Received: 1 May 2019 | Revised: 19 July 2019 | Accepted: 5 August 2019

DOI: 10.1111/mec.15218

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Geography is more important than host plant use for the population genetic structure of a generalist insect herbivore

Mayra C. Vidal[1,2] | Tom W. Quinn[1] | John O. Stireman III[3] | Robin M. Tinghitella[1] | Shannon M. Murphy[1]

[1]Department of Biological Sciences, University of Denver, Denver, CO, USA

[2]Department of Biology, Syracuse University, Syracuse, NY, USA

[3]Department of Biological Sciences, Wright State University, Dayton, OH, USA

Correspondence
Mayra C. Vidal, Department of Biology, Syracuse University, 452 Life Sciences Complex, Syracuse, NY 13244, USA.
Email: mcadorim@syr.edu

Funding information
University of Denver; Jefferson County Open Space; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 1198/13-0

Abstract

Population divergence can occur due to mechanisms associated with geographic isolation and/or due to selection associated with different ecological niches. Much of the evidence for selection-driven speciation has come from studies of specialist insect herbivores that use different host plant species; however, the influence of host plant use on population divergence of generalist herbivores remains poorly understood. We tested how diet breadth, host plant species and geographic distance influence population divergence of the fall webworm (*Hyphantria cunea*; FW). FW is a broadly distributed, extreme generalist herbivore consisting of two morphotypes that have been argued to represent two different species: black-headed and red-headed. We characterized the differentiation of FW populations at two geographic scales. We first analysed the influence of host plant and geographic distance on genetic divergence across a broad continental scale for both colour types. We further analysed the influence of host plant, diet breadth and geographic distance on divergence at a finer geographic scale focusing on red-headed FW in Colorado. We found clear genetic and morphological distinction between red- and black-headed FW, and Colorado FW formed a genetic cluster distinct from other locations. Although both geographic distance and host plant use were correlated with genetic distance, geographic distance accounted for up to 3× more variation in genetic distance than did host plant use. As a rare study investigating the genetic structure of a widespread generalist herbivore over a broad geographic range (up to 3,000 km), our study supports a strong role for geographic isolation in divergence in this system.

KEYWORDS
insects, landscape genetics, population genetics—empirical, species interactions

1 | INTRODUCTION

One of the core questions in biology is why so many different species exist. Selection for more efficient resource use can lead to ecological niche specialization (Futuyma & Moreno, 1988), and eventually to genetic divergence and diversification (Rundle & Nosil, 2005). Insect herbivores are an optimal group in which to study the role of niche specialization in species diversification, because they are so speciose, with at least 430,000 described species (~30% of all animal species; Roskov et al., 2013), and are primarily dietary specialists. Several factors have been proposed to drive such high levels of diversification of insect herbivores, including differences in the identity of host plants used (Ehrlich & Raven, 1964) and variation in the number of plant species used (i.e., diet breadth, Hardy & Otto, 2014). Herbivory has been shown to be an important driver of insect diversification at a broad phylogenetic scale (Wiens, Lapoint,

& Whiteman, 2015), and the use of different plant species has also been found to play a major role in the divergence of contemporary insect populations (e.g., Funk, 1998; Nosil et al., 2012; Powell et al., 2013). Diet breadth can also influence diversification of herbivores, as narrow diet breadth is associated with elevated speciation rates relative to generalist taxa (Hardy & Otto, 2014). However, wider diet breadth is expected to be associated with greater genetic diversity at the population level (Gloss, Nelson Dittrich, Goldman-Huertas, & Whiteman, 2013); compared to specialists, generalists are likely to have greater population sizes due to high resource availability, have larger geographic range as they are not constrained by one host species, and be subject to greater variability in selective pressures.

Host plant use and diet breadth are not the sole factors influencing divergence of herbivores. Geographic structure and isolation of populations are thought to play important roles in the diversification of herbivorous insects for both adaptive and nonadaptive macroevolutionary hypotheses (Hardy, Peterson, & Normark, 2016; Janz & Nylin, 2008). Indeed, geographic distance has been shown to influence host plant-related population differentiation of bogus yucca moths (Darwell, Fox, & Althoff, 2014). Isolation by distance has long been advocated as an important factor fostering evolutionary change in many species (Wright, 1943), as gene flow is expected to be negatively correlated with geographic distance. Therefore, evidence for as well as patterns of genetic divergence can strongly depend on the geographic scale being studied. For instance, Mascarene grey white-eye birds exhibit strong population structure over small geographic range (Milá, Warren, Heeb, & Thébaud, 2010), whereas populations of the specialist herbivore *Utetheisa ornatrix* (Lepidoptera) exhibit stable genetic divergence at large geographic scales (>3,000 km; Cogni, Trigo, & Futuyma, 2011). In addition to geography, the environment and the ecological context that populations experience can strongly influence levels of gene flow. Populations can become isolated from one another (i.e., experience reduced gene flow) because of adaptation to different environments or differences in species interactions (e.g., host use), despite geographic proximity (isolation by environment, IBE; Wang & Bradburd, 2014). Examining genetic population structure at multiple geographic scales can allow a more nuanced assessment of the ecological and geographic factors that influence population divergence (Hutchison & Templeton, 1999).

Diet breadth, host plant use and geographic distance can influence divergence of insect herbivores, but it is unclear how much each of these factors contributes to the divergence of generalist herbivores as most of the studies investigating the divergence of herbivores have focused on specialists (e.g., Funk, 1998; Nosil et al., 2012; Powell et al., 2013; but see Dopman, Sword, & Hillis, 2002). Generalist herbivores are expected to have relatively low fitness variation on different host plants (Vidal & Murphy, 2018a); thus, the pattern and mechanism of genetic divergence on generalist populations might be different than for specialists. For instance, we would expect population subdivision and speciation to be more often associated with geographic isolation than variation in host plant use for generalists. Indeed, a recent macroevolutionary hypothesis to explain insect herbivore diversification (Hardy et al., 2016) has proposed that nonadaptive processes are important contributors to diet breadth evolution and potentially speciation of herbivores; generalist populations tend to be more widespread and should continuously produce specialized, small and geographically isolated populations. Surprisingly, we still lack studies of the genetic structure of widespread generalist herbivores that span populations in a broad geographic range to test these predictions (but see Craft et al., 2010).

To tease apart the influence of diet breadth, host plant use and geographic distance on the genetic divergence of generalist herbivores, we used the widespread generalist herbivore, fall webworm (*Hyphantria cunea*, Erebidae, Lepidoptera; hereafter FW). FW is an excellent model organism to address this goal for several reasons. First, FW is one of the most generalized plant-feeding insect species known, feeding on more than 600 woody plant species worldwide (Warren & Tadić, 1967). Yet, its diet breadth varies considerably over its geographic range, with some populations clearly being more specialized than others (e.g., western Colorado, Murphy & Loewy, 2015 vs. northeastern coast of the United States, Mason, Wilkes, Lill, & Singer, 2011; but even the "specialized" populations remain generalists). Second, FW has a broad native range across North America. Nevertheless, the use of some host species is conserved across sites (e.g., *Prunus* sp., *Ulmus* sp. and *Malus* sp. are used in the west and east of the United States; Murphy & Loewy, 2015; Mason et al., 2011). Third, FW has previously been documented to consist of two morphologically and genetically distinct forms (Yang, Kawabata, Tufail, Brown, & Takeda, 2017) that differ in their life history traits, behaviour, host plant use and geographic distribution (e.g., Ito & Warren, 1973; Oliver, 1964). The two types are named by the colour of their larval head capsule, black-headed or red-headed (hereafter referred as black and red, respectively). Blacks and reds can be found in sympatry in the east and southeast of the United States, but they differ in host plant use even where they co-occur (e.g., Louisiana; Oliver, 1964). The recent genetic confirmation of the difference between the two types was done using mitochondrial CO1, which can be problematic to distinguish species (Hurst & Jiggins, 2005; Toews & Brelsford, 2012). Furthermore, the morphological differences are based solely on the larval stage. Thus, it is not completely clear whether the two types of FW can potentially be different species.

Using this extreme generalist species, we address the following goals: (a) to confirm genetic and morphological differentiation of red and black forms with morphometric data and population genomics using nuclear markers; (b) to assess the relative importance of geography and host plant use in explaining genetic variation among populations at broad geographic scales of both red and black FW; and (c) to assess the relative importance of geography, diet breadth and host plant use in the genetic variation among red FW at a fine geographic scale (Figure 1). By addressing these issues in this model system, we hope to provide insight into the patterns and processes of population divergence in generalist herbivores.

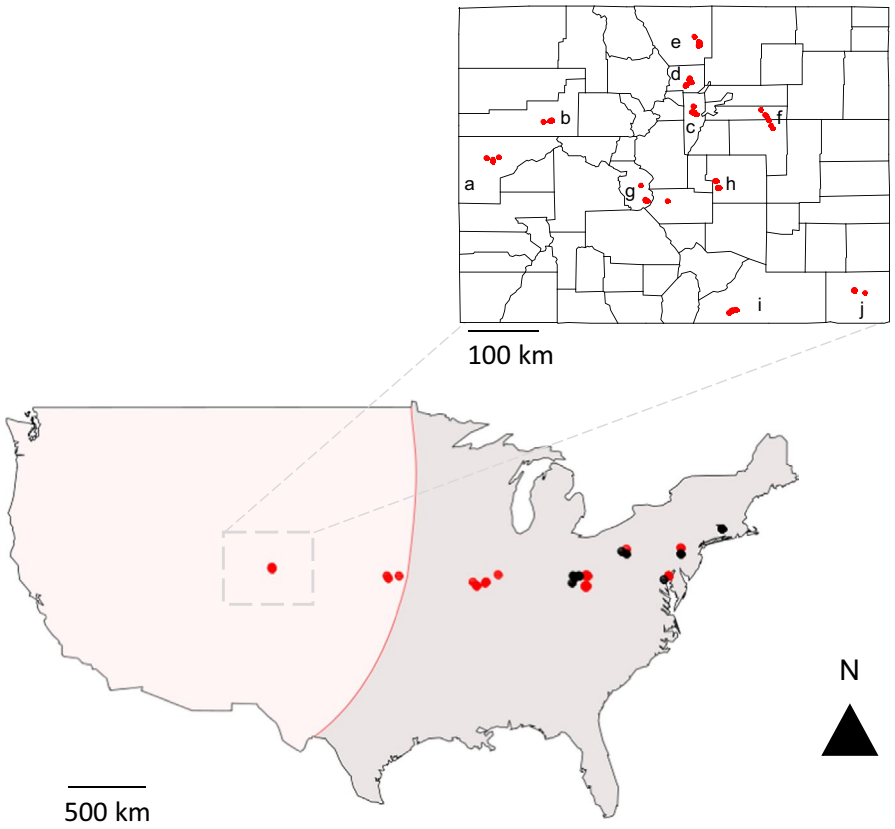

FIGURE 1 Map showing sampling locations. Red circles represent red fall webworm samples, while black circles represent black fall webworm samples. The map at lower left shows sampling locations in superposition with the occurrence of the two forms following Yang et al. (2017), where light red to the left indicates the presence of only the red type, and mauve to the right indicates the presence of both types. The map at upper right shows sampling locations within Colorado counties: a—Mesa County, b—Garfield County, c + d + e—Front Range (Jefferson, Boulder and Larimer Counties, respectively), f—Arapahoe and Ebert Counties, g—Chaffee and Fremont Counties, h—El Paso County, i—Las Animas County, j—Baca County [Colour figure can be viewed at wileyonlinelibrary.com]

2 | METHODS

2.1 | Morphometry

Larvae of the two types of FW are relatively easy to distinguish from one another, but it remains unclear whether adults are morphologically differentiated. Previous authors who have examined this issue focused on wing length (e.g., Yang et al., 2017); however, wing length can vary with environmental and dietary factors; for example, individuals are usually smaller when feeding on low-quality plants (e.g., Murphy & Loewy, 2015; Vidal & Murphy, 2018b). A more reliable way to measure such differences is to use morphological "landmarks" and to then calculate the ratio of distances between these landmarks as a measure of overall body shape (Zelditch, Swiderski, Sheets, & Fink, 2004). Therefore, to address part of our first goal, we measured adult females and males of FW from Colorado (red, n = 28), Missouri (red, n = 5), New Jersey (black, n = 12) and Maryland (red, n = 13; black, n = 12) following the criteria in Zelditch et al. (2004) to define our landmarks. We demarcated 13 landmarks as junctures between different wing veins and between wing veins and wing borders on the right forewing. Wing shape in Lepidoptera often varies strongly among species and can be used to distinguish taxa at the species and sometimes the family levels (Zhong et al., 2016). We used TPSUTIL and TPSDIG (Rohlf, 2005) to extract the landmark coordinates from photographs and MORPHOJ (Klingenberg, 2011) to analyse the resulting data using canonical variate analysis.

2.2 | Sample collection

We performed genetic analyses on two separate sets of samples to address our three goals. To address part of our first goal (the extent of genetic divergence between the two types) and the second goal, we used black and red FW over a 250–3,000 km range; thus, we define this data set as "broad geographic scale." To address our third goal, we used samples of red FW collected in Colorado over a range of 40–700 km; thus, we define this data set as "fine geographic scale." We analysed the broad and fine geographic scale data sets separately because we only had reliable within-population diet breadth data for Colorado, which has only red populations, and because patterns may vary depending on the geographic scale being analysed (e.g., Darwell et al., 2014). The broad-scale data set included 61 samples (41 of red, 20 of black) from a 3,000-km longitudinal transect, and the fine-scale data set included 126 samples of red FW only from Colorado. We consider a sample as distinct maternal lines. FW female moths oviposit a clutch of eggs on one leaf, and the sibling larvae feed together in a communal web. We collected and genotyped at least two larvae from each web, which constitute our sample per maternal line. The larvae were put in 95% ethanol immediately after collection, and the extracted DNA from two or more sibling larvae was combined for genotyping.

For the broad geographic scale analysis, we included samples from Colorado (n = 6 reds from Jefferson county), Connecticut (n = 7 blacks), Illinois (n = 4 reds), Kansas (n = 3 reds), Maryland (n = 5 reds), Missouri (n = 6 reds), New Jersey (n = 3 reds and three blacks), Ohio

(*n* = 13 reds and eight blacks) and Pennsylvania (*n* = 1 red and two blacks) (Figure 1). Hence, our sampling included allopatric populations of red FW (Colorado) and of black FW (Connecticut), as well as sympatric populations of reds and blacks (all the others). Although our broad geographic scale analysis had unbalanced sampling across sites, previous research has shown that even small sample sizes (two individuals) can accurately account for genetic divergence when there is a large number of SNPs (>1,500) (Nazareno, Bemmels, Dick, & Lohmann, 2017). For the fine geographic scale analysis, we used 10 locations of red FW from Colorado with 10–20 samples per location and used larvae from similar hosts across locations as much as possible, totalling 126 samples of which 6 were also used in the broad-scale analysis explained above (Figure 1). Larvae from Colorado were collected across the state in the following counties: Arapahoe, Baca, Boulder, Chaffee, El Paso, Garfield, Jefferson, Larimer, Las Animas, Mesa (letters a–j in Figure 1). Sample and host plant data were collected as explained in Murphy and Loewy (2015) and Vidal and Murphy (2018b), and we recorded host plant information for each sample (Tables 1 and A1).

2.3 | Genotyping by ddRAD-seq

We genotyped our 181 samples from both geographic scales using double-digest restriction site-associated DNA sequencing (ddRAD-seq) as described in Peterson, Weber, Kay, Fisher, and Hoekstra (2012), with slight modifications. We extracted DNA from head capsules of FW caterpillars (*n* = 178) and whole pupae (*n* = 3) using a Qiagen DNA extraction kit. We followed the manufacturer's instructions, omitting the RNA step. We quantified DNA using the QuantiFluor® dsDNA System (Promega Inc.) and digested 500 ng of DNA with *Msp*1 and *Mlu*C1 enzymes (New England Biolabs, Inc.) at 37°C for 3 hr. We ligated adaptors and selected 300 bp (±25 bp) fragments using a Pippin Prep PR00953 (Sage Science, Inc.) following the manufacturer's directions. Libraries were amplified for nine cycles in 4–8 20 µl reactions using Phusion High-Fidelity PCR® (Thermo Fisher, Inc.). Libraries were sequenced on an Illumina HiSeq 2500 by the Genomics and Microarray Core at the University of Colorado Anschutz. Samples from the two analytical sets were intermixed in the two lanes that we used for sequencing.

We demultiplexed, filtered and trimmed the raw reads using STACKS 1.46 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). We trimmed adaptors and removed low-quality bases (mean Phred score < 10 along a sliding window of 20 bp) and reads with uncalled bases or ambivalent barcodes. Since a reference genome is unavailable from FW or a closely related taxon, we performed de novo assembly using STACKS. We required a minimum of 3 identical reads to form a stack, distance allowed between stacks of 2, a maximum distance of 4 for secondary alignments and a maximum of 3 stacks allowed per de novo locus. We used the program POPULATIONS in STACKS to obtain a vcf file after filtering out loci with more than two alleles, as multiallelic sites tend to be rare in natural populations. We used VCFTOOLS 0.1.15 (Danecek et al., 2011) to remove sites with a minor allele frequency of fewer than 10% and more than 90% in the populations, and to remove sites that had 20% of missing data. We compared results for different values of the missing data threshold and found that 20%–60% yielded similar trends; therefore, we used the 20% value that retained more SNPs (following Huang & Knowles, 2014).

2.4 | Population analyses

We conducted population genomic analyses separately for the two sets of data (broad and fine geographic scales). Using the program POPULATIONS from STACKS, we obtained a Structure file from the cleaned vcf file. We used STRUCTURE 2.3.4 (Pritchard, Stephens, Rosenberg, & Donnelly, 2000) and STRUCTUREHARVESTER (Earl & VonHoldt, 2012) to define the most likely number of populations inferred from our data, and visualized the structure analysis with the most likely *K* value ± 1 using CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). For the structure analysis, we used three replicates per *K* value, and our *K* values ranged from 1 to 11, with 10,000 step burn-in followed by 50,000 Markov chain Monte Carlo repetitions. We evaluated genetic clustering with principal components analysis (PCA) and calculated pairwise Weir and Cockerham (1984) F_{ST} distances using the R package SNPRELATE 1.10.2 (Zheng et al., 2012) performed in the R environment 3.4.1 (R Development Core Team, 2011). To help visualize relationship among populations of blacks and reds for our first and second goals, we also constructed a splitstree using the program SPLITSTREE4 version 4.14.5 with the neighbour-net algorithm and UncorrectedP method (Huson, 1998; Huson & Bryant, 2005).

2.5 | Genetic distance at a broad geographic scale

We analysed the influence of host plant use and geographic distance on population structure in FW using red and black types across a broad geographic range (~2,500 apart; Figure 1). Since previous studies (Yang et al., 2017) and our own (results below) have indicated that black and red FW are genetically distinct, we performed the analyses using the two types separately. Colorado populations were also shown to be genetically distinct in the present study; thus, we removed Colorado samples when comparing eastern red and black FW.

We used Moran's eigenvectors, distance-based redundancy analysis (db-RDA) and matrix regression to analyse the importance of geographic distance and host plant on genetic distance. For these analyses, we used pairwise genetic distance between individual samples measured using identity by state (IBS), which we obtained using PLINK 1.07 (Purcell et al., 2007). To analyse how the geographic distance alone correlates with the genetic distance between individual samples, we used the R package MEMGENE 1.0 (Galpern, Peres-Neto, Polfus, & Manseau, 2014). This analysis takes into consideration the geographic coordinates and genetic distances to calculate Moran's eigenvectors and show how similar (same size and colour of circles) samples are to each other in a map view. Furthermore, it has the power to detect weak spatial genetic patterns and how landscape features can influence genetic patterns (Galpern et al., 2014). After analysing the influence of geographic distance, we tested for effects of host plant use on genetic distance using db-RDA. Assuming that geographic

distance influences host plant distribution and potentially genetic distance, we used the significant eigenvalues from principal coordinates of neighbourhood matrix (PCNM function from VEGAN) of the geographic distance as a condition in the model (Legendre & Fortin, 2010). Therefore, geographic distance was parsed out before the constrained variables. We selected the variables that best explained the variance in genetic data using stepwise model selection with the function ORDIR2STEP. Pcnm analysis and db-RDA were performed using the R package VEGAN 2.4-4 (Oksanen et al.., 2007). We further tested the influence of geographic distance and host plant use on genetic distance (IBS) by performing matrix regression using the function MRM from the package ECODIST 2.0.1 (Goslee & Urban, 2007). Because reds were sampled over a broader geographic range than blacks, we also removed samples of reds from locations where we did not sample blacks to see whether this difference in sampling range may have influenced the results of our matrix regression. To obtain the host plant distance used in matrix regression analysis and db-RDA, we used phylogenetic distance of the host plant species used by each of the larvae sampled. To obtain the phylogenetic distance, we first constructed a phylogenetic tree of host plant species using PHYLOMATIC from the package BRRANCHING version 0.4.0 (Chamberlain, 2018) based on the plant phylogeny of Zanne et al. (2014). Then, we used the function DISTTIPS from the package ADEPHYLO (Jombart & Dray, 2008) to produce a matrix of phylogenetic distances. For phylogenetic distance, we used patristic distance that considers the length of branches separating taxa.

2.6 | Genetic distance at a fine geographic scale

To test how host plant use, diet breadth and geographic distance influence genetic distance of FW at a finer geographic scale, we used red FW collected across the state of Colorado (from 40 to 700 km apart; Figure 1). To calculate fine-scale geographic distances, we used the R package GEOSPHERE with function DISTCOSINE, which estimates the shortest distance from one point to another. We repeated the above analyses (matrix regression, db-RDA and memgene) on this fine spatial scale. Memgene can be especially useful in this fine-scale analysis as it can detect spatial genetic patterns when gene flow is high and weaker genetic patterns are expected (Galpern et al., 2014). Since Colorado is divided by the Rocky Mountains, we included a topographic map of Colorado in our memgene graph to evaluate how the particular landscape of Colorado influences the possible geographic isolation of Colorado FW. We tested for the relative importance of host plant use on the genetic distance of Colorado FW using db-RDA (with eigenvalues from pcnm as geographic distance condition) and matrix regression. We coded the distance of host plants that were used as phylogenetic distance as explained above.

We used ordination diet breadth (Fordyce, Nice, Hamm, & Forister, 2016, ODB) to calculate diet breadth based on population information from Vidal and Murphy (2018b); this measure takes into consideration the number of plant species used in a population and also the similarity of hosts in one population as compared to all other hosts used by the other populations tested. Here, we consider similar hosts as the same species (or genus) of plants used by different individuals or populations. If a population uses few and similar plants compared to the other populations, it has a low value of ODB (close to 0, considered more specialist), whereas if a population uses many and different plants, it has a high value of ODB (close to 1, considered more generalist). ODB usually yields similar patterns as phylogenetic corrected diet breadth but was shown to outperform this type of measure (Fordyce et al., 2016). We used the diet breadth at the "population" level, as FW individuals typically feed on one host plant individual during their entire larval stage. The diet breadth of the populations of FW used in this analysis varied from 2 to 15 host plants being used: larvae from Arapahoe were feeding on 2 host species, Garfield on 3, Las Animas on 5, El Paso on 6, Chaffee on 8, Jefferson on 9, Baca, Larimer and Mesa on 10, Boulder on 15 (Vidal & Murphy, 2018b). To analyse the influence of diet breadth on the divergence of populations, we used partial Mantel test with the mean F_{ST} and diet breadth at the population level, while controlling for geographic distance between populations. We defined populations as the individuals occurring in the same location following Vidal & Murphy, 2018b (letters a–j in Figure 1), not necessarily correlated with gene flow. To obtain pairwise comparison between populations using ODB, we used the absolute difference between the two values being compared. Therefore, a lower value of this difference would mean that the two populations being compared have similar diet breadths, whereas if the values are high, the populations have very different diet breadths. We used the R package VEGAN 2.4-4 (Oksanen et al., 2007) to calculate the partial Mantel test. We further tested for a possible correlation between genetic diversity and diet breadth. As a measure of genetic diversity, we obtained the observed heterozygosity of each population from the program POPULATIONS from STACKS and used a linear regression between the observed heterozygosity and ordination diet breadth per population.

3 | RESULTS

3.1 | Morphometry

We found the adults of the red and black FW to be morphologically distinct (discriminant function analysis: $t^2 = 244.46$, $n = 70$, $p < .0001$); the two types form distinct groups, and the two first axes of the canonical variate analysis represent 81% of the variance in wing shape (Figure 2a). Pairwise discriminant comparison between all geographic locations revealed significant shape differences between reds from Colorado and reds from Missouri ($t^2 = 151.41$, $p = .02$) and Maryland ($t^2 = 93.46$, $p = .03$), but no significant shape differences between other locations within each type (all $p > .05$), possibly due to low sample size. A permutation test of Procrustes distances among groups resulted in $p < .05$ for all pairwise comparisons; therefore, when comparing the interposition of shapes formed by the landmarks, reds and blacks from different locations were different from each other, as well as reds from blacks overall.

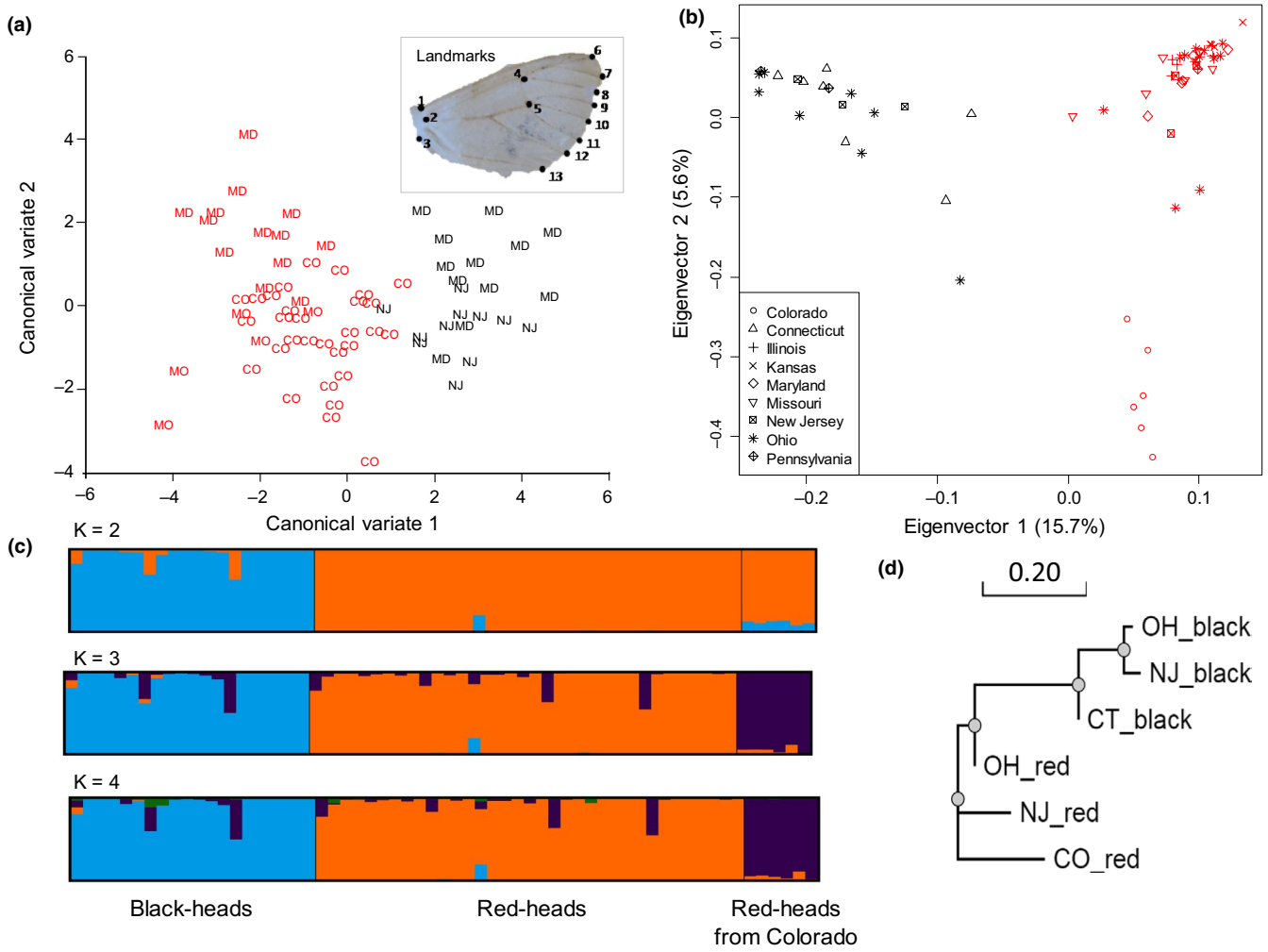

FIGURE 2 Morphometry and population genomics of the two colour types of fall webworm. (a) Morphometry of Colorado (CO), Maryland (MD), Missouri (MO) and New Jersey (NJ) fall webworm adults (using red for red type and black for black type); the image at top right shows the 13 landmarks in the forewings used. (b) Principal components analysis of the 61 genetic samples (using red for red type and black for black type) with symbols representing the sample locations. (c) Structure analysis for K = 2–4 showing black-heads, red-heads from across our geographic sampling except Colorado, and red-heads collected from Colorado alone. (d) Unrooted tree of Weir & Cockerham's F_{ST} pairwise comparisons for Colorado (CO), Connecticut (CT), New Jersey (NJ) and Ohio (OH), inferred with PRESTO [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Population genomics

After demultiplexing, we obtained 340,184–9,024,092 reads per individual (median = 2,245,657, total = 480,351,136 reads) from our 181 individuals. From the de novo assembly that included the 61 samples of red and black FW, we obtained 56,515 SNPs, and the depth of coverage from processed samples of de novo assembly ranged from 8.05 to 31.9× per sample. For the Colorado locations, we used 126 samples with 60,737 SNPs, and the depth of coverage ranged from 5.5 to 37.5×.

With our 61 samples of red and black FW from a broad geographic scale, we confirmed that the two types are genetically distinct and that the Colorado population is genetically divergent from black FW and other red FW (Figures 2b,c and 3). The two first axes of the principal components analysis explained more than 20% of the genetic variation, with clear separation between red and black samples along the first axis (Figure 2b). Although larvae from the different morphotypes used different host plant species with few overlaps (Table 1), samples did not cluster by host plant species (not shown) nor by geography, except for Colorado samples that formed a distinct group along the second axis (circles in Figure 2b). We found that the most likely number of populations (K) was 3 (delta K = 5,613) and that each type clearly formed distinct populations. Further, samples from Colorado were different from the two recognized types, even though in larval morphology, they resemble the eastern red FW (Figure 2c, K = 3). Based on pairwise F_{ST} values, the red samples from the sympatric populations (NJ and OH) were more similar to reds from other locations than to blacks from the same location (Figure 2d). The splitstree confirmed the same pattern as found with PCA and structure analysis, blacks and reds were split from each other, and reds from Colorado were also split from the other reds (Figure 3).

According to the genetic data of the fine geographic scale analysis, there was no clear differentiation of Colorado FW. There was no clear separation of populations in our PCA, and the two axes explained only

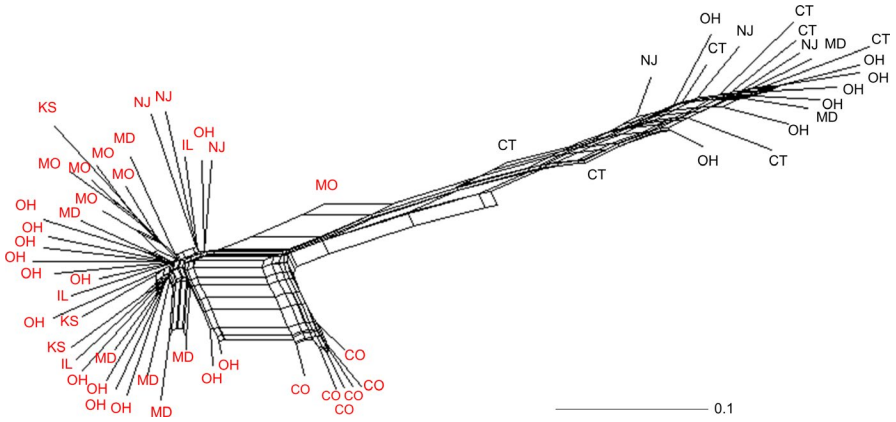

FIGURE 3 Splitstree of the broad geographic range data set (Fit = 95.026). Letters represent US state of origin of samples (CO, Colorado; CT, Connecticut; IL, Illinois; KS, Kansas; MD, Maryland; MO, Missouri; NJ, New Jersey; OH, Ohio), and colours represent the two fall webworm morphotypes (red = red-headed, black = black-headed) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of the host plants used by fall webworm that were included in our analyses, and the number of samples that were collected from each host plant species is in parentheses

Black type	Eastern red type	Colorado red type
Acer negundo (6)	*Ailanthus* sp. (2)	*Fraxinus americana* (2)
Cercis canadensis (4)	*Betula* sp. (3)	*Fraxinus pennsylvanica* (1)
Prunus serotina (2)	*Carya* sp. (2)	*Juglans nigra* (2)
Quercus sp. (1)	*Diospyros* sp. (4)	*Malus* sp. (12)
Salix nigra (2)	*Juglans nigra* (4)	*Populus deltoides* (39)
Salix sp. (1)	*Nyssa sylvatica* (1)	*Populus angustifolia* (30)
Vitis sp. (1)	*Oxydendrum* sp. (2)	*Populus tremuloides* (1)
Ulmus sp. (3)	*Platanus occidentalis* (4)	*Prunus virginiana* (16)
	Prunus serotina (9)	*Prunus virginiana var. Schubert* (1)
	Prunus sp. (1)	*Prunus* sp. (1)
	Quercus sp. (3)	*Quercus* sp. (1)
		Salix sp. (3)
		Ulmus sp. (17)

~7% of genetic variation (Figure 4a). There was also no clear host plant-related genetic structure. In the structure analysis, we found that the most likely *K* value to explain the genetic structure in Colorado was 2 (delta *K* = 46.3); Mesa, Garfield and the northern Front Range (letters a–e in Figure 4) comprise a mix of the two genetic groupings, while the other five sampling locations, which are found in eastern side of the Rocky Mountains and southern Colorado, are mostly composed of one genetic cluster (Figure 4b, light blue). However, the delta *K* value is low, and it is possible that the samples along Colorado form a single panmictic population (delta *K* is not possible to be calculated for *K* = 1).

3.3 | Host plant use and geographic distance as predictors of divergence at a broad geographic scale

We found that geographic distance is a better predictor of genetic distance than host plant use in our broad geographic scale analysis, but the magnitude explained by these two variables varied for each morphotype. Using memgene, we found that most samples that are close together geographically are genetically similar (circles have similar size and colour in Figure 5) for both red and black FW; however, these models explain only 1.2% and 0.4% of the genetic variation in the red and black populations, respectively. Furthermore, in both cases, landscape features could be acting as geographic barriers. For example, the Appalachian Mountain may be separating populations of the east coast (MD, NJ, CT) from other locations.

When we controlled for geographic distance in the db-RDA, host plant phylogenetic distance was not kept in the model for black FW (r^2 = −0.005), while it was maintained in the model for red FW (r^2 = 0.03, p = .03). When we tested for both geographic distance and host plant use together using matrix regression, geographic distance but not host plant distance influenced genetic distance for blacks and for reds (Table 2). Host plant and geographic distance explained 7.6% of the genetic variance for reds, while these variables in the matrix models explained <3% of the genetic variance for blacks. Host plant distance was correlated with geographic distance for black FW (r^2 = 0.066, p = .01) and for red FW (r^2 = 0.03, p = .01). Even when we removed samples of red FW from Kansas, Missouri and Illinois to have similar sampling area as black FW, the pattern remained the same, with only geographic distance influencing genetic distance and the two variables explaining ~6% of genetic divergence of reds. Taking these two analyses together, the matrix regression analysis confirms that geographic distance has an important influence on genetic divergence for both types; however, when we "control" for geographic distance in the db-RDA model selection, host plant distance was important only for red FW.

3.4 | Host plant use, diet breadth and geographic distance as predictors of divergence at a narrow geographic scale

For the Colorado samples, geographic distance was again a more important factor than host plant use in explaining genetic variation. We found that 11.3% of the genetic variation was explained by geographic distance using memgene (Figure 6a). In the db-RDA model

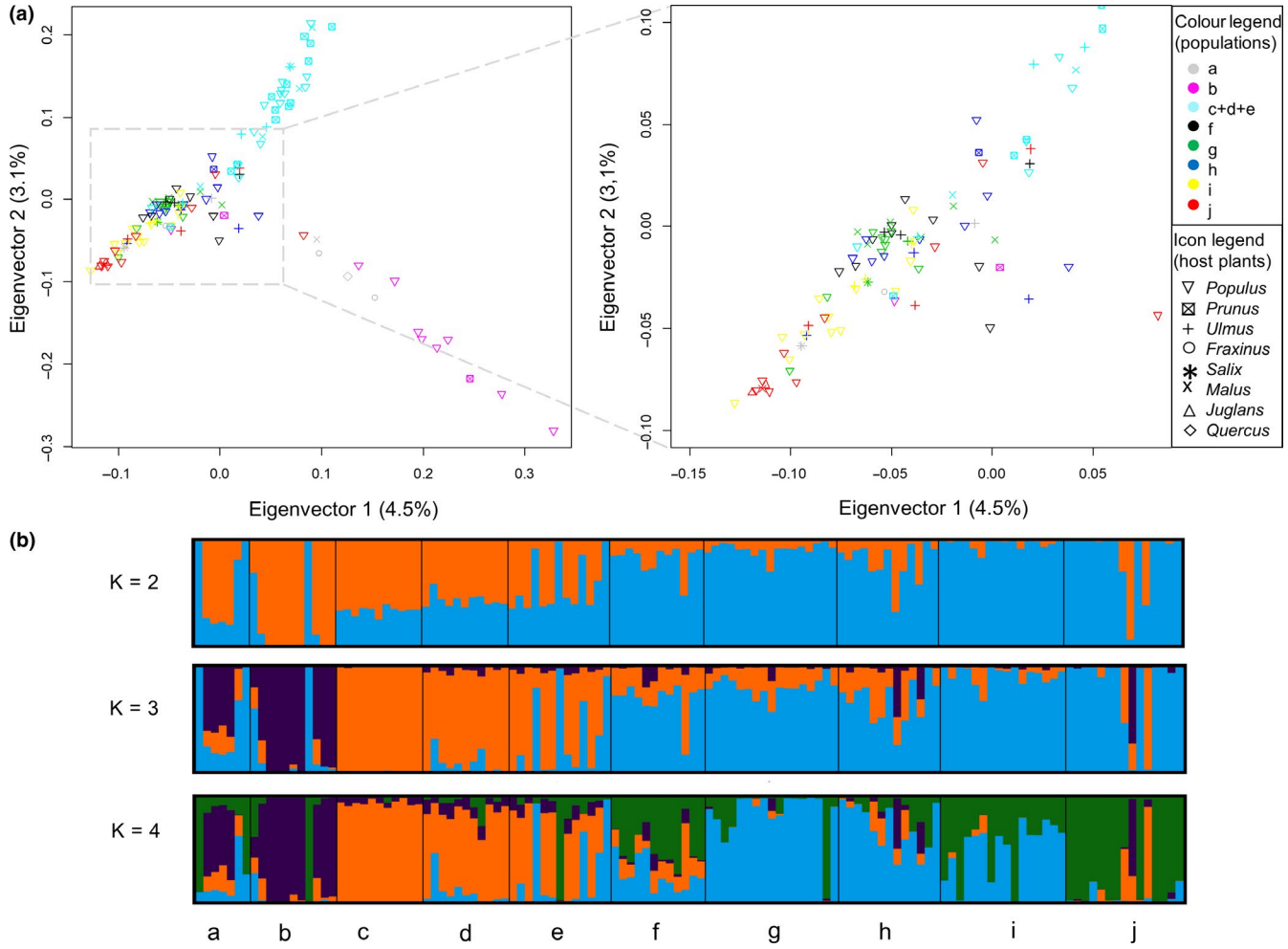

FIGURE 4 Population genomics of Colorado fall webworm populations. (a) Principal components analysis of the 126 genetic samples. Different colours represent geographic locations of fall webworm populations (same as Figure 1). Different symbols represent the host plant genus that the larvae were using when collected. The graph on the right is a zoom in of the region outlined by the dashed grey line in the graph on the left. (b) Structure analysis for K = 2–4, letters represent the same locations as in Figure 1 [Colour figure can be viewed at wileyonlinelibrary.com]

selection, host plant use was maintained in the model to explain genetic distance (adjusted R^2 = 0.036, F = 1.4, df = 9, p = .001); however, this variable explained only 3.6% of the genetic divergence. Thus, geographic distance was more than three times as important as host plant in explaining genetic structure in these subpopulations (11.3% vs. 3%). This result suggests that geography is important, a finding that is not altogether surprising as FW collected from western sites (Mesa and Garfield) is separated from the other subpopulations by the Continental Divide formed by the Rocky Mountains, which demarcates the point at which drainages from North America move towards the Atlantic or Pacific sides of the continent. Our matrix regression analysis confirms the importance of geographic distance as it showed that geographic distance was correlated with genetic distance, while phylogenetic distance of host plants marginally correlated with genetic distance (Table 2; r^2 = 0.1, p = .01).

For the diet breadth analysis using a partial Mantel test, we found no correlation between differences in diet breadth and F_{ST} among populations (Figure 6b, r = 0.08, p = .35). Furthermore, we found a marginal negative correlation between observed heterozygosity and diet breadth of populations (Figure 6c, r^2 = 0.33, t = −1.97, p = .08). We expected to find greater genetic diversity in more generalized populations, but our result suggests the opposite.

4 | DISCUSSION

Host plant use, diet breadth and geographic distance are some of the factors that can influence the genetic divergence of insect herbivores, but most studies that test these factors have focused on specialist herbivores. As generalist herbivores are differently affected by host plants than specialists (Vidal & Murphy, 2018a), it is not clear whether host plant use could influence the divergence of populations of generalist herbivores, nor how much this factor accounts for their divergence. Using an extremely generalist herbivore, fall webworm (FW), we found that the two types of FW are genetically and morphologically distinct. Furthermore, although both geographic distance and host plant use were correlated with genetic distance for the red type, geographic distance accounted for up to 3× more

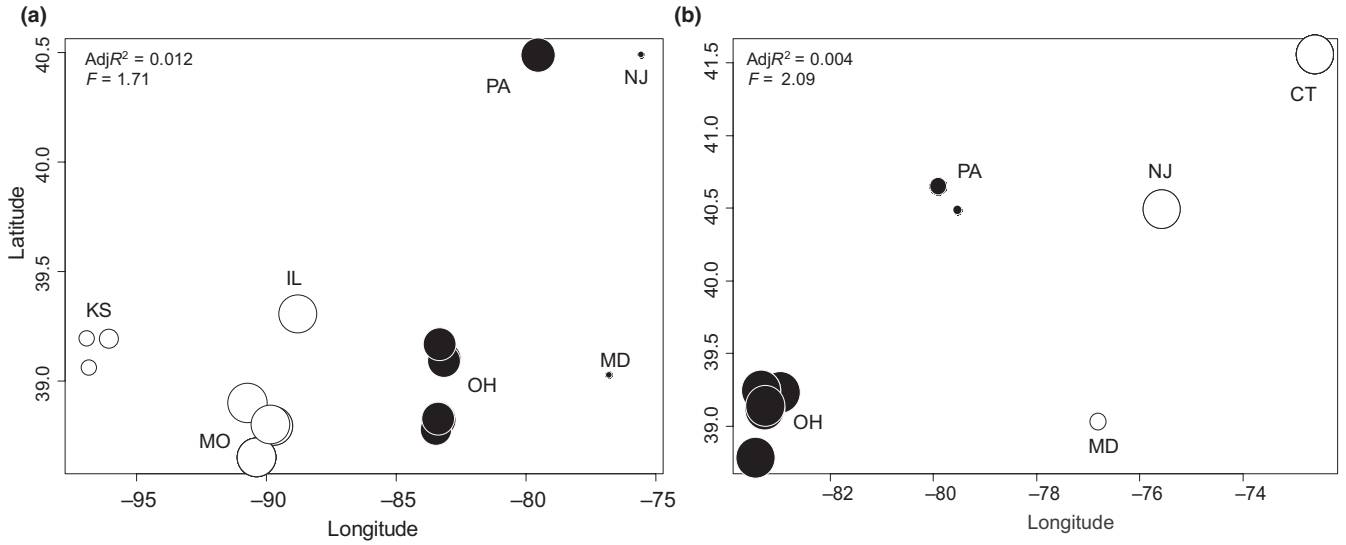

FIGURE 5 Memgene analysis showing genetic groupings of samples of red (a) and black (b) FW along the landscape for broad geographic scale, where letters represent states. Circles represent each sample (unless for cases where samples were very close to one another, e.g., CT), circles of same size and colour show individuals with similar memgene scores, while different sizes and colours indicate dissimilarity. Colour of circles represents the scores in the principal components axis from positive (black) to negative (white), and the size of the circles represents the location along the axis, from ±0.0125 (larger circles) to 0 (smaller circles)

TABLE 2 Summary of the influence of geographic distance and host plant use on genetic distance using matrix regression. Bold represents significant variables at $p < .05$

	Black type		Red type		Colorado red type	
	Coef.	***p***	**Coef.**	***p***	**Coef.**	***p***
Intercept	0.33	.87	0.34	.33	0.52	1
Geography	**0.03**	**.02**	**0.0004**	**.01**	**0.005**	**.01**
Host plant	-7×10^{-7}	.93	-8.1×10^{-6}	.19	6.67×10^{-6}	.05
Model R^2	0.023		.076		0.1	

variation in genetic distance than did host plant use. The greater influence of geographic distance than host plant use on FW genetic structure supports the view that not all generalists are composed of cryptic host-specialized populations or species, as was previously suggested (Bickford et al., 2006). However, other factors could also influence the genetic structure of FW that we did not include in this study. For example, a recent study found that top-down effects can also drive divergence in insect herbivores (Heath, Abbot, & Stireman, 2018). In the case of generalist herbivores that are more affected by top-down than bottom-up forces (Vidal & Murphy, 2018a), selection from natural enemies could indeed account for a good portion of their genetic divergence and remains to be investigated.

Our finding of black and red FW being morphologically and genetically different suggests that there must be extremely low levels of gene flow (or none) between the two types. This isolation is the case even where these types occur in sympatry, as local red FW were more similar to reds from other locations than to sympatric black FW. Recently, a study using mitochondrial DNA showed clear distinction between red and black types of FW (Yang et al., 2017); however, the use of mitochondrial DNA to infer species delimitations can be problematic in cases where there is introgression, and for arthropods, the pattern can also be biased by inherited symbionts (Hurst & Jiggins, 2005; Toews & Brelsford, 2012). Therefore, our nuclear data give additional and reliable evidence that the two forms likely represent distinct species. Besides morphological and genetic evidence, there are also natural history indications that the two types are reproductively isolated. In nature, reds and blacks can be reproductively isolated in time due to differences in generations per year and emergence time (Oliver, 1964; Takeda, 2005). The two types also use a different set of host plant species (Table 1, e.g., Oliver, 1964), and adult female moths have limited dispersal abilities (Yamanaka, Tatsuki, & Shimada, 2001). For example, in Ohio where the two types occur in sympatry, black-heads feed frequently on red bud (*Cercis canadensis*), but not red-heads (e.g., Table 1). Notably, there are also areas of host plant overlap as both types can be frequently found on Cherry plants (*Prunus* sp.). Finally, bioassays have shown that males are usually more attracted to pheromones of females of the same type than to the other type, resulting in more intratype than intertype matings (McLellan, Nordin, & Haynes, 1991). With the body of evidence from natural history and genetic analyses accumulated during the past four decades, and our data on morphometry and nuclear genomic analysis using thousands of SNPs, there is ample evidence that the two types of FW are divergent lineages and likely different species.

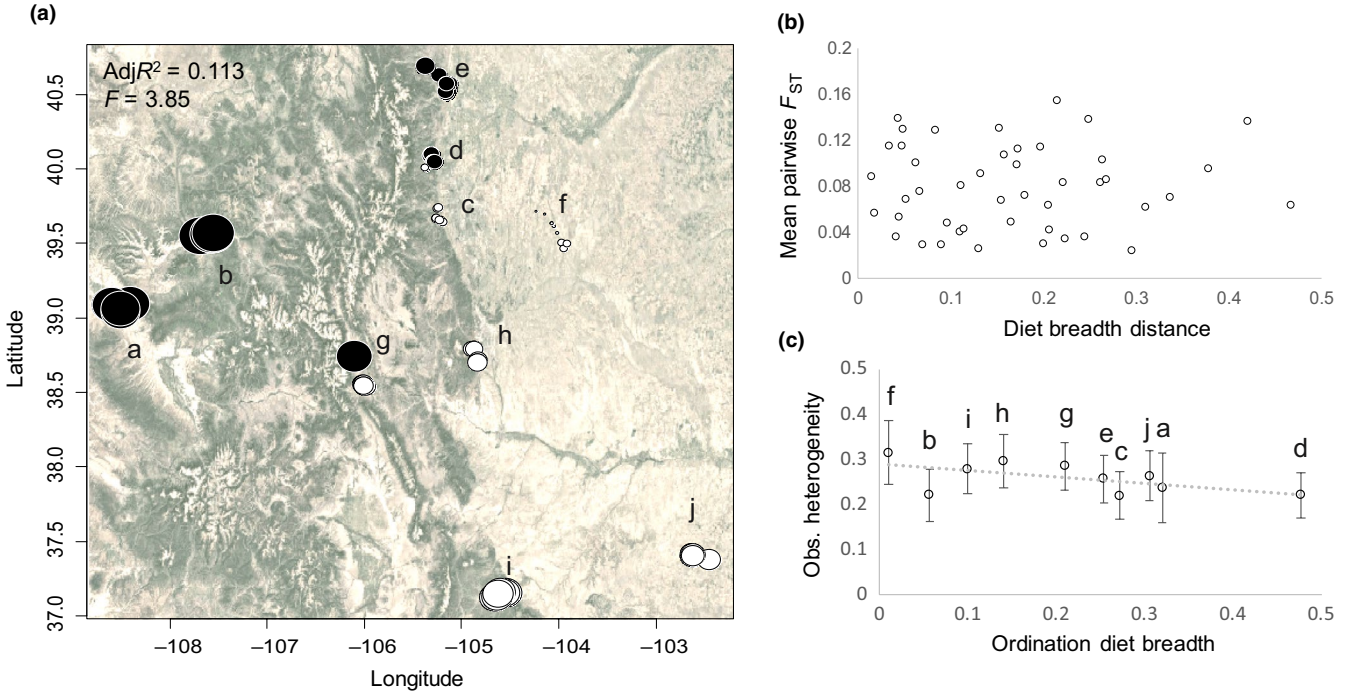

FIGURE 6 Influence of geography and diet breadth on population divergence of fall webworm in Colorado: (a) memgene analysis showing genetic groupings of samples across the Colorado landscape (superposition of satellite topography of Colorado from Google Earth). Circles represent each sample, circles of same size and colour show individuals with similar memgene scores, while different sizes and colours indicate dissimilarity. Colour of circles represents the scores in the principal components axis from positive (black) to negative (white), and the size of the circles represents the location along the axis, from ±0.075 (larger circles) to 0 (smaller circles). (b) Correlation between Weir & Cockerham's F_{ST} and diet breadth distance pairwise comparisons. (c) Correlation between observed heterozygosity (mean ± variance) and diet breadth of the 10 populations sampled. Letters in a and c represent the same subpopulations as in Figure 1 [Colour figure can be viewed at wileyonlinelibrary.com]

Our data provide evidence of factors that influence the divergence between the two types and among populations, but we should interpret these factors with caution. We showed that genetic divergence of populations is correlated with the use of different host plants and with geographic distance; however, a portion of the genetic divergence remained unexplained, and other factors such as competition between the two types, selective pressure from natural enemies or their colonization history could have also influenced their divergence. For the latter, for example, Yang et al. (2017) suggested that the separation of the two types started 1.2–1.6 million years ago; thus, it is possible that the two types began diverging genetically due to allopatric separation into different refugia during quaternary glaciation but then established overlapping ranges after the ice retreated. The different patterns of host plant use by the two FW types might have evolved due to the ancestral populations occupying different regions during a glaciation event, and thus, differing host plant use could be a consequence of geographic isolation and not the cause of divergence. In conclusion, although we found evidence that genetic distance was (weakly) correlated with host plant use, we cannot say that variation in host plant use was a cause of the divergence between black and red FW.

Besides the distinction between the two types, red FW from Colorado also formed a distinct genetic group from the other collection sites, which indicates that Colorado FW are distinct from eastern populations. Other species of *Hyphantria* are endemic from Central (*H. orizaba*, *H. panoezys*, *H. penthetria*) and South (*H. pictipupa*) America, which suggests a possible origin of *H. cunea* in the western or central part of its current range in the United States with subsequent expansions to the east. Colorado populations are isolated from eastern populations by the Central Plains, which is a dry region of grassland and where FW are rare (M. C. Vidal personal observation). FW usually feed on trees and shrubs and tend to fly along rivers and roads (e.g., Ito, Shibazaki, & Iwahasi, 1970). Therefore, the migration of FW between eastern populations and Colorado might be limited to the few riverine corridors of riparian vegetation across the Central Plains, resulting in genetic isolation of Colorado populations. The riparian vegetation in the Central Plains has cottonwood along river banks, which could make the corridors habitable for red FW but not for black FW. While red FW can use cottonwoods as a host plant, black FW are not known to use them in the wild and do not perform well on them in the laboratory (M. C. Vidal personal observation). In Colorado, the Rocky Mountains are clearly an important genetic barrier between populations to the east or west of the mountains; it would be interesting to compare the genetic structure of other western FW populations to see whether the pattern holds at a larger geographic scale. Other organisms have been shown to consist of populations on the western side of the Continental Divide that are genetically different from eastern populations (e.g., racers,

Burbrink, Fontanella, Pyron, Guiher, & Jimenez, 2008; rust fungus, Hamelin et al., 2000), but there are also cases of high gene flow across the continent (e.g., barn owls; Huang et al., 2016).

Our results show that divergence of a generalist herbivore is mostly associated with geographic distance. Other systems have also found stronger influence of geographic isolation than species interaction on taxa diversification. For example, in the yucca–yucca moth interaction, geographic isolation rather than co-evolution explained the pattern of diversification of host plants and yucca moths (Althoff, Segraves, Smith, Leebens-Mack, & Pellmyr, 2012). Similarly, ecological speciation was shown to be overestimated in sawflies (Nyman, Vikberg, Smith, & Boevé, 2010). Although geographic distance explained more genetic divergence than host plant use, this does not mean that divergence in FW is mostly nonadaptive. For example, geographic isolation explained more genetic variation when we considered a narrow geographic scale of samples from Colorado; this might be because host plants are not continuously distributed across the landscape in Colorado in comparison with the eastern United States, which could result is strong signal of isolation by distance in Colorado. In fact, host plant species had a marginal influence on genetic divergence in Colorado. Besides host plant distribution being potentially confounded by location, other environmental factors not included in our analysis could also vary geographically and influence the pattern of isolation by distance that we found (e.g., natural enemy communities).

In contrast to expectations, we found no correlation between broader diet breadth and higher genetic diversity nor between diet breadth and genetic divergence. Theoretical work predicts that generalist populations of insect herbivores should exhibit high genetic diversity, which may be matched by specialists only at the species level (Gloss et al., 2013). This was found to be the case for southern beech aphids, which have higher genetic diversity with broader diets (Gaete-Eastman, Figueroa, Olivares-Donoso, Niemeyer, & Ramírez, 2004). In the case of FW in Colorado, there is considerable gene flow among the subpopulations we sampled, which could have masked any pattern of genetic structure by diet breadth or host plant use at the subpopulation level. Furthermore, even though we consider some populations of fall webworm to be more specialized than others, they can still be considered generalists as all of the populations sampled fed on at least two plant species from distinct families. That being said, one of our subpopulations with narrowest geographic range and diet breadth (b in Figure 6c) had indeed the lowest genetic diversity (measured as observed heterogeneity). The influence of diet breadth on genetic structure deserves further analysis, preferably using closely related insect herbivores with distinctly differing diet breadths.

The relatively strong effects of geographic distance on divergence of a generalist herbivore support a recent macroevolutionary hypothesis of insect herbivore diversification. Hardy et al. (2016) proposed that generalist herbivores that are geographically widespread can form geographically isolated populations, and the diversification and specialization of herbivores may be a result of this isolation. For a widespread extreme generalist herbivore such as FW, geographic distance was strongly correlated with genetic distance, regardless of the geographic scale being analysed. Furthermore, it is very likely that the divergence of FW types involved geographic isolation of some form. In a rare population genomic analysis of a generalist insect herbivore, we confirm the existence of three distinct populations or races of FW and find that within these populations, genetic structure is primarily a function of geographic isolation rather than host plant use.

ACKNOWLEDGEMENTS

We thank the University of Denver Organismal Biology Group and three anonymous reviewers for insightful comments on previous versions of this manuscript. We would also like to thank Brent Horowitz, Erica Larson, John Lill, Scott Barbee, Jonathan Shortt, Kari Segraves and David Althoff for analytical and equipment support. We thank John Lill, Robert Marquis, Michael Singer and Laurel Cepero for providing samples for the genetic analysis. This research was supported by funding from Science without Borders (CAPES #1198/13-0) awarded to M. C. Vidal and funding from the University of Denver awarded to S. M. Murphy and R. M. Tinghitella. MCV also thanks the Jefferson County Open Space for allowing collection of specimens in Colorado.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

M.C.V., S.M.M., J.O.S. designed research, M.C.V. performed research and analysed data, S.M.M., J.O.S., R.M.T., T.W.Q. contributed new reagents or analytical tools, M.C.V. wrote the first draft and all authors contributed equally to the revisions.

DATA AVAILABILITY STATEMENT

Fastq read files can be accessed on NCBI SRA, Accession nos. SAMN12536603–SAMN12536784.

ORCID

Mayra C. Vidal https://orcid.org/0000-0003-3374-8050
Robin M. Tinghitella https://orcid.org/0000-0002-0049-5539
Shannon M. Murphy https://orcid.org/0000-0002-5746-6536

REFERENCES

Althoff, D. M., Segraves, K. A., Smith, C. I., Leebens-Mack, J., & Pellmyr, O. (2012). Geographic isolation trumps coevolution as a driver of yucca and yucca moth diversification. *Molecular Phylogenetics and Evolution*, *62*(3), 898–906. https://doi.org/10.1016/j.ympev.2011.11.024

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2006). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, *22*(3), 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Burbrink, F. T., Fontanella, F., Pyron, R. A., Guiher, T. J., & Jimenez, C. (2008). Phylogeography across a continent: The evolutionary and demographic history of the North American racer (Serpentes: Colubridae: Coluber constrictor). *Molecular Phylogenetics and Evolution*, *47*(1), 274–288. https://doi.org/10.1016/j.ympev.2007.10.020

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

Chamberlain, S. (2018). *brranching: Fetch 'Phylogenies' from many sources*. R package version 0.4.0. Retrieved from https://CRAN.R-project.org/package=brranching

Cogni, R., Trigo, J. R., & Futuyma, D. J. (2011). Varying herbivore population structure correlates with lack of local adaptation in a geographic variable plant- herbivore interaction. *PlosOne*, *6*(12), e29220. https://doi.org/10.1371/journal.pone.0029220

Craft, K. J., Pauls, S. U., Darrow, K., Miller, S. E., Hebert, P. D. N., Helgen, L. E., … Weiblen, G. D. (2010). Population genetics of ecological communities with DNA barcodes: An example from New Guinea Lepidoptera. *Proceedings of the National Academy of Sciences of the USA*, *107*(11), 5041–5046.

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

Darwell, C. T., Fox, K. A., & Althoff, D. M. (2014). The roles of geography and founder effects in promoting host-associated differentiation in the generalist bogus yucca moth *Prodoxus decipiens*. *Journal of Evolutionary Biology*, *27*(12), 2706–2718.

Dopman, E. B., Sword, G. A., & Hillis, D. M. (2002). The importance of the ontogenetic niche in resource-associated divergence: Evidence from a generalist grasshopper. *Evolution*, *56*(4), 731–740. https://doi.org/10.1111/j.0014-3820.2002.tb01384.x

Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution*, *18*(4), 586–608. https://doi.org/10.1111/j.1558-5646.1964.tb01674.x

Fordyce, J. A., Nice, C. C., Hamm, C. A., & Forister, M. L. (2016). Quantifying diet breadth through ordination of host association. *Ecology*, *97*(4), 842–849. https://doi.org/10.1890/15-0093.1

Funk, D. J. (1998). Isolating a role for natural selection in speciation: Host adaptation and sexual selection in *Neochlamisus bebbianae* leaf beetles. *Evolution*, *52*(6), 1744–1759.

Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1), 207–233. https://doi.org/10.1146/annurev.es.19.110188.001231

Gaete-Eastman, C., Figueroa, C., Olivares-Donoso, R., Niemeyer, H., & Ramírez, C. (2004). Diet breadth and its relationship with genetic diversity and differentiation: The case of southern beech aphids (Hemiptera: Aphididae). *Bulletin of Entomological Research*, *94*(3), 219–227. https://doi.org/10.1079/BER2004298

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

Gloss, A. D., Nelson Dittrich, A. C., Goldman-Huertas, B., & Whiteman, N. K. (2013). Maintenance of genetic diversity through plant–herbivore interactions. *Current Opinion in Plant Biology*, *16*(4), 443–450. https://doi.org/10.1016/j.pbi.2013.06.002

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, *22*(7), 1–9.

Hamelin, R. C., Hunt, R. S., Geils, B. W., Jensen, G. D., Jacobi, V., & Lecours, N. (2000). Barrier to gene flow between eastern and western populations of *Cronartium ribicola* in North America. *Phytopathology*, *90*(10), 1073–1078.

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*, 1–10. 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

Heath, J. J., Abbot, P., & Stireman, J. O. III (2018). Adaptive divergence in a defense symbiosis driven from the top down. *The American Naturalist*, *192*(1), E21–E36. https://doi.org/10.1086/697446

Huang, A. C., Elliott, J. E., Cheng, K. M., Ritland, K., Ritland, C. E., Thomsen, S. K., … Martin, K. (2016). Barn owls (*Tyto alba*) in western North America: Phylogeographic structure, connectivity, and genetic diversity. *Conservation Genetics*, *17*(2), 357–367. https://doi.org/10.1007/s10592-015-0787-0

Huang, H., & Knowles, L. L. (2014). Unforeseen consequences of excluding missing data from next-generation sequences: Simulation study of RAD sequences. *Systematic Biology*, *65*(3), 357–365. https://doi.org/10.1093/sysbio/syu046

Hurst, G. D. D., & Jiggins, F. M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proceedings. Biological Sciences*, *272*(1572), 1525–1534.

Huson, D. H. (1998). SplitsTree: Analyzing and visualizing evolutionary data. *Bioinformatics*, *14*(1), 68–73. https://doi.org/10.1093/bioinformatics/14.1.68

Huson, D. H., & Bryant, D. (2005). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, *23*(2), 254–267. https://doi.org/10.1093/molbev/msj030

Hutchison, D. W., & Templeton, A. R. (1999). Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, *53*(6), 1898–1914. https://doi.org/10.1111/j.1558-5646.1999.tb04571.x

Ito, Y., Shibazaki, A., & Iwahasi, O. (1970). Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan: XI. Results of road-survey. *Applied Entomology and Zoology*, *5*(3), 133–144.

Ito, Y., & Warren, L. O. (1973). Status of black-headed and red-headed types of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) I Biology of two types and results of crossing experiment. *Applied Entomological Zoology*, *8*(3), 157–171. https://doi.org/10.1303/aez.8.157

Janz, N., & Nylin, S. (2008). The oscillation hypothesis of host-plant range and speciation. In K. Tilmon (Ed.), *Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects* (pp. 203–215). Oakland, CA: University of California Press.

Jombart, T., & Dray, S. (2008). adephylo: Exploratory analyses for the phylogenetic comparative method. *Bioinformatics*, *26*, 1907–1909.

Klingenberg, C. P. (2011). MORPHOJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources*, *11*, 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191.

Legendre, P., & Fortin, M. J. (2010). Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Molecular Ecology Resources*, *10*(5), 831–844. https://doi.org/10.1111/j.1755-0998.2010.02866.x

Mason, P. A., Wilkes, S. R., Lill, J. T., & Singer, M. S. (2011). Abundance trumps quality: Bi-trophic performance and parasitism risk fail to

explain host use in the fall webworm. *Oikos*, *120*(10), 1509–1518. https://doi.org/10.1111/j.1600-0706.2011.19053.x

McLellan, K. A. M., Nordin, G. L., & Haynes, K. F. (1991). Chemical communication and reproductive isolation in two types of the fall webworm (Lepidoptera: Arctiidae). *Annals of the Entomological Society of America*, *84*(1), 118–123. https://doi.org/10.1093/aesa/84.1.118

Milá, B., Warren, B. H., Heeb, P., & Thébaud, C. (2010). The geographic scale of diversification on islands: Genetic and morphological divergence at a very small spatial scale in the Mascarene grey white-eye (Aves: *Zosterops borbonicus*). *BMC Evolutionary Biology*, *10*(1), 158. https://doi.org/10.1186/1471-2148-10-158

Murphy, S. M., & Loewy, K. J. (2015). Trade-offs in host choice of an herbivorous insect based on parasitism and larval performance. *Oecologia*, *179*(3), 741–751. https://doi.org/10.1007/s00442-015-3373-8

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

Nosil, P., Gompert, Z., Farkas, T. E., Comeault, A. A., Feder, J. L., Buerkle, C. A., & Parchman, T. L. (2012). Genomic consequences of multiple speciation processes in a stick insect. *Proceedings. Biological Sciences*, *279*(1749), 5058–5065.

Nyman, T., Vikberg, V., Smith, D. R., & Boevé, J. L. (2010). How common is ecological speciation in plant-feeding insects? A 'Higher' Nematinae perspective. *BMC Evolutionary Biology*, *10*(1), 266. https://doi.org/10.1186/1471-2148-10-266

Oksanen, J., Kindt, R., Legendre, P., O'hara, B., Henry, M., & Maintainer, H. S. (2007). The vegan package. *Community Ecology Package*, *10*, 631–637.

Oliver, A. D. (1964). A behavioral study of two races of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae), in Louisiana. *Annals of the Entomological Society of America*, *57*(2), 192–194. https://doi.org/10.1093/aesa/57.2.192

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. https://doi.org/10.1371/journal.pone.0037135

Powell, T. H. Q., Hood, G. R., Murphy, M. O., Heilveil, J. S., Berlocher, S. H., Nosil, P., & Feder, J. L. (2013). Genetic divergence along the speciation continuum: The transition from host race to species in *Rhagoletis* (Diptera:Tephritidae). *Evolution*, *67*(9), 2561–2576.

Pritchard, J. K., Stephens, M., Rosenberg, N. A., & Donnelly, P. (2000). Association mapping in structured populations. *The American Journal of Human Genetics*, *67*(1), 170–181. https://doi.org/10.1086/302959

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … 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 Development Core Team (2011). *R: A language and environment for statistical computing*, 1(2.11.1), p. 409. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.rproject.org

Rohlf, F. J. (2005). *tpsDig, digitize landmarks and outlines, version 2.05*. Department of Ecology and Evolution, State University of New York at Stony Brook.

Roskov, Y., Kunze, T., Paglinawan, L., Orrell, T., Nicolson, D., Culham, A., … De Wever, A. (Eds.) (2013). *Species 2000 & ITIS Catalogue of Life, 2013 Annual Checklist*. Species 2000, Reading, UK. Digital resource. Retrieved from http://www.catalogueoflife.org/annual-checklist/2013/Species2000

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Takeda, M. (2005). Differentiation in life cycle of sympatric populations of two forms of *Hyphantria* moth in central Missouri. *Entomological Science*, *8*(2), 211–218. https://doi.org/10.1111/j.1479-8298.2005.00117.x

Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*(16), 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Vidal, M. C., & Murphy, S. M. (2018a). Bottom-up vs. top-down effects on terrestrial insect herbivores: A meta-analysis. *Ecology Letters*, *21*(1), 138–150. https://doi.org/10.1111/ele.12874

Vidal, M. C., & Murphy, S. M. (2018b). Quantitative measure of tri-trophic fitness and its influence on diet breadth of insect herbivores. *Ecology*, *99*(12), 2681–2691.

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, *23*(23), 5649–5662. https://doi.org/10.1111/mec.12938

Warren, L. O., & Tadić, M. D. (1967). The fall webworm, *Hyphantria cunea*, its distribution and natural enemies: A world list. *Journal of the Kansas Entomological Society*, *40*, 194–202.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370.

Wiens, J. J., Lapoint, R. T., & Whiteman, N. K. (2015). Herbivory increases diversification across insect clades. *Nature Communications*, *6*(October), 1–7. https://doi.org/10.1038/ncomms9370

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114–138.

Yamanaka, T., Tatsuki, S., & Shimada, M. (2001). Flight characteristics and dispersal patterns of fall webworm (Lepidoptera: Arctiidae) males. *Environmental Entomology*, *30*(6), 1150–1157. https://doi.org/10.1603/0046-225X-30.6.1150

Yang, F., Kawabata, E., Tufail, M., Brown, J. J., & Takeda, M. (2017). r/K-like trade-off and voltinism discreteness: The implication to allochronic speciation in the fall webworm, *Hyphantria cunea* complex (Arctiidae). *Ecology and Evolution*, *7*(24), 10592–10603.

Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., … Beaulieu, J. M. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature*, *506*(7486), 89. https://doi.org/10.1038/nature12872

Zelditch, M., Swiderski, D., Sheets, H., & Fink, W. (2004). *Geometric morphometrics for biologists*. Cambridge, MA: Elsevier Academic Press.

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

Zhong, M., Hill, G. M., Gomez, J. P., Plotkin, D., Barber, J. R., & Kawahara, A. Y. (2016). Quantifying wing shape and size of saturniid moths with geometric morphometrics. *Journal of the Lepidopterists' Society*, *70*(2), 99–107. https://doi.org/10.18473/lepi.70i2.a4

How to cite this article: Vidal MC, Quinn TW, Stireman JO, Tinghitella RM, Murphy SM. Geography is more important than host plant use for the population genetic structure of a generalist insect herbivore. *Mol Ecol*. 2019;28:4317–4334. https://doi.org/10.1111/mec.15218

APPENDIX

TABLE A1 Host plant used by fall webworm larvae from our genetic analysis

Sample	Host plant species	Host plant family	Location	Morpho type	Geographic region analysis
AR-2016-22	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-23	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-24	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-25	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-26	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-27B	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-27C	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-28	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-32A	*Populus deltoides*	Salicaceae	Colorado – f	Red	Narrow
AR-2016-33	*Ulmus*	Ulmaceae	Colorado – f	Red	Narrow
CH-2016-63C	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-2016-66B	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-2016-67A	*Salix*	Salicaceae	Colorado – g	Red	Narrow
CH-2016-67B	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-2016-69	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-2016-70A	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-2016-70B	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-2016-71	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-2016-72	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-2016-75	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-93	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-94W1	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-95	*Populus deltoides*	Salicaceae	Colorado – g	Red	Narrow
CH-96	*Malus*	Rosaceae	Colorado – g	Red	Narrow
CH-97	*Populus angustifolia*	Salicaceae	Colorado – g	Red	Narrow
CH-98	*Populus deltoides*	Salicaceae	Colorado – g	Red	Narrow
CH-99	*Ulmus*	Ulmaceae	Colorado – g	Red	Narrow
EP-2016-13A	*Populus deltoides*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-13B	*Populus deltoides*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-13C	*Populus deltoides*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-14A	*Populus angustifolia*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-16	*Populus tremula*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-18	*Prunus*	Rosaceae	Colorado – h	Red	Narrow
EP-2016-50A	*Populus deltoides*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-50B	*Populus angustifolia*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-50C	*Populus angustifolia*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-50D	*Populus angustifolia*	Salicaceae	Colorado – h	Red	Narrow
EP-2016-83B	*Ulmus*	Ulmaceae	Colorado – h	Red	Narrow
EP-2016-85	*Ulmus*	Ulmaceae	Colorado – h	Red	Narrow
EP-2016-87	*Ulmus*	Ulmaceae	Colorado – h	Red	Narrow
GA-103	*Prunus virginiana*	Rosaceae	Colorado – b	Red	Narrow
GA-2016-34	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-35	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow

(Continues)

TABLE A1 (Continued)

Sample	Host plant species	Host plant family	Location	Morpho type	Geographic region analysis
GA-2016-37	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-38	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-39A	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-39B	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-40	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-41	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
GA-2016-44	*Prunus virginiana var Shubert*	Rosaceae	Colorado – b	Red	Narrow
GA-2016-45	*Populus angustifolia*	Salicaceae	Colorado – b	Red	Narrow
LA-2016-51C	*Populus angustifolia*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-51D	*Populus angustifolia*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-52A	*Populus angustifolia*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-52B	*Populus angustifolia*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-53	*Ulmus*	Ulmaceae	Colorado – i	Red	Narrow
LA-2016-54A	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-54B	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-54C	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-54D	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-55A	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-56	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-57	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-58B	*Populus angustifolia*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-59	*Populus deltoides*	Salicaceae	Colorado – i	Red	Narrow
LA-2016-60	*Ulmus*	Ulmaceae	Colorado – i	Red	Narrow
LA-2016-61	*Ulmus*	Ulmaceae	Colorado – i	Red	Narrow
MV-53	*Ulmus*	Ulmaceae	Colorado – f	Red	Narrow
MV-62	*Ulmus*	Ulmaceae	Colorado – f	Red	Narrow
BA-2016-100A	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-100B	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-100C	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-100E	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-100F	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-89	*Juglans nigra*	Juglandaceae	Colorado – j	Red	Narrow
BA-2016-90	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-91	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-92A	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-92B	*Populus deltoides*	Salicaceae	Colorado – j	Red	Narrow
BA-2016-94	*Ulmus*	Ulmaceae	Colorado – j	Red	Narrow
BA-2016-98	*Malus*	Rosaceae	Colorado – j	Red	Narrow
BA-58	*Juglans nigra*	Juglandaceae	Colorado – j	Red	Narrow
J-1L68	*Prunus virginiana*	Rosaceae	Colorado – c	Red	Narrow
J-2016-2	*Prunus virginiana*	Rosaceae	Colorado – c	Red	Narrow
J-71W2	*Prunus virginiana*	Rosaceae	Colorado – c	Red	Narrow
J-82	*Malus*	Rosaceae	Colorado – c	Red	Both
J-83	*Populus deltoides*	Salicaceae	Colorado – c	Red	Both

(Continues)

TABLE A1 (Continued)

Sample	Host plant species	Host plant family	Location	Morpho type	Geographic region analysis
J-84	*Ulmus*	Ulmaceae	Colorado – c	Red	Narrow
J-87	*Prunus virginiana*	Rosaceae	Colorado – c	Red	Both
J-88	*Salix*	Salicaceae	Colorado – c	Red	Both
J-90	*Populus angustifolia*	Salicaceae	Colorado – c	Red	Narrow
J-92	*Prunus virginiana*	Rosaceae	Colorado – c	Red	Both
J81	*Populus angustifolia*	Salicaceae	Colorado – c	Red	Both
KL-2	*Populus deltoides*	Salicaceae	Colorado – d	Red	Narrow
KL-21	*Prunus virginiana*	Rosaceae	Colorado – d	Red	Narrow
KL-3	*Prunus virginiana*	Rosaceae	Colorado – d	Red	Narrow
KL-30	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
KL-31	*Malus*	Rosaceae	Colorado – e	Red	Narrow
KL-33	*Populus deltoides*	Salicaceae	Colorado – d	Red	Narrow
KL-34	*Prunus virginiana*	Rosaceae	Colorado – d	Red	Narrow
KL-41	*Prunus virginiana*	Rosaceae	Colorado – d	Red	Narrow
KL-42	*Populus deltoides*	Salicaceae	Colorado – d	Red	Narrow
KL-48	*Populus deltoides*	Salicaceae	Colorado – d	Red	Narrow
KL-54A	*Populus angustifolia*	Salicaceae	Colorado – d	Red	Narrow
KL-61	*Populus deltoides*	Salicaceae	Colorado – e	Red	Narrow
KL-71A	*Populus deltoides*	Salicaceae	Colorado – d	Red	Narrow
KL-8A	*Populus angustifolia*	Salicaceae	Colorado – d	Red	Narrow
KL55	*Malus*	Rosaceae	Colorado – d	Red	Narrow
LR-03	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-10	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-11	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-12	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-6W1	*Malus*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-6W2	*Malus*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-7	*Prunus virginiana*	Rosaceae	Colorado – e	Red	Narrow
LR-2016-8	*Ulmus*	Ulmaceae	Colorado – e	Red	Narrow
LR-2016-9	*Ulmus*	Ulmaceae	Colorado – e	Red	Narrow
ME-106	*Salix*	Salicaceae	Colorado – a	Red	Narrow
ME-108	*Fraxinus pennsylvanica*	Oleaceae	Colorado – a	Red	Narrow
ME-30	*Fraxinus americana*	Oleaceae	Colorado – a	Red	Narrow
ME-32	*Malus*	Rosaceae	Colorado – a	Red	Narrow
ME-34	*Quercus*	Fagaceae	Colorado – a	Red	Narrow
ME-39	*Ulmus*	Ulmaceae	Colorado – a	Red	Narrow
ME19	*Fraxinus americana*	Oleaceae	Colorado – a	Red	Narrow
MV-55	*Ulmus*	Ulmaceae	Colorado – j	Red	Narrow
MV-59	*Ulmus*	Ulmaceae	Colorado – j	Red	Narrow
CT-01B	*Acer negundo*	Sapindaceae	Connecticut	Black	Broad
CT-02	*Acer negundo*	Sapindaceae	Connecticut	Black	Broad
CT-03	*Acer negundo*	Sapindaceae	Connecticut	Black	Broad
CT-04	*Salix nigra*	Salicaceae	Connecticut	Black	Broad
CT-06	*Salix*	Salicaceae	Connecticut	Black	Broad

(Continues)

TABLE A1 (Continued)

Sample	Host plant species	Host plant family	Location	Morpho type	Geographic region analysis
CT01C	*Acer negundo*	Sapindaceae	Connecticut	Black	Broad
CT05	*Salix nigra*	Salicaceae	Connecticut	Black	Broad
F-20NJ	*Prunus serotina*	Rosaceae	New Jersey	Black	Broad
F-22NJ	*Prunus serotina*	Rosaceae	New Jersey	Black	Broad
F19-NJ	*Quercus*	Fagaceae	New Jersey	Black	Broad
OH-01	*Cercis canadensis*	Fabaceae	Ohio	Black	Broad
OH-02	*Vitis*	Vitaceae	Ohio	Black	Broad
OH-05	*Cercis canadensis*	Fabaceae	Ohio	Black	Broad
OH-06	*Ulmus*	Ulmaceae	Ohio	Black	Broad
OH-10	*Cercis canadensis*	Fabaceae	Ohio	Black	Broad
OH-11	*Cercis canadensis*	Fabaceae	Ohio	Black	Broad
OH-12	*Acer negundo*	Sapindaceae	Ohio	Black	Broad
OH-7	*Acer negundo*	Sapindaceae	Ohio	Black	Broad
S45	*Ulmus*	Ulmaceae	Pennsylvania	Black	Broad
S47	*Ulmus*	Ulmaceae	Pennsylvania	Black	Broad
F-6NJ	*Quercus*	Fagaceae	New Jersey	Red	Broad
F-7NJ	*Prunus serotina*	Rosaceae	New Jersey	Red	Broad
F4-NJ	*Prunus serotina*	Rosaceae	New Jersey	Red	Broad
IL-01	*Carya*	Juglandaceae	Illinois	Red	Broad
IL-02	*Quercus*	Fagaceae	Illinois	Red	Broad
MD-01	*Platanus occidentalis*	Platanaceae	Maryland	Red	Broad
MD-02	*Prunus serotina*	Rosaceae	Maryland	Red	Broad
MD-03	*Diospyros*	Ebenaceae	Maryland	Red	Broad
MD-04	*Diospyros*	Ebenaceae	Maryland	Red	Broad
MD-05	*Diospyros*	Ebenaceae	Maryland	Red	Broad
MO-03	*Ailanthus*	Simaroubaceae	Missouri	Red	Broad
MO-04	*Diospyros*	Ebenaceae	Missouri	Red	Broad
MO-07	*Betula*	Betulaceae	Missouri	Red	Broad
MO-2	*Betula*	Betulaceae	Missouri	Red	Broad
MO-X	*Betula*	Betulaceae	Missouri	Red	Broad
MO-Y	*Ailanthus*	Simaroubaceae	Missouri	Red	Broad
OH-03	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
OH-04	*Platanus occidentalis*	Platanaceae	Ohio	Red	Broad
OH-08	*Platanus occidentalis*	Platanaceae	Ohio	Red	Broad
OH-13	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
OH-14	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
OH-15	*Juglans nigra*	Juglandaceae	Ohio	Red	Broad
OH-16	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
OH-18	*Oxydendrum*	Ericaceae	Ohio	Red	Broad
OH-19	*Oxydendrum*	Ericaceae	Ohio	Red	Broad
OH-20	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
OH-21	*Platanus occidentalis*	Platanaceae	Ohio	Red	Broad
OH-22	*Nyssa sylvatica*	Nyssaceae	Ohio	Red	Broad
OH17	*Prunus serotina*	Rosaceae	Ohio	Red	Broad
S-02	*Juglans nigra*	Juglandaceae	Kansas	Red	Broad

(Continues)

TABLE A1 (Continued)

Sample	Host plant species	Host plant family	Location	Morpho type	Geographic region analysis
S-03	*Juglans nigra*	Juglandaceae	Kansas	Red	Broad
S-23	*Carya*	Juglandaceae	Illinois	Red	Broad
S-25	*Quercus*	Fagaceae	Illinois	Red	Broad
S-48	*Prunus*	Rosaceae	Pennsylvania	Red	Broad
S01	*Juglans nigra*	Juglandaceae	Kansas	Red	Broad