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A test of genomic modularity among life-history adaptations promoting speciation with gene flow

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Abstract

Speciation with gene flow may require adaptive divergence of multiple traits to generate strong ecologically based reproductive isolation. Extensive negative pleiotropy or physical linkage of genes in the wrong phase affecting these diverging traits may therefore hinder speciation, while genetic independence or "modularity" among phenotypic traits may reduce constraints and facilitate divergence. Here, we test whether the genetics underlying two components of diapause life history, initial diapause intensity and diapause termination timing, constrain differentiation between sympatric hawthorn and apple-infesting host races of the fly *Rhagoletis pomonella* through analysis of 10,256 SNPs measured via genotyping-by-sequencing (GBS). Loci genetically associated with diapause termination timing were mainly observed for SNPs mapping to chromosomes 1–3 in the genome, most notably for SNPs displaying higher levels of linkage disequilibrium (LD), likely due to inversions. In contrast, selection on initial diapause intensity affected loci on all five major chromosomes of the genome, specifically those showing low levels of LD. This lack of overlap in genetically associated loci suggests that the two diapause phenotypes are largely modular. On chromosome 2, however, intermediate level LD loci and a subgroup of high LD loci displayed significant negative relationships between initial diapause intensity and diapause termination time. These gene regions on chromosome 2 therefore affected both traits, while most regions were largely independent. Moreover, loci associated with both measured traits also tended to exhibit highly divergent allele frequencies between the host races. Thus, the presence of nonoverlapping genetic modules likely facilitates simultaneous, adaptive divergence for the measured life-history components.

KEYWORDS
adaptation, apple maggot fly, diapause, genomics of speciation, inversions, linkage disequilibrium, *Rhagoletis pomonella*, speciation with gene flow

1 | INTRODUCTION

Speciation occurs as genetically based barriers to gene flow evolve between formerly interbreeding populations (Coyne & Orr, 2004). During ecological speciation, these barriers initially evolve as a consequence of divergent natural selection acting to differentially adapt populations to alternate habitats or environments (Nosil, 2012; Schluter, 2009). When ecological speciation occurs with gene flow in

sympatry, parapatry or following secondary contact, it is likely that multiple environmental factors differentially affect fitness through multiple phenotypic traits (Nosil & Sandoval, 2008; Rice & Hostert, 1993). Divergent selection along just one ecological axis or acting on just one trait may not be sufficient to overcome the homogenizing effects of gene flow and lead to speciation. But selection on multiple traits may effectively reduce gene flow such that much of the genome becomes impervious to introgression as speciation proceeds (Feder et al., 2014a; Flaxman, Feder, & Nosil, 2013; Flaxman, Wacholder, Feder, & Nosil, 2014; Nosil, Harmon, & Seehausen, 2009; Rice & Hostert, 1993).

Genetic and developmental correlations among traits, however, can affect their phenotypic responses to selection (Arnold, 1992). Developmental constraints may impede divergence if negative pleiotropy exists such that adaptive changes in one trait towards a fitness optimum result in correlated changes in other phenotypes that are maladaptive. Genetic constraints can impede divergence through pleiotropy or through linkage, wherein alleles underlying adaptation are tightly physically linked with one another in opposite phase. However, genetic independence or "modularity" among selected traits can facilitate adaptive divergence and ecological speciation (Wagner, Pavlicev, & Cheverud, 2007; Weber, Peterson, & Hoekstra, 2013). Here, we test for such modularity through a genomic analysis of different traits associated with diapause life-history timing between ancestral hawthorn and recently derived apple-infesting host races of the fly *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), a model for the study of the early stages of ecological speciation with gene flow (Funk, Filchak, & Feder, 2002).

Previous studies have shown that divergent selection acting on diapause timing is important to the ecological divergence of *R. pomonella*, causing allochronic reproductive isolation (RI) between populations infesting host plants fruiting at different times of the season (Feder, Hunt, & Bush, 1993; Data S1). *Rhagoletis pomonella* historically attacked hawthorn (*Crataegus* spp.) fruits, but novel populations, or host races, have formed in the last 160 years that emerge earlier to infest introduced, domesticated apple (*Malus pumila*) that fruit 3–4 weeks earlier in the season (Feder, Chilcote, & Bush, 1988; Feder & Filchak, 1999; McPheron, Smith, & Berlocher, 1988; Smith, 1988). *Rhagoletis* is univoltine, and adults live for about a month posteclosion in nature (Dean & Chapman, 1973). The fly overwinters in a metabolically and developmentally suppressed pupal diapause, although exposure to prolonged heating can cause *Rhagoletis* to forgo diapause (Prokopy, 1968; Figure 1a), which in turn leads to assured mortality due to lack of host fruit or harsh winter temperatures (Feder, Roethele, Wlazlo, & Berlocher, 1997).

The difference in host-related environmental conditions experienced by apple and hawthorn flies drives divergent selection on two diapause phenotypes. The first is initial diapause *intensity* or the ability to remain in diapause despite permissive conditions (i.e., refractoriness to nondiapause development). The apple race appears to have a greater initial diapause intensity because earlier phenology leads to prolonged exposure to warm temperatures, which selects more strongly against nondiapause development compared to the hawthorn race (Dambroski & Feder, 2007; Egan et al., 2015). The second phenotype is diapause *termination*, or the resumption of pharate adult (post pupal) development that sets the timing of adult eclosion. Natural selection favours earlier eclosion in apple vs. hawthorn flies to exploit the seasonally earlier apple fruit resource. The extent to which common molecular machinery affects each diapause developmental phase (i.e., the evolutionary independence or modularity of phases) is unknown. If the same sets of genes underlie such common mechanisms, evolutionary responses may be constrained, thereby hindering seasonal adaptation of insects to climate change or to novel hosts (Bale & Hayward 2010; Danks 1987; Tauber, Tauber, & Masaki, 1986).

Here, we examine the modular nature of genetic responses to "multifarious" divergent selection (Rice & Hostert, 1993) on diapause development and the resulting genomic footprint in *R. pomonella*. We address two primary questions: (i) Are loci associated with initial diapause intensity and diapause termination timing largely independent or do they have correlated phenotypic effects and (ii) to what extent do these two traits independently and jointly contribute to genome-wide differentiation between apple and hawthorn flies in nature. We combined a genotyping-by-sequencing (GBS) survey of 10,256 single nucleotide polymorphisms (SNPs) between co-occurring populations of apple and hawthorn flies at a field site near Grant, MI, with an association study of the timing of adult eclosion (Figure 1b), and an experiment artificially selecting on diapause intensity to identify loci associated with diapause intensity (Figure 1c; Egan et al., 2015). Previous studies suggest that both diapause phenotypes may have genome-wide consequences for divergence (Feder, Roethele, Filchak, Niedbalski, & Romero-Severson, 2003; Feder, Roethele et al., 1997; Feder, Stolz, et al., 1997; Michel et al., 2010). However, these inferences have, to date, been largely based on a relatively small number of markers (six allozymes and/or 33 microsatellites) or have considered only the single trait of diapause intensity (Egan et al., 2015). Here, we conduct a GBS survey of *R. pomonella* to investigate the relationship between diapause intensity and diapause termination timing, two critical phenotypes diverging between the fly host races.

Our rationale is that if initial diapause intensity and diapause termination timing are highly genetically correlated traits (either due to pleiotropy or physical linkage), then the same SNPs or gene regions should be associated with both phenotypes (Figure 1d). If not, then separate genetic modules may control different phases of diapause and different genomic regions should be associated with each phenotype (Figure 1d). Genetic associations with the two phenotypes can then be compared to allele frequency differences observed between apple and hawthorn fly populations at sympatric sites where both races co-occur with low but continuous gene flow (Feder et al., 1994). This comparison allows assessment of the degree to which divergent diapause adaptation generates genome-wide differentiation in nature, testing whether relationships of SNPs with life-history timing phenotypes in the laboratory translate into genetic divergence between the host races and, hence, reductions in gene flow that cause ecologically based allochronic reproductive isolation in the field.

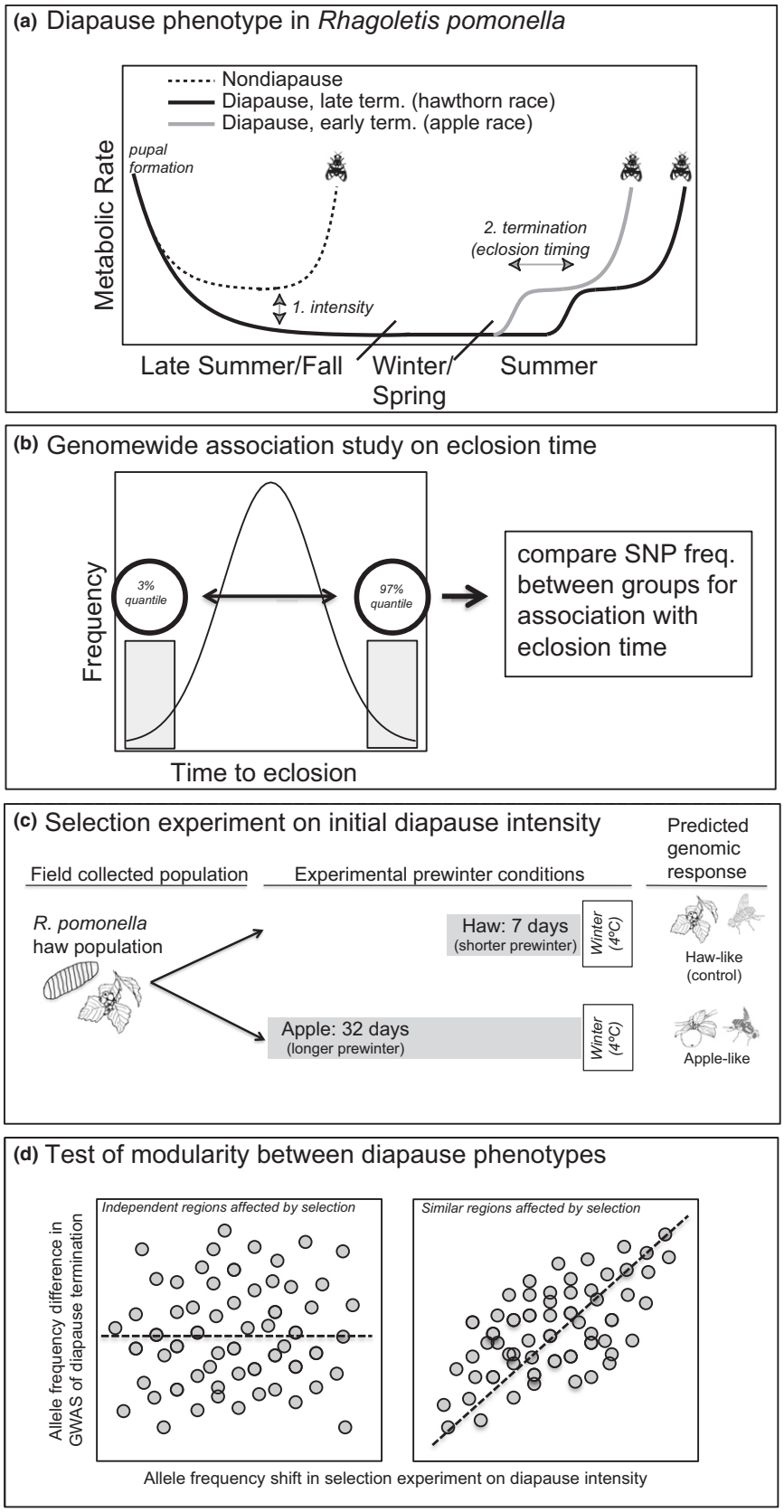

FIGURE 1 Conceptual diagram of diapause life-history phenotypes and experimental design. (a) Two diapause-related phenotypes are under divergent selection between host races: (1) initial diapause intensity, or the propensity to forego diapause and directly develop to adulthood under prolonged warm temperature exposure prior to winter (nondiapause phenotype) and (2) the timing of diapause termination and, thus, adult eclosion time, marked by a rapid, biphasic increase in metabolic rate (Ragland, Egan, Feder, Berlocher, & Hahn, 2011). (b) Eclosion time association study with the earliest and latest extremes of eclosing apple and hawthorn fly adults (the lower 3% and upper 97% quantiles) being genetically analysed for differences by GBS. (c) Design of selection experiment on hawthorn flies for greater initial diapause depth in which the prewinter period was lengthened from 7 to 32 days to emulate the difference between the later fruiting hawthorn and earlier fruiting apple hosts. (d) Test for modularity in diapause life-history traits. Eclosion time and initial diapause depth represent independent evolutionary modules if the two traits show limited genomic correlation (on left). Alternatively, eclosion time and initial diapause depth are potentially pleiotropically and/or developmentally constrained if they display a high degree of genomic correlation (on right)

Linkage disequilibrium (LD) will contribute to genetic correlations and will also affect adaptive divergence and restrict gene flow during ecological speciation, especially if structural features such as inversions inhibit recombination among selected blocks of genes (Feder, Flaxman, & Nosil, 2014; Joron et al., 2011; Lowry & Willis, 2010). This may be the case in *R. pomonella*, where many allozymes,

cDNA and microsatellites associated with host divergence appear to reside in regions inferred by genetic crosses to contain inversions (Feder, Chilcote, & Bush, 1989; Feder, Roethele, et al., 2003; Michel et al., 2010). Thus, we examined subsets of SNPs mapping to the five major linkage groups of the *R. pomonella* genome displaying high, intermediate and low levels of LD with each other to investigate the effects of linkage and potential inversion polymorphism on diapause phenotypes and host-related divergence. We note that the core conclusions of the study presented below do not rely on inversions, per se, just on LD. Thus, if future results find that some of the patterns we describe are due to particularly strong selection and low recombination occurring for certain colinear regions of the genome rather than inversions, this would not undermine arguments about genetic independence of diapause traits and associated genomic responses to selection.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Flies were collected directly from nature as eggs or early-instar larvae infesting apple (*Malus pumila*) or downy hawthorn (*Crataegus mollis*) fruit at two sympatric field sites in Grant and Fennville, Michigan (MI), USA., where host trees and the fly races co-occur. Field-collected flies were reared to the pupal diapause stage using standard *Rhagoletis* husbandry methods (see Egan et al., 2015). Flies used in the selection experiment on diapause intensity and in the comparison of sympatric host races in nature were collected from Grant, MI (43°21′5.6916″N, 85°53′23.3736″W), in the summer of 1989 and represent the material genotyped in Egan et al. (2015). Flies used in the diapause termination experiment were collected from Fennville, MI (42°35′54.5892″N, 86°09′5.0220″W; ~80 km south of Grant), in the summer of 2009. Apple and hawthorn flies from Grant and Fennville have been previously shown to display consistent diapause and genetic differences from one another when reared under standardized environmental conditions in the laboratory and nature (Dambroski & Feder, 2007; Feder & Bush, 1989; Feder, Chilcote, & Bush, 1990; Feder, Chilcote et al., 1988; Feder, Roethele et al., 1997; Michel et al., 2010).

2.2 | Diapause termination study

After overwintering as pupae in the laboratory, the samples of apple and hawthorn flies from Fennville were placed in a 21°C temperature-controlled room and monitored daily for newly eclosing adults. The timing of eclosion is determined by the duration of pupal diapause and thus the timing of diapause termination. The date of eclosion and sex of each individual were recorded and the fly subsequently frozen at −80°C for later genetic analysis (total n = 1,250 apple fly and 1,644 hawthorn fly adults). We used an extreme phenotyping design to increase the statistical power of the genomic analysis (Ehrenreich et al., 2010; Huang et al., 2012; Lai et al., 2007; Werner et al., 2005; Wolyn et al., 2004), in which the 48 (≤3% quantile) earliest eclosing apple (17 males and 31 females) and hawthorn flies (10 males and 38 females) and 48 latest eclosing (≥97% quantile) apple (20 males and 28 females) and hawthorn flies (15 males and 33 females) were genotyped (Figure 1b).

2.3 | Initial diapause intensity and host race comparisons

Previously, we investigated the effect that initial diapause intensity has on genomewide differentiation by GBS (Egan et al., 2015). Briefly, infested hawthorn fruit collected at Grant, MI, in 1989 were field-collected and brought to the laboratory and flies reared to pupation and placed under long (32-day) vs. control (7-day) prewinter conditions at 25°C emulating field conditions experienced by apple vs. hawthorn flies, respectively, in nature (Figure 1c). After 30 weeks of cold (4°C) followed by a return to 21°C to simulate postwinter (spring) warming, eclosing survivors were collected as adults and genotyped. Thus, the long prewinter treatment applied selection on initial diapause intensity, selecting against flies that underwent nondiapause development, whereas the shorter, benign, 7-day treatment provided a control sample under minimal selection.

2.4 | Genotyping-by-sequencing of the diapause termination study

Methods for GBS, filtering of sequence data, de novo genome assembly of contigs, variant SNP calling and allele frequency estimation were performed following Egan et al. (2015). Briefly, single-end (1989 Grant sample) and paired-end (2009 Fennville sample) sequencing of individually barcoded restriction amplified DNA libraries (restriction enzymes: EcoRI and MseI) was performed at the National Center for Genomic Resources (Santa Fe, NM) and the Beijing Genomics Institute Americas Corp. (Cambridge, MA), respectively, using Illumina GAII and HiSeq platforms, generating 1.2 billion 100- to 124-bp reads. Egan et al. (2015) used custom scripts and the Genome Analysis Toolkit (GATK version 2.5-2; DePristo et al., 2011) to identify 32,455 variable SNP sites passing stringent quality filters. First, 7-day apple and hawthorn control samples in the prewinter selection experiment were pooled to ensure that diagnostic SNPs were not excluded from subsequent analyses and that all SNPs were polymorphic, based on the criterion of having a combined frequency for the common allele of ≤0.95 at the Grant, MI site. In addition, SNPs with biased allele read counts (significant deviation from the binomial distribution assuming p = .5 with α = .05) in heterozygotes were excluded from further analysis, as they represent possible overassembly of paralogs (Egan et al., 2015). The 7-day apple and hawthorn samples were then separately tested for HWE; SNPs deviating from HWE ($p \leq .05$) within populations were excluded to limit the effects of allele dropout from cut site polymorphism (Arnold, Corbett-Detig, Hartl, & Bomblies, 2013). Treating the 2009 eclosion time paired-end data as single end (discarding the reverse read), we applied the same analysis pipeline, identifying 10,241 SNPs that were also genotyped in Egan et al. (2015). The

analyses presented below were performed on this common set of 10,241 SNPs shared between both experiments. All new, raw sequence data and SNP data are deposited in DRYAD (https://doi.org/10.5061/dryad.kn568/2).

2.5 | Building linkage groups

In Egan et al. (2015), we determined recombination distances among 2,352 SNPs that mapped to five of the six chromosomes constituting the *R. pomonella* genome based on five single-pair test crosses using the program JOIN MAP 4.1 (Kyazma BV, Wageningen, the Netherlands) (Note: no polymorphic marker has been found for the small, heterochromatic dot sixth chromosome). Of these 2,352 SNPs with recombination distance information, 563 were among the 10,241 SNPs genotyped in this study. Here, we determined chromosome assignments, but not recombination map distances, for an additional 3,711 SNPs based on (i) the assortment patterns of markers inherited from fathers in test crosses (recombination does not occur in males and so chromosomes are passed intact from fathers to their offspring) (Berlocher & Smith, 1983; Feder et al., 1989; Gethmann, 1988; Orr-Weaver, 1995; White, 1973) and (ii) the pattern of LD displayed among markers. In the latter case, a marker that showed a significant composite LD value (Weir, 1979) with at least three other SNPs assigned to a chromosome and to no other SNP residing elsewhere in the genome was considered to map to that chromosome.

2.6 | Measuring linkage disequilibrium

To assess genome structure, we estimated Burrow's composite measure of linkage disequilibrium (Δ) between pairs of SNPs in the 7-day (control) hawthorn and apple fly samples from Grant, MI, according to Weir (1979). Burrow's Δ does not assume HWE or require phased data, but instead provides a joint metric of intralocus and interlocus disequilibria based solely on genotype frequencies. Thus, Δ is equivalent to the LD parameter *D* under HWE (Weir, 1979). We used a Monte Carlo algorithm to incorporate uncertainty in genotype calling into our estimates of Δ, following Gompert et al. (2014). Composite Δ values standardized to *r* values between 1 and −1 following Weir (1979) were first calculated separately within the 7-day apple and 7-day hawthorn samples. Pairwise within-race LD for linked SNPs mapping to the same chromosome was highly correlated between apple and hawthorn flies ($r = .898$; $p < 10^{-16}$; 4,243 *df*). We therefore used the mean of within-race *r* values in subsequent analysis. We used the igraph package in R (Csardi & Nepusz, 2006) to visualize LD relationships among SNPs within each of the five major chromosomes constituting the *R. pomonella* genome. Specifically, we generated Fruchterman–Reingold network plots as in Kemppainen et al. (2015) which represent SNPs as nodes connected to one another in clusters by edges that exceed a given threshold pairwise LD (r^2) value. We generated network plots for r^2 values ranging from .9 to .1 for each chromosome in increments of .01. This allowed us to determine minimum threshold r^2 levels for chromosomes at which definitive clusters of SNPs could be identified that were in high LD with one another but remained distinct and not connected to all other linked loci. These high LD clusters of linked SNPs putatively represent inversions polymorphisms on chromosomes. We subsequently (see Results section) used the SNPs belonging to the high LD clusters together with mean pairwise *r* values of SNPs to all other linked loci and to the high LD cluster to categorize SNPs into high, medium and low LD classes for further analysis.

2.7 | Simulations

Throughout the discussion, we evaluate evidence that patterns of LD are consistent with the existence of inversions, but divergent selection with gene flow between host races could potentially generate similar patterns. We ran several simulations to evaluate this possibility.

First, we calculated pairwise LD values between SNPs mapping to different chromosomes to provide a baseline estimate of background disequilibrium within the host races due to divergent selection and gene flow in the absence of physical linkage between loci. To focus on variants likely strongly affected directly or indirectly by divergent selection, between chromosome pairwise LD values were calculated for a subset of 92 mapped SNPs that showed (i) significant allele frequency differences between the host races at the Grant, MI site (mean difference = 0.137 ± 0.0024 *SE*), as well as (ii) significant allele frequency differences in either the diapause termination time study and/or in the prewinter selection experiment (see results).

Next, we constructed a two-deme individual-based simulation model for host race divergence in the absence of physical linkage and with multiplicative fitness. In the model, a total of 400 unlinked loci were simulated, with each locus having a selection coefficient (*s*) of 0.0136, representative of a modest level of selection acting on each locus. This generated baseline results for inter-race LD and host-related allele frequency divergence similar to those observed at the Grant site for unlinked SNPs residing on different chromosomes (see Results section). We assumed a gross migration rate (*m*) of 0.04 per generation between the host races, equal to the rate estimated from a mark–release–recapture study conducted at the Grant, MI site (Feder et al., 1994). Genotypes of individuals were randomly created in both host races at the start of a simulation run (10 replicates were performed), with initial allele frequencies for all loci set to 0.5 in apple and hawthorn flies. A population size of 1,000 was used for each race and the simulation run for a total of 200 generations, approximating a likely upper bound of the time since the initial formation of the derived, apple host race. After 200 generations, 48 individuals from each race were randomly sampled and composite LD values calculated between each pairwise combination of loci ($n = 78{,}734$) within each race and then averaged between the races to generate mean values. Finally, to examine the effects of physical linkage on LD generated by divergent selection with gene flow, we also constructed a modified two-deme model by reducing the

recombination rate between SNPs for a subset of 50 of the 400 selected loci from 0.5 centi-Morgans to 0.0001 (modelling high LD regions).

2.8 | Tests for allele frequency differences

Probabilities of single locus genotypes and allele frequencies for SNPs were calculated following McKenna et al. (2010). Analyses conducted with raw and arcsine-square-root-transformed allele frequencies produced equivalent results; therefore, we present only the results generated using raw allele frequencies. Tests for significant allele frequency differences for SNPs were performed using a nonparametric, Monte Carlo approach between sample populations of (i) early vs. late eclosing flies (considering each host race separately and the means for the races combined) and (ii) 7-day vs. 32-day prewinter treatments for hawthorn flies. Two randomized samples of individual, whole fly genotypes were drawn with replacement from the pool of either early and late eclosing flies combined or 7-day and 32-day flies combined, corresponding to the respective numbers of flies genotyped in each treatment. Allele frequency differences were then calculated between the random samples of early vs. late eclosing or 7-day vs. 32-day prewinter flies and the process repeated 10,000 times to generate expected null probability distributions for SNPs, with the use of whole fly genotypes preserving LD relationships among loci. Observed differences for SNPs that exceeded the upper 97.5th quantile or were below the lower 2.5th quantile of their null Monte Carlo distribution were taken as statistically significant. We also assessed the direction of the frequency change in the experimental prewinter treatment under the hypothesis that hawthorn flies surviving the longer 32-day period were predicted to be more similar to apple flies in their SNP frequencies, consistent with the expectation that apple flies possess higher frequencies of alleles associated with deeper initial diapause intensity (Egan et al., 2015). We did not apply multiple comparison corrections on individual SNP tests because the purpose of the analysis was not to identify specific candidate loci, but rather to determine (i) whether similar sets of loci strongly associated with both initial diapause depth and diapause termination timing, and (ii) how associated loci were distributed across the genome. However, to determine whether the overall percentages of SNPs displaying associations with initial diapause depth and diapause termination timing were significantly greater than expected by chance for different categories of loci, we performed an additional set of Monte Carlo analyses resampling whole individual fly genotypes to determined how often observed percentages exceeded random simulated expectations.

2.9 | Associations with diapause termination

We applied a Bayesian sparse linear mixed model (BSLMM) relating eclosion phenotype-to-genotype probabilities in order to describe the total amount of phenotypic variation in eclosion time explained by all mapped SNPs. We implemented the following BSLMM in GEMMA (Zhou, Carbonetto, & Stephens, 2013), which accounts for LD and population structure:

$$y = \mu + X\beta + u + \epsilon$$

where y is the vector of phenotypes (eclosion timing), X is the matrix of genotype probabilities, β is a vector of effect sizes due to loci with measurable effects, u is a vector of random effects modelling the infinitesimal effects of each locus, and ϵ is the error term (prior specification also differs between measurable and infinitesimal effects). Posterior distributions for model parameters are estimated with MCMC, including a binomial vector (γ) whose posterior distribution estimates the inclusion probability (the probability that a given locus has a measurable effect on the phenotype). The GEMMA implementation also generates posterior distributions for the number of loci with measurable effects (based on γ), the per cent phenotypic variance explained by the combined measurable and infinitesimal genetic effects of all loci (per cent variance explained, or PVE), and the per cent genetic variance explained by loci of measurable effect (per cent genetic variance explained, or PGE).

3 | RESULTS

3.1 | Complex genome structure revealed by LD

Patterns of composite LD (Δ) among SNPs implied that the *R. pomonella* genome is highly structured. A total of 4,244 of the 10,241 SNPs genotyped were assigned to one of the five major chromosomes of the genome. Analysis of composite LD among linked SNPs mapping to the same chromosome revealed a sharp bimodal distribution, with a substantial number of SNPs on each chromosome displaying high mean $r \geq .3$ with all other loci on the same linkage group (Figure 2a). For chromosomes 1, 3, 4 and 5, sets of SNPs that clustered with one another and were distinct from all other loci at a r^2 threshold of .6 were designated as high LD SNPs (Table 1; Figure 3a, c, d, e, f; high LD clusters of SNPs highlighted in black or color). Note that member nodes (loci) of LD clusters in Figure 3 are in LD with at least one other member with an r^2 value surpassing the threshold. In contrast, the mean absolute r values represent the average pairwise LD value of a SNP to all members of the high LD cluster. Patterns of LD on Chromosome 2 were different from the other four major linkage groups, showing evidence for eight different clusters of high LD SNPs, rather than just one, that grouped differentially at an LD threshold of $r^2 = .55$ (Figure 3b).

For all chromosomes, we identified a set of "low" LD SNPs that did not cluster with any other locus mapping to the same chromosome at a r^2 value of .15 (Figure 3f–j). We defined the remainder of variants not falling into high or low categories as intermediate LD SNPs (filled grey circles in Figure 3f–j). Figure 2b shows the distribution of mean LD values between each locus and all high LD loci on the same chromosome. While the percentages of SNPs belonging to the three LD categories differed among chromosomes, each linkage group contained at least 9% of its loci in the high LD, 12% in the low LD and 36% in the intermediate LD class (Table 1). We note,

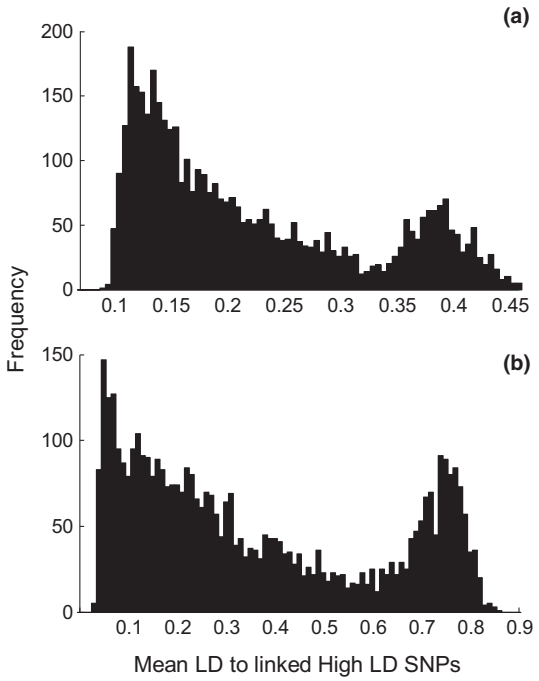

FIGURE 2 Histograms of mean pairwise composite LD values (*r*) for SNPs to (a) all other SNPs residing on the same chromosome and (b) the high LD class of SNPs residing on the same chromosome. Shown are combined histograms including values for both the apple and hawthorn races

however, that due to LD being a criterion used to assign SNPs to linkage groups, we likely overestimated the proportions of SNPs in the high and intermediate LD classes and underestimated the true proportion of low LD SNPs.

3.2 | Allele frequency distributions and LD

Loci colocalizing to high LD regions on chromosomes should segregate at very similar frequencies in a population. We constructed allele frequency distributions for SNPs at the Grant, MI site, to test for this signature. The allele frequency distributions for the most common variant in the hawthorn race at Grant for all 10,256 SNPs genotyped and for the 4,244 SNPs assigned to chromosomes are shown in Figure 4a and b, respectively (see Figure S1 for similar distributions for the apple race; the common allele is generally the same across the host races, with a high correlation of $r = .89$ for allele frequencies; Figure S2). The distributions exhibit an excess of SNPs with frequencies ranging between ~0.70 and 0.85 in the hawthorn race with a mode of ~0.78. Removal of the high LD class of SNPs essentially eliminated these "bulges" from the distributions (Figure 4c, S1C). Thus, the excess of SNPs with frequencies ranging from 0.70 to 0.85 was primarily due to SNPs displaying high LD with one another within chromosomes (Figure 4d, S1D).

The relationship between LD and SNP allele frequencies was not the same for all chromosomes, although SNPs in the high LD category within chromosomes tended to have very similar allele frequencies. Whereas allele frequency distributions for high LD SNPs on chromosomes 1, 3, 4 and 5 had similar, unimodal shapes centred between 0.7 and 0.85 (comparable to Figure 4d), the eight sets of high LD SNPs identified on chromosome 2 had a broader and discontinuous distribution (Figure 4e and Figure S2). These relationships are further illustrated in Figure 5, which plots mean pairwise LD of each locus to all high LD loci mapping to the same chromosomes vs. the allele frequency of the SNP in the hawthorn host race. In Figure 5, the high LD class of SNPs on chromosomes 1, 3, 4 and 5 (represented by black circles) clustered together in a frequency range between 0.70 and 0.85, while those on chromosome 2 (multicoloured star symbols) resided outside of this range, but still clustered by high LD group (1–8). Figure 5 also revealed that low and intermediate LD SNPs, particularly those with mean *r* values >.3, displayed a relative paucity of frequencies in the hawthorn population in the range of 0.70–0.85, yet a constriction in frequencies towards 0.70–0.85 with increasing mean LD. In general, allele frequencies tended to converge towards the 0.70–0.85 range with increasing mean LD values above 0.5.

3.3 | Distribution of LD classes along chromosomes

All three categories of SNPs (high, intermediate and low LD) appeared to be dispersed along the length of each of the five major chromosomes (Figure 6). A recombination linkage map previously developed for *R. pomonella* based on single-pair genetic crosses contained 563 of the 4,244 assigned SNPs scored in the present study (Egan et al., 2015). The recombination map showed that high LD SNPs were not clustered in one region of each chromosome (Figure 6). Rather, they were dispersed along chromosomes and interspersed with intermediate and low LD SNPs. Unfortunately, not enough markers were included in the recombination map for the eight different high LD groups on chromosome 2 to determine whether SNPs within each of these groups were localized or dispersed on chromosome 2 (Figure 6).

TABLE 1 Percentages of SNPs mapped to chromosomes belonging to high, intermediate (Int) and low linkage disequilibrium (LD) classes defined in the text. Data are given for each chromosome considered separately, as well as for all chromosomes together (chr 1–5)

	chr 1	chr 2	chr 3	chr 4	chr 5	chr1–5
Mapped SNPs	*n* = 949	*n* = 675	*n* = 996	*n* = 436	*n* = 1188	*n* = 4244
High LD	27.7	19.1	22.4	9.6	31.5	24.3
Int LD	58.8	68.0	60.1	36.5	49.9	55.8
Low LD	13.5	12.9	17.5	53.9	18.6	19.9
Ratio High/Low	2.1	1.5	1.3	0.2	1.7	1.2

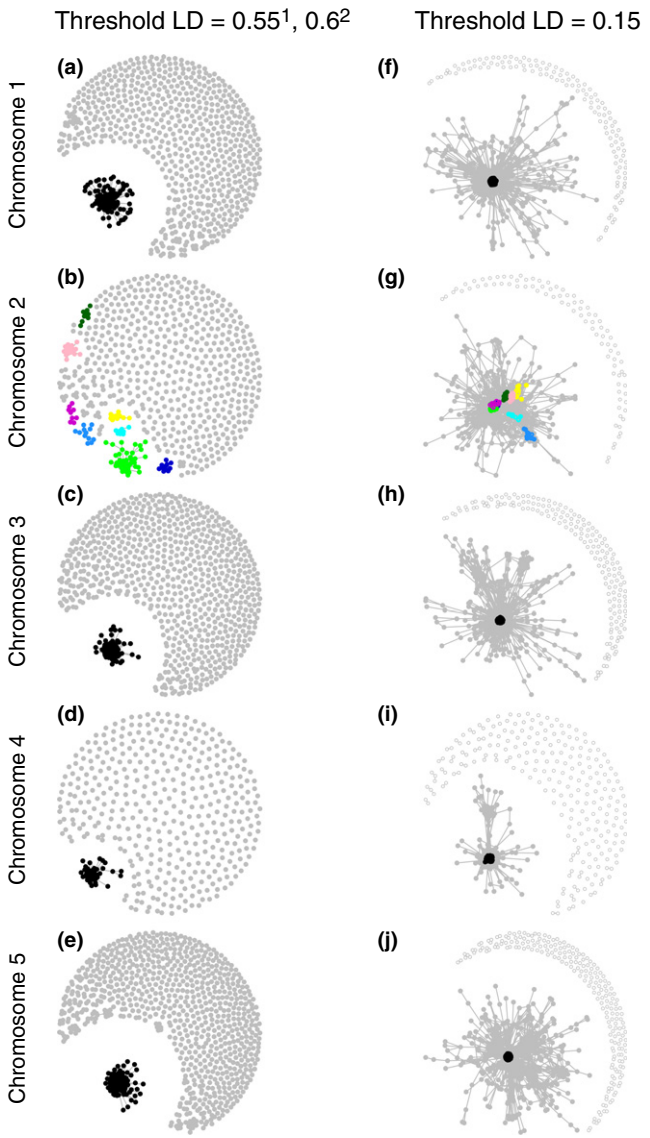

FIGURE 3 Visual representations (Fruchterman–Reingold layout) of pairwise LD networks for chromosomes 1–5. SNPs are represented as individual nodes (circles) that are connected by edges (lines) representing pairwise composite LD values (r^2) that surpass a threshold of $r^2 \geq .6$ (a–e) or $r^2 \geq .15$ (f–j); highly connected nodes (SNPs) appear clustered in the lower left, while nodes with low or absent connections (r^2 values below threshold) appear in the upper right of each panel. A threshold of $r^2 \geq .6$ (a, c–e) and $r^2 \geq .55$ (b) identifies a distinct sets of SNPs in high LD with each other (designated as the "High LD" class) depicted with black filled circles for chromosomes 1, 3, 4 and 5 and by eight sets of coloured circles for chromosome 2 that representing the following LD clusters identified in the main text: light green = group 1; pink = group 2; magenta = group 3; dark blue = group 4; cyan = group 5; dark green = group 6; yellow = group 7; light blue = group 8. A threshold of $r^2 \geq .15$ clusters the same set of high LD SNPs very tightly and identifies another class of SNPs connected by LD surpassing this lower threshold designated as "Intermediate LD" SNPs (filled grey circles; f–j). Open grey circles (f–j) depict "Low LD" class SNPs with LD values < 0.15. [1] Threshold for Chr 2; [2] threshold for Chr 1, 3–5

3.4 | Two-deme Simulation Results

The observed distribution of LD values for unlinked SNPs (on different chromosomes) fit predictions generated from a two-deme individual-based simulation model for host race divergence in the absence of physical linkage, with $s = 0.0136$ per locus and $m = 0.04$ (compare solid observed with the dashed predicted distribution in Figure 7). The simulation produced a mean LD value of 0.082 + 0.002 *SE* and frequency difference between the host races (0.137 ± 0.003 *SE*) closely matching the observed values for inter-chromosome LD between SNPs with pronounced frequency differences between host races. In a modified model, we reduced the recombination rate between SNPs for a subset of 50 of the 400 selected loci from 0.5 centi-Morgans to 0.0001 (modelling the high LD class of loci). We tested a variety of parameter values and found that only a limited set of conditions could yield the empirical levels of LD and allele frequency differences between host races observed in this study. In particular, the selection coefficients on the high LD loci had to be substantially lowered to $s = 0.0005$, and the simulations had to be started with an allele frequency difference of 0.98 between apple and hawthorn populations. This amounts to beginning with the races having alternate blocks of linked genes, as would be expected if the races differed in the frequency of an inversion. Under these conditions, the mean LD within the races averaged 0.72 ± 0.017 *SD* among the 50 linked genes and 0.082 ± 0.001 *SD* for the 350 unlinked genes, while the average frequency difference between the races for all 400 SNPs was 0.135 ± 0.002 *SD* ($n = 10$ replicates), paralleling observed values for SNPs putatively under selection (see discussion).

3.5 | Genomic associations with diapause termination timing

Allele frequency differences between the early and late eclosing quantiles of apple vs. hawthorn flies were significantly correlated with each other for all 10,241 SNPs ($r = .54$; $p < 10^{-16}$) and did not differ significantly between sexes (data not shown). Thus, similar sets of SNPs were associated with diapause termination time in both host races. We therefore used the mean frequency difference between early and late eclosing flies between the host races in subsequent analyses.

Overall, 2,196 of the total of 10,241 SNPs scored (21.4%) displayed significant allele frequency differences between early and late eclosing fly samples, significantly more than expected by chance alone as determined by nonparametric Monte Carlo sampling of whole fly genotypes ($p < 10^{-4}$). A large proportion of SNPs in the high LD category (570 of 1,031 = 52.7%) showed significant associations with diapause termination time (Table 2). The association was particularly pronounced for high LD SNPs mapping to chromosomes 1, 2 and 3 (95.1%, 68.2% and 90.1% of high LD SNPs on chromosomes 1–3, respectively, were significant), with mean allele frequency differences between early vs. late eclosing flies for high LD

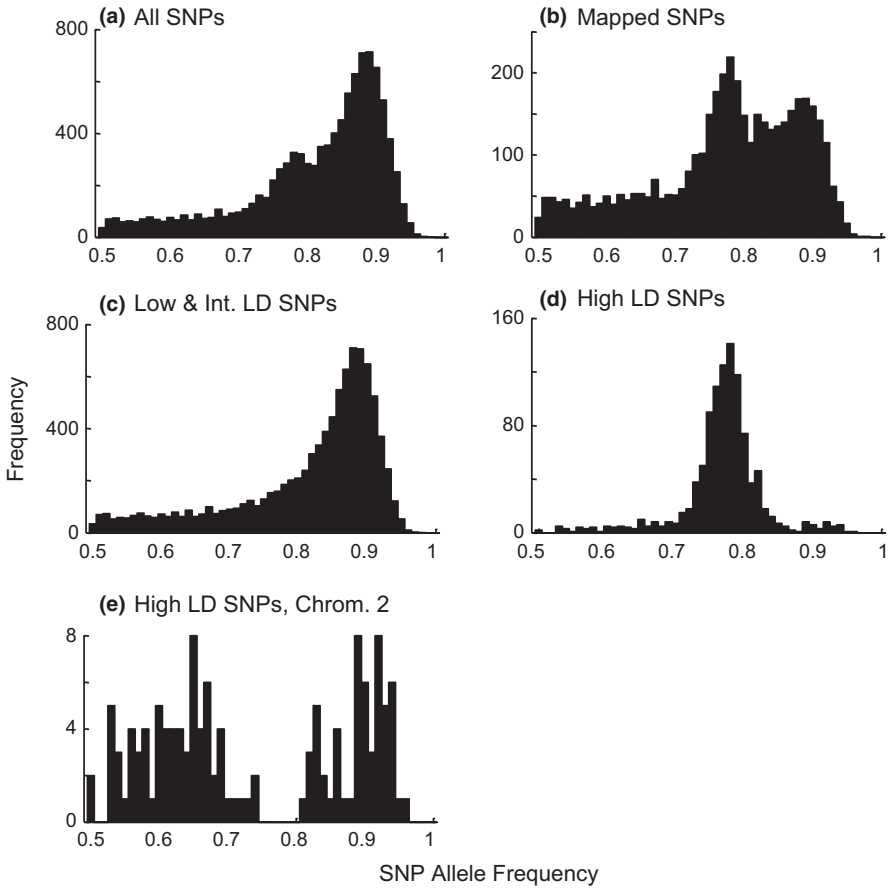

FIGURE 4 Allele frequency distributions for the hawthorn race at the Grant, MI site, for: (a) all 10,256 SNPs scored in the study; (b) 4,244 SNPs mapped to chromosomes 1–5; (c) low and intermediate classes of SNPs; (d) high LD class of SNPs; (e) high LD class of SNPs mapping to chromosome 2

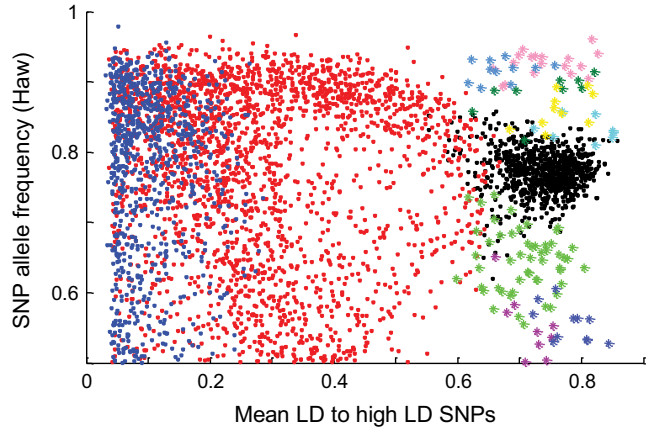

FIGURE 5 Allele frequencies of 4,244 mapped SNPs in the hawthorn race at the Grant, MI site, vs. the mean pairwise LD between a SNP and all high LD SNPs on the same chromosome. Black circles = high LD SNPs on chromosomes 1, 3, 4 and 5; red circles = intermediate LD SNPs on all chromosomes; blue circles = low LD SNPs on all chromosomes; stars = high LD SNPs on chromosome 2 (light green = group 1 loci; pink = group 2; magenta = group 3; dark blue = group 4; cyan = group 5; dark green = group 6; yellow = group 7; light blue = group 8)

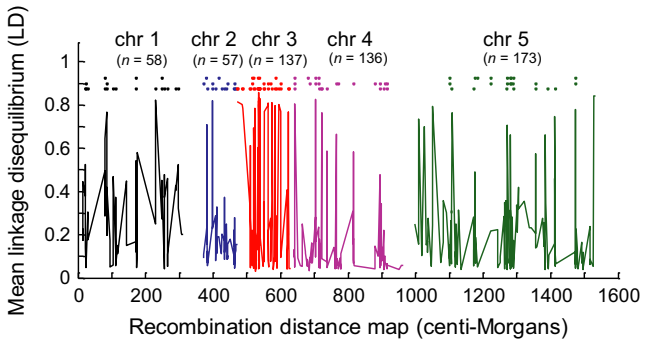

FIGURE 6 Pattern of composite linkage disequilibrium (LD) among mapped SNPs along the five major chromosomes constituting the *R. pomonella* genome. Shown are the recombination map positions for SNPs in centi-Morgans along chromosomes vs. their mean composite LD value (r) to the high LD class of SNPs residing on the same chromosome. Circles above the plot denote the map positions of SNPs displaying significant allele frequency differences in the diapause termination study (lower row), diapause intensity study (middle row) and between the host races at the Grant, MI site (upper row)

loci on chromosomes 1–3 of 0.25 + 0.005 *SE* (n = 263), 0.16 + 0.01 (n = 129) and 0.18 + 0.004 (n = 223), respectively (Table 2). Due to the significant LD among these SNPs, it is not possible to determine the exact number of loci directly affecting diapause termination time within the high LD (or intermediate LD) classes of loci on chromosome 1 and 3, only that at least one associated locus likely resides on each of these two chromosomes. Similar considerations apply to chromosome 2, except here each of the eight high LD groups of

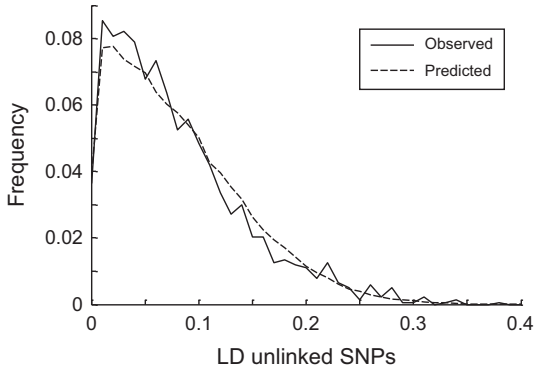

FIGURE 7 Observed (solid line) and predicted (dashed line) distribution of composite LD values (*r*) between unlinked SNPs displaying significant allele frequency differences between the host races at the Grant, MI site, and a significant difference in the diapause termination or diapause intensity study or both. The predicted curve was generated via a two-deme individual-based simulation model assuming 400 unlinked loci, each with a selection coefficient (*s*) of .0136, a gross migration rate (*m*) of .04 per generation, initial allele frequencies of 0.5, population sizes of 1,000 and a run-time of 200 generations (n = 10 replicates). See Discussion section of text for additional details

SNPs may be considered separately. In this case, five of the eight high LD groups on chromosome 2 (1, 3, 4, 5 and 8) contained more SNPs significantly associated with diapause termination time than random expectations. Indeed, every SNP in groups 1, 3 and 4 (n = 53, 9 and 11, respectively) and four of the eight loci in group 5 (= 50%, $p < .05$) and eight of 11 group 8 loci (=73%, $p < .01$) displayed a significant frequency difference between early and late eclosing flies. The low LD class of SNPs had more modest, but still significant, excesses of loci displaying associations with diapause termination time for chromosomes 1, 2 and 3 above null expectations (Table 2).

We used BSLMM to describe the total amount of phenotypic variation in diapause termination time explained by all mapped SNPs, accounting for LD and population structure. The 99% Bayesian credible interval for the per cent of the phenotypic variance explained by all genetic effects (PVE; includes measurable and infinitesimal effects) centred on 63% and varied between 48 and 78%. The 99% credible interval for the per cent of genetic variance explained only by loci with measurable effects (PGE) suggested a low and uncertain percentage, centred on 16% (range 0 – 99%). The 99% credible interval for the number of loci of measurable effect contributing to diapause termination time ranged from 0 to 5 (median = 3). Of the five loci with the highest inclusion probabilities (γ = .11–.36), one resided in the high LD class of each of chromosomes 1 and 3, and one each in the high LD clusters 1 and 8 and the intermediate LD class on chromosome 2. Because all of these loci map to high-intermediate LD regions, we can only infer that one or more loci in each high LD block on each of these chromosomes contribute to variance in diapause termination time. Given the relatively uncertain estimates of PGE and number of loci of measurable effect, yet a large estimate for PVE, polygenic (infinitesimal effect) variation appears to account for a sizeable amount of the variance in diapause termination time. The experimental design including only phenotypes on the extremes of the distribution improves statistical power to detect genetic associations, but my cause an overestimation of PVE.

3.6 | Diapause termination and population divergence in nature

Only allele frequency differences (i.e., no fixed differences) were observed between apple and hawthorn flies at the Grant, MI site, with the largest difference being 0.231. Allele frequency differences between Grant apple and hawthorn flies were correlated with the allele frequency differences between early and late eclosers in the diapause termination study (r = .29; $p < 10^{-4}$, as determined by Monte Carlo simulation of whole fly genotypes). Thus, the genome-wide pattern of host divergence for all 10,241 SNPs at Grant, MI, was significantly predicted by the genomewide pattern of genetic associations with diapause termination timing. The relationship was more pronounced for the 4,244 mapped SNPs (r = .41; $p < 10^{-3}$) and greatest for high LD SNPs (r = .56, $p < .05$). However, host differences within the high LD classes on chromosomes 1 and 3 were not significantly correlated with diapause termination timing because these loci show uniformly strong associations with diapause termination timing (low variance in allele frequency differences between early and late eclosion time groups; Table 3). Although the correlation between host-associated allele frequency differences and allele frequency differences between eclosion groups was nonsignificant when including all high LD loci on chromosome 2, high LD groups 4 and 6 on chromosome 2 individually showed significant correlations between diapause termination time associations and host differences (r = .86; $p < .05$; 10 *df*; and .70; $p < .05$; 8 *df*, respectively). For low LD SNPs, only chromosome 1 displayed a significant relationship between diapause termination time associations and host differences (r = .27; $p < 10^{-4}$; 127 *df*; Table 3).

3.7 | Genomic response to selection on initial diapause intensity

Overall, 731 (7.1%) of the total of 10,241 SNPs scored in the study displayed significant allele frequency differences between the 7-day control and 32-day prewinter treatments. This was not significantly greater than the null expectation predicted by Monte Carlo simulations of whole fly genotypes (p = .277). However, as predicted, there was a marked trend for significantly responding SNPs to shift frequency in the direction of the apple race in the 32-day experimental treatment applied to hawthorn flies (84.1% = 615/731; χ^2 = 360.4; $p \ll .001$; 1 *df* from 50:50 null expectation). Indeed, for the 107 SNPs displaying a significant frequency difference in the prewinter selection experiment and between the host races at Grant, MI, all responded in the direction of the apple race in the 32-day treatment. SNPs significantly responding in the direction of the apple race were not randomly distributed across the genome. In contrast to the diapause termination time study, only the low LD

TABLE 2 Percentages of SNPs showing significant frequency differences between early and late eclosing flies (Ecl. time) and between 7-day vs. 32-day treatments in the direction of the apple race in the selection experiment (Sel. exp.) for all mapped SNPs and for high, intermediate (Int) and low LD SNPs for each chromosome separately, as well as all together (chr 1–5)

Ecl. time	chr 1	chr 2	chr 3	chr 4	chr 5	chr1–5
All SNPs	n = 949	n = 675	n = 996	n = 436	n = 1188	n = 4244
	63.7****	47.4****	45.2****	4.6	4.5	34.2****
	(0.14)	(0.10)	(0.09)	(0.03)	(0.03)	(0.08)
High LD	n = 263	n = 129	n = 223	n = 42	n = 374	n = 1031
	95.1****	68.2****	90.1****	4.8	0.5	52.7****
	(0.25)	(0.16)	(0.18)	(0.03)	(0.03)	(0.13)
Int LD	n = 558	n = 459	n = 599	n = 159	n = 593	n = 2368
	59.3****	47.9****	37.1****	2.5	6.4	34.4****
	(0.10)	(0.09)	(0.07)	(0.03)	(0.04)	(0.07)
Low LD	n = 128	n = 87	n = 174	n = 235	n = 221	n = 845
	18.8***	13.8*	16.9***	5.9	6.3	10.9**
	(0.05)	(0.04)	(0.05)	(0.03)	(0.03)	(0.04)
Sel. exp.						
All SNPs	n = 949	n = 675	n = 996	n = 436	n = 1,188	n = 4,244
	3.7	6.8	3.9	7.1*	4.1	4.7
	(0.03)	(0.04)	(0.03)	(0.05)	(0.04)	(0.04)
High LD	n = 263	n = 129	n = 223	n = 42	n = 374	n = 1,031
	0.0	3.1	0.0	0.0	1.3	0.9
	(0.03)	(0.04)	(0.02)	(0.04)	(0.04)	(0.03)
Int LD	n = 558	n = 459	n = 599	n = 159	n = 593	n = 2,368
	4.6	7.4*	4.2	7.5	5.2	5.4
	(0.04)	(0.04)	(0.04)	(0.05)	(0.04)	(0.04)
Low LD	n = 128	n = 87	n = 174	n = 235	n = 221	n = 845
	7.0*	9.1**	8.0**	8.1***	5.9*	7.5***
	(0.04)	(0.04)	(0.05)	(0.04)	(0.04)	(0.04)

Significance levels are indicated where the number of SNPs in a category exceeded the number expected by chance alone, assessed by Monte Carlo sampling (see main text); *p < .01; **p < .01; ***p < .001; ****p < .0001; highlighted in grey. Average absolute allele frequency differences between early and late eclosing flies and 7-day vs. 32-day prewinter treatments are presented in parentheses.

class of loci on chromosomes 1–5 showed a significant excess of responding SNPs (Table 2). The only exception was the intermediate LD class of SNPs on chromosome 2. In comparison, no LD class of loci displayed a significant excess of SNPs responding in the prewinter experiment in the direction opposite the apple race (data not shown).

3.8 | Initial diapause intensity and population divergence in nature

As previously illustrated in Egan et al. (2015), the genomewide pattern of host divergence for all 10,241 SNPs at Grant, MI, was significantly predicted by the selection experiment on initial diapause intensity (r = .38; $p < 10^{-4}$). The same was also true for the 4,244 mapped loci (r = .35; p < .05; Table 3). The effect of initial diapause intensity on host-related divergence was genomewide, with almost every class of SNP (high, intermediate and low LD) on every chromosome showing a significant relationship (Table 3).

3.9 | Relationship between diapause termination and initial diapause intensity

Allele frequency differences in the diapause termination and diapause intensity experiments for all 10,241 SNPs were not significantly correlated (r = −.044; p = .41). The same was true for the 4,244 mapped loci (r = −.060; p = .53; Table 4), implying that diapause termination timing and initial diapause intensity represent largely independent genetic modules. However, the high LD and intermediate LD classes of SNPs on chromosome 2 displayed significant negative relationships between the genetic response in the diapause termination study and the prewinter selection experiment (r = −.67; p < .05; and r = −.33; p < .05; respectively; Table 4), such that alleles associated with earlier diapause termination were correlated with deeper initial diapause intensity. The significant negative relationship for high LD SNPs on chromosome 2 was primarily due to a single cluster of high LD loci (group 1; r = −.70; $p < 10^{-4}$).

TABLE 3 Correlation coefficients (*r*) of allele frequency differences for SNPs in the diapause termination time study (Ecl. time) and diapause intensity experiment (Sel. exp.) vs. differences between hawthorn and apple host races at the Grant, MI site, for all mapped SNPs and for high, intermediate (Int) and low LD SNPs. Mean absolute allele frequency differences between the host races at the Grant site appear in parentheses in the top (Ecl. time) panel

Ecl. time	chr 1	chr 2	chr 3	chr 4	chr 5	chr1–5
All SNPs	*n* = 949	*n* = 675	*n* = 996	*n* = 436	*n* = 1188	*n* = 4244
	0.63**	0.08	0.46**	−0.07	−0.02	0.41***
	(0.058)	(0.040)	(0.046)	(0.042)	(0.035)	(0.044)
High LD	*n* = 263	*n* = 129	*n* = 223	*n* = 42	*n* = 374	*n* = 1031
	0.08	0.09	0.09	0.14	−0.07	0.56*
	(0.087)	(0.041)	(0.059)	(0.045)	(0.026)	(0.051)
Int LD	*n* = 558	*n* = 459	*n* = 599	*n* = 159	*n* = 593	*n* = 2368
	0.50***	0.05	0.40**	−0.18	−0.03	0.28***
	(0.047)	(0.040)	(0.040)	(0.038)	(0.037)	(0.041)
Low LD	*n* = 128	*n* = 87	*n* = 174	*n* = 235	*n* = 221	*n* = 845
	0.27*	−0.05	0.06	0.02	−0.03	0.06
	(0.043)	(0.038)	(0.047)	(0.044)	(0.045)	(0.044)
Sel. exp.						
All SNPs	*n* = 949	*n* = 675	*n* = 996	*n* = 436	*n* = 1188	*n* = 4244
	0.37*	0.43*	0.38*	0.30*	0.40*	0.35*
High LD	*n* = 263	*n* = 129	*n* = 223	*n* = 42	*n* = 374	*n* = 1031
	0.31*	0.32	0.45***	0.59*	0.49****	0.04
Int LD	*n* = 558	*n* = 459	*n* = 599	*n* = 159	*n* = 593	*n* = 2368
	0.41*	0.48*	0.40**	0.23	0.35*	0.37***
Low LD	*n* = 128	*n* = 87	*n* = 174	*n* = 235	*n* = 221	*n* = 845
	0.39**	0.46**	0.41***	0.49****	0.46****	0.44****

Results are given for each chromosome considered separately, as well as for all chromosomes together (chr 1–5). *$p < .01$; **$p < .01$; ***$p < .001$; ****$p < .0001$; significant correlations highlighted in grey.

TABLE 4 Correlation coefficients (*r*) of allele frequency differences in diapause termination time study (Ecl. time) vs. differences in the diapause intensity experiment (Sel. exp.) for all mapped SNPs and for high, intermediate (Int) and low LD classes of SNPs. Results are given for each chromosome considered separately, as well as for all chromosomes together (chr 1–5)

	chr 1	chr 2	chr 3	chr 4	chr 5	chr1–5
All SNPs	*n* = 949	*n* = 675	*n* = 996	*n* = 436	*n* = 1188	*n* = 4244
	0.07	−0.40*	0.06	−0.04	0.11	−0.06
High LD	*n* = 263	*n* = 129	*n* = 223	*n* = 42	*n* = 374	*n* = 1031
	−0.09	−0.67*	−0.03	0.14	−0.01	−0.41
Int LD	*n* = 558	*n* = 459	*n* = 599	*n* = 159	*n* = 593	*n* = 2368
	0.05	−0.33*	0.10	−0.08	0.10	−0.05
Low LD	*n* = 128	*n* = 87	*n* = 174	*n* = 235	*n* = 221	*n* = 845
	0.03	0.01	0.03	−0.10	−0.05	−0.02

*$p < .01$; **$p < .01$; ***$p < .001$; ****$p < .0001$; significant tests highlighted in grey.

4 | DISCUSSION

Two major findings emerged from the current study. First, the genome of *R. pomonella* is highly physically structured, with clusters of SNP loci in high LD with each other on the same chromosome and displaying similar population allele frequencies. Inversions are the most likely cause of the observed LD patterns (see below), but we emphasize that other causes of high LD would not negate our conclusions regarding the genomic patterns or independence of diapause variation. Second, initial diapause intensity (refractoriness to nondiapause development) and diapause termination timing (measured as eclosion timing) largely represent different genomic modules free to evolve independently. Both traits predict genome-wide SNP divergence in nature between sympatric apple and

hawthorn host races at the Grant, MI site, suggesting that selection is driving ecological divergence in both aspects of diapause development.

4.1 | Linkage disequilibrium and evidence for inversions

Taken together, the presence of many SNPs displaying high LD with one another, their biased allele frequency distributions and their dispersion along chromosomes suggest that relatively large inversions exist on linkage groups 1, 3, 4 and 5. If we assume that alleles in the high LD class of SNPs largely reside on alternate rearrangements, then the frequencies for the common inversion on chromosomes 1, 3, 4 and 5 for fly populations at Grant, MI, would be predicted by the modes in their allele frequency distributions (Figure S2; modes are ~0.78 for all of chromosomes 1, 3, 4 and 5 in the haw host race and 0.67, 0.73, 0.82 and 0.74, respectively, in the apple host race). The interspersion of high LD with low and intermediate LD SNPs along chromosomes 1, 3, 4 and 5 may be explained by gene flux between rearrangements (transfer of alleles between inversion variants; Pegueroles, Aquadro, Mestres, & Pascual, 2013), although biases introduced by the composite method of linkage map construction (Egan et al., 2015) may also influence these results. Frequencies of SNPs tended to approach 0.78 with increasing LD to high LD SNPs (Figure 5), likely reflecting the increasing influence of evolutionary processes (e.g. stronger selection) affecting SNPs within inversions on SNPs just outside of these inversions. Consistent with the existence of inversion polymorphism, previous studies (Feder et al., 1989; Feder, Roethele, et al., 2003; Michel et al., 2010) reported evidence for significant variation in recombination rates and differences in linear gene order among allozymes, cDNA and microsatellite markers in genetic crosses. In contrast, the multiple high LD clusters identified on chromosome 2 (Figure 3b) suggests the presence of a series of smaller inversions dispersed along the chromosome that are segregating at varying frequencies at the Grant, MI site. However, additional mapping studies are needed to confirm this hypothesis.

There are alternative hypotheses besides inversions that could potentially account for the pattern of LD observed among SNPs. For example, elevated LD could be explained by reduced recombination associated with centromeric regions of chromosomes. However, the centromere hypothesis could not easily account for the dispersed nature of the high LD class of SNPs along chromosomes 1, 3, 4 and 5 or the eight different groups of high LD SNPs on chromosome 2.

Divergent selection with gene flow could also generate high LD within host races, but our two-deme simulation results suggest that this is unlikely in *R. pomonella*. The simulation model with moderate selection, migration rate equal to empirical estimates and no LD among loci generated a distribution of LD values that closely matched the observed distribution of interchromosome LD values for loci showing significant frequency differences between the host races (Figure 7). The simulations therefore implied a general lack of elevated LD and epistatic gene interactions between unlinked loci within the host races above the background expectation with no physical linkage. Modifying the model to include a subset of tightly linked loci (simulating the high LD class; 50 loci in high LD, 350 unlinked, with all loci experiencing moderate selection) and lowering the selection coefficients were the only conditions that produced LD values near to those observed in the LD analysis of empirical data. These simulations imply the need for additional structural features in the genome such as inversions to generate the observed patterns of LD and host race divergence. This conclusion is further strengthened when considering that high LD SNPs appear dispersed along chromosomes and interspersed with low and intermediate LD loci in *R. pomonella*, which cannot be easily explained by divergent selection and gene flow alone.

4.2 | Modularity of diapause developmental phenotypes

The modularity of diapause termination and initial diapause intensity traits therefore appear to be due, in part, to differences in their associations with linked blocks of loci. In this regard, diapause termination timing was most highly associated with SNPs on chromosomes 1–3 including SNPs in the high LD classes that we hypothesize to be due to inversions (Table 2). The BSLMM model confirmed the presence of segregating genetic variation underlying diapause termination timing (63% of phenotypic variance explained by SNP genotypes) and also identified the strongest genetic associations for loci in high LD blocks on chromosomes 1–3. In comparison, selection on initial diapause intensity primarily affected low LD SNPs on all five major chromosomes (Table 2). As a result, allele frequency differences associated with diapause termination timing and selection on initial diapause intensity were not correlated when considering all loci ($r = -.044$; $p = .41$; see also Table 4), suggesting genetic independence (Wagner et al., 2007; Weber et al., 2013). We cannot discount the possibility that reduced genetic correlation between the two traits could, in part, be a result of temporal and spatial differences in study samples (Fennville, MI 2009 and Grant, MI 1989, respectively). However, previous studies have shown a strong correspondence in the association of genetic markers with eclosion time between the Grant and Fennville sites, as well as geographic clines for these loci (Feder et al., 1990, 1993; Michel et al., 2010). Thus, the same genes appear to underlie diapause termination timing variation in both populations. Consequently, the overall low correlation between diapause termination timing and initial diapause depth cannot be primarily explained by population differences. The two traits are not completely genetically independent, however, as two SNP classes on chromosome 2 (high LD group and intermediate LD) displayed negative genetic relationships between the two traits (Table 4). We cannot determine at the present time whether the negative relationships for these two SNP classes are due to linkage or pleiotropy among loci affecting the traits.

There are few other data available to suggest whether genetic independence of seasonal life-history components is a general phenomenon. In the case of diapause regulation, similar signalling cascades play a role in the maintenance (related to intensity) and

termination (related to eclosion timing) of diapause (Denlinger, 2002), which may explain why some genomic regions (on chromosome 2) had correlated effects on both traits. Yet, both traits seem to be highly polygenic, and there is ample, unlinked variation available to respond to selection. In contrast, Donohue (2014) found that two traits regulating seasonal timing of flowering and seed germination in a plant were determined by largely overlapping loci that could potentially constrain independent evolution of each trait. We know of no other comparable studies in animals, but it is likely that developmental constraints, or lack thereof, contribute to the probability of host race formation requiring divergence along multiple ecological axes.

In particular, modularity can help facilitate genomic responses to selection along multiple ecological axes, as hypothesized for taxa prone to ecological speciation with gene flow. The responsiveness of the *R. pomonella* genome to selection also agrees with predictions that populations having the greatest potential for ecological speciation possess large stores of standing variation (Barrett & Schluter, 2008), with adaptive allelic combinations in such stores protected from recombination by inversions and/or physical linkage (Ayala, Guerrero, & Kirkpatrick, 2013; Feder, Berlocher, et al. 2003; Feder, Roethele, et al., 2003; Feder, et al., 2005; Joron et al., 2011; Lowry & Willis, 2010; Noor, Grams, Bertucci, & Reiland, 2001; Rieseberg, 2001). Divergence may be slower when responses to selection along multiple ecological axes are fuelled primarily through new mutations with small fitness effects. Consequently, environmental conditions could often cause populations to go extinct before adaptation and/or speciation occurs. Thus, standing, unconstrained variation is a key requirement for populations to respond quickly when ecological opportunity arises. *Rhagoletis* populations appear to be particularly well provisioned in this regard, with extensive, modular variation in diapause development likely present in the ancestral hawthorn race to track local, yearly and regional (latitudinal) variation in hawthorn fruiting time (Feder & Bush, 1989; Feder, Roethele et al., 1997; Feder, Stolz, et al., 1997). As a result, *R. pomonella* has the ability to shift and rapidly adapt to novel hosts with differing phenologies. This ability also appears to be a general pattern in insects, with many species exhibiting large stores of standing genetic variation in diapause regulation that may respond rapidly to changing seasonal environments (Bradshaw & Holzapfel, 2001; Gomi & Takeda, 1996; Urbanski et al., 2012).

4.3 | Diapause variation predicts host race differentiation

Despite their apparent genetic modularity, the strength of genetic associations with diapause termination timing and initial diapause intensity significantly predicted host-related differentiation between apple and hawthorn flies at the Grant, MI site. Genomewide, the pattern of host divergence for all 10,241 SNPs at Grant, MI, was significantly predicted by association with diapause termination timing ($r = .29$; $p < 10^{-4}$) and response to selection on initial diapause intensity ($r = .38$; $p < 10^{-4}$) (Table 3). Closer examination revealed contrasting associations with structural features of the genome; the correlation between host race differences and initial diapause intensity was fairly uniform across the genome, while significant correlations between host race differentiation and diapause termination timing were heavily biased towards chromosomes 1 and 3 (Table 3). Initial diapause intensity predicted host race differentiation consistently across the entire genome, including low, intermediate and high LD class SNPs, with only three subsets of SNPs failing to show significant relationships (Table 3; see also Egan et al., 2015). In contrast, the significant relationship between host race differentiation and diapause termination timing was limited to particular categories of SNPs, including chromosomes 1 and 3 (overall and intermediate LD), chromosome 1 low LD, high and intermediate LD SNPs overall, and high LD groups 4 and 6 on chromosome 2. Thus, the two diapause traits contributed differently to host-related divergence depending upon levels of LD displayed by SNPs and, by inference, their degree of association with inversions.

4.4 | Summary

Divergent ecological selection on standing, polygenic variation appears to have a large, genomewide impact on host-related divergence between the *R. pomonella* host races. This conclusion is based on analysis of two phenotypic components of diapause life-history that appear to be largely genetically modular, while sharing some common, causative genetic variation. Additional ecological differences besides diapause timing distinguish *Rhagoletis* host races and species, such as differential host choice (preference) (Feder et al., 1994; Linn et al., 2003; Prokopy, Diehl, & Cooley, 1988) and differential larval feeding performance and survivorship (Bierbaum & Bush, 1990; Diehl & Prokopy, 1986; Ragland et al., 2015; Smith, 1986). These ecological axes will likely further the effects of selection and the involvement of additional genes potentially acting as independent phenotypic modules in the adaptive divergence of *Rhagoletis* populations. As the field of speciation genomics develops, we need to test the degree to which other taxa prone to ecological speciation share genetic and phenotypic characteristics with *R. pomonella* such as modular standing variation for divergently selected traits, chromosomal rearrangements and other genome level processes generating biodiversity (Arnegard et al., 2014; Hohenlohe, Bassham, Currey, & Cresko, 2012; Jones et al., 2012; Keller et al., 2013; Kronforst et al., 2013; Peccoud, Ollivier, Plantegenest, & Simon, 2009; Renaut, Owens, & Rieseberg, 2014; Renaut et al., 2012; Soria-Carrasco et al., 2014; Strasburg et al., 2012; White, Cheng, Simard, Costantini, & Besansky, 2010).

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DATA ACCESSIBILITY

All raw sequence and SNP data generated from this study are deposited in DRYAD https://doi.org/10.5061/dryad.kn568/2. SNP data from Egan et al. (2015) used in this study are deposited in DRYAD https://doi.org/10.5061/dryad.mb2tj.

AUTHOR CONTRIBUTIONS

All authors developed the conceptual framework, G.J.R., P.J.M. and J.L.F. designed the experiments, G.J.R., P.J.M., M.M.D. and J.L.F. analysed the data, and all authors contributed to manuscript drafting.

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