold (XtX >26) for POD, we identified 8600 outlier SNPs (Figure S6). Our analysis that included presence/absence of *T. arvense* (as the environmental covariate) under the auxiliary covariate model identified 1008 SNPs that were associated with the presence of *T. arvense* (BF >20) (Figure S7). Overall, we identified nine SNPs that were shared by both the XtX outlier loci analysis and environmental association analysis (Figures 8, and S9),

13652394x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

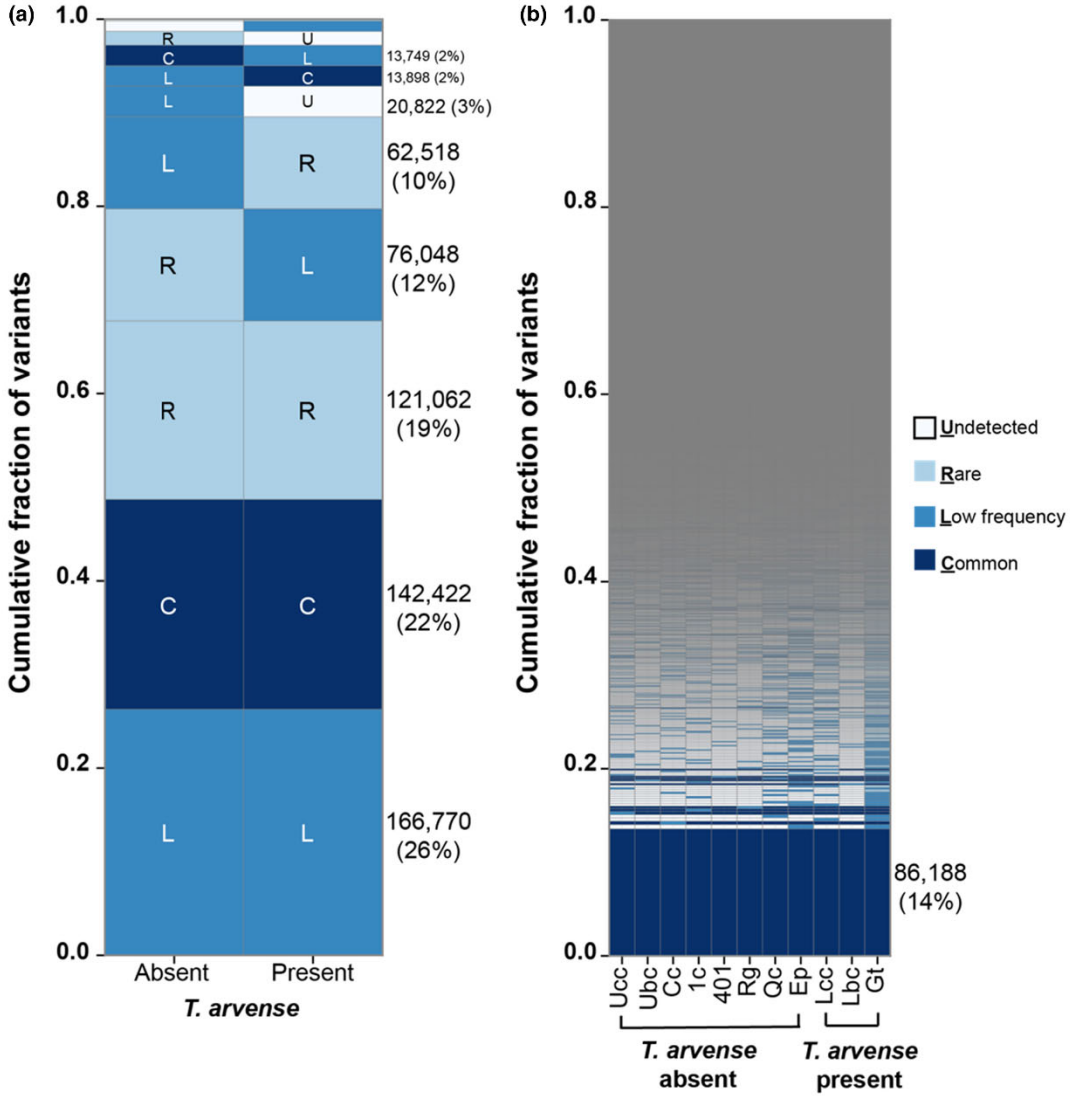


FIGURE 4 (a) Relative abundance of allele frequency variants of *P. macdunnoughii* in areas with *T. arvense* and areas without *T. arvense*. The letters depicted in the figure represent the frequency of the minor allele in the habitat (U-undetected: No alleles present, R-rare: <1% minor allele frequency (MAF), L-low frequency: 1%–5% MAF, C-common: >5% MAF). The percentages and their corresponding numbers indicate the number of variants in each class and are grouped based on SNP identity. (b) Relative abundance of allele frequency variants of *P. macdunnoughii* in our study area. 1c, 1st Crossing; 401, 401 site; Cc, Copper Creek; p, Elko Park; Gt, Gothic townsite; Lbc, Lower Brush Creek; Lcc, Lower Cement Creek; Qc, Quigley Creek; Rg, Rustler's Gulch; Ubc, Upper Brush Creek; Ucc, Upper Cement Creek. Greyed area represent alleles whose contribution was not significant to the differences between comparisons.

consistently more than detected using randomly assigned associations (BayPass permutations, $p < .04$; Figure S8).

Our SNPeff analysis indicated that of the nine SNPs, one was identified as a low-impact protein-coding change in the exon (unlikely to change protein behaviour) with the rest of the SNPs impacts classified as modifiers (effects on non-coding regions and/or effects of gene regulation). The BlastX analysis of these regions revealed that these genes were primarily involved in cytoskeletal organization, DNA damage repair, lipidation of chylomicrons in the intestines, eye development, epithelial development, and catalysis of phosphoric acid. Importantly, regions that were under selection and associated with the presence of *T. arvense* have previously been linked to larval development and metabolism (Tables 1 and S3).

4 | DISCUSSION

Pieris macdunnoughii butterflies in the East River Valley comprise a single admixed population. Allele frequency differences between habitats (i.e. presence or absence of *Thlaspi arvense*) were largely driven by localized alleles, whereas pairwise allele frequency differences among sites were driven by geographically widespread alleles. Additionally, heterozygosity of butterflies was lower in areas where *T. arvense* occurred compared to areas where the plant was absent. Examining relative and effective gene flow patterns revealed bidirectional, asymmetric relative gene flow and low effective gene flow across the East River valley. Finally, we identified signatures of selection in *P. macdunnoughii* in response to *T. arvense*. Based on our

13652994x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

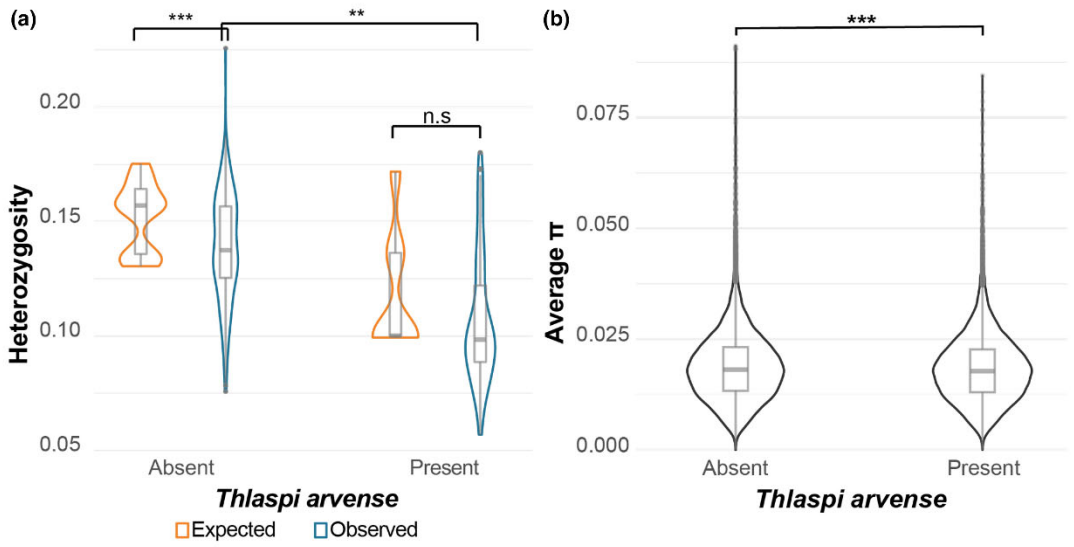


FIGURE 5 (a) Genome-wide variance in expected and observed heterozygosity of *P. macdunnoughii* in the East River valley. Bartlett's tests were used to compare observed heterozygosity between habitats where *T. arvense* was present or absent, and observed versus expected heterozygosity within each of these habitat types. (b) Genome-wide variance in nucleotide diversity (π) of *P. macdunnoughii* in habitats without and with *T. arvense* (Bartlett's test, $^{***}p < .001$).

annotation of the assembly, it appears that selection is affecting loci involved in larval ability to feed on *T. arvense*, not the adult females' recognition and discrimination of potential hosts. In sum, we identified genetic signatures of local selection in a native herbivore in response to a novel toxic hostplant at a fine geographic scale despite the absence of strong population structure and genome-wide differentiation.

4.1 | Evidence for a single population among sites

Our results did not separate individuals into distinct clusters and indicated that all sites comprise a single population, which accords with previous dispersal estimates for *P. macdunnoughii* in this area. Mark–release–recapture surveys in the 1970s and early 2000s estimated *P. macdunnoughii* mean dispersal between 400 m and 700 m (among dispersants, with approximately 0.42% of recaptures being dispersants) (Nakajima, 2014) which generally exceeds the extent of *T. arvense* in invaded areas (Nakajima et al., 2013). *P. macdunnoughii* disperses along large elevational gradients (Nakajima, 2014), occurring up to 4500 m.a.s.l. Several species of butterflies are known to disperse larger distances, thus increasing gene flow and blurring population boundaries across heterogeneous environments (Kitahara, 2016; Spieth & Cordes, 2012; Takami et al., 2004).

4.2 | Distinct allele frequency distribution patterns between habitats and among sites

Recent research has highlighted the utility of allele or site frequency spectra (AFS/SFS) to identify outlier loci, understand population structure, infer demographic changes and identify signatures of positive selection. In our study, AFS differences of *P. macdunnoughii* between areas with and without *T. arvense* were largely driven by alleles that were localized or private to each habitat, even in the absence of strong population structure. However, overall differences among sites in general were largely due to differences in shared, common alleles. This is in contrast with theoretical and empirical work in other systems, which showed that pairwise differences among sites within a population were driven by shared, common alleles (Biddanda et al., 2020; Gutenkunst et al., 2009). For instance, analysis of large-scale human genomic data from the 1000 Genomes Project (1KGP) showed that allele differences between populations were due to localized and rare alleles and differences between pairs of individuals regardless of population origin were due to common variants found globally (Biddanda et al., 2020). Allele frequency differences between populations can result from local adaptation and/or new mutations specific to the population (Günther & Coop, 2013). In our case, the abundance of localized, low-frequency alleles between the habitats could be due to the selection imposed by *T. arvense* leading to putative adaptive alleles.

4.3 | Mechanism underlying local adaptation with high gene flow and low genetic variation

Our results highlight the potential of adaptation to occur in a panmictic population with low genetic differentiation and genetic variation in the presence of strong selection. Population genetics theory suggests that local adaptation occurs in the absence of gene flow and that high gene flow often leads to homogenization and maladaptation (Bachmann et al., 2020; Farkas et al., 2016; Gandon et al., 1996; Garant et al., 2007; Kirkpatrick & Barton, 1997; Lenormand, 2002). However, recent studies showed that local adaptation occurs in

1365294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

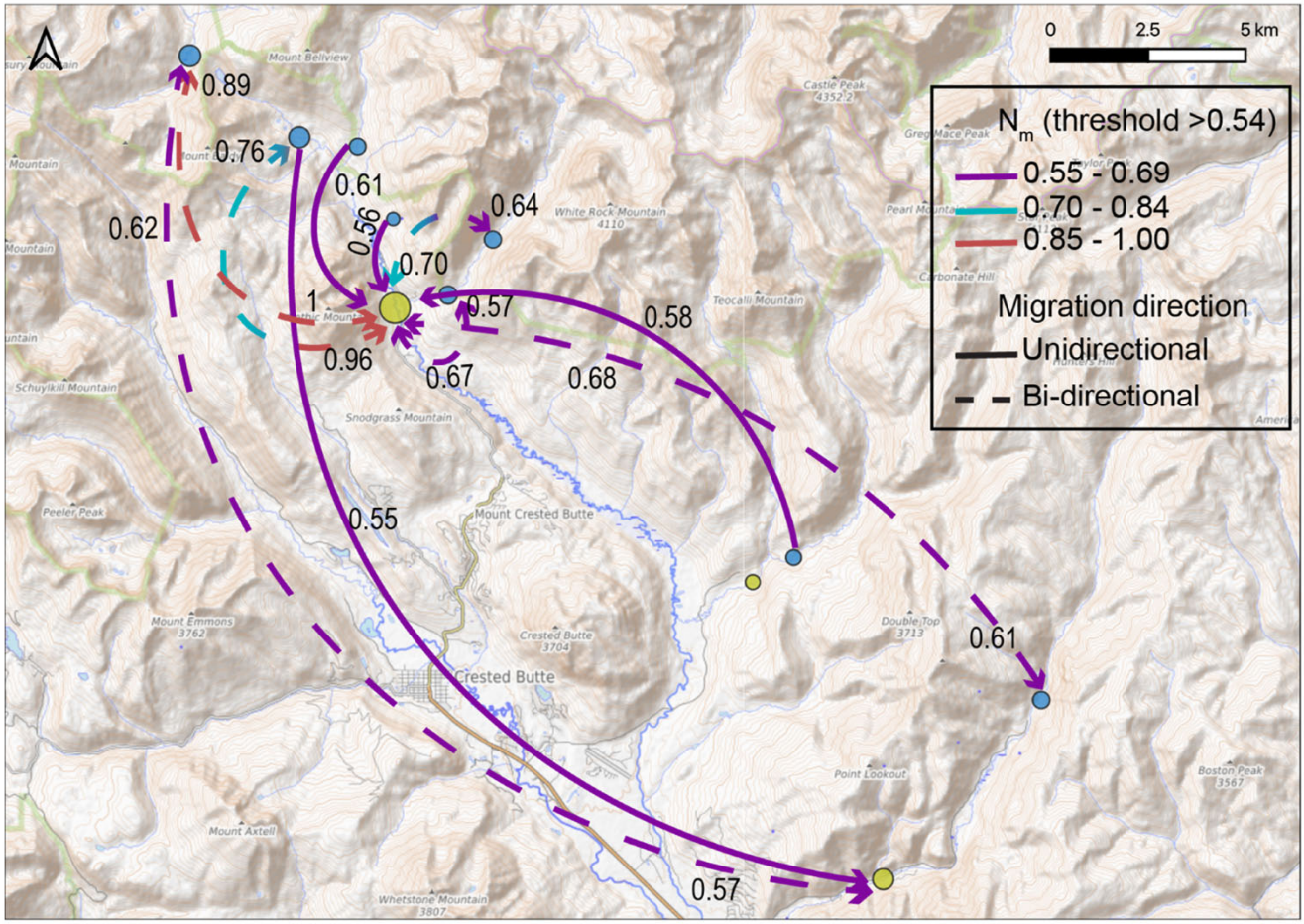


FIGURE 6 Relative migration rates of *P. macdunnoughii* among the 11 sites in the East River valley estimated using divMigrate based on the number of migrants per generation (Nm). Colours indicate migration levels (low = blue, high = red), and arrows indicate direction of migration. Solid lines represent unidirectional migration and dashed lines represent bi-directional migration. Colours in bidirectional migration correspond to the migration level of the closest arrow. Migration routes are only shown for Nm >0.55 and migration routes from areas with *T. arvense* to areas without *T. arvense* and vice-versa. Sites in blue represent areas where *Thlaspi arvense* does not occur and sites in yellow represent areas where *T. arvense* is present. The size of the circle represents the magnitude of sample sizes from each location.

the presence of strong gene flow in certain scenarios (Fitzpatrick et al., 2015; Tigano & Friesen, 2016). For example, in spatially and temporally varying habitats, gene flow augments standing genetic variation and thus increases local adaptation (Blanquart et al., 2012, 2013). Local adaptation can also occur in the presence of gene flow through adaptive introgression and strong selection against the immigrant alleles (Griffiths et al., 2021; Leroy et al., 2020; Rendón-Anaya et al., 2021).

Our results indicated that all sites in the East River Valley were panmictic, with high gene flow, low genetic differentiation and low genetic variation within the population. Nonetheless, we were able to identify strong signatures of local adaptation in *P. macdunnoughii* where *T. arvense* occurred. Eggs laid on *T. arvense* die before pupation, which introduces fitness costs for immigrant individuals that prefer *T. arvense* (Nakajima, 2014; Nakajima & Boggs, 2015). Similarly, local adaptation in lava flow lizards (melanism) occurred in the presence of strong gene flow and low genetic variation (Krohn et al., 2019). This is in line with recent theoretical and empirical work that suggests that environmentally driven local adaptation does not lead to genome-wide differences or require substantial standing genetic variation unless the underlying traits are linked to reproduction (Feder et al., 2012; Krohn et al., 2019; Savolainen et al., 2013).

4.4 | Selection on oviposition versus larval performance

We did not find that the fitness costs for naïve females (Nakajima et al., 2013) ovipositing on *T. arvense* translated into signatures of selection at the genomic level. Rather, loci under selection were near or in genes annotated with functions that support larval feeding and performance. Recent studies have found that genomic bases of herbivorous insects' response to novel hostplants are polygenic, involving genes underlying oviposition, larval feeding, larval metabolism and detoxification (Egan et al., 2015; Gompert et al., 2022; Vertacnik & Linnen, 2017). Similarly, we identified a polygenic response to selection from *T. arvense*, including nine loci distributed across

1365294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

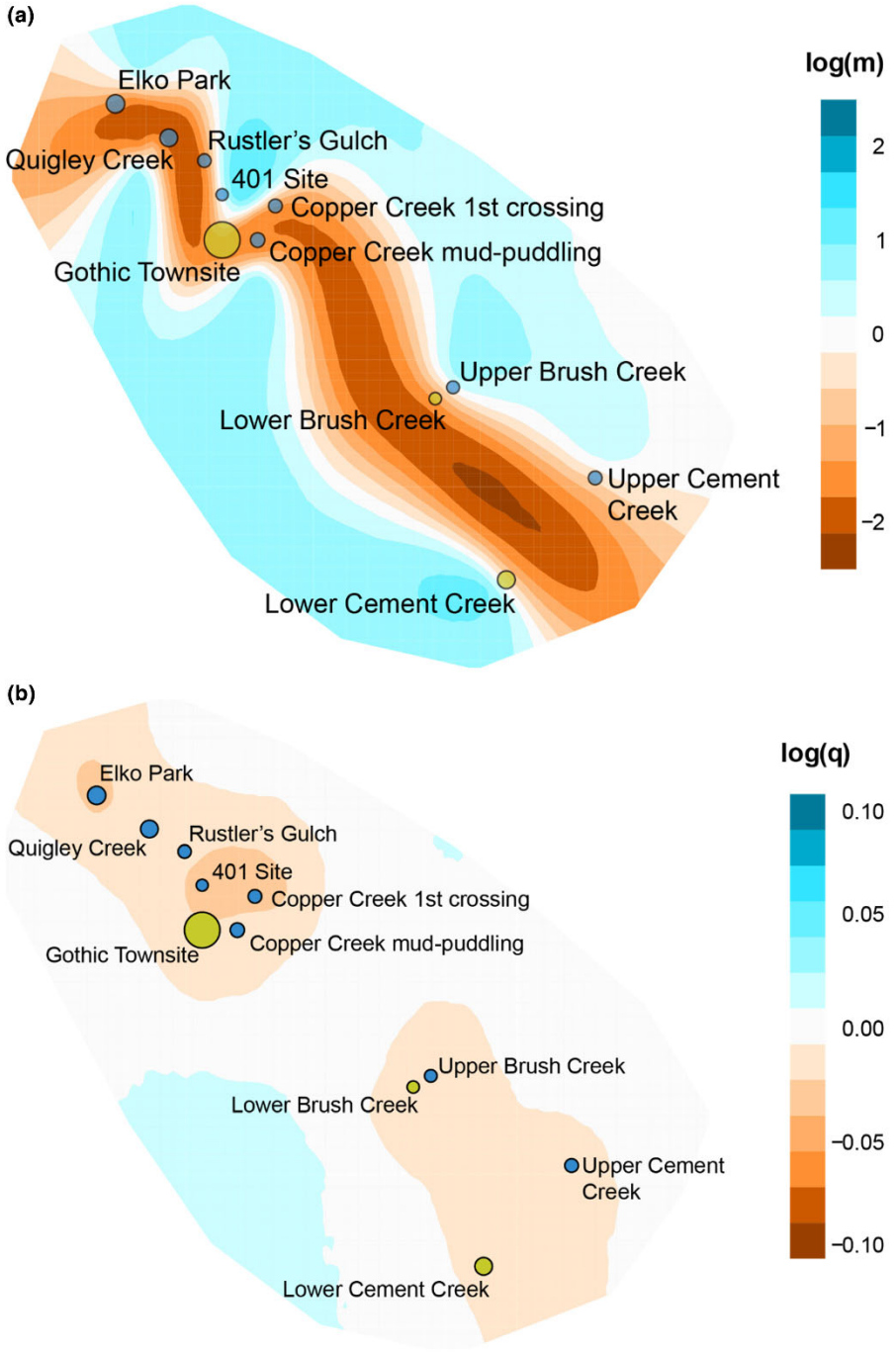


FIGURE 7 (a) Estimated effective migration surfaces and (b) Estimated effective diversity surfaces for *P. macdunnoughii* in the East River Valley. The migration and diversity rates, log(m) and log(q) represent gene flow or genetic diversity barriers and corridors in the habitat respectively. Each value corresponds to a 10-fold increase (blue) or decrease (orange/brown) in migration or genetic diversity compared to the null hypothesis of isolation by distance (white). Sites in blue represent areas where *Thlaspi arvense* does not occur and sites in yellow represent areas where *T. arvense* is present.

five chromosomes, causing 25 variant effects. Identification using SNPeff and BlastX revealed that the majority of the genes were involved in larval development and metabolism and most of the variant changes affected non-coding regions. Non-coding regions in the genome contain regulatory elements that play a critical role in protein assembly, gene expression and regulation and are under purifying selection (Andolfatto, 2005; Bird et al., 2006; Loehlin et al., 2010). Therefore, these variant changes might have significant effects in larval feeding and detoxification.

We did not identify any loci under selection that were associated with sensory (olfactory, gustatory, or visual) functions that underlie female oviposition choice (de Fouchier et al., 2017; Engsontia et al., 2014; Ramaswamy et al., 1987; Yang et al., 2020). Lepidopteran females use a combination of sensory receptors to

13652394x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

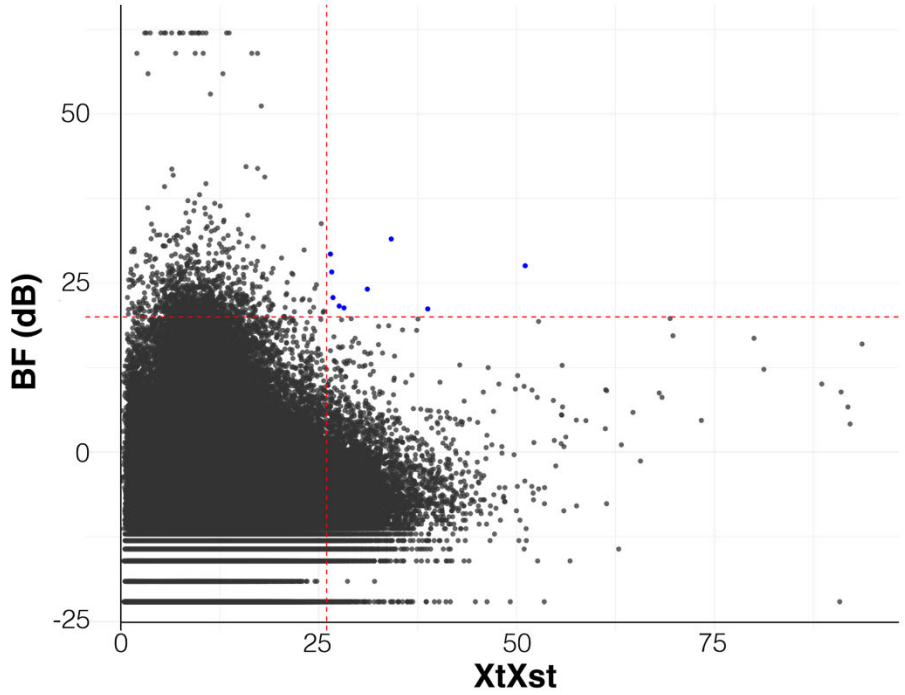


FIGURE 8 Pairwise comparison of median SNP XtX and BFmc values from three independent BayPass analyses for adaptive differentiation associated with *T. arvense* presence in the habitat. The vertical dashed line represents the 1% POD (pseudo observed dataset) significance threshold (XtX = 26), and the horizontal dashed line represents the 20-dB threshold for BFmc. Blue dots represent the nine loci of interest (outliers for both XtX and BFmc).

identify a potential hostplant (Haverkamp et al., 2018; Renwick & Chew, 1994; Thompson & Pellmyr, 1991). Females use olfaction and visual cues for long-range detection of hostplants and the final decision is made after gustatory tactile contact with the hostplant using the first pair of fore tarsi in their legs (Ozaki et al., 2011; Ryuda et al., 2013). Differences in oviposition choice in females are driven by underlying differences in their chemosensory repertoire. Thus, our failure to identify chemosensory genes in our outlier analysis further supports the conclusion that selection might be acting on larvae instead of the females.

Existing evidence suggests that error-prone oviposition in Lepidoptera females can drive hostplant range expansions and adaptation to new hosts since repeated oviposition on less suitable/non hostplants imposes selection on the larvae to evolve to feed on the plant (Janz et al., 1994; Nylin et al., 2000; Nylin & Janz, 2009; Stefanescu et al., 2012). We envision several similar scenarios for how signatures of selection on larval performance might have accumulated in this population.

First, females of *P. macdunnoughii* distribute their eggs on multiple host plants while laying eggs (Chew, 1977a). Larvae from eggs laid on *T. arvense* can survive if suitable native host plants are in the vicinity of *T. arvense*. Indeed, individual-based models have suggested that plant abundance, patch shape and distance to nearest native host plant can affect larval survival (Nakajima & Boggs, 2015). Although all early instar larvae reared in the laboratory on *T. arvense* eventually died, these studies limited access to other hosts or counted missing larvae as deaths rather than dispersals (Chew, 1977b; Nakajima et al., 2013). Additionally, there is considerable variation in the time to death of the larvae when feeding on *T. arvense*, suggesting that some larvae could survive longer on *T. arvense* compared to others (Boggs C.L., unpublished data). These factors suggest that larvae that survive longer on *T. arvense* and are in the vicinity of a native host plant could be rescued from the evolutionary trap. This is corroborated by evidence from host switching trials in the laboratory that have demonstrated that larvae that survive to later instars can be rescued when they are provided with suitable host plants (*C. cordifolia* or *D. incana*) (Ravikanthachari, N., and Boggs, C. L., pers. obs.).

Second, late instar *Pieris* larvae are shown to be more robust to host switches in general (Augustine & Kingsolver, 2018; Kumar et al., 2021; Ohata et al., 2011). Thus, larvae which encounter *T. arvense* at a later stage (especially in cases when native hostplants are scarce) could potentially survive to pupation and adulthood.

Third, temporal and spatial changes in *T. arvense* chemical defences may impact larval survival in ways that are still not understood. As is the case for many Brassicaceae species, *T. arvense* growth, morphology and defensive chemistry are highly sensitive to abiotic conditions (Cousins, 2022). For example, drought stress can impact foliar sinigrin concentrations, and recent research involving *Boechera stricta* (a native hostplant of *P. macdunnoughii* that co-occurs with *C. cordifolia* and *T. arvense*) and *Trichoplusia ni* (a model herbivore on Brassicaceae) has demonstrated that plants that survived drought stress were more susceptible to herbivory by *T. ni* (Carley et al., 2021). Additionally, *P. macdunnoughii* larvae that were reared throughout their entire larval stage on *T. arvense* plants which experienced extreme drought stress in the greenhouse successfully developed into

1365294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Annotation of outlier loci identified by BayPass XtX (XtX > 26) and BF(dB > 20), associated isoforms and predicted effects. Annotation was carried out using SNPeff and BlastX. Loci that have multiple functional annotations result from multiple transcripts and/or effects on more than one gene that are affected by the variant under selection.

Loci (SNP) position	Chromosome	Codon affected	Intervals affected	BlastX identification	Annotation
156,011	1	STOP	153,581–153,583	Cilia- and flagella-associated protein 410 isoform	Regulation of cell morphology and cytoskeletal organization (Bai et al., 2011); Involved in DNA damage repair (Lai et al., 2011).
3,430,127	7	STOP	3,428,853–3,428,855	Uncharacterized protein	
11,523,752	9	START	11,545,167–11,545,169	ARFRP 1	Lipidation of chylomicrons in the intestine and required for VLDL lipidation in the liver (Jaschke et al., 2012).
4,595,201	9	START	4,592,359–4,592,361	Homeobox protein Hox-A3-like	Part of a developmental regulatory system that provides cells with specific positional identities on the anterior–posterior axis (Gaudet et al., 2011).
4,595,201	9	STOP	4,595,024–4,595,026	Homeobox protein Hox-A3-like	Part of a developmental regulatory system that provides cells with specific positional identities on the anterior–posterior axis (Gaudet et al., 2011).
4,595,201	9	START	4,597,927–4,597,929	Retinal homeobox protein Rax-like	Plays a critical role in eye formation by regulating the initial specification of retinal cells and/or their subsequent proliferation (Kimura et al., 2000).
4,595,201	9	STOP	4,599,618–4,599,620	Retinal homeobox protein Rax-like	Plays a critical role in eye formation by regulating the initial specification of retinal cells and/or their subsequent proliferation (Kimura et al., 2000).
11,703,864	14	START	11,697,968–11,697,970	DOCK 4	Functions as a guanine nucleotide exchange factor (GEF) that promotes the exchange of GDP to GTP, converting inactive GDP-bound small GTPases into their active GTP-bound form (Yan et al., 2006).
11,703,864	14	STOP	11,701,979–11,701,981	inx3	Structural components of the gap junctions. Essential for proper epithelial development of the epidermis (Lehmann et al., 2006).
5,741,973	14	START	5,730,927–5,730,929	Mediator of RNA polymerase II transcription subunit 15	Required for activated transcription of the MtnA, MtnB and MtnD genes. Negatively regulates sex comb development (Boube et al., 2000); Required for cholesterol-dependent gene regulation. Positively regulates the Nodal signalling pathway (F. Yang et al., 2006).
5,741,973	14	STOP	5,742,219–5,742,221	Tripartite motif-containing protein 45	May act as a transcriptional repressor in mitogen-activated protein kinase signalling pathway (Wang et al., 2004).
261,573	xfSc0000009	STOP	261,794–261,796	Protein ALP1-like	Encodes an alkaline phosphatase. Alkaline phosphatases catalyse the hydrolysis of monoesters of phosphoric acid and a transphosphorylation reaction in the presence of large concentrations of phosphate acceptors (Harper & Armstrong, 1972).
261,573	xfSc0000009	STOP	263,610–263,612	Transposase	
577,356	xfSc0000009	START	573,908–573,910	Uncharacterized protein	

13652940, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

adults (N. Ravikanthchari N. and Boggs C.L, pers. obs.). Thus, abiotic variation between and within growing seasons has the potential to influence the interaction between *P. macdunnoughii* and *T. arvense*, potentially mitigating this evolutionary trap and opening a window for future escape through improved larval performance.

4.5 | Evolutionary constraints on oviposition preference and larval performance

Pieris macdunnoughii and its sister taxa in North America are derived from the Eurasian *P. napi* during a recent Holarctic speciation event (Chew & Watt, 2006). *Pieris napi* larvae can develop successfully on *T. arvense* and other invasive Eurasian mustards found in the Eastern United States, including *Alliaria petiolata* (Forsberg, 1987; Friberg & Wiklund, 2019; Prasai & Karlsson, 2011). *Pieris oleracea*, another North American species in the *Pieris* species complex has reduced fitness when feeding on *A. petiolata* due to its toxicity to the larvae (Chew, 1977b; Haribal et al., 2001; Haribal & Renwick, 1998; Huang et al., 1994; Keeler & Chew, 2008). Thus, the North American *Peiris* larvae have lost the ability to develop or have reduced fitness on introduced mustards from Eurasia while females have retained the ancestral preference for hostplants. After decades of maladaptation of *P. oleracea* on *A. petiolata*, the larvae are now able to develop on the plant during its bolting stage but not the rosette stage (Keeler & Chew, 2008). This highlights the importance of understanding the interactions between plant phenology, plant chemical composition and larval performance. Our results suggest that a similar outcome may occur in *P. macdunnoughii* in the event of rapid evolution due to selection. Future research quantifying fine-scale spatial and temporal patterns of larval performance coupled with quantifying *T. arvense* distribution and variation in plant chemical profiles might provide insights into the processes of escape from or persistence of evolutionary traps.

5 | CONCLUSIONS

Instances of interactions between native and non-native species are projected to increase across the globe due to range shifts, competition from invasive species and climate change. Understanding the effects of these interactions requires careful dissection of the ecological and molecular processes that mediate these interactions. Our results build upon decades of research aimed at understanding the causes and maintenance of evolutionary traps, to elucidate the molecular response and potential for adaptation to novel resources (Brady, Bolnick, Barrett, et al., 2019; Farkas et al., 2015; Gilroy & Sutherland, 2007; Robertson et al., 2013; Schlaepfer et al., 2002, 2005). As insects are currently experiencing a global decline (Hallmann et al., 2017; Nakajima & Boggs, 2015; Wagner et al., 2021), the *Pieris macdunnoughii–Thlaspi arvense* system can serve as a model to understand and predict the outcomes of these interactions in insects even at a fine geographical scale. This work identifies putative molecular mechanisms under selection by an evolutionary trap, thus pioneering *Pieris macdunnoughii–Thlaspi arvense* as a model system to understand maladaptation and evolutionary traps in the face of climate change (Chew, 1977b; Nakajima, 2014; Steward et al., 2019, 2021, 2022; Steward & Boggs, 2020). Our work also highlights the potential of adaptation to occur in a fine-grained landscape in the absence of segregating genetic variation and in instances of high gene flow.

AUTHOR CONTRIBUTIONS

NR and CLB designed the research. RAS collected specimens in 2016; NR collected specimens in 2019. NR did the molecular work and analysed the data. NR wrote the draft manuscript. All authors contributed to manuscript revisions. CLB provided oversight for all stages of the work.

ACKNOWLEDGEMENTS

We thank W. Watt, J. Quattro, P. Andolfatto, B. Hollis and C. Wessinger for providing feedback on the manuscript. We also thank the two anonymous reviewers and the subject editor for their constructive feedback. We thank J. Nicklas, L. Tran, L. Fisher, L. Hubbard, T. Feemster, M. Brown, M. Olson, S. McDaniel, H. Walton, E. Wagner and O. Shrestha for assisting with fieldwork. We thank K. Deitz and A. Pinharanda for assistance and advice on Tn5 library preparation. We thank K. Correa Veléz, J. Rivera-Hernandez, B. Stokes, J. Stewart and D. Reisman for advice on PCR and sequencing. M. Kuwalekar, S. Baral, R. Deshmukh, M. Stager, O. Lindestad, D. Speiser and C. Wessinger provided feedback on bioinformatic data analysis. Field research permits from the US Forest Service were arranged by the Rocky Mountain Biological Laboratory (RMBL). Funding was provided by the University of South Carolina College of Arts & Sciences to CLB, an NSF Graduate Research Fellowship to RAS and RMBL graduate fellowships to NR and RAS.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequence reads are deposited in the NCBI SRA database and are available under the BioProject ID PRJNA1081113. The source code for data analysis is available on Github on https://github.com/nitinra/population_genomics_pieris

BENEFITS GENERATED

Benefits from this research will ensue from the data and results shared from this study on public databases as described above.

ORCID

Nitin Ravikanthachari https://orcid.org/0000-0002-9474-7951
Rachel A. Steward https://orcid.org/0000-0001-8610-334X
Carol L. Boggs https://orcid.org/0000-0001-7601-6277

13652394x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

REFERENCES

Agosta, S. J. (2006). On ecological fitting, plant–insect associations, herbivore host shifts, and host plant selection. *Oikos*, *114*(3), 556–565. https://doi.org/10.1111/j.2006.0030-1299.15025.x

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Andolfatto, P. (2005). Adaptive evolution of non-coding DNA in *drosophila*. *Nature*, *437*(7062), 1149–1152.

Andolfatto, P., Davison, D., Erezyilmaz, D., Hu, T. T., Mast, J., Sunayama-Morita, T., & Stern, D. L. (2011). Multiplexed shotgun genotyping for rapid and efficient genetic mapping. *Genome Research*, *21*(4), 610–617. https://doi.org/10.1101/gr.115402.110

Augustine, K. E., & Kingsolver, J. G. (2018). Biogeography and phenology of oviposition preference and larval performance of Pieris virginiensis butterflies on native and invasive host plants. *Biological Invasions*, *20*(2), 413–422. https://doi.org/10.1007/s10530-017-1543-9

Bachmann, J. C., Jansen van Rensburg, A., Cortazar-Chinarro, M., Laurila, A., & Buskirk, J. V. (2020). Gene flow limits adaptation along steep environmental gradients. *American Naturalist*, *195*(3), E67–E86. https://doi.org/10.1086/707209

Bai, S. W., Herrera-Abreu, M. T., Rohn, J. L., Racine, V., Tajadura, V., Suryavanshi, N., Bechtel, S., Wiemann, S., Baum, B., & Ridley, A. J. (2011). Identification and characterization of a set of conserved and new regulators of cytoskeletal organization, cell morphology and migration. *BMC Biology*, *9*(1), 54. https://doi.org/10.1186/1741-7007-9-54

Best, K. F., & McIntyre, G. I. (1975). The biology of Canadian weeds: 9. *Thlaspi arvense* L. *Canadian Journal of Plant Science*, *55*(1), 279–292. https://doi.org/10.4141/cjps75-039

Biddanda, A., Rice, D. P., & Novembre, J. (2020). A variant-centric perspective on geographic patterns of human allele frequency variation. *eLife*, *9*, e60107. https://doi.org/10.7554/eLife.60107

Bird, C. P., Stranger, B. E., & Dermitzakis, E. T. (2006). Functional variation and evolution of non-coding DNA. *Current Opinion in Genetics & Development*, *16*(6), 559–564. https://doi.org/10.1016/j.gde.2006.10.003

Blanquart, F., Gandon, S., & Nuismer, S. L. (2012). The effects of migration and drift on local adaptation to a heterogeneous environment. *Journal of Evolutionary Biology*, *25*(7), 1351–1363. https://doi.org/10.1111/j.1420-9101.2012.02524.x

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195–1205. https://doi.org/10.1111/ele.12150

Bolnick, D. I., & Nosil, P. (2007). Natural selection in populations subject to a migration load. *Evolution*, *61*(9), 2229–2243. https://doi.org/10.1111/j.1558-5646.2007.00179.x

Boube, M., Faucher, C., Joulia, L., Cribbs, D. L., & Bourbon, H. M. (2000). Drosophila homologs of transcriptional mediator complex subunits are required for adult cell and segment identity specification. *Genes & Development*, *14*(22), 2906–2917. https://doi.org/10.1101/gad.17900

Brady, S. P., Bolnick, D. I., Angert, A. L., Gonzalez, A., Barrett, R. D. H., Crispo, E., Derry, A. M., Eckert, C. G., Fraser, D. J., Fussmann, G. F., Guichard, F., Lamy, T., McAdam, A. G., Newman, A. E. M., Paccard, A., Rolshausen, G., Simons, A. M., & Hendry, A. P. (2019). Causes of maladaptation. *Evolutionary Applications*, *12*(7), 1229–1242. https://doi.org/10.1111/eva.12844

Brady, S. P., Bolnick, D. I., Barrett, R. D. H., Chapman, L., Crispo, E., Derry, A. M., Eckert, C. G., Fraser, D. J., Fussmann, G. F., Gonzalez, A., Guichard, F., Lamy, T., Lane, J., McAdam, A. G., Newman, A. E. M., Paccard, A., Robertson, B., Rolshausen, G., Schulte, P. M., … Hendry, A. P. (2019). Understanding maladaptation by uniting ecological and evolutionary perspectives. *American Naturalist*, *194*(4), 495–515. https://doi.org/10.1086/705020

Carley, L. N., Mojica, J. P., Wang, B., Chen, C.-Y., Lin, Y.-P., Prasad, K. V. S. K., Chan, E., Hsu, C.-W., Keith, R., Nuñez, C. L., Olson-Manning, C. F., Rushworth, C. A., Wagner, M. R., Wang, J., Yeh, P.-M., Reichelt, M., Ghattas, K., Gershenzon, J., Lee, C.-R., & Mitchell-Olds, T. (2021). Ecological factors influence balancing selection on leaf chemical profiles of a wildflower. *Nature Ecology & Evolution*, *5*(8) Article 8., 1135–1144. https://doi.org/10.1038/s41559-021-01486-0

Chaturvedi, S., Gompert, Z., Feder, J. L., Osborne, O. G., Muschick, M., Riesch, R., Soria-Carrasco, V., & Nosil, P. (2022). Climatic similarity and genomic background shape the extent of parallel adaptation in Timema stick insects. *Nature Ecology & Evolution*, *6*(12), 1952–1964. https://doi.org/10.1038/s41559-022-01909-6

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Chew, F. S. (1977a). Coevolution of pierid butterflies and their cruciferous foodplants. II. The distribution of eggs on potential foodplants. *Evolution*, *31*(3), 568–579. https://doi.org/10.2307/2407522

Chew, F. S. (1977b). The effects of introduced mustards (Cruciferae) on some native north American cabbage butterflies (lepidoptera: Pieridae). *Atalaya*, *5*(2), 13–19.

Chew, F. S. (1980). Foodplant preferences of Pieris caterpillars (lepidoptera). *Oecologia*, *46*(3), 347–353. https://doi.org/10.1007/BF00346263

Chew, F. S., & Watt, W. B. (2006). The green-veined white (*Pieris napi* L.), its Pierine relatives, and the systematics dilemmas of divergent character sets (lepidoptera, Pieridae). *Biological Journal of the Linnean Society*, *88*(3), 413–435. https://doi.org/10.1111/j.1095-8312.2006.00630.x

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly*, *6*(2), 80–92. https://doi.org/10.4161/fly.19695

Cotto, O., & Ronce, O. (2014). Maladaptation as a source of senescence in habitats variable in space and time. *Evolution*, *68*(9), 2481–2493. https://doi.org/10.1111/evo.12462

Cousins, E. (2022). Ecology and phytochemistry of the non-native plant Thlaspi arvense (Brassicaceae) in subalpine meadows of Colorado, USA. https://scholarworks.umass.edu/dissertations_2/2607

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

de Fouchier, A., Walker, W. B., Montagné, N., Steiner, C., Binyameen, M., Schlyter, F., Chertemps, T., Maria, A., François, M.-C., Monsempes, C., Anderson, P., Hansson, B. S., Larsson, M. C., & Jacquin-Joly, E. (2017). Functional evolution of lepidoptera olfactory receptors revealed by deorphanization of a moth repertoire. *Nature Communications*, *8*(1), 15709. https://doi.org/10.1038/ncomms15709

Edger, P. P., Heidel-Fischer, H. M., Bekaert, M., Rota, J., Glöckner, G., Platts, A. E., Heckel, D. G., Der, J. P., Wafula, E. K., Tang, M., Hofberger, J. A., Smithson, A., Hall, J. C., Blanchette, M., Bureau, T. E., Wright, S. I., dePamphilis, C. W., Eric Schranz, M., Barker, M. S., … Wheat, C. W. (2015). The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences*, *112*(27), 8362–8366. https://doi.org/10.1073/pnas.1503926112

Egan, S. P., Ragland, G. J., Assour, L., Powell, T. H. Q., Hood, G. R., Emrich, S., Nosil, P., & Feder, J. L. (2015). Experimental evidence of

1365294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

genome-wide impact of ecological selection during early stages of speciation-with-gene-flow. *Ecology Letters*, *18*(8), 817–825. https://doi.org/10.1111/ele.12460

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution*, *18*(4), 586–608.

Engsontia, P., Sangket, U., Chotigeat, W., & Satasook, C. (2014). Molecular evolution of the odorant and gustatory receptor genes in lepidopteran insects: Implications for their adaptation and speciation. *Journal of Molecular Evolution*, *79*(1–2), 21–39. https://doi.org/10.1007/s00239-014-9633-0

Farkas, T. E., Hendry, A. P., Nosil, P., & Beckerman, A. P. (2015). How maladaptation can structure biodiversity: Eco-evolutionary Island biogeography. *Trends in Ecology & Evolution*, *30*(3), 154–160. https://doi.org/10.1016/j.tree.2015.01.002

Farkas, T. E., Mononen, T., Comeault, A. A., & Nosil, P. (2016). Observational evidence that maladaptive gene flow reduces patch occupancy in a wild insect metapopulation. *Evolution*, *70*(12), 2879–2888. https://doi.org/10.1111/evo.13076

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation-with-gene-flow. *Trends in Genetics*, *28*(7), 342–350. https://doi.org/10.1016/j.tig.2012.03.009

Fitzpatrick, S. W., Gerberich, J. C., Kronenberger, J. A., Angeloni, L. M., & Funk, W. C. (2015). Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, *18*(1), 37–47. https://doi.org/10.1111/ELE.12388

Forister, M. L., & Jenkins, S. H. (2017). A neutral model for the evolution of diet breadth. *The American Naturalist*, *190*(2), E40–E54. https://doi.org/10.1086/692325

Forsberg, J. (1987). Size discrimination among conspecific hostplants in two pierid butterflies; *Pieris napi* L. and *Pontia daplidice* L. *Oecologia*, *72*(1), 52–57. https://doi.org/10.1007/BF00385044

Franch-Gras, L., Hahn, C., García-Roger, E. M., Carmona, M. J., Serra, M., & Gómez, A. (2018). Genomic signatures of local adaptation to the degree of environmental predictability in rotifers. *Scientific Reports*, *8*(1), 16051. https://doi.org/10.1038/s41598-018-34188-y

Friberg, M., & Wiklund, C. (2019). Host preference variation cannot explain microhabitat differentiation among sympatric *Pieris napi* and *Pieris rapae* butterflies. *Ecological Entomology*, *44*(4), 571–576. https://doi.org/10.1111/een.12728

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y., & Olivieri, I. (1996). Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proceedings of the Royal Society B: Biological Sciences*, *263*(1373), 1003–1009. https://doi.org/10.1098/rspb.1996.0148

Garant, D., Forde, S. E., & Hendry, A. P. (2007). The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, *21*(3), 434–443. https://doi.org/10.1111/j.1365-2435.2006.01228.x

Gaudet, P., Livstone, M. S., Lewis, S. E., & Thomas, P. D. (2011). Phylogenetic-based propagation of functional annotations within the gene ontology consortium. *Briefings in Bioinformatics*, *12*(5), 449–462. https://doi.org/10.1093/bib/bbr042

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics*, *201*(4), 1555–1579. https://doi.org/10.1534/genetics.115.181453

Gilroy, J. J., & Sutherland, W. J. (2007). Beyond ecological traps: Perceptual errors and undervalued resources. *Trends in Ecology & Evolution*, *22*(7), 351–356. https://doi.org/10.1016/j.tree.2007.03.014

Gimbernat-Mayol, J., Mantes, A. D., Bustamante, C. D., Montserrat, D. M., & Ioannidis, A. G. (2022). Archetypal analysis for population genetics. *PLoS Computational Biology*, *18*(8), e1010301. https://doi.org/10.1371/journal.pcbi.1010301

Gompert, Z., Saley, T., Philbin, C., Yoon, S. A., Perry, E., Sneck, M. E., Harrison, J. G., Buerkle, C. A., Fordyce, J. A., Nice, C. C., Dodson, C. D., Lebeis, S. L., Lucas, L. K., & Forister, M. L. (2022). Additive genetic effects in interacting species jointly determine the outcome of caterpillar herbivory. *Proceedings of the National Academy of Sciences*, *119*(36), e2206052119. https://doi.org/10.1073/pnas.2206052119

Gomulkiewicz, R., Thompson, J. N., Holt, R. D., Nuismer, S. L., & Hochberg, M. E. (2000). Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist*, *156*(2), 156–174. https://doi.org/10.1086/303382

Griffiths, J. S., Kawji, Y., & Kelly, M. W. (2021). An experimental test of adaptive introgression in locally adapted populations of splash Pool copepods. *Molecular Biology and Evolution*, *38*(4), 1306–1316. https://doi.org/10.1093/molbev/msaa289

Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, *195*(1), 205–220. https://doi.org/10.1534/genetics.113.152462

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, *5*(10), e1000695. https://doi.org/10.1371/journal.pgen.1000695

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., & de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One*, *12*(10), e0185809. https://doi.org/10.1371/journal.pone.0185809

Hardy, N. B., & Otto, S. P. (2014). Specialization and generalization in the diversification of phytophagous insects: Tests of the musical chairs and oscillation hypotheses. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795), 20132960. https://doi.org/10.1098/rspb.2013.2960

Hardy, N. B., Peterson, D. A., & Normark, B. B. (2016). Nonadaptive radiation: Pervasive diet specialization by drift in scale insects? *Evolution*, *70*(10), 2421–2428. https://doi.org/10.1111/evo.13036

Haribal, M., & Renwick, J. A. A. (1998). Isovitexin 6″-O-β-d-glucopyranoside: A feeding deterrent to *Pieris napi oleracea* from *Alliaria petiolata*. *Phytochemistry*, *47*(7), 1237–1240. https://doi.org/10.1016/S0031-9422(97)00740-1

Haribal, M., Yang, Z., Attygalle, A. B., Renwick, J. A. A., & Meinwald, J. (2001). A Cyanoallyl glucoside from *Alliaria petiolata*, as a feeding deterrent for larvae of *Pieris napi oleracea*. *Journal of Natural Products*, *64*(4), 440–443. https://doi.org/10.1021/np000534d

Harper, R. A., & Armstrong, F. B. (1972). Alkaline phosphatase of Drosophila melanogaster. I. Partial purification and characterization. *Biochemical Genetics*, *6*(1), 75–82. https://doi.org/10.1007/BF00485968

Haverkamp, A., Hansson, B. S., & Knaden, M. (2018). Combinatorial codes and labeled lines: How insects use olfactory cues to find and judge food, mates, and oviposition sites in complex environments. *Frontiers in Physiology*, *9*, 49. https://doi.org/10.3389/fphys.2018.00049

Hedrick, P. W. (2011). *Genetics of populations* (4th ed.). Jones and Bartlett Publishers.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist*, *173*(5), 579–588. https://doi.org/10.1086/597611

Horváth, G., Blahó, M., Egri, Á., Kriska, G., Seres, I., & Robertson, B. (2010). Reducing the maladaptive attractiveness of solar panels to polarotactic insects. *Conservation Biology*, *24*(6), 1644–1653. https://doi.org/10.1111/j.1523-1739.2010.01518.x

Horváth, G., Malik, P., Kriska, G., & Wildermuth, H. (2007). Ecological traps for dragonflies in a cemetery: The attraction of *Sympetrum* species (Odonata: Libellulidae) by horizontally polarizing black gravestones. *Freshwater Biology*, *52*(9), 1700–1709. https://doi.org/10.1111/j.1365-2427.2007.01798.x

Huang, X. P., Renwick, J. A. A., & Chew, F. S. (1994). Oviposition stimulants and deterrents control acceptance of *Alliaria petiolata* by *Pieris*

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

rapae and *P. Napi oleracea*. *Chemoecology*, *5*(2), 79–87. https://doi.org/10.1007/BF01259436

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, *21*, 243–273.

Janz, N., Nylin, S., & Wedell, N. (1994). Host plant utilization in the comma butterfly: Sources of variation and evolutionary implications. *Oecologia*, *99*(1), 132–140. https://doi.org/10.1007/BF00317093

Jaschke, A., Chung, B., Hesse, D., Kluge, R., Zahn, C., Moser, M., Petzke, K.-J., Brigelius-Flohé, R., Puchkov, D., Koepsell, H., Heeren, J., Joost, H.-G., & Schürmann, A. (2012). The GTPase ARFRP1 controls the lipidation of chylomicrons in the Golgi of the intestinal epithelium. *Human Molecular Genetics*, *21*(14), 3128–3142. https://doi.org/10.1093/hmg/dds140

Jeffreys, H. (1961). *Theory of probability* (3rd ed.). Oxford University Press.

Joshi, A., & Thompson, J. N. (1995). Trade-offs and the evolution of host specialization. *Evolutionary Ecology*, *9*(1), 82–92. https://doi.org/10.1007/BF01237699

Keeler, M. S., & Chew, F. S. (2008). Escaping an evolutionary trap: Preference and performance of a native insect on an exotic invasive host. *Oecologia*, *156*(3), 559–568. https://doi.org/10.1007/s00442-008-1005-2

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Kimura, A., Singh, D., Wawrousek, E. F., Kikuchi, M., Nakamura, M., & Shinohara, T. (2000). Both PCE-1/RX and OTX/CRX interactions are necessary for photoreceptor-specific gene expression*. *Journal of Biological Chemistry*, *275*(2), 1152–1160. https://doi.org/10.1074/jbc.275.2.1152

Kirkpatrick, M., & Barton, N. H. (1997). Evolution of a Species' range. *The American Naturalist*, *150*(1), 1–23. https://doi.org/10.1086/286054

Kitahara, H. (2016). Oviposition plants and seasonal migratory movements of sympatric *Pieris melete* and *P. Napi japonica* (lepidoptera, Pieridae). *Lepidoptera. Science*, *67*(1), 32–40.

Korunes, K. L., & Samuk, K. (2021). Pixy: Unbiased estimation of nucleotide diversity and divergence in the presence of missing data. *Molecular Ecology Resources*, *21*(4), 1359–1368. https://doi.org/10.1111/1755-0998.13326

Krohn, A. R., Diepeveen, E. T., Bi, K., & Rosenblum, E. B. (2019). Local adaptation does not lead to genome-wide differentiation in lava flow lizards. *Ecology and Evolution*, *9*(12), 6810–6820. https://doi.org/10.1002/ECE3.5231

Kumar, P., Akhter, T., Bhardwaj, P., Kumar, R., Bhardwaj, U., & Mazumdar-Leighton, S. (2021). Consequences of 'no-choice, fixed time' reciprocal host plant switches on nutrition and gut serine protease gene expression in Pieris brassicae L (Lepidoptera: Pieridae). *PLoS One*, *16*(1), e0245649. https://doi.org/10.1371/journal.pone.0245649

LaForgia, M. L., Harrison, S. P., & Latimer, A. M. (2020). Invasive species interact with climatic variability to reduce success of natives. *Ecology*, *101*(6), e03022. https://doi.org/10.1002/ecy.3022

Lai, C. K., Gupta, N., Wen, X., Rangell, L., Chih, B., Peterson, A. S., Bazan, J. F., Li, L., & Scales, S. J. (2011). Functional characterization of putative cilia genes by high-content analysis. *Molecular Biology of the Cell*, *22*(7), 1104–1119. https://doi.org/10.1091/mbc.e10-07-0596

Lan, D., Tobler, R., Souilmi, Y., & Llamas, B. (2021). Genozip: A universal extensible genomic data compressor. *Bioinformatics*, *37*(16), 2225–2230. https://doi.org/10.1093/bioinformatics/btab102

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution*, *30*(2), 314–334. https://doi.org/10.2307/2407703

Lehmann, C., Lechner, H., Löer, B., Knieps, M., Herrmann, S., Famulok, M., Bauer, R., & Hoch, M. (2006). Heteromerization of innexin gap junction proteins regulates epithelial tissue Organization in Drosophila. *Molecular Biology of the Cell*, *17*(4), 1676–1685. https://doi.org/10.1091/mbc.e05-11-1059

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, *17*(4), 183–189. https://doi.org/10.1016/S0169-5347(02)02497-7

Leroy, T., Louvet, J.-M., Lalanne, C., Le Provost, G., Labadie, K., Aury, J.-M., Delzon, S., Plomion, C., & Kremer, A. (2020). Adaptive introgression as a driver of local adaptation to climate in European white oaks. *New Phytologist*, *226*(4), 1171–1182. https://doi.org/10.1111/nph.16095

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*, 1303.3997. http://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., 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

Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*(2), 298–299. https://doi.org/10.1093/bioinformatics/btr642

Loehlin, D. W., Oliveira, D. C. S. G., Edwards, R., Giebel, J. D., Clark, M. E., Cattani, M. V., van de Zande, L., Verhulst, E. C., Beukeboom, L. W., Muñoz-Torres, M., & Werren, J. H. (2010). Non-coding changes cause sex-specific wing size differences between closely related species of *Nasonia*. *PLoS Genetics*, *6*(1), e1000821. https://doi.org/10.1371/journal.pgen.1000821

Nakajima, M. (2014). Dynamics and structure of a native Pieris population in the presence of a non-native, toxic larval host plant. *The Journal of the Lepidopterists' Society*, *68*(3), 175–184. https://doi.org/10.18473/lepi.v68i3.a4

Nakajima, M., & Boggs, C. L. (2015). Fine-grained distribution of a non-native resource can alter the population dynamics of a native consumer. *PLoS One*, *10*(11), 1–17. https://doi.org/10.1371/journal.pone.0143052

Nakajima, M., Boggs, C. L., Bailey, S., Reithel, J., & Paape, T. (2013). Fitness costs of butterfly oviposition on a lethal non-native plant in a mixed native and non-native plant community. *Oecologia*, *172*(3), 823–832.

Nosil, P., Soria-Carrasco, V., Feder, J. L., Flaxman, S. M., & Gompert, Z. (2019). Local and system-wide adaptation is influenced by population connectivity. *Conservation Genetics*, *20*(1), 45–57. https://doi.org/10.1007/s10592-018-1097-0

Nylin, S., Bergström, A., & Janz, N. (2000). Butterfly host plant choice in the face of possible confusion. *Journal of Insect Behavior*, *13*(4), 469–482. https://doi.org/10.1023/A:1007839200323

Nylin, S., & Janz, N. (2009). Butterfly host plant range: An example of plasticity as a promoter of speciation? *Evolutionary Ecology*, *23*(1), 137–146. https://doi.org/10.1007/s10682-007-9205-5

Ohata, M., Furumoto, A., & Ohsaki, N. (2011). Hatchling morphology and adaptation to host plants during juvenile stages in the butterfly Pieris napi. *Ecological Research*, *26*(1), 59–66. https://doi.org/10.1007/s11284-010-0758-3

Ozaki, K., Ryuda, M., Yamada, A., Utoguchi, A., Ishimoto, H., Calas, D., Marion-Poll, F., Tanimura, T., & Yoshikawa, H. (2011). A gustatory receptor involved in host plant recognition for oviposition of a swallowtail butterfly. *Nature Communications*, *2*(1), 542. https://doi.org/10.1038/ncomms1548

Perrier, C., Rougemont, Q., & Charmantier, A. (2020). Demographic history and genomics of local adaptation in blue tit populations. *Evolutionary Applications*, *13*(6), 1145–1165. https://doi.org/10.1111/eva.13035

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Petkova, D., Novembre, J., & Stephens, M. (2015). Visualizing spatial population structure with estimated effective migration surfaces. *Nature Genetics*, *48*(1), 94–100. https://doi.org/10.1038/ng.3464

Picard toolkit. (2019). *In broad institute, GitHub repository*. Broad Institute. https://broadinstitute.github.io/picard/

Prasai, K., & Karlsson, B. (2011). Variation in immune defence in relation to developmental pathway in the green-veined white butterfly, Pieris Napi. *Evolutionary Ecology Research*, *13*(3), 295–305.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ramaswamy, S. B., Ma, W. K., & Baker, G. T. (1987). Sensory cues and receptors for oviposition by *Heliothis virescens*. *Entomologia Experimentalis et Applicata*, *43*(2), 159–168. https://doi.org/10.1111/j.1570-7458.1987.tb03600.x

Ravikanthachari, N., & Boggs, C. L. (2024). Genetic mechanisms underlying preference-performance mismatches: Insights from a specialized native herbivore on an invasive toxic plant. *bioRxiv*, 2024.02.14.580222. https://doi.org/10.1101/2024.02.14.580222

Reed, D. H., Lowe, E. H., Briscoe, D. A., & Frankham, R. (2003). Fitness and adaptation in a novel environment: Effect of inbreeding, prior environment, and lineage. *Evolution*, *57*(8), 1822–1828. https://doi.org/10.1111/j.0014-3820.2003.tb00589.x

Remington, C. l. (1954). A new name for the colorado race of pieris napi. *The Lepidopterists' News*, *8*(3–4), 75.

Rendón-Anaya, M., Wilson, J., Sveinsson, S., Fedorkov, A., Cottrell, J., Bailey, M. E. S., Ruņģis, D., Lexer, C., Jansson, S., Robinson, K. M., Street, N. R., & Ingvarsson, P. K. (2021). Adaptive introgression facilitates adaptation to high latitudes in European Aspen (*Populus tremula* L.). *Molecular Biology and Evolution*, *38*(11), 5034–5050. https://doi.org/10.1093/molbev/msab229

Renwick, J. A. A., & Chew, F. S. (1994). Oviposition behavior in lepidoptera. *Annual Review of Entomology*, *39*(1), 377–400. https://doi.org/10.1146/annurev.en.39.010194.002113

Richard, M., Tallamy, D. W., & Mitchell, A. B. (2019). Introduced plants reduce species interactions. *Biological Invasions*, *21*(3), 983–992. https://doi.org/10.1007/s10530-018-1876-z

Robertson, B. A., & Chalfoun, A. D. (2016). Evolutionary traps as keys to understanding behavioral maladapation. *Current Opinion in Behavioral Sciences*, *12*, 12–17. https://doi.org/10.1016/j.cobeha.2016.08.007

Robertson, B. A., & Hutto, R. L. (2006). A framework for understanding ecological traps and an evaluation of existing evidence. *Ecology*, *87*(5), 1075–1085. https://doi.org/10.1890/0012-9658(2006)87[1075:AFFUET]2.0.CO;2

Robertson, B. A., Rehage, J. S., & Sih, A. (2013). Ecological novelty and the emergence of evolutionary traps. *Trends in Ecology & Evolution*, *28*(9), 552–560. https://doi.org/10.1016/j.tree.2013.04.004

Rodman, J. E., & Chew, F. S. (1980). Phytochemical correlates of herbivory in a community of native and naturalized Cruciferae. *Biochemical Systematics and Ecology*, *8*(1), 43–50. https://doi.org/10.1016/0305-1978(80)90024-1

Ryuda, M., Calas-List, D., Yamada, A., Marion-Poll, F., Yoshikawa, H., Tanimura, T., & Ozaki, K. (2013). Gustatory sensing mechanism coding for multiple oviposition stimulants in the swallowtail butterfly, *Papilio xuthus*. *Journal of Neuroscience*, *33*(3), 914–924. https://doi.org/10.1523/JNEUROSCI.1405-12.2013

Saul, W.-C., & Jeschke, J. M. (2015). Eco-evolutionary experience in novel species interactions. *Ecology Letters*, *18*(3), 236–245. https://doi.org/10.1111/ele.12408

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, *14*(11), 807–820. https://doi.org/10.1038/nrg3522

Schlaepfer, M. A., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*(10), 474–480. https://doi.org/10.1016/S0169-5347(02)02580-6

Schlaepfer, M. A., Sherman, P. W., Blossey, B., & Runge, M. C. (2005). Introduced species as evolutionary traps. *Ecology Letters*, *8*(3), 241–246. https://doi.org/10.1111/j.1461-0248.2005.00730.x

Schweiger, O., Biesmeijer, J. C., Bommarco, R., Hickler, T., Hulme, P. E., Klotz, S., Kühn, I., Moora, M., Nielsen, A., Ohlemüller, R., Petanidou, T., Potts, S. G., Pyšek, P., Stout, J. C., Sykes, M. T., Tscheulin, T., Vilà, M., Walther, G.-R., Westphal, C., … Settele, J. (2010). Multiple stressors on biotic interactions: How climate change and alien species interact to affect pollination. *Biological Reviews*, *85*(4), 777–795. https://doi.org/10.1111/j.1469-185X.2010.00125.x

Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1712), 1601–1609. https://doi.org/10.1098/rspb.2011.0176

Singer, M. C., & Parmesan, C. (2010). Phenological asynchrony between herbivorous insects and their hosts: Signal of climate change or pre-existing adaptive strategy? *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *365*(1555), 3161–3176. https://doi.org/10.1098/rstb.2010.0144

Singer, M. C., & Parmesan, C. (2018). Lethal trap created by adaptive evolutionary response to an exotic resource. *Nature*, *557*(7704), 238–241. https://doi.org/10.1038/s41586-018-0074-6

Singer, M. C., & Parmesan, C. (2019). Butterflies embrace maladaptation and raise fitness in colonizing novel host. *Evolutionary Applications*, *12*(7), 1417–1433. https://doi.org/10.1111/eva.12775

Spieth, H. R., & Cordes, R. (2012). Geographic comparison of seasonal migration events of the large white butterfly. *Pieris Brassicae*. *Ecological Entomology*, *37*(6), 439–445. https://doi.org/10.1111/j.1365-2311.2012.01385.x

Stefanescu, C., Jubany, J., & Dantart, J. (2012). Egg–laying by the butterfly *Iphiclides podalirius* (lepidoptera, Papilionidae) on alien plants: A broadening of host range or oviposition mistakes? *Animal Biodiversity and Conservation*, *29*(1), 83–90.

Steward, R. A., & Boggs, C. L. (2020). Experience may outweigh cue similarity in maintaining a persistent host-plant-based evolutionary trap. *Ecological Monographs*, *90*(3), e01412. https://doi.org/10.1002/ecm.1412

Steward, R. A., Epanchin-Niell, R. S., & Boggs, C. L. (2022). Novel host unmasks heritable variation in plant preference within an insect population. *Evolution*, *76*, 2634–2648. https://doi.org/10.1111/EVO.14608

Steward, R. A., Fisher, L. M., & Boggs, C. L. (2019). Pre- and post-ingestive defenses affect larval feeding on a lethal invasive host plant. *Entomologia Experimentalis et Applicata*, *167*(4), 292–305. https://doi.org/10.1111/eea.12773

Steward, R. A., Okamura, Y., Boggs, C. L., Vogel, H., & Wheat, C. W. (2021). The genome of the margined white butterfly (*Pieris macdunnoughii*): Sex chromosome insights and the power of polishing with PoolSeq data. *Genome Biology and Evolution*, *13*(4), evab053. https://doi.org/10.1093/GBE/EVAB053

Takami, Y., Koshio, C., Ishii, M., Fujii, H., Hidaka, T., & Shimizu, I. (2004). Genetic diversity and structure of urban populations of Pieris butterflies assessed using amplified fragment length polymorphism. *Molecular Ecology*, *13*(2), 245–258.

Thompson, J. N. (1999a). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, *153*(SUPPL), S1–S14. https://doi.org/10.1086/303208

Thompson, J. N. (1999b). The evolution of species interactions. *Science*, *284*(5423), 2116–2118. https://doi.org/10.1126/science.284.5423.2116

Thompson, J. N. (Ed.). (2005). The geographic mosaic of coevolution. In *The Geographic Mosaic of Coevolution*. University of Chicago Press. https://doi.org/10.7208/9780226118697

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Thompson, J. N., & Pellmyr, O. (1991). Evolution of oviposition behavior and host preference in lepidoptera. *Annual Review of Entomology*, *36*(1), 65–89. https://doi.org/10.1146/annurev.en.36.010191.000433

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K. V., Altshuler, D., Gabriel, S., & DePristo, M. A. (2013). From FastQ data to high-confidence variant calls: The genome analysis toolkit Best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11.10.1–11.10.33. https://doi.org/10.1002/0471250953.bi1110s43

Vertacnik, K. L., & Linnen, C. R. (2017). Evolutionary genetics of host shifts in herbivorous insects: Insights from the age of genomics. *Annals of the new York Academy of Sciences*, *1389*(1), 186–212. https://doi.org/10.1111/nyas.13311

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the National Academy of Sciences*, *118*(2), e2023989118. https://doi.org/10.1073/pnas.2023989118

Wang, Y., Li, Y., Qi, X., Yuan, W., Ai, J., Zhu, C., Cao, L., Yang, H., Liu, F., Wu, X., & Liu, M. (2004). TRIM45, a novel human RBCC/TRIM protein, inhibits transcriptional activities of EIK-1 and AP-1. *Biochemical and Biophysical Research Communications*, *323*(1), 9–16. https://doi.org/10.1016/j.bbrc.2004.08.048

Warwick, S. I., Francis, A., & Susko, D. J. (2011). The biology of Canadian weeds. 9. *Thlaspi arvense* L. (updated). *Canadian Journal of Plant Science*, *82*(4), 803–823. 10.4141/P01-159.

Wheat, C. W., Vogel, H., Wittstock, U., Braby, M. F., Underwood, D., & Mitchell-Olds, T. (2007). The genetic basis of a plant–insect co-evolutionary key innovation. *Proceedings of the National Academy of Sciences*, *104*(51), 20427–20431. https://doi.org/10.1073/pnas.0706229104

Whitlock, M. C. (2015). Modern approaches to local adaptation. *American Naturalist*, *186*, S1–S4. https://doi.org/10.1086/682933

Yan, D., Li, F., Hall, M. L., Sage, C., Hu, W.-H., Giallourakis, C., Upadhyay, G., Ouyang, X. M., Du, L. L., Bethea, J. R., Chen, Z. Y., Yajnik, V., & Liu, X. Z. (2006). An isoform of GTPase regulator DOCK4 localizes to the stereocilia in the inner ear and binds to Harmonin (USH1C). *Journal of Molecular Biology*, *357*(3), 755–764. https://doi.org/10.1016/j.jmb.2006.01.017

Yang, F., Vought, B. W., Satterlee, J. S., Walker, A. K., Jim Sun, Z.-Y., Watts, J. L., DeBeaumont, R., Mako Saito, R., Hyberts, S. G., Yang, S., Macol, C., Iyer, L., Tjian, R., van den Heuvel, S., Hart, A. C., Wagner, G., & Näär, A. M. (2006). An ARC/mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature*, *442*(7103), 700–704. https://doi.org/10.1038/nature04942

Yang, K., Gong, X.-L., Li, G.-C., Huang, L.-Q., Ning, C., & Wang, C.-Z. (2020). A gustatory receptor tuned to the steroid plant hormone brassinolide in *Plutella xylostella* (lepidoptera: Plutellidae). *eLife*, *9*, e64114. https://doi.org/10.7554/eLife.64114

Yoon, S., & Read, Q. (2016). Consequences of exotic host use: Impacts on lepidoptera and a test of the ecological trap hypothesis. *Oecologia*, *181*(4), 985–996. https://doi.org/10.1007/s00442-016-3560-2

Zhang, C., Dong, S.-S., Xu, J.-Y., He, W.-M., & Yang, T.-L. (2019). PopLDdecay: A fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics*, *35*(10), 1786–1788. https://doi.org/10.1093/BIOINFORMATICS/BTY875

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ravikanthachari, N., Steward, R. A., & Boggs, C. L. (2024). Patterns of genetic variation and local adaptation of a native herbivore to a lethal invasive plant. *Molecular Ecology*, *00*, e17326. https://doi.org/10.1111/mec.17326

13652294x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/mec.17326 by Stanford University, Wiley Online Library on [22/03/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License