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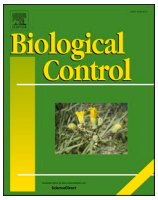

Perspective

Shared patterns of population genomic variation and phenotypic response across rapid range expansions in two invasive lady beetle species

Angela G. Jones [a,d], John J. Obrycki [b], Arun Sethuraman [c], David W. Weisrock [a,*]

[a] Department of Biology, University of Kentucky, Lexington KY 40506 USA
[b] Department of Entomology, University of Kentucky, Lexington KY 40546 USA
[c] Department of Biology, San Diego State University, San Diego CA 92182 USA
[d] Current Address: University Program of Genetics and Genomics, Duke University, Durham, NC 27708 USA

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ABSTRACT

Non-native lady beetle species have often been introduced, with variable success, into North America for biological control of aphids, scales, whiteflies, and other agricultural pests. Two predatory lady beetle species, *Propylea quatuordecimpunctata* and *Hippodamia variegata*, both originating from Eurasia, were first discovered near Montreal, Quebec, in North America in 1968 and 1984, respectively, and have since expanded into northeastern North America and the midwestern United States. In this study, we estimate the range-wide population structure, establishment and range-expansion, and recent evolutionary history of these lady beetle species using reduced-representation genotyping-by-sequencing via ddRADseq. In addition, we quantified the responses to a key abiotic factor, photoperiod, that regulates adult reproductive diapause in these two species and may influence their geographical range in North America. Our analyses detect: (1) non-significant genetic differentiation and divergence among North American populations that likely originated from a single accidental introduction, (2) evidence of reduced contemporary gene flow within the continental US, and (3) minor phenotypic differences in diapause induction between populations of the two species from the same location.

1. Introduction

Understanding how species establish new populations in previously unoccupied geographic regions is important in predicting future evolutionary success, especially during times of increased environmental change and possible resulting range shifts. This could apply to species that are undergoing rapid shifts in their geographic ranges, often as a response to a changing environment and global climate change (e.g., Collevatti et al., 2011; Karban & Strauss, 2004; Melles et al., 2011), and also to species that have been intentionally introduced to novel geographic ranges (e.g., Gillis & Walsh, 2017; Ochocki & Miller, 2017; Osborne et al., 2013). For this latter category, the general factors responsible for, or resulting from, the establishment and expansion of exotic species (e.g., genetic diversity, population structure, local adaptation) are often difficult to discern due to variability between species (Lodge, 1993). For example, some introduced species display significant population structure within twenty years of establishment (Wang et al., 2017), while others have remained genetically undifferentiated across their invasive range (Tsutsui et al., 2000).

The evolutionary potential for nascent populations to survive in new locations is expected to be influenced by many factors, including environmental conditions in the non-native ranges, and the plastic or adaptive phenotypic responses to novel environments (Jardeleza et al., 2022). Often, one of the first steps in understanding how populations expand into new ranges involves studying the spatial distribution of genetic variation as a means to understand how evolutionary processes structure populations and their phenotypic variation. For example, multiple introductions, resulting in increased genetic diversity and higher adaptive potential have been described in several species (e.g., seven-spotted lady beetles, Kajita et al., 2012; data in Dlugosch & Parker, 2008). Similarly, numerous studies have described plasticity in invasive species that have shifted their ranges or microgeographic niches because of changing environments in their previously native ranges (e.g., leafminer flies, Rodríguez-Castañeda et al., 2017; tiger mosquitos, Lounibos et al., 2003; fritillary butterflies, Zheng et al., 2009; summarized in Moran & Alexander, 2014). With increased access to

* Corresponding author.
E-mail address: asethuraman@sdsu.edu (D.W. Weisrock).

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large-scale genomic data and complementary phenotypic and geographical data, studying invasive species has turned an important corner in elucidating: (1) establishment and adaptation of species to novel environments (Vandepitte et al., 2014), (2) extent of genomic population structure resultant from rapid range expansion, and (3) links between genomic and phenotypic plasticity, and resultant adaptive potential to previously uninvaded locations (Mathieu-Bégné et al., 2021).

Biological control organisms such as predatory lady beetles (Coleoptera: Coccinellidae) provide an ideal study system to investigate the establishment, success, and future adaptive potential of invasive species (Sethuraman et al., 2020; Szűcs et al., 2019). Numerous species of lady beetles have been introduced to North America for biological control of agricultural and forest pests, with several becoming established and invasive in their introduced ranges (Gordon, 1985; Goryacheva & Blekhman, 2017; Obrycki & Kring, 1998; Sethuraman et al., 2018, 2020).

To date, the majority of studies exploring the evolutionary and population genetics of invasive lady beetles have been restricted to allozyme (Krafsur et al., 1992, 1996), nuclear microsatellite (Lombaert et al., 2010, 2014), or mitochondrial (Kajita et al., 2012) DNA data. More recently, large-scale genotyping by sequencing studies, complemented with relevant phenotypic information, are now yielding important insights into range expansions and establishment in biological control organisms (e.g., Hopper et al., 2019; Stahlke et al., 2021).

Here, we combine genomic and ecological approaches to study the establishment and range expansion of two exotic species of Palearctic lady beetles, *Propylea quatuordecimpunctata* and *Hippodamia variegata,* (Coleoptera: Coccinellidae) for biological control of aphid pests in North America. During the 20th century, agriculturalists attempted multiple times to introduce and establish these two predatory species for biological control of aphid pests (Rogers et al., 1972; Gordon, 1985, 1987; Prokrym et al., 1998). Collections of these two predatory species were made from a wide range of locations in the Palearctic region, mass-reared and released in several locations in the USA; however, no documented establishment of either species as a result of these efforts has

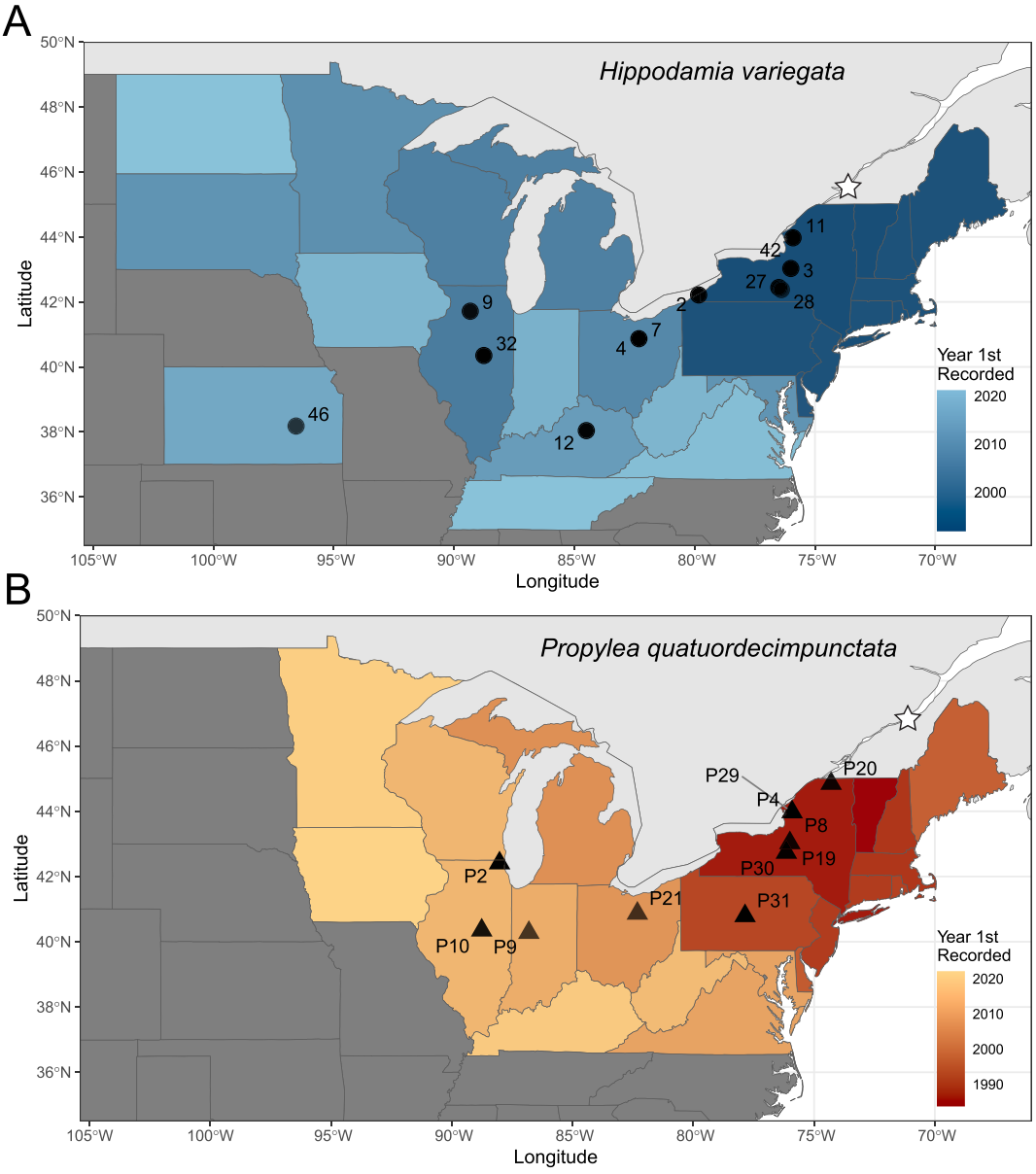

Fig. 1. Sampling of **(A)** *H. variegata* and **(B)** *P. quatuordecimpunctata* across their invasive United States ranges with numerical locality identifiers (Table S2, S3). Parenthetical numbers denote the number of sequenced samples retained for genetic analyses. Color gradient displays the year of first recorded specimen in each state. Star icon shows approximate location of North American introduction.

been reported (Gordon, 1985, Prokrym et al., 1998; Rogers et al., 1972; Wheeler, 1993). Instead, the establishment of North American populations of both species presumably resulted from a single passive introduction via shipping in the Saint Lawrence Seaway, with the first known collection of non-introduced individuals near Montreal, Quebec (Dysart, 1988; Gordon, 1987). Both species have since spread across northeastern North America and into the midwestern US (Fig. 1, Table S1), with *H. variegata* displaying a more rapid and widespread range expansion than *P. quatourdecimpunctata* (Hesler & Hatfield, 2018; Hesler & Lundgren, 2011). However, little is known about the genetic impact of this rapid expansion in either species.

Using a genome-wide dataset of single nucleotide polymorphisms (SNPs) we estimated the population structure of *P. quatuordecimpunctata* and *H. variegata* in their invasive North American ranges through non-spatial and spatially-informed analyses. To complement these genomic studies, we also quantified the responses to photoperiod regulating adult reproductive diapause in both species. Previous studies have characterized the responses of North American populations of *P. quatuordecimpunctata* and *H. variegata* to photoperiods as long-day species, in which females under long daylengths avert diapause and short daylengths induce diapause (Obrycki, 2018; Obrycki et al., 1993). Variation in responses to photoperiod has previously been shown to contribute to the invasion of several continents by two non-native lady beetles, *Coccinella septempunctata* and *Harmonia axyridis* (Belyakova et al., 2021; Hodek, 2012; Reznik et al., 2015). In this study, we compare responses to photoperiods for populations of both species from the same location in North America to determine levels of interspecific variation. We also compared the response of two *P. quatuordecimpunctata* populations from eastern North America, collected approximately 30 years apart, to examine if the response to photoperiod has changed. The response to photoperiods may play a role in the phenology of these species and influence the boundaries of their latitudinal ranges in North America. Specifically, time to onset of diapause is an adaptive plastic trait that influences generation times, fecundity, and overall reproductive fitness of the species (Phoofolo & Obrycki, 2000). We predict that a plastic response to induction of diapause would support rapid range expansion across regions with varying photoperiods. Here, we use genomic and phenotypic datasets of both species to investigate: (1) the standing genomic diversity of invasive populations, (2) signatures of single or multiple releases on population structure, and (3) phenotypic plasticity in the adult reproductive diapause response to photoperiod.

2. Methods

2.1. Sampling, ddRAD Sequencing, and SNP genotyping

The SNP data were generated from 37 *H. variegata* individuals from across their known US range (Fig. S1A; Table S2) collected between 2005–2017. Additionally, *H. variegata* samples were included from the Czech Republic, which is within the native range of this species. The specific geographic origins of the North American populations of *H. variegata* and *P. quatuordecimpunctata* are not known, but presumably, because of the initial discovery near the Saint Lawrence Seaway, are likely from Europe. Thus, the samples from the Czech Republic serve as an example of the genetic diversity within European populations. *H. variegata* sampling also included four samples from a population in Australia with unknown origin of establishment and two samples from a population in Chile that likely originated through a biological control program (Grez et al., 2012). SNP data were generated from 45 *P. quatuordecimpunctata* individuals from across their known US range collected from 2010 to 2019 and included four *P. quatuordecimpunctata* samples from the Czech Republic, which is also in the native range of this species (Fig. S1B; Table S3).

Double digest restriction-site associated DNA (ddRAD) sequencing was performed using the protocol outlined in Peterson, et al. (2012). Briefly, DNA was extracted from whole individuals using a Qiagen DNEasy tissue kit (Qiagen, Valencia, CA, USA). DNA was quantified with a Qubit 2.0 fluorometer (Thermo-Fisher) and quality-checked with agarose gel electrophoresis. The restriction enzymes *Eco*RI and *Nla*III were used to digest approximately 250 ng of DNA per individual. Custom inline barcodes and P1/P2 adapters were ligated onto the digested DNA fragments. The uniquely barcoded samples were pooled and size-selected between 473 and 579 bp using a Pippin Prep (Sage Science) machine. Size-selected DNA was then amplified using High-Fidelity DNA Polymerase (Bio-Rad), unique Illumina index sequences, and Illumina sequencing primers for 10 cycles, ensuring that each individual contained a unique set of inline and index sequences. Samples were then pooled into two libraries and sequenced on a single lane of an Illumina HiSeq X at Novogene using 150 bp paired-end (PE) reads. The combined libraries were sequenced using an additional 1 % spike of PhiX control library.

Resulting sequence data were subjected to standard Illumina chastity filtering and reads were demultiplexed with STACKS v. 2.53 (Catchen et al., 2013). Downstream processing of demultiplexed reads was performed using ipyrad v. 0.9.19 (Eaton & Overcast, 2020). Reads were then *denovo* assembled using standard settings (described in the code repository). Samples with more than 50 % missing data were removed. SNP data were filtered at the population level in ipyrad to retain SNPs present in at least half of the total populations and in at least half of the total number of individuals in that population. Subsets of U.S. samples were created using the −-branch option. VCFTools v. 0.1.16 (Danecek et al., 2011) was used to sample a single SNP per locus (−-thin 5000), resulting in a dataset of putatively unlinked SNPs (O'Leary et al., 2018). To avoid biases in estimation of population structure, we filtered the data in PLINK v. 1.9 (Chang et al., 2015) to remove non-biallelic SNPs, as well as SNPs with a minor allele frequency (MAF) less than 0.05 (Linck & Battey, 2019).

2.2. Non-spatially informed population structure

Pairwise F_{ST} between sampling localities was calculated according to Weir & Cockerham (1984) in the R package StaMMP v.1.6.3 (Pembleton et al., 2013). Bootstrap replicates (10,000) were used to create a 95% confidence interval for calculated F_{ST} values and test for significance. As a non-model-based test of isolation by distance (IBD) in US populations of *H. variegata* and *P. quatuordecimpunctata*, a Mantel test was performed in the R package vegan v.1.4.1 with a normalized F_{ST} matrix and a Haversine distance geographic matrix (Legendre & Legendre, 2012).

Two distinct analyses were used to estimate range-wide subpopulation assignment and admixture of *H. variegata* and *P. quatuordecimpunctata* individuals. First, discriminant analysis of principal components (DAPC) was used to infer genetic clusters using adegenet (Jombart & Ahmed, 2011; Jombart et al., 2010). DAPC constructs genetic clusters from a SNP data matrix that have the largest between-group variance and the smallest within-group variance. A range of proposed cluster numbers (K = 1–10) was tested and the Bayesian Informative Criterion (BIC) method was used to estimate the best fit of K to the data (Jombart et al., 2010).

Second, the program ADMIXTURE v1.3.0 (Alexander & Lange, 2011) was used to assign individuals to one of K genetic clusters. ADMIXTURE jointly estimates subpopulation allele frequencies and admixture proportions under a maximum likelihood framework to quantify deviations from Hardy-Weinberg Equilibrium. A range of cluster numbers (K = 1–10) was tested across 10 individual replicates and the optimal K was inferred using a cross-validation (CV) approach (Alexander & Lange, 2011).

2.3. Spatially informed population structure

Three spatially informed methods were used to estimate population structure across both species in the U.S. Spatial principal component analysis (sPCA) was performed in adegenet to estimate genetic

dissimilarity on a geographical landscape (Jombart et al., 2008). Briefly, sPCA uses Moran's *I* index to correlate spatial information to genetic allele frequencies. To create the input GENIND file, we used the R tool *vcfR* (Knaus & Grünwald, 2017). The analyses used a nearest-neighbor connection network accounting for multiple samples at each sampling site (type = 6) with k = 20 and k = 25 neighbors for *P. quatuordecimpunctata* and *H. variegata,* respectively. This connection network scheme was created to maximize connectivity across spatially distinct samples while accounting for sampling differences between species and potential long-distance dispersal events among sampling sites (Maigret et al., 2020). We estimated the significance of global and local structure with 999 permutations through adegenet (Montano & Jombart, 2017).

Second, MEMGENE was used to identify spatial neighborhoods in genetic distance data. MEMGENE combines Moran's eigenvalue maps with a regression framework where genetic distances are spatially independent. Samples are mapped based on independent location, and only significant eigenvalues are retained for analysis (Galpern et al., 2014).

Lastly, conStruct (Bradburd et al., 2018) was used to investigate if genetic structure was due to IBD. ConStruct is a model-based method that simultaneously infers continuous and discrete patterns of population structure by estimating ancestry proportions for each sampled individual from a two-dimensional population layer, while estimating the rate at which relatedness decays with geographic distance. This method also allows for cross-validation procedure for model selection, between both spatial and nonspatial models. The R package *fields* was used to create the geographic distance matrix that conStruct utilizes. Cross-validation analyses from $K = 1$–10 with 10,000 iterations and 5 replications per K value were then conducted.

2.4. Estimation of migratory history

To estimate evolutionary history of the structured populations identified by ADMIXTURE analyses across *H. variegata* and *P. quatuordecimpunctata*, the Bayesian MCMC tool, BayesAss3-SNPs (Mussmann et al., 2019; Wilson & Rannala, 2003) was used. Briefly, BayesAss estimates contemporary migration rates from multilocus genotype data using a Bayesian MCMC method. 48-hour runs of each MCMC were performed, discarding 10 % of all sampled states as burn-in, resulting in a total of 4.94e7 states for the *H. variegata* data, and 1.18e7 states for the *P. quatuordecimpunctata* data. Convergence of the MCMC was assessed using Tracer v1.7.1 and 95 % confidence intervals around migration rate estimates were constructed using the marginal posterior density distributions.

2.5. Effect of photoperiod on induction of adult reproductive diapause

Data for the reproductive responses to different photoperiod conditions for *H. variegata* were taken from Obrycki (2018). Briefly, adult *H. variegata*, collected in Jefferson County, New York, USA (43.98°N, 75.91°W) were placed in LD 16:8, 22 °C and fed daily. Eggs were collected from 4 to 6 females and placed systematically into one of four constant photoperiod treatments (LD 16:8, 14:10, 12:12, or 10:14 at 22 °C). Larvae were reared individually and F1 adults were paired and maintained at the same photoperiod. The date of first oviposition was recorded for each female (Obrycki, 2018). The reproductive response of female *H. variegata* from Jefferson County, New York to photoperiod was characteristic of a long-day species; females at long daylengths reproduce, whereas females at short daylengths enter diapause (Obrycki, 2018). Data on the length of the preoviposition period (days) and the percentage of females ovipositing at each photoperiod from the *H. variegata* experiment (Obrycki, 2018) were compared with data collected for *P. quatuordecimpunctata* in the present study.

Data for *P. quatuordecimpunctata* were generated using adults collected from Jefferson County, New York, USA (43.98°N, 75.91°W). Individual females or mating pairs were placed in 0.24 L (8 oz.) paper containers (webstaurantstore.com), maintained at a photoperiod of L:D 16:8 (light:dark), a temperature of 22 ± 1 °C, and provided water, a Wheast (GreenMethods.com)-honey mixture, and a daily supply of pea aphids, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). Eggs were collected daily from 4 to 6 females and placed in L:D 16:8, 14:10, 12:12,10:14, at 22 °C ± 1.0 °C. These photoperiods approximate the seasonal range of photoperiods in the northeastern United States. F1 offspring were individually reared in glass vials at each photoperiod on *A. pisum* and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Beneficial Insectary, Redding, CA).

Pairs of F1 adults were placed in 0.24 L paper containers at the same larval photoperiod, provided water, a Wheast-honey mixture, and pea aphids. Pairs were fed and maintained at each photoperiod for 120 days, when the experiment was ended. The date of first oviposition was recorded for each female. If a female died, the date of her death was recorded. If a male died, a male from the same L:D condition was used as a replacement. Voucher specimens of *P. quatuordecimpunctata* are accessioned in the University of Kentucky Insect Museum.

The length of the pre-oviposition period (days) was recorded to quantify the proportion of *P. quatuordecimpunctata* females at a given photoperiod that was in diapause and estimate the duration of diapause in females at the four photoperiods. A prolonged pre-oviposition period was observed in some females at long daylengths (L:D 16:8) and excess prey availability, conditions that typically do not induce diapause (Hodek 2012). The diapause or non-diapause condition of each *P. quatuordecimpunctata* female at the four photoperiods was based on twice the median pre-oviposition period (days) observed at L:D 16:8. This type of classification has been used to separate females into diapause and non-diapause groups in previous studies of adult reproductive diapause in predatory Hemipterans (Ruberson et al., 2000, 2001) and coccinellids (Obrycki, 2018, Obrycki et al., 2024).

2.6. Photoperiod statistical analyses

The days from female eclosion to first oviposition (pre-oviposition period) at each photoperiod was compared within each species and between species for populations collected in Jefferson County using event-time analysis (JMP Pro 14.0.0). Females that died or did not oviposit within 120 days, the duration of the experiment, were censored, because the pre-oviposition period for these individuals was not measured. A non-parametric log-rank analysis of the response to photoperiod within each population was used to examine if the pre-oviposition period varied among females at each photoperiod (JMP Pro 14.0.0). Comparisons of responses to the four photoperiods between *P. quatuordecimpunctata* from Jefferson County to *P. quatuordecimpunctata* from Montreal, Canada were also conducted using the non-parametric log-rank test (JMP Pro 14.0.0). Data for the Montreal, Canada population are from Obrycki et al., (1993).

3. Results

3.1. Sampling, ddRAD Sequencing, and SNP genotyping

A total of ~ 996 million 150 bp PE reads were generated, with a mean of 10,379,052 reads per individual. After demultiplexing and standard Illumina filtering, an average of 82.3% of reads were retained per individual. Two sub-libraries sequenced poorly and retained an average of 25.1% of reads after demultiplexing. The average percentage retained excluding those poorly sequenced was 96.6%. After filtering, we recovered genotypes for 70 individuals (41 *H. variegata*; 29 *P. quatuordecimpunctata*) from 26 localities (16 *H. variegata*; 10 *P. quatuordecimpunctata*). Filtering by a minor allele frequency of 0.05 resulted in a total of 7,478 SNPs in 634 loci in *H. variegata* and 24,062 SNPs in 2,196 loci in *P. quatuordecimpunctata*. Data sets restricted to US samples resulted in a total of 11,317 SNPs in 876 loci in 32 *H. variegata*

individuals and 25,475 SNPs in 2,359 loci in 25 *P. quatuordecimpunctata* individuals. For downstream analyses, a filtered dataset consisting of a single SNP per locus was used.

3.2. Non-spatial population structure

Pairwise F_{ST} analyses of *H. variegata* revealed significant ($p < 0.05$) genetic distance between continental groups (Table S4). In addition, we detected small ($F_{ST} < 0.1$) but significant effects between some North American populations which roughly follow an east–west cline. Parallel analysis of *P. quatuordecimpunctata* again revealed significant separation by continent and small differences along an east–west pattern between North American populations (Table S5). Mantel tests did not detect significant signatures of IBD in North American samples of *H. variegata* ($p = 0.25$) or *P. quatuordecimpunctata* ($p = 0.39$). Neither DAPC nor ADMIXTURE identified distinct geographic genetic clusters in the North American range of either species. The BIC value of DAPC and the cross-validation of ADMIXTURE were lowest for $K = 1$ (Fig. S2). When $K = 2$ was considered for *H. variegata*, ADMIXTURE (Fig. 2A) and DAPC (Fig. S2) assignment plots supported a separation of North American and Czech samples from Australian and Chilean samples. ADMIXTURE assignment plots of $K = 2$ for *P. quatuordecimpunctata* clustered the Czech samples with an assortment of North American samples (Fig. 3A), but did not reveal any clear geographic patterning in the North American samples.

3.3. Spatial population structure

MEMGENE did not detect any significant eigenvectors in either *H. variegata* or *P. quatuordecimpunctata*, and thus no results were produced. Cross-validation conStruct analyses of both *H. variegata* (Fig. 2B) and *P. quatuordecimpunctata* (Fig. 3B) indicated minor differences in the predictive accuracy of spatial and nonspatial models, suggesting that geographic distance does not significantly contribute to genetic differences. Interestingly, conStruct cross-validation analyses indicate that a $K = 2$ has the highest predictive accuracy of tested *K*s in both *H. variegata* and *P. quatuordecimpunctata*. However, further investigations revealed that only a single *K* is notably contributing to spatial model layers (Fig. S3). Thus, these results do not detect a signature of population structure affected by IBD.

For the U.S. dataset of *H. variegata*, sPCA detected an eigenvector delineating genetic differentiation along an east–west axis (Fig. 2C, Fig. S4A). However, neither global ($p = 0.61$) nor local structure ($p = 0.17$) was significant. SPCA of *P. quatuordecimpunctata* yielded similar results with a single eigenvector delimiting an east–west division (Fig. 3C, Fig. S4B). Again, there was no significant structure on the global ($p = 0.96$) or local ($p = 0.37$) scales.

3.4. Contemporary migratory history

Estimates of contemporary migration using BayesAss3-SNPs reaffirmed our admixture analyses using ADMIXTURE, with negligible gene flow estimated between populations (Tables S6, S7) in both populations of *P. quatuordecimpunctata* ($K = 2$ of individuals in the USA, and Czech Republic) and *H. variegata* ($K = 3$, separated into individuals in the USA, Czech Republic, and Australia + Chile). All MCMC runs were assessed for convergence and mixing using Tracer v.1.7.1 by observing the traceplots for migration rates (to ensure mixing), large effective sample sizes

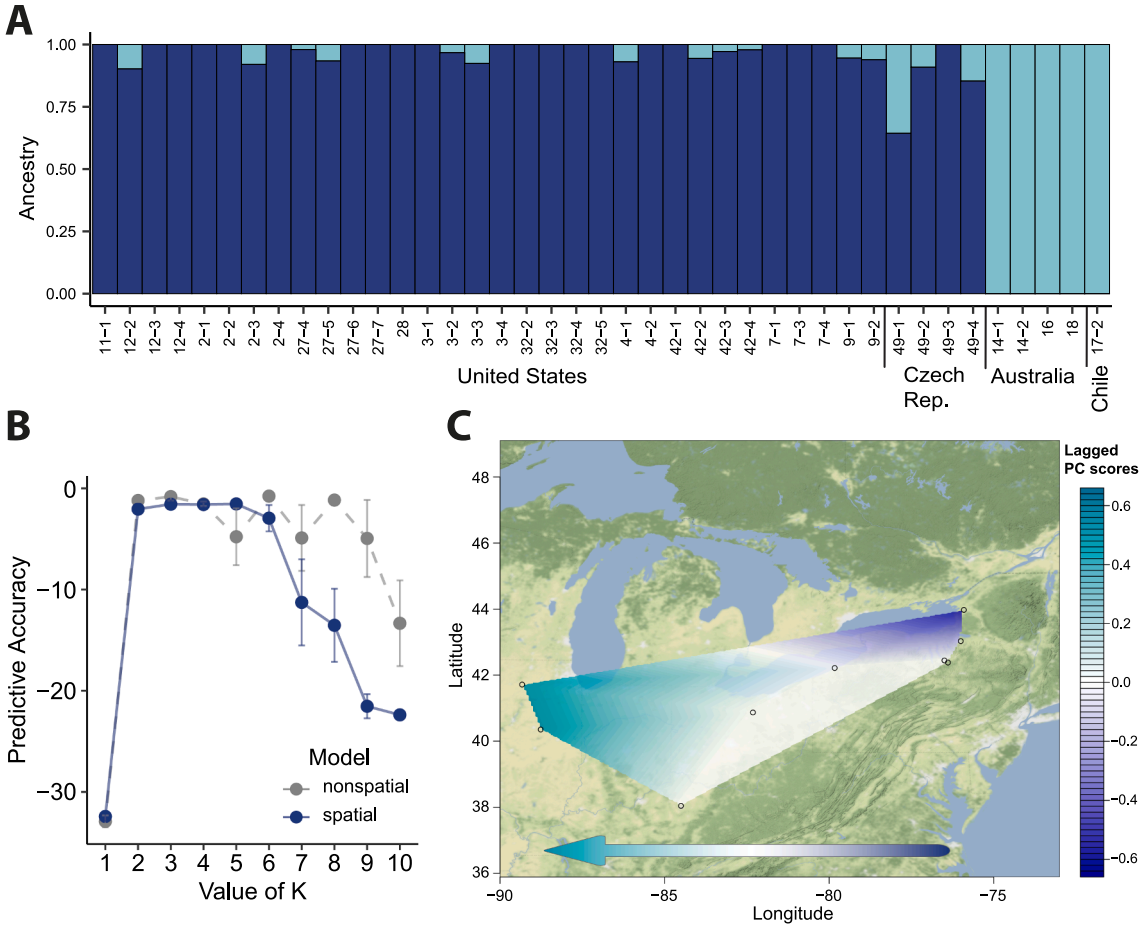

Fig. 2. Population structure analyses of *H. variegata* in the United States. **(A)** ADMIXTURE assignment plot for $K = 2$. **(B)** conStruct cross-validation results for $K = 1$–10 in both spatial and non-spatial analyses. **(C)** sPCA lagged principal component scores across populations.

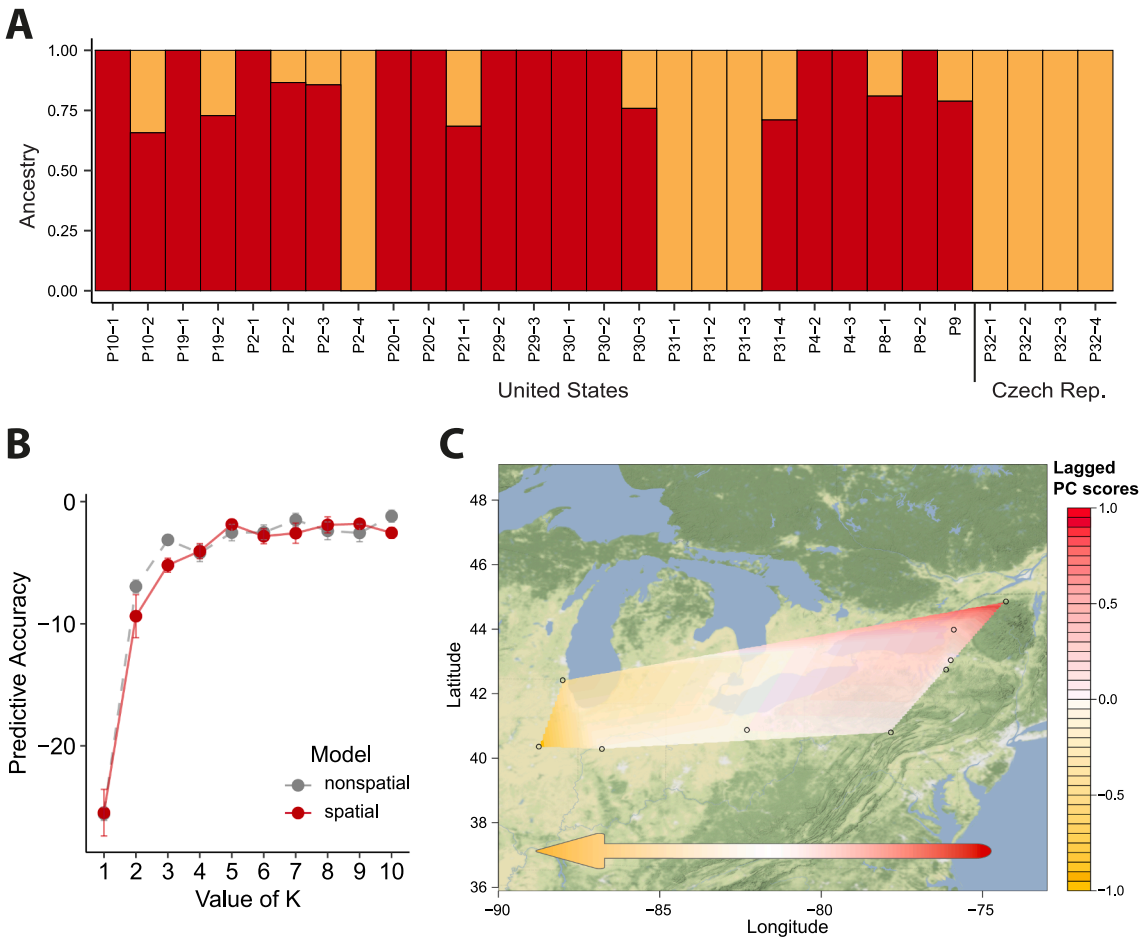

Fig. 3. Population structure analyses of *P. quatuordecimpunctata* in the United States. **(A)** ADMIXTURE assignment plot for $K = 2$. **(B)** conStruct cross-validation results for $K = 1$–10 in both spatial and non-spatial analyses. **(C)** sPCA lagged principal component scores across populations.

(ESS), and autocorrelations.

3.5. Photoperiodic effects on diapause induction

The adult reproductive responses to photoperiod, as measured by the pre-oviposition period varied significantly among the four photoperiods (Table S8, *P. quatuordecimpunctata* analyses within row). The median pre-oviposition period for *P. quatuordecimpunctata* females at L:D 16:8 was 10 days; no oviposition during the 120-day experiment was observed at L:D 14:10, 12:12 or 10:14).

3.6. Intraspecific and interspecific comparisons of responses to photoperiod

The responses of two *P. quatuordecimpunctata* populations from Montreal, Quebec, Canada and Jefferson County, New York, USA to the four constant photoperiods were similar (Table S9: analyses within columns). The non-parametric log-rank analysis of pre-oviposition periods indicated significant differences in responses by *P. quatuordecimpunctata* and *H. variegata* collected in Jefferson County, New York to L:D 14:10 and L:D 12:12, but similar responses to L:D 16:8 and L:D 10:14 (Table 1, analyses within rows). At each of the four constant photoperiods, a similar percentage of female *P. quatuordecimpunctata* and *H. variegata* from Jefferson County, NY and *P. quatuordecimpunctata* (*P. quatuordecimpunctata*CAN) females from Montreal, Quebec, Canada were induced into diapause (Table 1).

4. Discussion

Here, we analyze genetic diversity, distribution, and patterns of phenotypic variation in two different invasive species of lady beetles utilized in biological control, with shared patterns of importation and

Table 1
Percentage of female *P. quatuordecimpunctata* and *H. variegata* from Jefferson County, NY and *P. quatuordecimpunctata* females from Montreal, Quebec, Canada in diapause at four constant photoperiods: L:D 16:8; 14:10; 12:12; and 10:14 at 22 °C ± 1 °C. The criteria for reproductive diapause in a female was 2X the median pre-oviposition period (days) observed at L:D 16:8 for each population.

Population	Reproductive diapause	Daylength Treatment (L:D) 16:8	14:10	12:12	10:14	Reference
P. quatuourdecimpunctata Jefferson County, NY	20 days	8 %	100 %	100 %	100 %	Present Study
P. quatuourdecimpunctata Montreal, Canada	18 days	22 %	80 %	90 %	90 %	Obrycki et al., 1993
H. variegata Jefferson County, NY	15 days	15 %	100 %	100 %	100 %	Obrycki, 2018

augmentation history. Genetic analyses indicate seemingly rapid westward expansion of both species, with little discernible population structure evolving across its non-native range. In contrast, experimental analyses of phenotypic variation in photoperiod response identified minor differences between populations of *H. variegata* and *P. quatuordecimpunctata* (Table S8), suggesting the potential for either local adaptation or phenotypic plasticity.

4.1. Genetic differences in native and introduced populations

Non-spatial estimates of population structure showed little difference between European and North American samples in both species. This result parallels previous allozyme results in *H. variegata* (Krafsur et al., 1996) and *P. quatuordecimpunctata* (Krafsur & Obrycki, 1996), suggesting minimal significant genome-wide genetic differentiation between populations from the native and introduced ranges. In addition, no significant differences have been detected in physiological mechanisms such as response to photoperiod and net reproductive rates between North American and European samples of *P. quatuordecimpunctata* (Obrycki et al., 1993; Phoofolo & Obrycki, 2000). This suggests that all non-native populations of both species in North America were derived from a small number of founders with little successful augmentation from secondary introductions, as discovered in other invasive lady beetle species (e.g. *Harmonia axyridis* – Lombaert et al., 2014, 2011).

In contrast, Chilean and Australian samples of *H. variegata* appear to be genetically distinct from European and North American samples. This may be due to a founder effect after a bottleneck and accumulation of genetic drift. This pattern also may suggest that the founding individuals of Chile and Australia originated from a different source population than those of North America. During the 1970s, *H. variegata* from South Africa were released into Chile; the origins of the Australian populations are not known (Franzmann, 2002; Grez et al., 2012). Previous studies have suggested eastern Asia as the origin of *H. variegata* due to higher levels of genetic diversity found in that region (Krafsur et al., 1996). Our analyses do not include samples from Asia, but further investigation could determine the source populations of invasions into Chile and Australia.

Within the U.S., weak signatures of gene flow were detected across the ranges of both *H. variegata* and *P. quatuordecimpunctata*. sPCA eigenvectors support an east–west divide in both species, indicating signatures of isolation by distance (IBD). These differences are likely due to genetic drift associated with increasing geographic distance (Krafsur et al., 1996). Non-spatial analyses and IBD-informed analysis through conStruct however were unable to detect any significant population structure in the invasive ranges of both *P. quatuordecimpunctata* and *H. variegata*.

Our results do not show significant genetic structure within the invasive range, which supports a rapid expansion of both species from a single introduction.. *H. variegata* and *P. quatuordecimpunctata* were both introduced to North America within the last 55 years. Within this time, these species have rapidly expanded and have proved successful in colonization of new territory, in spite of a likely small founder group. Invasive species are often able to spread quickly with few genetic signatures of divergence (Eyer et al., 2018; Tsutsui et al., 2000). This is particularly true of coccinellid species where single females establish new colonies when there is sufficient prey density (Krafsur & Obrycki, 1996). These colonizing events cause successive founder effects that may be responsible for the lack of significant structure and within population genetic diversity in the U.S. populations of *H. variegata* and *P. quatuordecimpunctata*.

The analyses of spatial structure and dispersal indicate that both species are showing natural dispersal from their source populations in a generally western direction. This is evident even in our widespread sampling of invasive populations that show limited genomic diversity. We (Sethuraman et al., 2024) and others (Nazareno et al., 2017; Li et al., 2020, Qu et al., 2020) have previously shown that even limited sampling across diverse populations is sufficient to recover genetic differences in population structure. In addition, through the use of ddRADseq, we are able to identify thousands of SNPs across the entire genome, as opposed to previous work with these species that were limited to allozyme or microsatellite results. However, to uncover fine-scale structure, further sampling of both previously sampled and additional populations is necessary.

H. variegata disperses at a more rapid rate than *P. quatuordecimpunctata*; the former species was first reported in Canada in 1984, whereas *P. quatuordecimpunctata* was first collected in Canada in 1968, but *H. variegata* currently has a larger North American distribution. *H. variegata* has also recently been found in Oregon, in the western U.S. (Jessie et al., 2020). Additional study across a broader geographical range is required to determine that this western collection of *H. variegata* does not represent a new introduction of this species.

4.2. Photoperiod response during a rapid range expansion

Despite genetic similarities, we observed some phenotypic variability in invasion-related traits in *P. quatuordecimpunctata* and *H. variegata* which may indicate that the expanding distribution of both species is related to abiotic factors, such as photoperiodic induction of diapause. Phenotypic plasticity (Shearer et al., 2016), pre-adaptation (Reznik et al., 2015), and/or post-colonization evolution (Bean et al., 2012) in responses to the abiotic factors temperature and photoperiod have contributed to the geographic expansion of invasive insect species. North American populations of *P. quatuordecimpunctata* and *H. variegata* from the northeastern U.S. and southeastern Canada generally responded similarly to four constant photoperiods at 22 °C, with significant number of ovipositing females, and diapause induction across diurnal periods. In our comparisons of *P. quatuordecimpunctata* populations from Montreal, Quebec, Canada collected in 1989 (Obrycki et al., 1993) to the population from Jefferson County, New York, U.S. in this study (collected in 2019), we did not detect any significant ($p > 0.05$) differences in the photoperiodic responses of *P. quatuordecimpunctata*. Previous studies suggest that reproductive diapause in *H. variegata* might end by January and that initiation of vernal activity may be regulated by temperature (Obrycki, 2018). Data for *P. quatuordecimpunctata* presented in this study indicates that this species may have a prolonged diapause regulated by increasing daylengths in the spring. A possible explanation for the more widespread distribution of *H. variegata* is the opportunistic production of two generations/year compared to a single generation of *P. quatuordecimpunctata* (Day & Tatman, 2006). These photoperiodic response results suggest that there are minor differences between non-native populations within each species, and further investigation in additional populations across the non-native range may reveal ecological adaptation in both species.

Based on current understanding of the genomic and ecological characteristics of the North American populations of *H. variegata* and *P. quatuordecimpunctata*, it appears that both species were unintentionally introduced into southeastern Canada near Montreal, Quebec in the latter half of the 20th Century. Multiple geographic populations of both species were mass reared and released in large numbers for biological control of aphid pests in the U.S., but no established populations were ever confirmed based on these releases (Gordon, 1985; Prokrym et al., 1998). Our genomic analysis indicates little genetic structure in the North American populations of *H. variegata* and *P. quatuordecimpunctata* supporting the argument of natural spread from the original accidentally established Canadian populations.

5. Summary

In summary, these findings indicate shared patterns of introductory history of two species of invasive lady beetles, leading to serial founder effects, and similar phenological response among non-native

populations in the United States. Our study indicates a possible rapid response to shorter diurnal cycles in lower latitudes, either through adaptation or phenotypic plasticity, and a generally westward invasion by both species, despite low genomic diversity and population structure. This suggests that the success of invasive species need not necessarily be mediated by higher genomic diversity of founding populations, or bridgehead effects from secondary augmentations, but perhaps contingent on standing genetic diversity and adaptive evolution to local environments.

CRediT authorship contribution statement

Angela G. Jones: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **John.J. Obrycki:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Arun Sethuraman:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis. **David W. Weisrock:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Sequence data are available on the NCBI Sequence Read Archive (BioProject accession PRJNA911772). Input files for all population genetic analyses are available via figshare (10.6084/m9.figshare.21896991). Scripts used for all analyses are available via figshare (10.6084/m9.figshare.21897075). Data from the photoperiodic induction of reproductive diapause portion of this study are deposited at UKnowledge (https://doi.org/10.13023/h7da-7055).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105519.

References

Alexander, D.H., Lange, K., 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics 12 (1), 246. https://doi.org/10.1186/1471-2105-12-246.

Bean, D.W., Dalin, P., Dudley, T.L., 2012. Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (Tamarix spp.). Evolutionary Applications 5 (5), 511–523. https://doi.org/10.1111/j.1752-4571.2012.00262.x.

Belyakova, N.A., Ovchinnikov, A.N., Bezman-Moseyko, O.S., Reznik, S.Y., 2021. Comparative Study of the Phenotypic Structure and Photoperiodic Responses of Female Asian Ladybirds Harmonia axyridis (Pallas) (Coleoptera, Coccinellidae) from Moscow, Belgorod, and Sochi. Entomological Review 101 (6), 733–742. https://doi.org/10.1134/S0013873821060014.

Bradburd, G.S., Coop, G.M., Ralph, P.L., 2018. Inferring Continuous and Discrete Population Genetic Structure Across Space. Genetics 210 (1), 33–52. https://doi.org/10.1534/genetics.118.301333.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: An analysis tool set for population genomics. Molecular Ecology 22 (11), 3124–3140. https://doi.org/10.1111/mec.12354.

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015. Second-generation PLINK: Rising to the challenge of larger and richer datasets. GigaScience 4 (1). https://doi.org/10.1186/s13742-015-0047-8.

Collevatti, R.G., Nabout, J.C., Diniz Filho, J., a. F., 2011. Range shift and loss of genetic diversity under climate change in *Caryocar brasiliense*, a Neotropical tree species. Tree Genetics and Genomes 7 (6), 1237–1247.

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.

Day, W. H., & Tatman, K. M. (2006). Changes in abundance of native and adventive Coccinellidae (Coleoptera) in alfalfa fields, in northern New Jersy (1993-2004) and Delaware (1999–2004), U.S.A. *Entomological News*, *117*(5), 491–502. https://doi.org/10.3157/0013-872X(2006)117[491:CIAONA]2.0.CO;2.

Day, W.H., Prokrym, D.R., Ellis, D.R., Chianese, R.J., 1994. The known distribution of the predator Propylea *quatuordecimpunctata* (Coleoptera: Coccinellidae) in the United States, and thoughts on the origin of this species and five other exotic lady beetles in eastern North America. Entomological News 105 (4), 244–256.

Dlugosch, K.M., Parker, I.M., 2008. Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17 (1), 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x.

Dysart, R. J. (1988). The European Lady Beetle *Propylea quatuordecimpunctata*: New Locality Records for North America (Coleoptera: Coccinellidae). *Journal of the New York Entomological Society*, *96*(1), 119–121. JSTOR.

Eaton, D.A.R., Overcast, I., 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. Bioinformatics 36 (8), 2592–2594. https://doi.org/10.1093/bioinformatics/btz966.

Ellis, D., Prokrym, D., Adams, R., 1999. Exotic lady beetle survey in northeastern United States: *Hippodamia variegata and Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae). Entomological News (USA).

Eyer, P.-A., McDowell, B., Johnson, L.N.L., Calcaterra, L.A., Fernandez, M.B., Shoemaker, D., Puckett, R.T., Vargo, E.L., 2018. Supercolonial structure of invasive populations of the tawny crazy ant Nylanderia fulva in the US. BMC Evolutionary Biology 18 (1), 209. https://doi.org/10.1186/s12862-018-1336-5.

Franzmann, B.A., 2002. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology 41 (4), 375–377. https://doi.org/10.1046/j.1440-6055.2002.00318.x.

Galpern, P., Peres-Neto, P.R., Polfus, J., Manseau, M., 2014. MEMGENE: Spatial pattern detection in genetic distance data. Methods in Ecology and Evolution 5 (10), 1116–1120. https://doi.org/10.1111/2041-210X.12240.

Gardiner, M.M., Parsons, G.L., 2005. *Hippodamia variegata* (Goeze)(Coleoptera: Coccinellidae) detected in Michigan soybean fields. The Great Lakes Entomologist 38 (3 & 4), 8.

Gillis, M.K., Walsh, M.R., 2017. Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. Proceedings of the Royal Society b: Biological Sciences 284 (1858), 20170814. https://doi.org/10.1098/rspb.2017.0814.

Gordon, R.D., 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93 (1).

Gordon, R.D., 1987. The first North American records of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). Journal of the New York Entomological Society 307–309.

Goryacheva, I., Blekhman, A., 2017. Genetic structure of native and invasive populations of *Harmonia axyridis* Pall. In the light of global invasion. Russian Journal of Genetics 53 (1), 9–20.

Grez, A.A., Viera, B., Soares, A.O., 2012. Biotic interactions between *Eriopis connexa* and *Hippodamia variegata*, a native and an exotic coccinellid species associated with alfalfa fields in Chile. Entomologia Experimentalis Et Applicata 142 (1), 36–44. https://doi.org/10.1111/j.1570-7458.2011.01202.x.

Heidel, T. T., & Morey, A. C. (2011). First collection records of *Hippodamia variegata* (Coleoptera: Coccinellidae) in Minnesota Corn and Soybean. *Great Lakes Entomologist*, *44*(1–2), 83–85. Scopus.

Hesler, L.S., Hatfield, M., 2018. First records of *Hippodamia variegata* (Goeze)(Coleoptera: Coccinellidae) for Iowa. Insecta Mundi, USA.

Hesler, L.S., Lundgren, J.G., 2011. *Hippodamia variegata* (Goeze)(Coleoptera: Coccinellidae) Found in South Dakota, USA. The Coleopterists Bulletin 65 (1), 78–79.

Hesler, L.S., Lundgren, J.G., 2017. First record of *Hippodamia variegata* (Coleoptera: Coccinellidae) in Illinois, USA, and relation to its other Midwestern collection records. The Great Lakes Entomologist 50 (1), 9.

Hodek, I. 2012. Diapause / Dormancy, pp 275-342. In I, Hodek, H.F. van Emden, and A. Honek (eds). Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). Wiley-Blackwell, New York.

Hopper, J.V., McCue, K.F., Pratt, P.D., Duchesne, P., Grosholz, E.D., Hufbauer, R.A., 2019. Into the weeds: Matching importation history to genetic consequences and pathways in two widely used biological control agents. Evolutionary Applications 12 (4), 773–790. https://doi.org/10.1111/eva.12755.

Jardeleza, M.-K.-G., Koch, J.B., Pearse, I.S., Ghalambor, C.K., Hufbauer, R.A., 2022. The roles of phenotypic plasticity and adaptation in morphology and performance of an invasive species in a novel environment. Ecological Entomology 47 (1), 25–37. https://doi.org/10.1111/een.13087.

Jessie, C.N., Reich, I., Mc Donnell, R., 2020. First Oregon record of *Hippodamia variegata* (Goeze, 1777)(Coleoptera: Coccinellidae). The Pan-Pacific Entomologist 96 (4), 259–262.

Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics 27 (21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521.

Jombart, T., Devillard, S., Dufour, A.-B., Pontier, D., 2008. Revealing cryptic spatial patterns in genetic variability by a new multivariate method. Heredity 101 (1), Article 1. https://doi.org/10.1038/hdy.2008.34.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics 11 (1), 94. https://doi.org/10.1186/1471-2156-11-94.

Kajita, Y., O'Neill, E.M., Zheng, Y., Obrycki, J.J., Weisrock, D.W., 2012. A population genetic signature of human releases in an invasive ladybeetle. Molecular Ecology 21 (22), 5473–5483.

Karban, R., Strauss, S.Y., 2004. Physiological tolerance, climate change, and a northward range shift in the spittlebug. Philaenus Spumarius. *Ecological Entomology* 29 (2), 251–254.

Knaus, B.J., Grünwald, N.J., 2017. vcfr: A package to manipulate and visualize variant call format data in R. Molecular Ecology Resources 17 (1), 44–53. https://doi.org/10.1111/1755-0998.12549.

Krafsur, E.S., Obrycki, J.J., Flanders, R.V., 1992. Gene Flow in Populations of the Seven-Spotted Lady Beetle, *Coccinella septempunctata*. Journal of Heredity 83 (6), 440–444. https://doi.org/10.1093/oxfordjournals.jhered.a111248.

Krafsur, E.S., Obrycki, J., Nariboli, P., 1996. Gene flow in colonizing *Hippodamia variegata* ladybird beetle populations. Journal of Heredity 87 (1), 41–47.

Krafsur, E., Obrycki, J.J., 1996. Gene flow in the exotic 14-spotted ladybird beetle. *Propylea Quatuordecimpunctata*. *Genome* 39 (1), 131–139. https://doi.org/10.1139/g96-018.

Legendre, P., Legendre, L., 2012. Numerical ecology. Elsevier.

Li, H., Qu, W., Obrycki, J.J., Meng, L., Chu, D., Zhou, X., Li, B., 2020. Optimizing sample size for genomic study of *Harmonia axyridis* populations from native and novel ranges. Insects 11 (5), 290. https://doi.org/10.3390/insects 11050290.

Linck, E., Battey, C.J., 2019. Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. Molecular Ecology Resources 19 (3), 639–647. https://doi.org/10.1111/1755-0998.12995.

Lodge, D.M., 1993. Biological invasions: Lessons for ecology. Trends in Ecology & Evolution 8 (4), 133–137. https://doi.org/10.1016/0169-5347(93)90025-K.

Lombaert, E., Guillemaud, T., Cornuet, J.-M., Malausa, T., Facon, B., Estoup, A., 2010. Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird. PLOS ONE 5 (3), e9743.

Lombaert, E., Guillemaud, T., Thomas, C.E., Lawson Handley, L.J., Li, J., Wang, S., Pang, H., Goryacheva, I., Zakharov, I.A., Jousselin, E., Poland, R.L., Migeon, A., Van Lenteren, J., De Clercq, P., Berkvens, N., Jones, W., Estoup, A., 2011. Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: Case study of the invasive ladybird *Harmonia axyridis*. Molecular Ecology 20 (22), 4654–4670. https://doi.org/10.1111/j.1365-294X.2011.05322.x.

Lombaert, E., Estoup, A., Facon, B., Joubard, B., Grégoire, J.-C., Jannin, A., Blin, A., Guillemaud, T., 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27 (3), 508–517. https://doi.org/10.1111/jeb.12316.

Losey, J., Allee, L., Smyth, R., 2012. The Lost Ladybug Project: Citizen spotting surpasses scientist's surveys. American Entomologist 58 (1), 22–24.

Lounibos, L.P., Escher, R.L., Lourenço-De-Oliveira, R., 2003. Asymmetric Evolution of Photoperiodic Diapause in Temperate and Tropical Invasive Populations of Aedes albopictus (Diptera: Culicidae). Annals of the Entomological Society of America 96 (4), 512–518. https://doi.org/10.1603/0013-8746(2003)096[0512:AEOPDI]2.0.CO;2.

Maigret, T.A., Cox, J.J., Weisrock, D.W., 2020. A spatial genomic approach identifies time lags and historical barriers to gene flow in a rapidly fragmenting Appalachian landscape. Molecular Ecology 29 (4), 673–685. https://doi.org/10.1111/mec.15362.

Mathieu-Bégné, E., Loot, G., Mazé-Guilmo, E., Mullet, V., Genthon, C., Blanchet, S., 2021. Combining species distribution models and population genomics underlines the determinants of range limitation in an emerging parasite. Ecography 44 (2), 307–319. https://doi.org/10.1111/ecog.05301.

Melles, S.J., Fortin, M.-J., Lindsay, K., Badzinski, D., 2011. Expanding northward: Influence of climate change, forest connectivity, and population processes on a threatened species' range shift. Global Change Biology 17 (1), 17–31. https://doi.org/10.1111/j.1365-2486.2010.02214.x.

Montano, V., Jombart, T., 2017. An Eigenvalue test for spatial principal component analysis. BMC Bioinformatics 18 (1), 562. https://doi.org/10.1186/s12859-017-1988-y.

Moran, E.V., Alexander, J.M., 2014. Evolutionary responses to global change: Lessons from invasive species. Ecology Letters 17 (5), 637–649. https://doi.org/10.1111/ele.12262.

Mussmann, S.M., Douglas, M.R., Chafin, T.K., Douglas, M.E., 2019. BA3-SNPs: Contemporary migration reconfigured in BayesAss for next-generation sequence data. Methods in Ecology and Evolution 10 (10), 1808–1813.

Nazareno, A.G., Bemmels, J.B., Dick, C.W., Lohmann, L.G., 2017. Minimum sample sizes for population genomics: An empirical study from an Amazonian plant species. Molecular Ecology Resources 17 (6), 1136–1147. https://doi.org/10.1111/1755-0998.12654.

O'Leary, S.J., Puritz, J.B., Willis, S.C., Hollenbeck, C.M., Portnoy, D.S., 2018. These aren't the loci you'e looking for: Principles of effective SNP filtering for molecular ecologists. Molecular Ecology 27 (16), 3193–3206. https://doi.org/10.1111/mec.14792.

Obrycki, J., 2018. Reproductive Diapause in North American Populations of the Introduced Lady Beetle *Hippodamia variegata* (Coleoptera: Coccinellidae). Environmental Entomology 47 (5), 1337–1343. https://doi.org/10.1093/ee/nvy118.

Obrycki, J., Kring, T.J., 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology 43, 295–321. https://doi.org/10.1146/annurev.ento.43.1.295.

Obrycki, J., Orr, D., Orr, C., Wallendorf, M., Flanders, R., 1993. Comparative developmental and reproductive biology of three populations of Propylea quatuordecimpunctata (Coleoptera: Coccinellidae). Biological Control 3 (1), 27–33.

Obrycki, J.J., Tauber, C.A., Tauber, M.J., 2024. Diapause in eastern North American populations of *Coccinella septempunctata*. Eur. J. Entomol. 121, 98–108.

Ochocki, B.M., Miller, T.E.X., 2017. Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nature*. Communications 8 (1), Article 1. https://doi.org/10.1038/ncomms14315.

Org User, G.B.I.F., 2022. Occurrence Download. The Global Biodiversity Information Facility. Https://. https://doi.org/10.15468/DL.Y5H5WX.

Osborne, M.J., Diver, T.A., Turner, T.F., 2013. Introduced populations as genetic reservoirs for imperiled species: A case study of the Arkansas River Shiner (Notropis girardi). Conservation Genetics 14 (3), 637–647.

Pavuk, D.M., Sundermeier, A., Stelzer, S., Wadsworth, A.M., Keeler, D.M., Bergolc, M.L., Hughes-Williams, L., 2007. First occurrence of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) in Ohio. The Great Lakes Entomologist 40 (3 & 4), 12.

Pembleton, L.W., Cogan, N.O.I., Forster, J.W., 2013. StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Molecular Ecology Resources 13 (5), 946–952. https://doi.org/10.1111/1755-0998.12129.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLOS ONE 7 (5), e37135.

Phoofolo, M.W., Obrycki, J.J., 2000. Demographic analysis of reproduction in Nearctic and Palearctic populations of *Coccinella septempunctata* and *Propylea quatuordecimpunctata*. BioControl 45 (1), 25–43. https://doi.org/10.1023/A:1009918610088.

Prokrym, D. R., Pike, K., & Nelson, D. (1998). Biological control of *Diuraphis noxia* (Homoptera: Aphididae): Implementation and evaluation of natural enemies. *A Response Model for an Introduced Pest-the Russian Wheat Aphid. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD*, 183–208.

Qu, W.-M., Liang, N., Wu, Z.-K., Zhao, Y.-G., Chu, D., 2020. Minimum sample sizes for invasion genomics: Empirical investigation in an invasive whitefly. Ecology and Evolution 10 (1), 38–49. https://doi.org/10.1002/ece3.5677.

Reznik, S.Y., Dolgovskaya, M.Y., Ovchinnikov, A.N., Belyakova, N.A., 2015. Weak photoperiodic response facilitates the biological invasion of the harlequin ladybird Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). Journal of Applied Entomology 139 (4), 241–249. https://doi.org/10.1111/jen.12158.

Rodríguez-Castañeda, G., MacVean, C., Cardona, C., Hof, A.R., 2017. What Limits the Distribution of *Liriomyza huidobrensis* and Its Congener *Liriomyza sativae* in Their Native Niche: When Temperature and Competition Affect Species' Distribution Range in Guatemala. Journal of Insect Science 17 (4), 88. https://doi.org/10.1093/jisesa/iex059.

Rogers, C., Jackson, H., Angalet, G., Eikenbary, R., 1972. Biology and life history of Propylea 14-punctata (Coleoptera: Coccinellidae), an exotic predator of aphids. Annals of the Entomological Society of America 65 (3), 648–650.

Ruberson, J.R., Shen, Y.J., Kring, T.J., 2000. Photoperiodic sensitivity and diapause in the predator Orius insidiosus (Heteroptera: Anthocoridae). Annals of the Entomological Society of America 93 (5), 1123–1130.

Ruberson, J.R., Yeargan, K.V., Newton, B.L., 2001. Variation in diapause responses between geographic populations of the predator Geocoris punctipes (Heteroptera: Geocoridae). Annals of the Entomological Society of America 94 (1), 116–122.

Sethuraman, A., Janzen, F.J., Rubio, M.A., Vasquez, Y., Obrycki, J.J., 2018. Demographic histories of three predatory lady beetles reveal complex patterns of diversity and population size change in the United States. Insect Science 25 (6), 1065–1079. https://doi.org/10.1111/1744-7917.12481.

Sethuraman, A., Janzen, F.J., Weisrock, D.W., Obrycki, J.J., 2020. Insights from Population Genomics to Enhance and Sustain Biological Control of Insect Pests. Insects 11 (8), 462. https://doi.org/10.3390/insects11080462.

Sethuraman, A., Nunziata, S.O., Jones, A., Obrycki, J., Weisrock, D.W., 2024. Go west: Population genomics reveals unexpected population fluctuations and little gene flow in Western Hemisphere populations of the predatory lady beetle. Hippodamia Convergens. Evol. Appl. 17, e13631.

Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., Chiu, J.C., 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC Ecology 16 (1), 11. https://doi.org/10.1186/s12898-016-0070-3.

Stahlke, A.R., Bitume, E.V., Özsoy, Z.A., Bean, D.W., Veillet, A., Clark, M.I., Clark, E.I., Moran, P., Hufbauer, R.A., Hohenlohe, P.A., 2021. Hybridization and range

expansion in tamarisk beetles (Diorhabda spp.) introduced to North America for classical biological control. Evolutionary Applications 15 (1), 60–77. https://doi.org/10.1111/eva.13325.

Szűcs, M., Vercken, E., Bitume, E.V., Hufbauer, R.A., 2019. The implications of rapid eco-evolutionary processes for biological control—A review. Entomologia Experimentalis Et Applicata 167 (7), 598–615. https://doi.org/10.1111/eea.12807.

Tsutsui, N.D., Suarez, A.V., Holway, D.A., Case, T.J., 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences 97 (11), 5948–5953. https://doi.org/10.1073/pnas.100110397.

Vandepitte, K., de Meyer, T., Helsen, K., van Acker, K., Roldán-Ruiz, I., Mergeay, J., Honnay, O., 2014. Rapid genetic adaptation precedes the spread of an exotic plant species. Molecular Ecology 23 (9), 2157–2164. https://doi.org/10.1111/mec.12683.

Wang, G., Hou, Y., Zhang, X., Zhang, J., Li, J., Chen, Z., 2017. Strong population genetic structure of an invasive species, Rhynchophorus ferrugineus (Olivier), in southern China. Ecology and Evolution 7 (24), 10770–10781. https://doi.org/10.1002/ece3.3599.

Weir, B.S., Cockerham, C.C., 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution 38 (6), 1358–1370. https://doi.org/10.2307/2408641.

Wheeler, A., 1993. Establishment of *Hippodamia variegata* and new records of Propylea *quatuordecimpunctata* (Coleoptera: Coccinellidae) in the eastern United States. Entomological News 104 (2), 102–110.

Williams, A.H., Young, D.K., 2009. The alien *Hippodamia variegata* (Coleoptera: Coccinellidae) quickly establishes itself throughout Wisconsin. The Great Lakes Entomologist 42 (1 & 2), 2.

Wilson, G.A., Rannala, B., 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163 (3), 1177–1191.

Zheng, C., Ovaskainen, O., Hanski, I., 2009. Modelling single nucleotide effects in phosphoglucose isomerase on dispersal in the Glanville fritillary butterfly: Coupling of ecological and evolutionary dynamics. Philosophical Transactions of the Royal Society b: Biological Sciences 364 (1523), 1519–1532. https://doi.org/10.1098/rstb.2009.0005.