INTRODUCTION

A principal aim in ecology and evolutionary biology is to resolve factors and understand processes that influence genetic divergence at both the individual and population level (Coyne & Orr, 2004; Mayr, 1963; Nosil, 2012). When genetic divergence has a strong spatial component, causes are generally attributed to spatial variation in evolutionary processes, such as gene flow, genetic drift,
and selection (Bohonak, 1999; Rousset, 1997; Schwartz McKelvey, Cushman, & Luikart, 2010; Slatkin, 1987). However, inferring the relative contributions of these processes is challenging. Landscape genetics addresses this by quantitatively relating patterns of genetic divergence to geographic and environmental landscape factors (Cushman, McKelvey, Hayden, & Schwartz, 2006; Manel, Schwartz, Luikart, & Taberlet, 2003; Richardson, Brady, Wang, & Spear, 2016; Shirk, Wallin, Cushman, Rice, & Warheit, 2010; Sork, Nason, Campbell, & Fernandez, 1999).

Multiple heuristics have been invoked to conceptualize relationships between genetic divergence and landscape factors, with each implicating specific evolutionary processes. The first, isolation-by-distance (IBD; Wright, 1943), predicts that geographic distance or physical barriers to dispersal reduce gene flow and permit drift between spatially separated individuals or populations. Because dispersal is limited in most species (Greenwood, 1980), Euclidean and genetic distances are often positively correlated, supporting IBD (Rousset, 1997; Vekemans & Hardy, 2004; but see Meirmans, 2012). A second heuristic, isolation-by-resistance (IBR; McRae, 2006), may be seen as a modification of IBD and predicts that patterns of genetic divergence will be best explained by geographic distances accounting for variation in the relative resistance organisms experience when dispersing through heterogeneous landscapes. To assess IBR, resistance surfaces are often parameterized as the inverse of habitat suitability, generally modelled using occurrences of focal taxa and geographic and environmental/ecological predictor variables (McRae & Beier, 2007; Wang, Yang, Bridgman, & Lin, 2008; Storfer, Murphy, Spear, Holderegger, & Waits, 2010; Wang, Glor, & Losos, 2012, but see Peterman, Connette, Semlitsch, & Egger, 2014). Optimal pathways (e.g., least-cost path distances) or a multitude of possible pathways of varying probability derived from circuit theory (circuit distances) may then be estimated across resistance surfaces and related to patterns of genetic divergence (McRae & Beier, 2007). When resistance surfaces are parametrized in this way, positive correlations between genetic distance and resistance-based distances suggest that organisms are more likely to disperse within suitable habitat and experience high movement resistance/cost within unsuitable habitat. IBR thereby equates the concept of habitat suitability to that of landscape permeability (the ease with which organisms move through a landscape), or in circuit theory terms, conductance. A third heuristic, isolation-by-environment (IBE; Wang & Summers, 2010), predicts that spatial variation in environmental/ecological conditions contributes to genetic divergence via the combination of (a) reduced fitness and negative selection on individuals that have dispersed across environmental gradients; (b) reduced fitness and negative selection on dispersers’ offspring in non-natal habitats (outbreeding depression); or (c) reduced tendency of individuals to disperse due to local adaptation to environmental conditions (Crispo, Bentzen, Reznick, Kinnison, & Hendry, 2006; Dobzhansky, 1937; Lee & Mitchell-Olde, 2011; Nosil, 2004, 2012; Nosil, Vines, & Funk, 2005; Wang & Bradburd, 2014). After controlling for geographic distance, positive correlations between genetic distance and differences in environmental/ecological conditions confer support for IBE.

Although often framed as competing hypotheses, complementarity of IBD, IBR, and IBE has been documented in multiple studies (Coyne & Orr, 2004; Crispo et al., 2006; Sánchez-Ramírez et al., 2018; Thorpe, Surget-Groba, & Johansson, 2008; Van Buskirk & van Rensburg, 2020; Wang, Glor, et al., 2012). Within such investigations, it can be instructive to invoke Euclidean, least-cost path, and circuit distances as measures of geographic isolation (IBD + IBR) for contrast with measures of environmental/ecological isolation (IBE) estimated as differences in biotic or abiotic conditions. For example, Wang, Glor, et al. (2012) compared the effects of geographic isolation (estimated as least-cost path and circuit distances) and ecological isolation (estimated as differences in values of 24 environmental variables) on genetic divergence for 17 Anolis species. Genetic divergence was significantly related to geographic and ecological isolation for 15 and 13 species, respectively, with inferred effects of geographic isolation being, on average, more than twice as strong as those of ecological isolation. Similar results have been reported for a variety of other vertebrate taxa, including the Trinidad Guppy (Poecilia reticulata; Crispo et al., 2006), Agassiz’s Desert Tortoise (Gopherus agassizii; Sánchez-Ramírez et al., 2018), and the Common Frog (Rana temporaria; Van Buskirk & van Rensburg, 2020), suggesting that geographic distance, spatial features, and arrangements of suitable habitat are of greater importance to genetic divergence than variation in environmental/ecological conditions (but see support for IBE in epigenetic data, Wogan, Yuan, Mahler, & Wang, 2020).

Across these studies, greater support for IBD + IBR than IBE contrasts with the hypothesis that factors contributing to genetic divergence are predominantly environmental/ecological (Foll & Gaggiotti, 2006; Nosil, 2012; Thorpe et al., 2008). However, past comparisons of geographic and environmental isolation have tended to address vertebrate taxa with relatively specific habitat associations that are maintained through their life cycles. It is

**FIGURE 1** Visual representation of the methods used to generate geographic and environmental distances between the 161 sequenced *Papilio machaon dodi* included in this study. Various spatial data layers (a–g) and 375 *P. m. dodi* records (161 sequenced individuals and 214 georeferenced *P. m. dodi* Global Biodiversity Information Facility (GBIF) records) were used to build a Maxent habitat suitability model, which was then used to predict habitat suitability across the study area, with higher values indicating greater suitability. High habitat suitability generally followed the eroding banks of major rivers in southern Alberta and Saskatchewan, Canada (Red Deer, Old Man, South Saskatchewan, and Milk Rivers). Euclidean distance (i) represents the minimum distance between sequenced individuals. A resistance surface was parameterized as the inverse of habitat suitability and used to estimate least-cost path and circuit distances (j and k). The background in inset j is a projected cost surface, representing the cumulative costs incurred by individuals moving across the landscape from each occurrence point. Environmental distances (e–g) were estimated by taking the absolute difference between values of environmental variables extracted from the occurrences of sequenced individuals. Inset pictures are the adult and larval stage of *P. m. dodi* [Colour figure can be viewed at wileyonlinelibrary.com]
therefore intuitive that patterns of genetic divergence are generally best explained by resistance-based geographic distances, as configurations of suitable habitat typically moderate spatial variation in movement and dispersal in such taxa (Broquet, Ray, Petit, Fryxell, & Burel, 2006; Coulon et al., 2004; Crispo et al., 2006; Epps, Wehausen, Bleich, Torres, & Brashares, 2007; McRae, 2006; McRae & Beier, 2007; Sánchez-Ramírez et al., 2018; Vignieri, 2005; Wang, Savage, & Bradley Shaffer, 2009; Wang et al., 2008). But other taxa, including many terrestrial invertebrates, have discrete dispersal life stages (generally the adult) with broader habitat tolerances...
than larval stages, which may have important consequences for processes affecting genetic divergence (Phillipsen et al., 2015). For example, Keller and Holderegger (2013) found that short-distance movements of the southern damselfly (Coenagrion mercuriale) generally followed corridors of reproductive and larval habitat (streams), while long-distance dispersal, inferred from patterns of genetic divergence, was best explained by Euclidean distance across unsuitable habitat (agricultural land). Although IBE was not evaluated for C. mercuriale, genetic divergence within this taxon and similar taxa with discrete dispersal life stages may be expected to show stronger relationships to environmental isolation than geographic isolation, as evolutionary processes predicted by IBD and IBR become subsidiary to those predicted by IBE.

The aim of our study was to evaluate IBD, IBR, and IBE for a taxon with high habitat specificity, a discrete dispersal stage, and distribution across a variable environment. The Old World swallowtail butterfly (Papilio machaon L.) species group has been the subject of considerable study in North America (e.g., Dupuis, Cullingham, Nielsen, & Sperling, 2019; Dupuis, Mori, & Sperling, 2016; Dupuis & Sperling, 2015, 2016; Sperling, 1987, 1990). One subspecies in particular, P. m. dodi McDunnough, 1939, is well suited for this investigation. Adult P. m. dodi search for mates by hilltopping along prominent edges of river valleys, leading to clustering of occurrence records along the Red Deer, South Saskatchewan, Old Man, and Milk Rivers in southern Alberta and Saskatchewan, Canada (Sperling, 1987; Bird, Hilchke, Kondla, Pike, & Sperling, 1995; Dupuis et al., 2019; occurrence records are visualized in Figure 1, inset h). After mating, females travel downslope from hilltops to oviposit on their larval host plant, Artemisia dracunculus L., which is generally restricted to south-facing eroding slopes of river valleys. We therefore hypothesize that these river valleys constitute suitable habitat under the functional resource-based concept (sensu Dennis, Shreeve, & Van Dyck, 2003), providing resources sufficient for mate location, reproduction, resting, roosting, and feeding. This unique habitat configuration may be described as a dendritic ecological network of suitable habitat corridors situated in a matrix of unsuitable agricultural and prairie habitat. Such configurations have proven practical for decoupling Euclidean and resistance-based distances (e.g., Keller & Holderegger, 2013). Additionally, occurrences of P. m. dodi in Canada span an area of approximately 53,000 km² that is sufficiently variable in environmental conditions to assess IBE (environmental gradients are visualized in Figure 1, insets e–g).

2 | MATERIALS AND METHODS

We used a series of causal models to assess and contrast the effects of geographic isolation (IBD + IBR) and environmental isolation (IBE) on genetic divergence within P. m. dodi. Resistance-based distances were estimated using a resistance surface parameterized as the inverse of predicted habitat suitability to assess whether adult butterflies are more likely to disperse within suitable habitat. Such a result would suggest that (a) configurations of suitable habitat are important considerations for predicting gene flow on heterogeneous landscapes; and (b) habitat suitability may be used as a proxy of landscape permeability. However, although habitat associations of P. m. dodi appear to be specific and geographically restricted, the dispersal ability of P. machaon is estimated to be among the greatest of all Canadian butterflies (Burke, Fitzsimmons, & Kerr, 2011). We therefore hypothesize that, considering only measures of geographic isolation, relationships between genetic divergence and Euclidean distance (IBD) will be stronger than those between genetic divergence and least-cost path or circuit distances (IBR), despite a resistance surface predicting greatest landscape permeability (lowest resistance) along the dendritic ecological network of suitable habitat.

Environmental isolation may also play a significant role in structuring genetic divergence within P. m. dodi. Sperling (1987) noted distinct differences in the butterfly’s phenology, diapause propensity, and voltinism across its Canadian range, possibly implicating divergent selection related to variation in summer temperatures as a driver of spatial genetic divergence. If IBE is detected, mechanisms by which environmental isolation structures genetic divergence may be inferred from patterns of population clustering, and we can make several subsequent predictions. First, if IBE is primarily driven by reduced fitness and negative selection on individuals that have dispersed across environmental gradients, genetic clustering should indicate some spatial discordance of individual cluster assignments (i.e., migrants found in non-natal populations) without admixture between clusters indicative of successful hybridization. Second, if IBE operates via reduced fitness and negative selection on genetically intermediate individuals, some admixture indicative of F1 hybridization between migrant and natal individuals may be evident, but substantial admixture among clusters should be absent due to selection against these admixed genotypes. Third, if individuals exhibit a reduced tendency to disperse across environmental gradients due to local adaptation, there should be little or no spatial discordance of individual cluster assignments or admixture among clusters. Finally, we also identified loci under putative divergent selection between genetic clusters (FST outliers) and assessed environmental associations of allele frequencies for individual loci. If population structure is driven by local adaptation to environmental conditions, we hypothesize that FST outlier and environmental association analyses will identify similar sets of candidate loci under putative selection.

2.1 | Sample collection

We collected adult and larva P. m. dodi with aerial net surveys and host plant searches, respectively, between 15 May and 31 August 2017. We visited most known P. m. dodi occurrence locations in Canada and aimed to collect 5–10 individuals every 25–50 km along the Red Deer, South Saskatchewan, Old Man, and Milk Rivers, where suitable habitat is present (collection locations are visualized in Figure 1, inset h). As with previous studies (Dupuis et al., 2016, 2019; Dupuis & Sperling, 2015, 2016; Sperling, 1987, 1990),
we attempted to locate and collect P. m. dodi between major river valleys (e.g., smaller eroding slopes with A. dracunculus and hilltopping features). However, consistent with our past work and historical records (e.g., Bird et al., 1995), we did not observe any individuals outside of known suitable habitat along major river valleys. Adults were generally collected on prominences along major river valleys used as hilltopping features. Adult females descend from hilltops immediately after mating while males remain in search of additional mates, which means that males are more frequently encountered during sampling (Dupuis et al., 2019). Larvae were collected from patches of A. dracunculus on eroding slopes, typically 100–500 m² in area, below hilltopping features. Individuals collected within 500 m of each other were given the same collection location, recorded as the centroid of the hilltopping feature or A. dracunculus patch. After collection, adult individuals were frozen live and stored at -20°C. Larvae were raised to 4th or 5th instar on clippings of A. dracunculus, preserved in 95% ethanol, and stored at -20°C.

2.2 DNA extraction and library preparation

We extracted genomic DNA from thoracic tissue of adults (n = 148) and larvae (n = 32) using DNeasy Kits (Qiagen, Hilden, Germany). Extractions followed the manufacturer’s protocol, with the addition of bovine pancreatic ribonuclease A treatment (RNaseA, 4 μl at 100 mg/ml; Sigma-Aldrich Canada Co., Canada). Genomic DNA was then ethanol precipitated and stored in 50 μl Millipore water at -20°C. Double digest restriction-site associated DNA sequencing (ddRADseq) libraries were prepared from 200 ng input DNA and MspI and PstI. We followed a modified version of Poland, Brown, Sorrells, and Jannink (2012) for wet laboratory procedures and used a standard dual index Illumina adapter system following Peterson, Weber, Kay, Fisher, and Hoecker (2012). Details of our library preparation protocol and adapters are provided in Data S1 and S2, respectively. A final, pooled library of 180 individuals was sequenced with single-end, 75 bp sequencing on a single high output flowcell of an Illumina NextSeq 500.

2.3 Bioinformatic processing

Following Illumina sequencing, we used “process_radtags” in the program Stacks 2.0 (Rochette, Rivera-Colón, & Catchen, 2019) to demultiplex FASTQ reads and filter those with quality scores below 20 within a sliding window 15% of the read length. All reads were truncated to 67 bp after removing the 8 bp Illumina index sequences, identified with one mismatch permitted. We then searched for and removed remnant Illumina adaptor sequences and removed the first 5 bp from the 5’ end of each read (corresponding to the PstI restriction site) using the program Cutadapt 1.9.1 (Martin, 2011). Filtered and trimmed reads were aligned to a P. machaon reference genome comprised of 63,187 scaffolds (NCBI Accession GCA_001298355.1) using Burrows-Wheeler Aligner 0.7.17 (BWA-MEM) (Li, 2013; Li & Durbin, 2009). We then converted files from SAM to BAM format using SAMtools 1.9 (Li et al., 2009) and used “gstacks” and “populations” within Stacks 2.0 to call SNPs and generate output files, stipulating a single population containing all individuals. Genotype calls were exported in variant call format (VCF), and individuals with more than 50% missing data were removed from the data set (three adults and three larvae). Finally, we used VCFtools 0.1.14 (Danecek et al., 2011) to filter genotypes with read depths less than five and filter loci with minor allele frequencies less than 0.05, percentages of missing data greater than 5%, and those within <10 kb of each other to reduce the probability of retaining loci that are in physical linkage. This thinning interval was based on linkage decay documented in other butterfly species; e.g., linkage decays to baseline within 1 kb–10 kb in Heliconius spp. (Martin et al., 2013) and within 100 bp in Danaus plexippus (Zhan et al., 2014).

Multiple larval samples were often collected from single or adjacent A. dracunculus plants. Full-sibling relationships between individuals are therefore probable due to females ovipositing multiple eggs on single plants, which may bias inferences of genetic divergence and population structure (O’Connell, Mulder, Maldonado, Currie, & Ferraro, 2019). To identify full sibs, we used the package “SNPRelate” (Zheng et al., 2012) implemented in the R environment (v3.5.1; R Core Team) to estimate kinship coefficients for all pairs of sequenced individuals. For diploid organisms, the expected kinship coefficient between full sibs is 0.25. Only pairs of larvae collected from single locations had kinship coefficients greater than 0.22, where a natural break in values occurred. For each of these larval pairs, we removed the individual with the greater percentage of missing data (13 individuals total). We then reverted to the original BAM files, recalled SNPs with Stacks, and filtered VCF files as above using the reduced data set of 161 individuals comprised of 136 adult males, eight adult females, and 17 unsexed larvae.

2.4 Population structure

Two independent methods were used to quantify population structure. We first used the model-based clustering program Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) in a hierarchical fashion (Vähä & Primmer, 2006) to assess K-values ranging from 1 to 10. For the first set of runs in the hierarchical analysis, 10 independent runs were completed for each value of K using the admixture model and correlated allele frequencies. The burnin period and number of Markov chain Monte Carlo (MCMC) repetitions were set to 100,000 and 1,000,000, respectively. Location priors (n = 27 collection sites) were used to inform the MCMC algorithm. The alpha prior (relative admixture levels between populations) was set to 0.5, based on the inverse of the expected value of K = 2 informed by overt clustering in preliminary principal component analysis (PCA; see Figure S1) (Wang, 2017). Two approaches were then used to determine the optimal K from Structure outputs; the ΔK method (Evanno, Regnaut, & Goudet, 2005), implemented in the program Structure Harvester (Earl & vonHoldt, 2012), and the rate of change in the likelihood of
K across K = 1:10 (Pritchard et al., 2000). For the second set of runs in the hierarchical analysis, we completed independent Structure analyses on each cluster identified in the first analysis using settings identical to the first set of runs. Q-values > 0.8 from the first set of runs denoted assignment of individuals to specific clusters, effectively excluding individuals with substantial admixture.

In addition to Structure analyses, we also assessed population structure using discriminant analysis of principal components (DAPC), which conducts discriminant analysis (DA) on principal components (PCs) generated in PCA (Jombart, Devillard, & Balloux, 2010). Assignments of individuals to a priori population clusters for DAPC were inferred using the “find.clusters” function (adegenet package) with default parameters, retaining all PCs, to find the optimal K value based on Bayesian Information Criterion (BIC) scores in successive K-means clustering analysis across K = 1:20. DAPC was then completed using the R package “adegenet” v2.1.1 (Jombart, 2008). We used the “xvalDapc” function (adegenet package), stipulating 100 replicates, to determine the optimal number of PCs to retain in DAPC using stratified cross-validation of DAPC across increasing numbers of principal components (PCs). Missing genotypes were imputed as the mean of the available data per locus for this cross-validation.

2.5 | Habitat suitability

To map habitat suitability for P. m. dodi within our study landscape, we created a habitat suitability model using Maxent software (Phillips, Anderson, & Schapire, 2006), implemented through the R package “dismo” (Hijmans, Phillips, Leathwick, & Elith, 2011). Briefly, Maxent uses machine-learning maximum entropy modelling to predict habitat suitability across a landscape using georeferenced occurrence locations and a set of geographic information systems (GIS) predictor variables (spatial data layers). For georeferenced occurrence locations, we used both the collection locations of the 161 sequenced individuals from this study and georeferenced P. m. dodi occurrences downloaded from the Global Biodiversity Information Facility (GBIF; accessed from https://doi.org/10.15468/dl.axez0s, 5 December 2018). Of the 259 occurrences available from GBIF, 214 were within the study landscape. Geographic and environmental GIS data layers included elevation, a terrain ruggedness index, a heat load index (based on terrain slope and aspect), land cover (12 categories), and three Worldclim 2 (Fick & Hijmans, 2017) bioclimatic variables: mean temperature of warmest quarter (temp.warm), mean temperature of coldest quarter (temp.cool), and mean annual precipitation (precip.). These environmental variables were selected based both on biological relevance and to minimize collinearity (see Data S1 for further details). Each GIS data layer was reprojected to an equal-area projection (NAD83(CSRS98)/UTM zone 12N) at 30-m resolution using the R package “raster” (Hijmans & van Etten, 2012). Further details and sources for GIS data layers may be found in Data S1. Correlation coefficients were less than 0.7 for all pairs of GIS data layers, and so all seven were included in the habitat suitability model.

To evaluate the predicative power of the habitat suitability model, 20% of occurrence localities were withheld for cross-validation and receiver operating characteristic (ROC) analysis (Phillips et al., 2006). Following evaluation, we used the model to predict habitat suitability across the study landscape using the “predict” function (raster package), with each grid cell receiving habitat suitability values ranging from 0–1, where higher values indicate greater habitat suitability. Information on the validity of the Maxent process and its application to P. m. dodi is available in Data S1.

2.6 | Geographic distance

We estimated geographic isolation between sequenced individuals using three different pairwise distance metrics; Euclidean distance, least-cost path distance, and circuit distance. Euclidean distances represent minimal distances required to travel between locations and do not account for landscape characteristics. In contrast, least-cost path distances are estimated by searching for single, optimal routes that minimize cumulative costs associated with travelling through heterogeneous landscapes (Wang et al., 2009). Least-cost path analysis thereby assumes that organisms have complete knowledge of such landscapes and are able to consistently navigate optimal routes. Circuit-based analyses relax this assumption, with distances estimated by summarizing costs associated with all possible paths through heterogeneous landscapes (McRae & Beier, 2007).

Pairwise Euclidean distances between the collection locations of the 161 sequenced individuals were estimated using the “spDists” function in the R package “sp” ( Pebesma & Bivand, 2005). To estimate least-cost path and circuit distances, we first parameterized a resistance surface as the inverse of habitat suitability scores predicted by the habitat suitability model (McRae & Beier, 2007; Storfer et al., 2010; Wang et al., 2008; Wang, Glor, et al., 2012). We then estimated pairwise least-cost path distances using the “costDistance” function in the R package “gdistance” (van Etten, 2018) and pairwise circuit distances using the program Circuitscape 5.0 (McRae, 2006), both with an eight-neighbour connection scheme. To increase computational efficiency, we aggregated the resolution of the resistance surface to 300 m, as connectivity inferences are shown to be generally robust to such aggregations (McRae & Beier, 2007). Collectively, these analyses produced three pairwise matrices of geographic distances.

2.7 | Environmental distance

Environmental isolation between sequenced individuals was estimated using the same Worldclim 2 bioclimatic variables included in the habitat suitability model (temp.warm, temp.cool, and precip.). We extracted values for each of the three bioclimatic variables for collection locations of sequenced individuals using the “extract” function in the R package “raster”. Following Wang, Glor, et al. (2012), environmental distances were estimated by taking absolute
2.8 | Determinants of genetic divergence

We used two types of causal models and linear mixed effects models, each implementing an individual-based (c.f. population-based) approach, to evaluate how variation in geographic and environmental isolation relates to genetic divergence within *P. m. dodi*. To quantify genetic divergence, we first used the "dist" function within the R package "adegenet" to estimate pairwise genetic distance (sum of squared Euclidean distances between *i*th and the *j*th genotype) between sequenced individuals, commensurate with the geographic and environmental distance matrices generated above. This simple, individual-based measure of genetic distance has been shown to effectively quantify genetic divergence in a variety of simulations (e.g., Shirk, Landguth, & Cushman, 2017) and in field studies (e.g., Sánchez-Ramírez et al., 2018).

2.8.1 | Reciprocal causal modelling (RCM)

Our first set of causal models addressed relationships between genetic distance and geographic and environmental distances using reciprocal causal modelling (RCM) with partial Mantel tests (Cushman et al., 2006; Cushman, Wasserman, Landguth, & Shirk, 2013). We used the "mantel.partial" function within the R package "vegan" (Oksanen et al., 2007) to perform partial Mantel tests (999 permutations) for genetic distance and each combination of the six geographic and environmental distances, totalling to 30 tests organized into 15 reciprocal causal models. For each comparison of relationships between genetic distance and two geographic/environmental distances (one reciprocal model), we first estimated the partial Mantel's *R* coefficient (*R*~PM~) between genetic distance and one geographic/environmental distance (focal variable) conditioned on the other geographic/environmental distance (alternative variable), comprising partial Mantel test A. We then estimated the reciprocal *R*~PM~ comprising partial Mantel test B. If *R*~PM-A~ > *R*~PM-B~, the focal variable from partial Mantel test A is better supported, and vice versa. Results of *R*~PM-A~ - *R*~PM-B~ were summarized in a heatmap similar to Ruiz-Gonzalez Cushman, Madeira, Randim, and Gómez-Moliner (2015). Notwithstanding this RCM framework, if both *R*~PM-A~ and *R*~PM-B~ were significant, we inferred partial support for relationships between genetic distance and each of the two geographic or environmental distances used in the reciprocal model.

2.8.2 | Structural equation modelling (SEM)

Our second set of causal models employed structural equation modelling (SEM), a method originally developed by Wright (1921), to quantify the relative strength of effects of geographic distance and environmental distance on genetic distance according to an a priori causal path network. SEM analyses have proven particularly useful for distinguishing effects of multiple collinear variables (e.g., geographic and environmental distances; Grace, 2006; Wang, Glor, et al., 2012). Our causal path network included two regression pathways: one from geographic distance to genetic distance and one from environmental distance to genetic distance. Geographic and environmental distance were linked by a covariance pathway. Results of the RCM analysis were used to infer which single measures of geographic and environmental distance were most strongly related to genetic distance. To test whether geographic and environmental distance contributed meaningfully to observed variation in genetic distance, we compared Akaike’s information criterion (AIC) scores for the full model, a model excluding geographic distance, and a model excluding environmental distance (sensu Wang, Glor, et al., 2012). Lower AIC scores indicated superior model fit. Models with AIC scores exceeding the best supported model by 10 or more points were unsupported (Burnham & Anderson, 1998). SEM analyses were completed using maximum-likelihood estimation in the R package "lavaan" (Rosseel, 2012). To account for nonindependence among pairwise data, we randomly permuted rows and columns of distance matrices to generate null distributions for path coefficients assuming no relationships between variables exist (Fourtune et al., 2018). Unbiased standard errors and *p*-values for path coefficients were then calculated by comparing observed coefficients to their null distributions. This was completed using a modification of the "permutation-based.pathanalysis" R code provided by Fourtune et al. (2018).

2.8.3 | Validation of the causal model framework

To investigate whether inferences from causal models were contingent on method of analysis, we also employed linear mixed effects models with maximum likelihood population effects (MLPE; Clarke, Rothery, & Raybould, 2002). MLPE linear mixed effects models have been shown to be one of the highest performing methods for quantifying relationships between distance matrices while controlling for nonindependence among pairwise data (Shirk et al., 2017). Relationships between genetic distance and each of the six measures of geographic and environmental isolation were quantified in independent models. The identities of sequenced individuals involved in pairwise distance values were included in models as mixed effects to control for nonindependence within distance matrices (Clarke et al., 2002). MLPE linear mixed effects models were fit using the "MLPE.imm" function within the R package "ResistanceGA" (Peterman, 2018). We set REML = FALSE to allow for the estimation of valid AIC scores that were used to evaluate relative model support (Peterman, 2018; Row, Knick, Oyler-McCance, Lougheed, & Fedy, 2017; Shirk et al., 2017).

2.9 | Population divergence of candidate loci

To identify candidate loci under putative divergent selection, we used Bayescan 2.1 (Foll & Gaggiotti, 2008) to estimate allele frequencies and *F*~ST~ values for 1,382 loci. When population assignment
of individuals is sensible, Bayescan is generally recognized as the most effective method for identification of \( F_{ST} \) outlier loci (De Mita et al., 2013; Lotterhos & Whitlock, 2014; Narum & Hess, 2011). Q-values >0.8 from the Structure analysis (\( K = 2 \)) denoted assignment of individuals to either the northern or southern cluster, effectively excluding individuals with substantial admixture from this analysis. We used default parameters to run Bayescan (prior odds set to 10, thinning interval to 10, number of pilot runs to 20, length of pilot runs to 5,000, and burnin length to 50,000), except for the number of outputted iterations, set to 10,000. To reduce the likelihood of false positives associated with multiple tests, we assessed the significance of \( F_{ST} \) outliers using q-values generated by Bayescan according to the False Discover Rate (FDR) criterion (Benjamini & Hochberg, 1995). \( F_{ST} \) outlier loci were identified using a q-value-threshold of 0.05. Fifteen Bayescan runs were completed using this protocol and a union of the resulting lists of \( F_{ST} \) outlier loci was taken to generate a final list of loci under putative divergent selection.

We also used the "snpzip" function (adegenet package) with default settings to identify which loci contributed most significantly to between-population structure in DAPC, with population assignment based on K-means clustering analysis. This analysis uses the relative contribution of each SNP to DAPC to perform hierarchical clustering and classify loci as either “structural” or “nonstructural”.

### 2.9.1 Environmental associations of individual loci

While Bayescan is effective for identifying loci under putative divergent selection among discrete populations, an individual-based approach may be more effective for identifying candidate loci potentially under selection across environmental gradients (Frichot, Schoville, Bouchard, & François, 2013). To accomplish this, we used latent factor mixed modelling (LFMM), implemented in LFMM 1.3 (Frichot et al., 2013) via the R package “LEA” (Frichot & François, 2015). LFMM assesses correlations between allele frequencies of individual loci and environmental variables (each included in an independent model as a fixed effect) while controlling for background population structure using latent factors equal in number to the optimal value of \( K \). This reduces the likelihood of false positives arising from spurious relationships between allele frequencies and environmental variables due to autocorrelation of space, demography, and the environment (Frichot et al., 2013; Lotterhos & Whitlock, 2014), which can be problematic for other analysis methods, such as those employed in BayEnv2 (Günther & Coop, 2013; Lotterhos & Whitlock, 2014).

Environmental variables included in LFMM analyses were temp. warm, temp.cool, and precip.. We completed five independent LFMM runs with 10,000 iterations and a burnin of 5,000, stipulating two latent factors (\( K = 2 \) inferred from both Structure and DAPC/K-means clustering analyses). Results were then combined by calculating the median \(|z|\) scores across the five LFMM runs, which represent the strength of the genetic-environment association for each locus. To validate the number of latent factors used in LFMM, we visually inspected adjusted \( p \)-values histograms for each environmental variable, estimated using the genomic inflation factor (\( \lambda \)) procedure (Devlin & Roeder, 1999). Distributions that are relatively flat with a peak near zero indicate the selected number of latent factors adequately controlled for potentially confounding effects of spatial genetic structure (Frichot & François, 2015). Finally, to control for false positives associated with multiple tests, we again used the FDR criterion (Benjamini & Hochberg, 1995), producing q-values for each association. Loci with q-values <0.05 were inferred as having significant environmental associations.

### 2.9.2 Genomic contexts of candidate loci

To map the location of candidate loci within the \( P. \ m. \ dodi \) genome, we used BEDTools v2.27.1 (Quinlan & Hall, 2010) to extract 5 kb of flanking sequence on the 5’ and 3’ ends of each candidate locus identified by either Bayescan or LFMM analyses. This length of flanking sequence was selected in reference to previous thinning of loci within 10 kb of each other. We then used the BLAST function within Lepbase (Challis, Kumar, Dasmahapatra, Jiggins, & Blaxter, 2016) to match resulting sequences to annotated genes within Lepbase’s butterfly and moth CDS databases. As this search queried multiple species’ genomes, we evaluated possible interspecific matches based on percent match of the query, phylogenetic distance to the matched species, and the number of distinct genomes (multiple species) in which each gene was reported. Putative gene functions were compiled from the UniProt Consortium (2018) using gene accession codes included within the Lepbase output.

### 3 RESULTS

ddRAD sequencing resulted in a total of 293,036,249 single-end, 75-bp reads across the original set of 180 sequenced individuals. After running “process_radtags” and associated filters, 273,664,014 reads remained, of which, 192,902,810 were aligned to the \( P. \ machaon \) reference genome. After removing individuals with > 0% missing data and putative full-sibs, 108,049,831 reads were used to call 104,038 SNPs for the final set of 161 sequenced individuals. Filtering of loci resulted in a total of 1,382 SNPs with a mean read depth of 71.99 (min = 11.76, max = 1,950.7), comprising the data set used in all subsequent analyses.

#### 3.1 Population structure

Our first set of Structure runs predicted an optimal \( K \)-value of \( K = 2 \) using both the \( \Delta K \) method (Evanno et al., 2005) and the rate of change in the likelihood of \( K \) across \( K = 1:10 \) (Pritchard et al., 2000; see Data S1, Figure S2a). Individuals collected near and north of Dorothy, Alberta, were generally assigned to a northern cluster, while individuals collected within and south of Dinosaur Provincial Park, Alberta, were assigned to a southern cluster (Figure 2). Spatial discordance of
two individuals’ cluster assignments (i.e., migrants found in non-natal populations) suggests that dispersal between the regions occurs. Nine individuals had an approximate 50/50 split of Q-values, suggesting that some hybridization between migrant and natal individuals occurs. However, little admixture was observed beyond these putative F1 hybrids. In the second set of Structure runs, no subclustering was evident in the northern cluster, while the existence of two subclusters was best supported within the southern cluster (Data S1, Figure S2b and c). Q-values for individuals of these two subclusters (K = 2 in the southern cluster only analysis) were very similar to those for K = 3 in the first set of runs (including all individuals). For simplicity, we therefore present admixture plots from the first set of Structure runs (all individuals) for both K = 2 and K = 3 (Figure 2).

Similar to the first set of Structure runs, K-means clustering analysis suggested K = 2 was best supported. An optimal number of 20 PCs was retained for DAPC. Assignments of individuals to northern and southern clusters were nearly identical between the first set of Structure runs and DAPC/K-means clustering analysis, save four admixed individuals with Q-values around 0.5 that were assigned to the southern cluster by Structure (based on Q-values > 0.5) and the northern cluster by DAPC/K-means clustering analysis.

3.2 | Habitat suitability

The Maxent habitat suitability model adequately predicted habitat suitability across our study landscape, indicated by an out-of-sample AUC score of 0.948. As hypothesized, high suitability generally followed the eroding banks of major rivers, taking on the form of a dendritic ecological network (Figure 1, inset h). Contributions of each variable to the habitat suitability model were estimated by measuring the drop in AUC after values of each variable were randomly permuted (permutational importance): terrain ruggedness = 47.7, temp. cool = 34.2, elevation = 4.8, precip. = 4.4, temp.warm = 0.2, and heat load = 0.1. A resistance surface parameterized as the inverse of habitat suitability scores permitted estimation of least-cost path and circuit distances (Figure 1, insets j and k).

3.3 | Determinants of genetic divergence

3.3.1 | Reciprocal causal modelling (RCM)

Results of RCM are summarized in a heatmap (Figure 3), with red and blue colours indicating positive and negative values for \( R_{PM-A} - R_{PM-B} \) respectively (sensu Ruiz-Gonzalez et al., 2015). Focal and alternative variables used in partial Mantel test A for each reciprocal model are on the y- and x-axes, respectively. For ease of interpretation, variables on the y-axis with more positive (red) values in their corresponding rows are better supported. Overall, the strongest correlates of genetic distance after partialling out relationships with alternative variables were Euclidean distance and temp.warm distance. Euclidean distance was significantly correlated with genetic distance after partialling out temp.warm distance (\( R_{PM} = 0.23; \)

[FIGURE 2 Population genetic structure within Papilio machaon dodi in Alberta and Saskatchewan, Canada, inferred using the model-based clustering program Structure. An optimal K value of 2 was best supported by the \( \Delta K \) method and rate of change in the likelihood of K across K = 1:10 for the first set of Structure runs addressing all individuals. Hierarchical runs addressing the northern and southern clusters independently suggested no overt subclustering within the northern cluster and the existence of two subclusters within the southern cluster. We present admixture plots derived from the first set of Structure runs for both K = 2 (exhibiting the two primary clusters) and K = 3 (including the two southern subclusters) for simplicity. For K = 2, individuals collected near and north of Dorothy, Alberta, were generally assigned to a northern cluster, while individuals collected within and south of Dinosaur Provincial Park, Alberta, were assigned to a southern cluster [Colour figure can be viewed at wileyonlinelibrary.com] ]
and the reciprocal partial Mantel test was also significant ($R_{PM} = 0.19; p = .001$). All other partial Mantel tests using either the Euclidean or temp.warm distances as alternative variables were not significant, indicating other measures of geographic and environmental distances were unsupported.

### 3.3.2 Structural equation modelling (SEM)

Based on results of RCM analysis, Euclidean distance and temp.warm distance were used as single measures of geographic and environmental distance, respectively, in our causal path network. The full model, including direct paths from both geographic distance (Euclidean distance) and environmental distance (temp.warm distance) to genetic distance, was better supported than alternative models excluding either geographic or environmental distance (Table 1). Path coefficients for Euclidean distance and temp.warm distance were 0.120 ($p < .001$) and 0.331 ($p < .001$), respectively, suggesting each is positively related to genetic distance. Covariance of the two predictor variables in the model was 0.592; thus, relative effect sizes should be interpreted with caution. However, model selection based on AIC indicated that variation in Euclidean distance and temp.warm distance each have important relationships to variation in genetic distance beyond that associated with their covariance structure.

### 3.3.3 Validation of the causal model framework

Results of MLPE linear mixed effects models aligned with those of our causal models. Overall, the best supported variable affecting genetic distance was temp.warm distance followed by Euclidean distance (Table 2). This result supported the use of Euclidean and temp.warm distances as single distance measures for geographic and environmental distances, respectively, in SEMs. AIC scores for MLPE linear mixed effects models with Euclidean and temp.warm distances were >2 points lower than all other models, suggesting that alternative measures of geographic and environmental isolation were unsupported (Burnham & Anderson, 1998).

### 3.4 Population divergence of candidate loci

All 15 independent Bayescan runs identified the same list of 33 outlier loci based on elevated $F_{ST}$ values (2.39% of 1,382 SNPs). Positive alpha values for each of these 33 loci indicated they were under putative divergent selection, rather than purifying or balancing selection. To visualize the data, $-\log_{10}$ q-values were plotted against $F_{ST}$ values estimated in the first Bayescan run for all 1,382 SNPs (see Data S1, Figure S3). The mean of $F_{ST}$ values for putative outlier loci was 0.169 ($SD = 0.092$), ranging from 0.030 to 0.272. The “snpzip” function (adegenet package) identified 17 structural loci that significantly contributed to between-population structure in DAPC. Each of these 17 structural loci were contained within the list of 33 candidate loci identified by Bayescan.

### 3.5 Environmental associations of individual loci

Histograms of adjusted $p$-values were uniformly distributed with a peak near zero for all three environmental variables, suggesting

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**FIGURE 3** Pairwise heatmap visualizing reciprocal causal modelling (RCM) results. Values in each cell represent results of $R_{PM,A} - R_{PM,B}$, with red and blue colours indicating positive and negative values, respectively. Rows and columns contain the focal and alternative variables, respectively, for partial Mantel test A within each reciprocal model. Therefore, the figure should be interpreted by rows and not columns; variables on the y-axis with more positive (red) values in their corresponding rows are better supported [Colour figure can be viewed at wileyonlinelibrary.com]
associations. While Bayescan identified 8 candidate loci with temp.warm, 0 with temp.cool, and 2 with precip. LFMM identified 56 loci with temp.warm distance, respectively). Single loci often had multiple significant environmental associations, probably due to spatial correlation of environmental variables. We therefore only considered the strongest association for each locus based on median z-scores (De Kort, Vandepitte, Mergeay, Mijnsbrugge, & Honnay, 2015; Martins et al., 2018). In total, 52 of 57 variables were most strongly related to genetic distance and used in place of geographic and environmental distance (Euclidean distance and temp. warm distance, respectively).

### Table 1: Relative support for structural equation models (SEMs) inferred using Akaike's information criterion (AIC)

<table>
<thead>
<tr>
<th>SEM structure</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model</td>
<td>34,147.52</td>
<td>0</td>
</tr>
<tr>
<td>Environment only</td>
<td>34,288.77</td>
<td>141.25</td>
</tr>
<tr>
<td>Geography only</td>
<td>35,203.77</td>
<td>1,056.25</td>
</tr>
</tbody>
</table>

Note: Akaike's information criterion (AIC) scores are reported for each model.

K = 2 adequately controlled for confounding effects of spatial genetic structure (see Data S1, Figure S4). LFMM identified a total of 78 loci with significant environmental associations (q-values <0.05; Figure 4). Single loci often had multiple significant environmental associations, probably due to spatial correlation of environmental variables. We therefore only considered the strongest association for each locus based on median z-scores (De Kort, Vandepitte, Mergeay, Mijnsbrugge, & Honnay, 2015; Martins et al., 2018). In total, 52 of 57 loci significantly associated with temp.warm were more strongly associated than any other environmental variable, 12 of 27 for temp.cool, and 14 of 43 for precip. A total of 25 loci were identified by both LFMM and Bayescan analyses as being under putative selection; 23 of these loci were most strongly associated with temp. warm, 0 with temp.cool, and 2 with precip. LFMM identified 56 loci with significant environmental associations that were not identified as candidate loci by Bayescan, while Bayescan identified 8 candidate loci that were not identified by LFMM as having significant environmental associations.

#### Table 2: Relative support for effects of geographic and environmental distances on genetic distance inferred using linear mixed effects models with maximum likelihood population effects (MLPE)

<table>
<thead>
<tr>
<th>Distance measure</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.warm</td>
<td>32,955.55</td>
<td>0</td>
</tr>
<tr>
<td>Euclidean</td>
<td>33,147.24</td>
<td>191.69</td>
</tr>
<tr>
<td>Circuit</td>
<td>33,152.41</td>
<td>196.86</td>
</tr>
<tr>
<td>Least-cost</td>
<td>34,623.89</td>
<td>1,668.34</td>
</tr>
<tr>
<td>Precip.</td>
<td>35,055.58</td>
<td>2,100.03</td>
</tr>
<tr>
<td>Temp.cool</td>
<td>35,590.9</td>
<td>2,635.35</td>
</tr>
</tbody>
</table>

Note: Akaike's information criterion (AIC) scores are reported for each model.

In addition to mating and reproduction associated with adult life stages of invertebrates, it may be instructive to think of adult P. m. dodi as dispersal specialists within the taxon’s life cycle, with greater vagility and more general habitat tolerances than larval stages. Such traits enable long-distance dispersal of adults across considerable stretches of unsuitable habitat, implicated in the spatial discordance

### 4. Discussion

#### 4.1 Geographic isolation and dispersal machines

Overt clustering of P. m. dodi occurrences along major river valleys in southern Canada is generally understood to be a function of both the ecologically restricted occurrences of its larval host plant, A. dracunculus, and the presence of hilltopping features along river valley edges (Bird et al., 1995; Dupuis et al., 2019; Sperling, 1987). Accordingly, results of our habitat suitability model support the inference that suitable habitat takes on the form of a dendritic ecological network, with the intervening landscape primarily comprised of unsuitable habitat. Examination of a corresponding cost surface (Figure 1, inset j) shows that individuals dispersing between river valleys are predicted to experience high resistance/costs. IBR based on habitat suitability therefore predicts that dispersal and resultant gene flow should follow the dendritic ecological network (Figure 1, insets j and k). However, both RCM and MLPE linear mixed effects models suggested that variation in genetic distance was better explained by Euclidean distance than by resistance-based distances accounting for arrangements of suitable habitat. It is possible that other landscape features, beyond configurations of suitable habitat, influence dispersal of P. m. dodi and thereby support IBR. For example, Peterman et al. (2014) outlines a methodological approach for optimizing resistance surfaces using a nonlinear optimization algorithm and any combination of spatial variables. However, we did not have a priori mechanistic hypotheses predicting what variables might influence P. m. dodi dispersal beyond those associated with habitat suitability. Lack of support for IBR based on habitat suitability in this system was of principal interest and demonstrates a clear decoupling of landscape permeability from habitat suitability (c.f. Coyne & Orr, 2004; Crispo et al., 2006; McRae, 2006; McRae & Beier, 2007; Thorpe et al., 2008; Wang et al., 2009; Sánchez-Ramírez et al., 2018).

In addition to matting and reproduction associated with adult life stages of invertebrates, it may be instructive to think of adult P. m. dodi as dispersal specialists within the taxon’s life cycle, with greater vagility and more general habitat tolerances than larval stages.
of the cluster assignments of three adult individuals (i.e. migrants found in non-natal populations; see Figure 2 and Data S1, Figure S1). Indeed, for many vagile butterfly species occupying fragmented landscapes, instances of long-distance dispersal between patches or corridors of suitable habitat are rare, but are known to have important consequences for gene flow, metapopulation dynamics, and emergent diversity patterns (Hanski, 1998; MacDonald, Anderson, Acorn, & Nielsen, 2018; Nowicki et al., 2014; Wiens 2001). We therefore propose that habitat suitability and landscape permeability should be evaluated as distinct concepts for taxa with discrete dispersal life stages, even if they are habitat specialists. As a mental shortcut, we suggest a “dispersal machine” concept, similar to Dawkins’s (1976) selfish gene perspective on evolution by natural selection, in which genes metaphorically build “survival machines” (i.e., bodies of organisms) to facilitate their own stability and replication. In landscape genetics, it may be instructive to understand adult life stages of many terrestrial invertebrates as not only the life stage in which mating and reproduction occur, but also as “dispersal machines,” exhibiting greater vagility and broader habitat tolerances than larval life stages. Such characteristics are likely to facilitate long-distance dispersal resulting in gene flow across heterogeneous landscapes. As a consequence of the dispersal machine concept, considerable support for IBR in past research cannot necessarily be extrapolated to organisms with disparate life histories; particularly, if the life cycles of focal taxa include a discrete dispersal life stage with substantially different habitat constraints than other life stages.

4.2 Environmental isolation

Beyond the effects of geographic isolation on genetic divergence, this study provides multiple lines of evidence for strong associations between genetic variation and environmental conditions. First, analyses of population structure within P. m. dodi indicated the presence of two prominent genetic clusters, with their spatial separation corresponding to a cline in mean temperature of the warmest quarter (temp.warm). Although any correlative relationship between spatial genetic structure and an environmental variable may be a product of spatial autocorrelation with a third unconsidered but causative variable, our results accord our a priori hypotheses relating summer temperatures to variation in phenology, diapause propensity, and voltinism within P. m. dodi, suggesting this relationship is both
meaningful and worthy of further consideration. Second, RCM, SEM, and MLPE linear mixed effects models indicated that genetic distance among individuals was strongly associated with environmental distance (specifically, temp.warm distance) beyond its covariance structure shared with geographic distance (specifically, Euclidean distance). Finally, Bayescan identified 33 $F_{ST}$ outlier loci inferred to be under putative spatially divergent selection between the two primary genetic clusters. Of these 33 $F_{ST}$ outlier loci, 23 were also identified by LFMM analysis as having allele frequencies that were significantly associated with temp.warm values, meaning most loci identified as being under putative spatially divergent selection were also significantly associated with variation in summer temperatures.

Referring back to our original hypotheses, mechanisms by which environmental isolation structures genetic divergence may be inferred from patterns of population structure. Spatial discordance of a few individuals’ cluster assignments suggests that some dispersal of adult individuals between natal and non-natal regions of the study area occurs (and see Dupuis & Sperling, 2016). Additionally, some admixture indicative of F1 hybridization between migrant and natal individuals is evident (individuals with ~ 50/50 split of $Q$-values in Structure plots). However, further admixture among the two primary genetic clusters was not prevalent, possibly due to reduced fitness and negative selection on admixed genotypes. We therefore infer that mechanisms by which environmental isolation contributes to genetic divergence in *P. m. dodi* may be a combination of reduced fitness and negative selection on both individuals that have dispersed across environmental gradients and genetically intermediate individuals resulting from hybridization.

### 4.3 Adaptation to local environmental conditions

Our combination of analyses suggests that variation in environmental conditions has significant effects on genetic structure within *P. m. dodi*, possibly attributed to divergent selection across environmental gradients resulting in local adaptation. BLAST searches of candidate loci (both $F_{ST}$ outliers and loci with significant associations) and their flanking sequences did not resolve annotated genes with biological functions sufficient to justify specific narratives of local adaptation. However, a total of 52 loci were significantly and most strongly associated with mean temperature of the warmest quarter (temp.warm)—the period in which development, reproduction, and diapause initiation occur. Nineteen of these 52 loci were evenly distributed along a single scaffold (NW_014538813.1, length 6.9 Mb) within the *P. machaon* reference genome, corresponding to the spike in $-\log_{10}$ $q$-values observed in LFMM analysis (Figure 1, inset a). Seventeen of these 19 loci were identified by Bayescan as being under putative divergent selection based on elevated $F_{ST}$ values. Considered together, these results suggest the existence of an island of genomic differentiation (sensu Harr, 2006; Turner, Hahn, & Nuzhdin, 2005) between northern and southern *P. m. dodi* populations, possibly corresponding to local adaptation to environmental conditions. However, the relatively low quality of the *P. machaon* genome assembly (>60 thousand scaffolds) precludes more in-depth comparative genomic analyses of this region.

Local adaptation to environmental conditions has been documented in a number of butterfly species, with variation in voltinism and diapause propensity across environmental gradients being a focal point of past work (e.g., Aalberg Haugen & Gotthard, 2015; Friberg, Bergman, Kullberg, Wahlberg, & Wiklund, 2008; Pruisscher, Nylin, Gotthard, & Wheat, 2018; Ryan, Vailla, Thivierge, Aardema, & Scriber, 2018). *Papilio machaon dodi* is known to exhibit variation in voltinism and diapause propensity across its Canadian range, with northern and southern populations exhibiting one and two generations per year, respectively (Bird et al., 1995; Sperling, 1987). Additionally, we have noted that a proportion (~25%) of pupae reared from northern populations (collected near Drumbelle, AB) require two distinct cooling cycles before emergence occurs, while pupae reared from southern populations (collected near Taber, AB) consistently emerge after a single cooling cycle (unpublished data, and see Dupuis et al., 2016; Sperling, 1987). We hypothesize that this facultative second diapause in northern populations represents an ecological “hedging of bets” (sensu Seger & Brockmann, 1987), distributing risks of high mortality and low fecundity due to poor environmental conditions across multiple years (Dupuis et al., 2016; Hanski, 1988; Tuljapurkar, 1990). Specific mechanisms by which variation in voltinism and diapause propensity might contribute to divergent selection between northern and southern *P. m. dodi* populations are not entirely clear, but there are several possibilities. Following hybridization between natal and migrant individuals in the northern extent of the study area, genetically intermediate offspring may experience high mortality if over-winter diapause is not induced in the first generation, as individuals of a second generation will lack sufficient day-degree accumulation and resources to complete their life cycle and enter over-winter diapause before temperatures drop to lethal levels. Conversely, genetically intermediate individuals emerging in the southern extent of the study area may experience reduced fecundity and fitness relative to natal individuals if only one generation of genetically intermediate offspring emerge annually and/or a proportion of genetically intermediate individuals undergo a second diapause, resulting in a partially semivoltine lifecycle. By these mechanisms, divergent selection on voltinism and diapause propensity may maintain the integrity of the two genetic clusters. However, further work is required to evaluate the validity of these hypotheses.

### 4.4 Environmental determinants of genetic diversity versus species occurrence

The environmental variables that were most strongly associated with genetic variation in *P. m. dodi* (temp.warm, followed by precip., followed by temp.cool) differed from those that best predicted *P. m. dodi* occurrences in our habitat suitability model (temp.cool, followed by precip., followed by temp.warm). While summer temperatures may influence population structure in *P. m. dodi* via spatially
divergent selection related to phenology, diapause propensity, and voltinism, winter temperatures may influence habitat suitability and limit the range of *P. m. dodi* due to limited cold tolerance of pupae. Indeed, winter temperatures have been inferred to limit ranges of other congeneric species (e.g., Kukal, Ayres, & Scriber, 1991; Scriber, Maher, & Aardema, 2012; Yoshio & Ishii, 2001). Considered together, our inferences suggest that the environmental/ecological conditions that influence divergent selection and possibly facilitate ecological speciation (Foll & Gaggiotti, 2006; Nosil, 2012; Thorpe et al., 2008) may differ from those that limit species’ ranges and structure emergent patterns of species diversity (e.g., due to environmental filtering; sensu Kraft et al., 2015). Landscape genetic analyses, comparing the environmental/ecological conditions that influence divergent selection to those that limit species’ ranges, are required for multiple species to assess the generality of this finding.

### 4.5 Anticipated changes to genetic structure in *Papilio machaon dodi*

Currently, no members of the *P. machaon* species group are listed as being of conservation concern in Canada. However, recognition of cryptic evolutionary significant units (sensu Ryder; 1986), such as the northern and southern genetic clusters identified in this study, may affect future conservation directives in light of continued climate change. Our study resolved that a northern genetic cluster, which includes the type specimen for *P. m. dodi* (Kondla, 1981), is geographically restricted and occupies a climatic niche that is distinct from more southerly populations in Alberta and Saskatchewan. If genetic divergence between the northern and southern genetic clusters is indeed driven by local adaptation to environmental conditions, continued climate change and rising summer temperatures may lead to the displacement of the northern genetic cluster as genotypes and associated phenological traits of southern populations become more advantageous across the northern extent of the range of *P. m. dodi*.

Quantitative data support these predictions. Mean temperature of the warmest quarter differed by an average of 1.60°C between collection locations of individuals belonging to the northern and southern genetic clusters (excluding migrant individuals). Based on the ClimateWNA model (Wang, Hamann, Spittlehouse, & Murdock, 2012), which provides climate data from 24 general circulation models, mean annual temperature for Alberta is predicted to rise by 2.8°C–4.2°C by the end of the century, contingent on emission scenarios (Schneider, 2013). Accordingly, growing degree-days, based on a break point of 5°C, are estimated to increase 33%-56% (Schneider, 2013). Within Alberta and Saskatchewan, changes in mean annual temperature, growing degree-days, and vegetation composition are expected to be most pronounced in central and southern regions, including the present-day range of *P. m. dodi* (Barber, Nielsen, & Hamann, 2016; Schneider, 2013; Zhang, Nielsen, Stolar, Chen, & Thuiller, 2015). There is some evidence that vagile North American butterfly species may track their climatic niches poleward as temperatures warm; however, more often than not, these range expansions are not sufficient to offset contractions toward the equator (Lewthwaite et al., 2018). Indeed, there exist few opportunities for *P. m. dodi* to track its climatic niche northward as temperatures rise. Steep south-facing riverbanks that might provide adequate *A. dracunculus* habitat are sparse north of the current range of *P. m. dodi* and successional changes to the composition of riverbank vegetation that could provide suitable habitat are unlikely to match the pace of the southern genetic cluster’s northward expansion. We therefore hypothesize that genotypes unique to the northern genetic cluster may be displaced by the end of the century.

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### DATA AVAILABILITY STATEMENT

DNA sequences in fastq format: Genbank accessions: SAMN13811887 - SAMN13812047; NCBI SRA: PRJNA600290. Geographic data, environmental data, and Maxent input files: Dryad https://doi.org/10.5061/dryad.w0v4b8ms

Sampling locations of sequenced individuals: Dryad https://doi.org/10.5061/dryad.w0v4b8ms

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### REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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