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The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation

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Abstract

The genetic and ecological factors that shape the evolution of animal diets remain poorly understood. For herbivorous insects, the expectation has been that trade-offs exist, such that adaptation to one host plant reduces performance on other potential hosts. We investigated the genetic architecture of alternative host use by rearing individual *Lycaeides melissa* butterflies from two wild populations in a crossed design on two hosts (one native and one introduced) and analysing the genetic basis of differences in performance using genomic approaches. Survival during the experiment was highest when butterfly larvae were reared on their natal host plant, consistent with local adaptation. However, cross-host correlations in performance among families (within populations) were not different from zero. We found that *L. melissa* populations possess genetic variation for larval performance and variation in performance had a polygenic basis. We documented very few genetic variants with trade-offs that would inherently constrain diet breadth by preventing the optimization of performance across hosts. Instead, most genetic variants that affected performance on one host had little to no effect on the other host. In total, these results suggest that genetic trade-offs are not the primary cause of dietary specialization in *L. melissa* butterflies.

Keywords: antagonistic pleiotropy, diet breadth, *Lycaeides melissa* genome, polygenic modelling, specialization, standing genetic variation

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Introduction

Considerable progress has been made in understanding the evolutionary origins of novelty, particularly for morphological features of organisms and also for physiological traits (Carroll 2008; Martin & Orgogozo 2013). More complex features, such as behaviour or life history traits, have historically been less tractable, at least in part because of previous limitations in our ability to connect genetic variation to phenotypic differences among individuals. A prominent axis of life history diversity among heterotrophs is the number and type of dietary resources consumed, and a key challenge has been to understand the factors that constrain or facilitate the utilization of new types of food or prey (Futuyma & Moreno 1988; Devictor *et al.* 2010; Poisot *et al.* 2011). All else being equal, the ability to utilize a wide variety of food types should confer an individual fitness advantage. Thus, we can ask: Why do all species not evolve to utilize a wider range of resources?

The most common answer to this question has been that specialists have higher individual fitness and population growth rates than generalists (i.e. 'a jack of all trades is a master of none'; Futuyma & Moreno 1988; Roff & Fairbairn 2007; Hereford 2009). For herbivorous

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insects, the expectation has been that the ability to utilize a specific host plant and detoxify secondary metabolites will involve physiological or behavioural adaptations that are associated with reduced success on a different potential host (Jaenike 1990; Joshi & Thompson 1995; Scriber 2010). The validity of this simple model for constraints on the evolution of diet breadth in herbivorous insects has far-reaching consequences, both basic and applied, from models of ecological, host-associated speciation that incorporate genetic trade-offs (Berlocher & Feder 2002; Fry 2003; Nosil 2012) to our understanding of the origin and management of crop pests (Via 1990; Higginson *et al.* 2005).

Experiments that have addressed constraints on diet breadth in herbivorous insects have mostly used tools from classical quantitative genetics. These include artificial selection treatments (e.g. Agrawal 2000) and assays of natural and manipulated genetic variation (e.g. Fox 1993) in which insect larvae are reared on different host plants and performance is examined for the presence of negative cross-host correlations among families, which would be indicative of genetic trade-offs or antagonistic pleiotropy. In contrast to expectations, these studies have most often failed to detect any correlation or have revealed positive, rather than negative, cross-host genetic correlations: some genotypes simply do better in all environments (Joshi & Thompson 1995; Fry 1996; Scheirs *et al.* 2005; but see Hawthorne & Via 2001). These results, however, do not necessarily falsify the hypothesis that genetic trade-offs constrain or slow diet breadth evolution (Charlesworth 1990; Joshi & Thompson 1995). In particular, if performance is polygenically controlled, genetic variants with host-specific effects (i.e. conditionally neutral loci) or common effects across hosts (i.e. genetic variants with general effects on resource acquisition; Agrawal *et al.* 2010) could persist in populations (Houle 1991; Joshi & Thompson 1995). Such variants could even be maintained indefinitely by mutation-selection balance, by gene flow (Charlesworth 1990; Yeaman & Jarvis 2006), or through epistatic interactions with other loci (Remold 2012). The resultant standing variation would comprise a mixture of loci with various effects on performance on different hosts. This could complicate attempts to detect antagonistically pleiotropic loci by causing overall cross-host genetic correlations to be weak or positive (Houle 1991; Joshi & Thompson 1995). Thus, we need to know whether and how often alleles at individual loci increase performance on one host while decreasing performance on another to better evaluate the trade-off hypothesis (Agrawal *et al.* 2010).

Alternatively, the ability to utilize novel resources could be constrained by low levels of standing genetic variation for ecologically relevant traits even in the absence of genetic trade-offs. Insufficient genetic variation is thought to constrain adaptation in other systems (e.g. Jump & Penuelas 2005; Hoffmann & Sgro 2011). And, although functional genetic variation has been documented for many traits in natural and experimental populations (Barton & Keightley 2002; Bridle & Vines 2007; Gompert *et al.* 2014a), diet breadth could still be constrained by a lack of genetic variation in one or a few key traits or trait combinations (Blows & Hoffmann 2005). Thus, this hypothesis also warrants serious consideration along with the more commonly tested trade-off hypothesis.

Contemporary cases of host range expansion provide opportunities for examining factors that constrain or promote the successful utilization of novel resources, and here, we take advantage of the well-studied colonization of alfalfa (*Medicago sativa*) by the Melissa blue butterfly (*Lycaeides melissa*) in western North America (Forister *et al.* 2009; Gompert *et al.* 2010, 2013). *Lycaeides melissa* utilizes a small number of native legume hosts (fewer than five species) in the region of our focal populations in the western Great Basin. These butterflies can be found in many locations in association with naturalized alfalfa (also a legume), often persisting solely on this novel host, which has only been available to the butterfly within the last 200 years since introduction to the region (Michaud *et al.* 1988). Alfalfa is a relatively poor larval host, conferring low survival, small adult size and reduced fecundity (Forister *et al.* 2009; Scholl *et al.* 2012). The novel host appears to support populations of *L. melissa* under a narrow set of ecological conditions that includes flowering phenology and the presence of ants that engage in a protective mutualism with caterpillars (Forister *et al.* 2011). The relatively inferior quality of alfalfa as a host allows us to investigate recent adaptation in the ability of caterpillars to develop on the novel resource and ask to what extent evolution has the potential to improve performance and survival on the currently inferior novel host. Females in alfalfa-associated populations appear to have evolved an increased acceptance of the otherwise less preferred host (Forister *et al.* 2013), and larvae from a small number of alfalfa populations have reduced ability to feed on a native host (Scholl *et al.* 2012), but that is all that is currently known about evolution or the potential for evolution on the novel resource in this system.

Here, we analyse juvenile performance and genomic variation in *L. melissa* from two wild populations experimentally reared in a crossed-host design to test for constraints on the evolution of diet breadth. We take advantage of recent advances in statistical genetics (e.g. Ober *et al.* 2012; Zhou *et al.* 2013) and a draft *L. melissa* genome, which is reported here for the first time, to overcome some of the limitations inherent in earlier

studies of this topic. This allows us to address the following specific questions: (i) Are *L. melissa* populations adapted to their natal hosts (native or introduced)? (ii) Is there standing genetic variation for larval performance (including survival) and does this variation include loci with major phenotypic effects? (iii) Is there evidence of genetic trade-offs, loci with positive effects on one host and negative effects on the other host, or do loci have independent, host-specific effects? and (iv) Finally, do variants in genes, or those in or near genes with specific molecular functions contribute disproportionately to performance? We ask about the molecular function of genes associated with performance not to test existing hypotheses (too few clear expectations exist), but rather to generate hypotheses about the mechanistic basis of host adaptation that could be tested in future studies.

Methods

The two wild *Lycaeides melissa* populations that were used for the rearing experiment were Silver Lake (SLA_{Ac}, 39.6497°N, −119.9263°W) and Goose Lake (GLA_{Ms}, 41.9858°N, −120.2931°W). Both populations are on the western edge of the Great Basin, but they are separated by about 260 km. The populations exhibit weak genetic differentiation (Nei's $D = 0.03$; Gompert *et al.* 2014b). The former (SLA_{Ac}) is in typical, low elevation Great Basin *L. melissa* habitat associated with a native host, *Astragalus canadensis* (Fabaceae), on the edge of a seasonally dry lake bed. The latter (GLA_{Ms}) is on the weedy margin of a cultivated alfalfa (Fabaceae) field. Alfalfa is the only known host at GLA_{Ms} and is not present at SLA_{Ac}. *Astragalus canadensis* is the main host plant available at SLA_{Ac}; a second potential host, *Astragalus agrestis*, occurs there too, but is quite rare. Both populations appear to be large and stable and have been studied previously (Forister *et al.* 2013; Gompert *et al.* 2014b).

Larval rearing experiment

In early July of 2011, 50 females were captured at each location and caged individually with natal hosts in small oviposition cages (see Gompert *et al.* 2013 for a description of these cages). Fifty males were also captured from each population and frozen for genetic analyses. After 48 h in cages in an outdoor facility, females were frozen and eggs were removed from plants and stored in petri dishes (keeping families in separate dishes) under bright lights at room temperature for 7–10 days until hatching. Neonate larvae were placed individually in petri dishes (90 mm × 12 mm) for rearing on either the natal host plant or the host plant of the other population (the mean number of larvae per family was 14.61, SD 8.63). Host assignment alternated as larvae from a given family emerged from eggs. Plants were collected from the field once a week and fed to larvae every four days (or sooner if needed) as sprigs with leaf petioles wrapped in damp laboratory tissues (Kimwipes). All dishes were cleaned daily, and water in laboratory tissues was refreshed. Rearing was conducted at room temperature on benchtops under 12-h light/dark cycles as we have done for other experiments using *Lycaeides* butterflies (Scholl *et al.* 2012; Forister *et al.* 2013).

Rearing *L. melissa* caterpillars on alfalfa results in very high mortality, which is useful in an experimental evolution context but also challenging (because of low sample sizes). From previous work, we knew that mortality could be ameliorated on alfalfa if caterpillars had access to flowers, particularly in the earliest developmental stages. Thus, for the first 12 days of the experiment (which is approximately $\frac{1}{3}$ of the development time from egg hatch to adult), alfalfa dishes contained sprigs with flowers; then after 12 days, only foliage was provided. Caterpillars feeding on the native host were only given foliage throughout the experiment. While alfalfa flowers improve performance on that host, the presence of flowers on the native host has no effect on performance (Forister *et al.* 2009). Furthermore, *L. melissa* caterpillars are frequently found eating flowers on alfalfa in the field, but rarely found eating flowers of the native host *A. canadensis* (Forister *et al.* 2013).

During daily dish cleaning, plants were searched for living or dead caterpillars, any dead caterpillars were immediately frozen for genetic study, and the date of death was noted. For caterpillars that survived development, date of eclosion as an adult (and sex) was noted, and newly emerged adults were weighed to the nearest 0.01 mg on a Mettler Toledo XP26 microbalance on the day of emergence and subsequently frozen.

Analysis of larval performance

We calculated cross-host correlations among families for survival and adult weight to test for genetic trade-offs based on mean family performance and as a basis of comparison with our genomic analyses (described below). Because our families were the progeny of wild-caught females, we do not know the proportion of families composed of full- vs. half-siblings. Family means were based on an average of 14.61 (SD 8.63) and 6.75 (SD 5.00) larvae for survival and adult weight, respectively. Pearson product-moment correlation coefficients were calculated for mean survival and adult weight in each population using the R statistical computing environment (R Core Team 2013).

We consider the two *L. melissa* populations to be adapted to their natal host if their performance is higher on their natal host compared to the non-natal host. We tested this prediction by estimating the effects of butterfly population and plant treatment on survival to adult and adult weight using Bayesian generalized linear mixed models. We modelled performance as a function of an intercept, butterfly population (a binary variable), plant treatment (a binary variable), a population by plant interaction and butterfly family (assigned a zero-centred hierarchical prior with family nested within population). We assumed normal errors for adult weight, but modelled survival as a binary response variable with Bernoulli errors and a logit link function. We placed uninformative normal priors on the nonhierarchical regression coefficients ($\mu = 0$, $\tau = 0.001$) and uninformative gamma hyper-priors ($\alpha = 1$, $\beta = 0.01$) on the hierarchical precision parameters for the family effects. We specified and fit these models using the R interface with JAGS provided by the RJAGS package (Plummer 2003; R Core Team 2013). We ran three Markov chain Monte Carlo (MCMC) analyses for each performance metric, and each chain consisted of a 5000 iteration burn-in and 10 000 additional iterations with samples retained from every other iteration. We verified adequate MCMC mixing by inspecting sample history plots and calculating parameter effective sample sizes (we used the R package CODA to calculate effective sample sizes, which were >2000 for all parameters; Plummer *et al.* 2006).

Genome assembly and annotation

We sequenced, assembled and annotated a draft *L. melissa* genome to serve as a reference for statistical and functional genetic analyses of larval performance. For genome sequencing, we utilized the shotgun sequencing and assembly approach that is implemented in ALLPATHS-LG (version snapshot 47 417 downloaded and compiled August 2013; Gnerre *et al.* 2011). All library construction and DNA sequencing were performed by Macrogen (Seoul, Korea). Three jumping libraries were constructed (with inserts of 3, 5 and 10 kb) and sequenced on individual lanes of Illumina HiSeq 2000 with 100-bp paired-end reads ($\sim 400 \times 10^6$ reads each, of which only 3.6–7.5% were retained as uniquely informative in the assembly). These libraries were constructed from a single DNA extraction of 25 SLA_{Ac} females collected on 16th June 2013 (total wet weight of 284.53 mg). The DNA for these libraries was extracted using Qiagen's genomic-tip kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's standard protocol. Likewise, we made a genomic DNA library from a single individual female that was collected on the same day as the females above, but extracted with the Qiagen DNeasy Blood & Tissue kit (Qiagen Inc.). The DNA was sonicated to an average size of 180 bp and was sequenced on one Illumina HiSeq 2000 lane with 100-bp paired-end reads (457 815 530 reads, of which 47.4% were used in assembly). The ALLPATHS-LG pipeline was run with default settings except for setting the 'HAPLOIDIFY' option to true (to improve performance for an outcrossed, heterozygous individual used in the fragment library) and 'MIN_CONTIG' set to 250 bp (after testing 1000, 500, 350 and 250) as this allowed a much greater fraction of the genome to be assembled in a similar number of scaffolds and without an increase in the number of bases left unresolved ('*N*').

We annotated structural genetic elements in the draft *L. melissa* genome with the MAKER pipeline (version 2.8b; Holt & Yandell 2011; Yandell & Ence 2012). This bioinformatics pipeline is designed for de novo annotation of genomes from nonmodel organisms. The pipeline includes repeat masking, *ab initio* gene prediction, protein and RNA alignment to facilitate evidence-informed gene prediction, and integrating evidence to produce synthetic annotations with evidence-based quality scores. We identified and masked tandemly repeated sequences (with low complexity) and complex repetitive elements (e.g. transposons and retrotransposons) with REPEATMASKER 4.0.1 (Smit *et al.* 1996–2010). Complex repeats were identified from the hexapoda repeat library in REPBASE 18.05 and a curated library of transposable element protein sequences. We used SNAP 2006-07-28 to generate *ab initio* gene predictions from a hidden Markov model (HMM) of exon and intron boundaries (Korf 2004). CEGMA 2.4 (Parra *et al.* 2007) was used to identify conserved eukaryotic genes to train the HMM in SNAP. Because we lacked mRNA data from *Lycaeides*, we used expressed sequences from three other butterfly species as RNA evidence: *Danaus plexippus* (19 577 ESTs from brain tissue; GenBank Accession no. EY274705), *Heliconius erato* (9394 wing disc ESTs; GenBank Accession nos. DV570503–DV570535, CV525684–CV526458, and EL595871–EL604456) and *Heliconius melpomene* (6004 wing disc ESTs; GenBank accessions CO729474–CO729979, CV126116–CV126118, CV133737–CV133811, CX700422–CX700907, DN048384–DN172770, EE743387–EE743533, ES584599–ES587832 and GE842152–GE843131). We combined these data with 990 934 insect protein sequences from the UniProt Knowledgebase (Magrane & Consortium, 2011) for evidence-informed gene predictions. We then refined intron–exon boundaries with EXONERATE 2.2.0 (Slater & Birney 2005). We used MAKER to calculate the edit distance for each structural annotation as a measure of the evidence supporting the annotation (Eil-

beck *et al.* 2009). We then used INTERPROSCAN 5.4-47 to functionally annotate the 13 518 predicted protein sequences from MAKER. We scanned these protein sequences against 12 databases: COILS, GENE3D, HAMAP, PANTHER, PFAM-A, PIRSF, PRINTS, PRODOM, PROSITE, SMART, SUPERFAMILY and TIGRFAM.

Genotyping by sequencing and alignment

We extracted genomic DNA from each of the 515 adult butterflies, 473 of the 685 butterflies that died as larvae (we were unable to extract DNA or generate sequence data from 212 larvae that died early in development), and 98 adult butterflies collected from GLA_{Ms} (40 butterflies) and SLA_{Ac} (58 butterflies). Genomic DNA was extracted with Qiagen's DNeasy 96 Blood & Tissue kit (Qiagen Inc.) according to the manufacturer's recommendations. Double-digest genomic libraries were made for each butterfly following previously published laboratory methods (Gompert *et al.* 2012; Parchman *et al.* 2012, with modifications described in Gompert *et al.* 2014b). In brief, we reduced the complexity of the whole genome by sequencing DNA associated with restriction fragments. We digested 0.5 μg of genomic DNA from each butterfly with the restriction endonucleases *Eco*RI and *Mse*I and then attached double-stranded adaptor oligonucleotides to the sticky ends of the digested DNA with T4 DNA ligase. The oligonucleotides were similar to the Illumina adaptor molecules, but included unique 8–10 base pair (bp) barcode sequences for multiplexing. We PCR amplified the restriction fragment library with the Illumina PCR primers and iProof high-fidelity polymerase (Bio-Rad Laboratories Inc., Hercules, CA, USA). We then pooled the genomic libraries and size-selected those fragments between 300 and 450 bp in length with Qiagen's QiaQuick gel extraction kit (Qiagen Inc.). Detailed information about reaction conditions can be found in the online supporting information associated with Gompert *et al.* (2014b). The genomic libraries were sequenced at the University of Texas Genomic Sequencing and Analysis Facility (Austin, TX, USA) on the Illumina HiSeq 2500 platform.

We used BWA 0.7.5a-r405 to align these sequences to the draft *L. melissa* genome. We used the ALN and SAMSE algorithms for the alignments and allowed a maximum of four differences between each sequence and the reference (no more than two differences were allowed in the first 20 bp of the sequence). We trimmed all bases with a phred-scaled quality score lower than 10 and only placed sequences with a unique best match. We then used SAMTOOLS and BCFTOOLS versions 0.1.19-44428cd to compress, sort and index the alignments.

Genetic variation in the experiments

We conducted a series of analyses to describe genetic variation in the *L. melissa* experimental populations. We first identified a set of single nucleotide variants (SNVs) from the aligned DNA sequence data. This was done using a larger data set that included an additional 483 wild-caught *L. melissa* collected from across western North America (this includes data from Gompert *et al.* 2014b and an additional data set that will be described and published later), but we restrict our analyses in this study to the subset of SNVs that were variable in the experimental populations. We used SAMTOOLS and BCFTOOLS to calculate the Bayesian posterior probability that each nucleotide was variable. We used the full prior with $\theta = 0.001$, removed sequences with phred-scaled mapping qualities lower than 15 and removed nucleotides with quality scores lower than 10, and only considered SNVs where we had sequence data for 80% or more of the butterflies. We called a nucleotide position variable if (i) the posterior probability of the data under the null model that the position was invariant was <0.01, (ii) there were at least 20 sequences supporting the nonreference allele, (iii) we observed no more than one reverse orientation read and (iv) no more than one nonreference allele, and (v) we had a minimum of 1500 sequence reads covering the nucleotide position. We identified 206 047 SNVs in the wild-caught *L. melissa* based on these criteria.

We used a hierarchical Bayesian model to estimate genotypes ($\mathbf{g}$) and population allele frequencies ($\boldsymbol{\pi}$). We computed the probability of each individual's sequence data ($\mathbf{x}$) conditional on its genotype and the sequence quality scores ($\boldsymbol{\varepsilon}$) as described by Li (2011). We assumed that the prior probability of an individual's genotype was a function of the population allele frequencies, such that $\Pr(g_{ij}|\pi_j) \sim \text{binomial}(p = \pi_i, n = 2)$. Here, g_{ij} is individual i's genotype at SNV j and denotes the number of nonreference alleles. In other words, g_{ij} is 0 if individual i is homozygous for the reference allele, 1 if individual i is a heterozygote and 2 if individual i is homozygous for the nonreference allele. π_j denotes the frequency of the reference allele in the population. We estimated this parameter from butterflies collected from GLA_{Ms} (40 individuals) or SLA_{Ac} (58 individuals) rather than the experimental butterflies. We placed a Jeffry's prior on each π_j, that is $\Pr(\pi_j) \sim \text{beta}(\alpha = 0.5, \beta = 0.5)$. Thus, we estimated the joint posterior distribution of the genotypes and allele frequencies as $\Pr(\mathbf{g},\boldsymbol{\pi} \mid \mathbf{x},\boldsymbol{\varepsilon}) \propto \Pr(\mathbf{x} \mid \mathbf{g},\boldsymbol{\varepsilon})\Pr(\mathbf{g} \mid \boldsymbol{\pi})\Pr(\boldsymbol{\pi})$. We implemented a MCMC algorithm to estimate the parameters in this model in a computer program written in C++ using the GNU Scientific Library (Galassi *et al.* 2009). We ran two MCMC replicates for each population, and each chain was run

for 4600 iterations, with a 100 iteration burn-in. Samples were retained every third iteration. We used the posterior mean, $\hat{g_{ij}} = \sum_{g_{ij} \in \{0,1,2\}} g_{ij} \Pr(\mathbf{g}, \boldsymbol{\pi} | \mathbf{x}, \boldsymbol{\epsilon})$, as the point estimate of the genotype for each individual and SNV for downstream analyses, and we estimated the sample allele frequency in each experimental population as $\hat{p_j} = \frac{1}{2N} \sum_{i=1}^{N} \hat{g_{ij}}$.

We estimated pairwise linkage disequilibrium (LD) in each experimental population for pairs of SNVs on the same genome scaffold and with a sample minor allele frequency (MAF) >5% (considering all pairs of SNVs would be computationally prohibitive and provide little additional information). We used BCFTOOLS to estimate r^2 LD statistics via an expectation-maximization algorithm that incorporates uncertainty in genotypes and haplotype configurations (Li 2011).

Genetic variation for larval performance

Polygenic models (a.k.a. genomic prediction models) have been developed over the past decade to better understand the relationship between genetic and phenotypic variation, particularly for quantitative or complex traits. Such models are well known in animal and plant breeding where they have been used for genomic prediction of phenotypes (e.g. Meuwissen *et al.* 2001; Goddard & Hayes 2007; Hayes *et al.* 2009; Heffner *et al.* 2009; Thomasen *et al.* 2014), but have received much less attention in molecular ecology (but see, Ober *et al.* 2012; Resende *et al.* 2012). Polygenic models include a kinship or genetic relatedness matrix as a predictor of phenotypic similarity among individuals (Kang *et al.* 2008; de los Campos *et al.* 2013). The genetic relatedness matrix is estimated from genomewide genetic marker data (i.e. tens of thousands to millions of SNVs) and captures the overall genetic similarity among individuals. This matrix allows one to estimate genomic breeding values for individuals and to infer the proportion of phenotypic variance explained by all of the genetic data without a known pedigree, which is an advantage relative to traditional quantitative genetic approaches (de los Campos *et al.* 2013). As the number of genetic markers assayed increases, the proportion of variation explained by the genetic data should approach a trait's narrow-sense heritability.

Some polygenic models allow a subset of genetic markers to contribute to phenotypic variation through main effects (i.e. not just via their contribution to the genetic relatedness matrix), which can be particularly important when major effect loci exist (Guan & Stephens 2011; Zhou *et al.* 2013). In practice, Bayesian model averaging is often used to estimate these main effects while accounting for the possibility that each genetic marker does or does not tag a major functional variant (Zhou *et al.* 2013). This approach avoids the stringent significance thresholds that limit the utility of genomewide association methods that test one marker at a time (Meuwissen *et al.* 2001; Yang *et al.* 2010; de los Campos *et al.* 2013). Likewise, estimates of quantitative genetic parameters obtained with polygenic models, such as the proportion of phenotypic variation explained (PVE) and the total number of functional variants tagged by the genetic marker data, fully incorporate uncertainty in whether and to what extent each genetic marker is associated with phenotypic variation (Guan & Stephens 2011; Zhou *et al.* 2013). More information on polygenic models and genomic prediction can be found in de Koning & McIntyre (2012), de los Campos *et al.* (2013) and Zhou *et al.* (2013).

We used the polygenic model described by Zhou *et al.* (2013) to quantify standing genetic variation for larval performance in the experimental population. The particular method we used fits a Bayesian sparse linear mixed model that includes a genetic relatedness matrix and the genotype of each individual at each SNV as predictors of each individual's phenotype. Models with and without a main effect for each SNV are evaluated. We analysed each of the four butterfly population × host plant treatments separately and additionally analysed five combined treatments: (i) larvae from either population reared on *A. canadensis* ('Ac'), (ii) larvae from either population reared on *Medicago sativa* ('Ms'), (iii) GLA_{Ms} larvae reared on either plant species ('GLA_{Ms}'), (iv) SLA_{Ac} larvae reared on either plant species ('SLA_{Ac}') and (v) and all reared larvae ('all'). The combined treatments allowed us to identify ecologically important genetic variants segregating in both populations or variants that affected performance on both hosts. We fit the polygenic models for each single and combined treatment in GEMMA.

We were primarily interested in three parameters or parameter vectors that described aspects of the genetic architecture of larval performance: (i) the total proportion of PVE by the model, (ii) the total number of SNVs with main effects (which is an estimate of the number of functional variants tagged by the SNV data set) and (iii) the model-averaged effect estimates for each SNV. The model-averaged effects are given by $\hat{b_j} = \beta_j \gamma_j + \alpha_j$, where β_j is SNV j's main effect if it is included in the model (i.e. if it has a main effect), γ_j is the posterior inclusion probability for SNV j (the probability that SNV j has a main effect, i.e. that it tags a functional variant), and α_j is SNV j's contribution to the phenotypic variation via the genetic relatedness matrix (Zhou *et al.* 2013). BSLMM in GEMMA does not readily incorporate covariates, so we analysed residuals from regressing performance metrics on population or plant species in the combined treatments. This effectively removes the

effect of these covariates on phenotype. We fit linear models for adult weight (all treatments) and survival (residuals after accounting for plant and population) in the combined treatments, but probit models for survival in the population × plant treatments (survival is binary in this case). Model parameters were estimated using MCMC, with 5 (combined treatments) or 10 (population × plant treatments) MCMC replicates per performance metric. Each MCMC replicate consisted of a 1 million iteration burn-in and 5 million sampling iterations with samples retained every 40th iteration.

We used repeated random subsampling cross-validation to assess the predictive performance of the polygenic models. We generated 20 cross-validation data sets for each analysis where 80% of individuals (10 replicates) or families (10 replicates) were used to train the model and the other 20% were used to validate the model. Family-based cross-validation is expected to be a more difficult problem as individuals in the training and validation data sets are not each other's close relatives. We ran two MCMC replicates per cross-validation data set (MCMC conditions were as described in the preceding paragraph). We measured predictive performance by calculating the area under the receiver operating characteristic (ROC) curve (AUC; Wray *et al.* 2010) or cross-validated r^2 (denoted q^2).

Genetic trade-offs in performance across hosts

We tested for genetic trade-offs in performance (survival and weight) by quantifying the association between the estimated effect of each locus on survival or adult weight across the two hosts. We used model-averaged locus effects from the BSLMMs for these analyses because these estimates account for uncertainty in whether or not each variant had a measurable affect on performance. We analysed each population separately and both populations combined. We first tested for pervasive genetic trade-offs by estimating the correlation between the effects of all SNVs on *A. canadensis* vs. alfalfa. We then conducted a separate correlation analysis focused solely on the 0.1% of SNVs with the greatest model-averaged effects on performance on either host plant. Correlation coefficients were estimated in R (R Core Team 2013).

Additionally, considering only those SNVs with the greatest positive or negative (top 0.1% of SNVs for each) model-averaged effects on either host, we classified SNVs as antagonistically pleiotropic: positive effect and in the top 0.1% for one host and negative effect and in the top 0.1% for the other or vice versa; conditionally neutral: positive or negative effect and in the top 0.1% for one host and not in the top 0.1% for the other host; or having similar effects on both hosts: in the top 0.1% with a positive or negative effect on each host. We then enumerated each class of SNV. We focused on the top 0.1% of SNVs because this corresponds approximately with the upper bounds of our estimates of the number of variants affecting performance (about 150 SNVs for each population by host plant combination, see Results). However, we repeated these analyses with the top 0.01% (about 15 SNVs per population by host plant combination) and top 0.5% (about 750 SNVs per population by host plant combination) of SNVs to assess the sensitivity of our results to this cut-off.

Functional genetic analysis of variation in larval performance

We asked whether SNVs with the greatest model-averaged effect-size estimates were disproportionately associated with specific genomic features. We first tested whether the top 0.1% or 0.05% performance-associated SNVs (based on the absolute value of the model-averaged effect estimates) were found in genes (exons or introns), coding sequences or repeat regions (low-complexity tandem repeats or complex repetitive elements) more often than expected by chance. Null expectations were based on the proportion of typed SNVs assigned to each of these classes. We based tests of functional enrichment on 14 713 PFAM-A matches from INTERPROSCAN. We extracted gene ontology (GO) terms for matches and assigned each SNV within 1000 bp of an annotated gene the GO term for that gene (SNVs more distant from annotated genes were not assigned GO terms). We focused tests of functional enrichment on the top 0.1% of performance-associated SNVs and only considered molecular function GO terms that appeared two or more times among the top SNVs for a treatment (other GO terms were not observed often enough among the top SNVs to warrant meaningful enrichment tests). We used randomization tests (1000 randomizations per test) to generate null expectations for the proportion of SNVs assigned each GO term. We used the false discovery rate procedure described by Benjamini & Hochberg (1995) to control for false positives. With that said, we view these tests as a means to generate new, testable hypotheses, not as a robust tests of the functional genomic basis of host adaptation in *L. melissa*.

Results

Analysis of larval performance

Five hundred and fifteen of the 1200 *Lycaeides melissa* larvae survived through development to adulthood,

and the mean adult weight was 7.04 mg (SD 3.36 mg; Figs S1 and S2, Supporting information). Correlations between family survival proportions on *Medicago sativa* vs. *Astragalus canadensis* were not significantly different from zero in either population (GLA_{Ms}: $r = -0.17$, $P = 0.33$; SLA_{Ac}: $r = -0.02$, $P = 0.90$; Fig. 1). Mean adult weight was negatively correlated between hosts in GLA_{Ms} ($r = -0.68$, $P < 0.01$), but this was due to a single outlier observation. After removing this observation, family adult weight was not correlated between hosts in either population (GLA_{Ms}: $r = 0.22$, $P = 0.36$; SLA_{Ac}: $r = 0.05$, $P = 0.80$; Fig. 1).

Butterfly population affected survival directly [$\beta_{population} = -0.558$, 95% equal-tail probability intervals (ETPIs) = −1.000 to −0.148] and via an interaction with plant species ($\beta_{pop. \times plant} = 3.589$, 95% ETPIs = 2.951–4.250), such that survival was higher when larvae were reared on their natal host but with a more pronounced difference between populations on *A. canadensis* (Table S1, Supporting information, Fig. 2). In contrast, adult weight was affected by plant species but not butterfly population ($\beta_{plant} = 6.436$, 95% ETPIs = 5.951–6.917), with greater weight on *A. canadensis* (Table S1, Supporting information, Fig. 2).

Genome assembly and genetic variation in the experiments

The assembled *L. melissa* genome included 14 029 scaffolds with a total length of 360×10^6 bp (158×10^6 bp excluding *N*'s). Thus, the assembly includes 80% (35% excluding *N*'s) of the 450×10^6 bp *L. melissa* genome. The median scaffold length was 9651 bp; 718 scaffolds were over 10^5 bp long, and two scaffolds exceeded 10^6 bp. We identified 13 158 putative genes (16.1% of the assembled genome), including complete (49.2%) or partial (68.6%) matches to about half of the 248 core eukaryotic genes in CEGMA, 62 064 putative coding sequences (3.5% of the genome), 2094 UTR sequences and 167 432 repeat regions. The number of matches for the 13 158 *L. melissa* predicted protein sequences to known protein domains varied by database: 31 115 in COILS, 13 464 in GENE3D, 109 in HAMAP, 14 713 in PANTHER, 14 713 in PFAM-A, 239 in PIRSF, 6448 in PRINTS, 1 in PRODOM, 13 493 in PROSITE, 10 006 in SMART, 12 501 in SUPERFAMILY and 372 in TIGRFAM.

We identified 206 047 *L. melissa* SNVs with an average sequencing depth of 17.2× (SD = 11.0) per individual from partial, reduced complexity, genome sequences. Many of these SNVs were variable (MAF > 0%) in the experimental populations: 142 128 in GLA_{Ms}, 137 356 in SLA_{Ac} and 102 583 in both. The sample site allele frequency distribution in each population was dominated by low-frequency variants, with about half of the MAFs <5% (Fig. 3a), and most SNVs exhibited similar allele frequencies in the two experimental populations ($r = 0.84$, Fig. 3b). Average LD between common variants (MAF > 5%) was modest for SNVs within 100 bp of each other (GLA_{Ms}: $\bar{r}^2 = 0.10$; SLA_{Ac}: $\bar{r}^2 = 0.10$) and decayed rapidly with physical distance (Fig. 3c,d).

Genetic architecture of larval performance

We found that the SNVs explained a non-negligible proportion of the variation in survival and adult weight via their combined polygenic and main effects [range of posterior median proportion of PVE across treatments: survival 0.12–0.57, adult weight 0.07–0.47]. However, PVE estimates were characterized by considerable uncertainty, particularly for population × plant treatments, and thus, we cannot rule out considerably smaller or larger PVE values (Fig. 4). Point estimates for combined treatments were generally lower, but with less uncertainty (Fig. S3, Supporting information). Similarly, point estimates (posterior median) for the number of SNVs with main (i.e. sparse) effects on performance ranged from 11 to 29, but we could not confidently rule out many

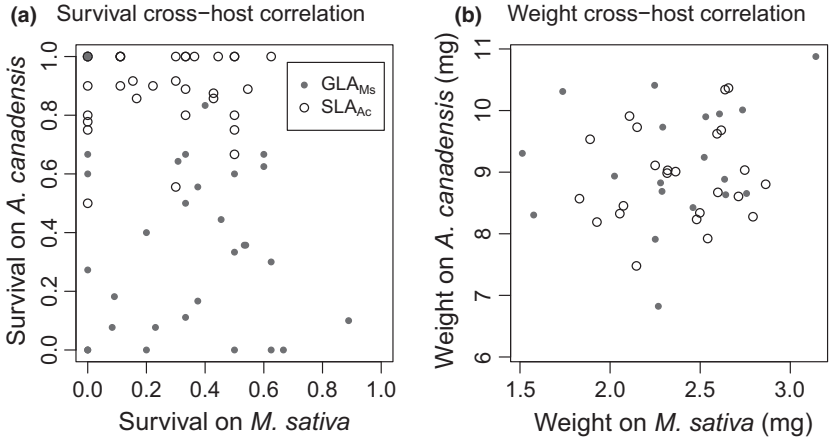

Fig. 1 Cross-host genetic correlations for survival and adult weight on *Medicago sativa* vs. *Astragalus canadensis*. Points show (a) the proportion of larvae surviving to pupation or (b) mean adult weight for each family. Two outlying points were excluded from pane (b) for improved visualization. Neither of the correlations was significantly different from zero.

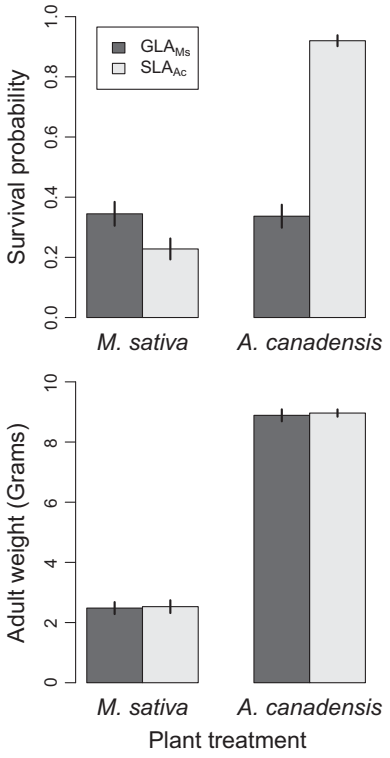

Fig. 2 Larval survival and adult weight as a function of population and host plant. The barplots show the posterior mean (bar) and standard deviation (vertical line, analogous to the standard error) from Bayesian regression analysis.

more or fewer SNVs (Figs 4 and S3, Supporting information). We also conducted a set of complementary single-SNV association analyses using linear mixed models to obtain maximum-likelihood PVE estimates and identify any SNVs with major effects on performance (see Appendix S1, Supporting information for details). Maximum-likelihood PVE estimates were mostly similar to those from the BSLMMs, and we detected few SNVs with substantial and significant individual associations with performance (Table S2, Figs S5 and S6, Supporting information).

Based on cross-validation goodness-of-prediction analyses, the polygenic BSLMMs had little predictive power despite their moderate explanatory power (Fig. S4, Supporting information). Indeed, in most cases, the BSLMM did not perform differently than a model with no predictive ability. The most notable exception was the model for survival in the GLA$_{Ms}$ × *A. canadensis* and GLA$_{Ms}$ × *M. sativa* treatments, where the BSLMM showed some (albeit still quite limited) predictive ability with both individual [mean area under the receiver operating characteristic curve (AUC) on *A. canadensis* = 0.55; mean AUC on *M. sativa* = 0.65] and family-based cross-validation (mean AUC on *A. canadensis* = 0.55; mean AUC on *M. sativa* = 0.55).

Genetic trade-offs in performance across hosts

We found no evidence of negative correlations between SNV effects on the different hosts (Fig. 5). Instead, these correlations were zero (survival and adult weight for SLA$_{Ac}$ on *A. canadensis* vs. *M. sativa* and adult weight for both populations combined, $P > 0.05$) or very weakly positive (GLA$_{Ms}$ on *A. canadensis* vs. *M. sativa*: survival, $r = 0.014$, $P < 0.0001$; adult weight, $r = 0.033$, $P = 0004$; both populations combined: survival, $r = 0.014$, $P = 0.0002$). We obtained similar results when only considering the 0.1% of SNVs with the greatest model-averaged effects on performance ($P > 0.05$ in all cases).

More than 99% of the SNVs with the largest positive or negative effects (top 0.1% of SNVs for each) were classified as conditionally neutral for each population and performance metric (Table 1). Whereas, in total, only four SNVs had common effects across hosts, and three exhibited antagonistic pleiotropy. The proportion of SNVs exhibiting conditional neutrality was robust to the different cut-offs we used for classification (Table 1). Moreover, while the number of SNVs in each category was higher when considering the top 0.5% of SNVs, about twice as many SNVs exhibited common effects across hosts as exhibited antagonistic pleiotropy under this condition.

Functional genetic basis of variation in performance

Those SNVs with the largest model-averaged effects on performance did not occur in genes or coding regions more often then expected by chance ($P > 0.05$ for all treatments), and this was true for both the top 0.1% and top 0.05% of SNVs. But these SNVs were over-represented in repeat regions in multiple treatments and for both survival and adult weight (significant enrichments ranged from 2.3 to 4.7×; Table 2). This included 21 SNVs in transposable elements (seven of which were in the Zenon family of retrotransposons), and seven in low-complexity tandem repeats.

Nonetheless, the subset of these performance-associated SNVs that were in or near genes, were in or near genes with a variety of molecular functions (Appendix S2, Supporting information). A significant excess of the top survival-associated SNVs were in or near genes with catalytic or motor activity in several treatments (no. of SNVs = 2–3, enrichment = 8–18×), and in or near genes with binding or DNA binding functions in one treatment each (two SNVs each, enrichment = 6–14×; Table S3, Supporting information). Likewise, genes with DNA helicase activity (enrichment = 90×), G-protein-coupled receptor activity (enrichment = 7–9×), oxidoreductase activity (enrichment = 9×), binding (enrichment = 10×) and calcium binding (enrichment = 5×) functions were

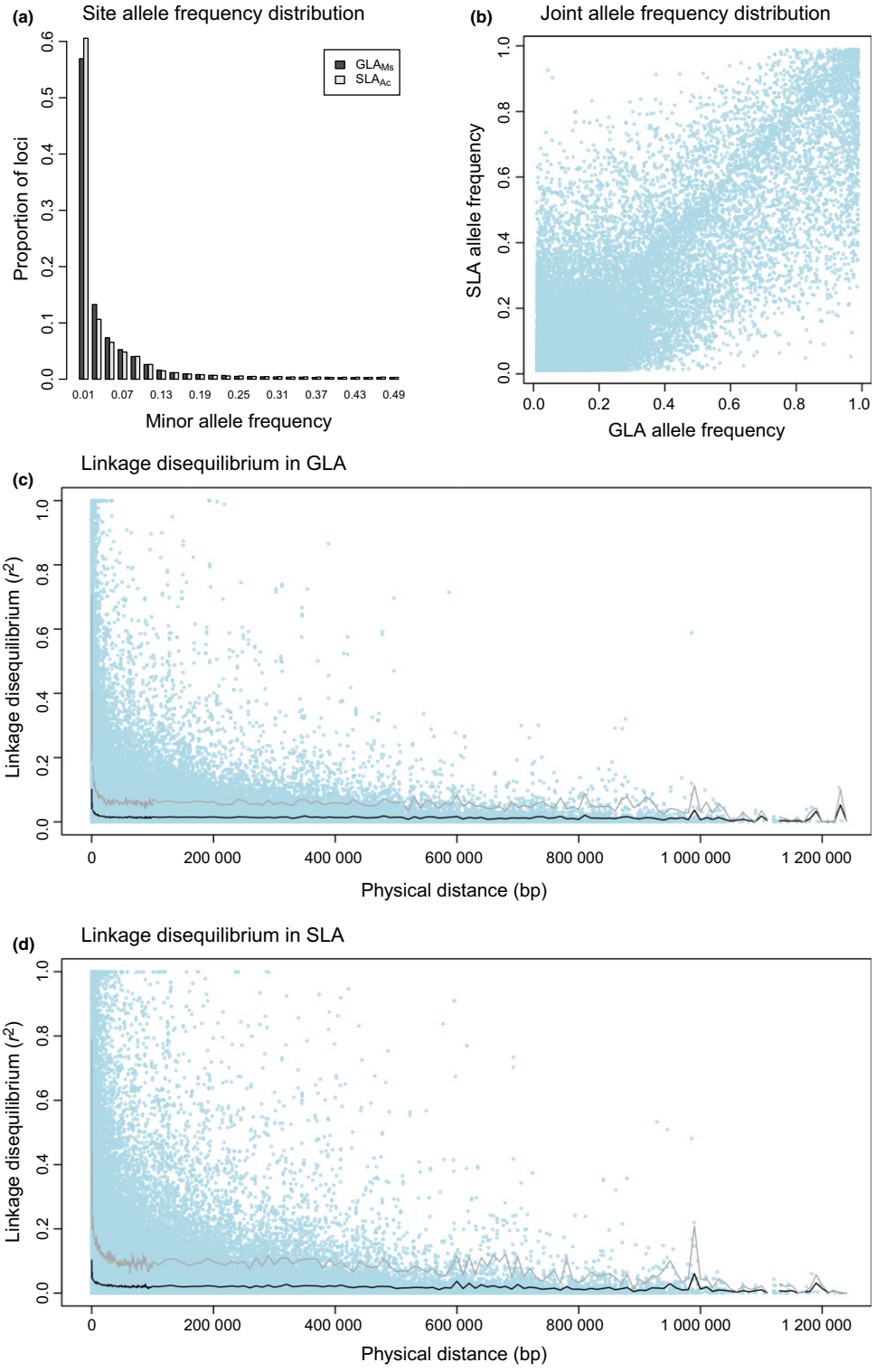

Fig. 3 Summary of genetic variation in the experimental populations. The histogram (a) and scatterplot (b) show the marginal and joint site allele frequency distribution in GLA_{Ms} and SLA_{Ac}. Invariant single nucleotide variants (SNVs) in each (a) or either (b) experimental population are excluded. (c, d) Linkage disequilibrium (calculated as r^2) as a function of physical distance for pairs of common variants (MAF > 5%) on the same genome scaffold. Blue points denote r^2 for individual SNVs, and lines denote the mean (black) and 95th percentile (grey) for different physical distance bins (we used 100-bp bins from 0 to 1000 bp, 1000-bp bins from 1000 to 10 000 bp and 10 000-bp bins beyond 10 000 bp). MAF, minor allele frequency.

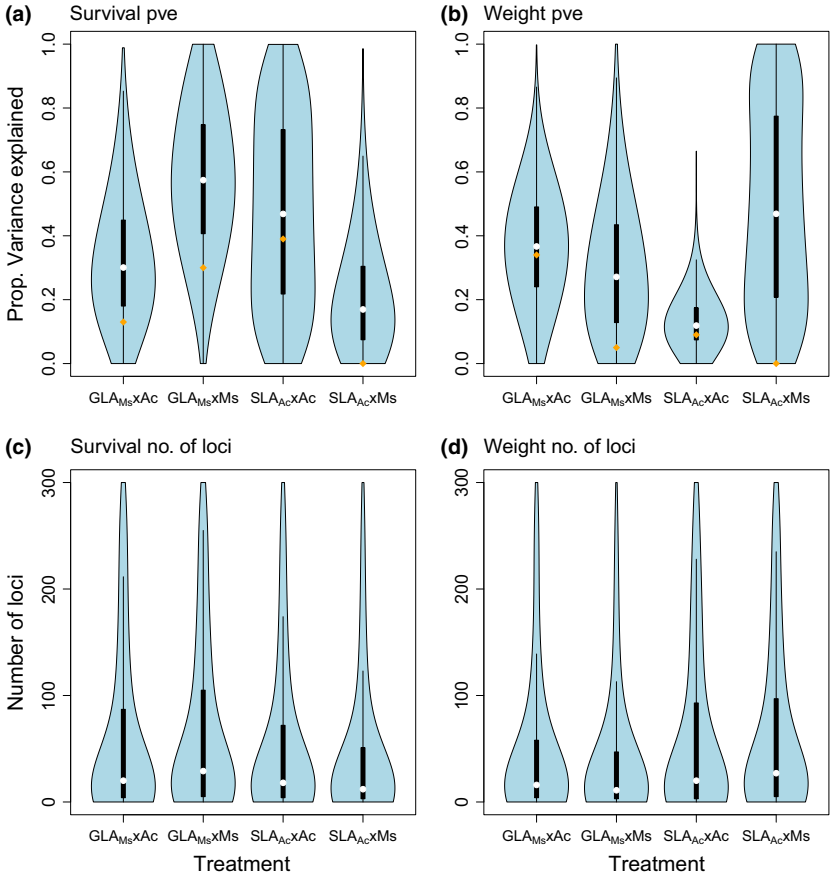

Fig. 4 Violin plots of the posterior probability distributions of the proportion of phenotypic variance explained by the polygenic model (PVE; a, b), and the number of loci affecting phenotypic variation (c, d). Estimates from individual host plant by population treatments are shown for survival (a, c) and adult weight (b, d). Violin plots combine the information conveyed by box plots and density plots. The plot shows the median (white dot), the first through third interquartile range (solid vertical box) and kernel density estimator of the posterior distribution (blue area). Maximum-likelihood estimates of PVE from null models without sparse effects are depicted with orange diamonds (see Appendix S1, Supporting information for details).

over-represented among the top weight-associated SNVs in one or two treatments (two SNVs were assigned each function in each case; Table S4, Supporting information). In several cases, the observed functional enrichment was caused by a single pair of SNVs associated with the same gene in one (DNA helicase activity) or two treatments (catalytic activity in GLA_{Ms} and all, G-coupled receptor activity).

Discussion

Dietary specialization is common in herbivorous insects and persists despite factors that should favour generalists: greater resource availability and versatility (Via 1990; Mopper 1996; Scriber 2010). Here, we showed that dietary specialization has evolved in *Lycaeides melissa* butterflies, such that survival was higher when larvae were reared on their population's natal host than on a novel host (Fig. 2). With that said, alfalfa appears to be a relatively poor larval host, as butterflies reared on alfalfa were considerably smaller than those reared on *Astragalus canadensis*, and many more GLA_{Ms} caterpillars died when reared on their natal host (alfalfa) than did SLA_{Ac} caterpillars reared on their natal host (*A. canadensis*) (also see, Forister *et al.* 2009, 2011; Scholl *et al.* 2012). Adaptation to this novel resource is unlikely to be constrained by a lack of ecologically important genetic variation or genetic trade-offs with performance on a native host. Instead, our results suggest that *L. melissa* populations harbour substantial genetic variation for larval performance (Fig. 4), that variants affecting performance on alfalfa do not affect performance on *A. canadensis* (Figs 1 and 5) and that genetic variants affecting performance are enriched for particular structural and perhaps some functional genetic features (Tables 2, S3 and S4, Supporting information). Thus, existing hypotheses (trade-offs or a lack of genetic variation) do not explain constraints on diet breadth in *L. melissa*, and poor overall performance on alfalfa may simply reflect the fact that *L. melissa* has only recently colonized this resource and that adaptation is ongoing. We discuss each of these findings in more detail below.

Standing genetic variation for larval performance

We found that the alfalfa-feeding Goose Lake population (GLA_{Ms}) harboured considerable genetic variation (relative to phenotypic variation among individuals) for

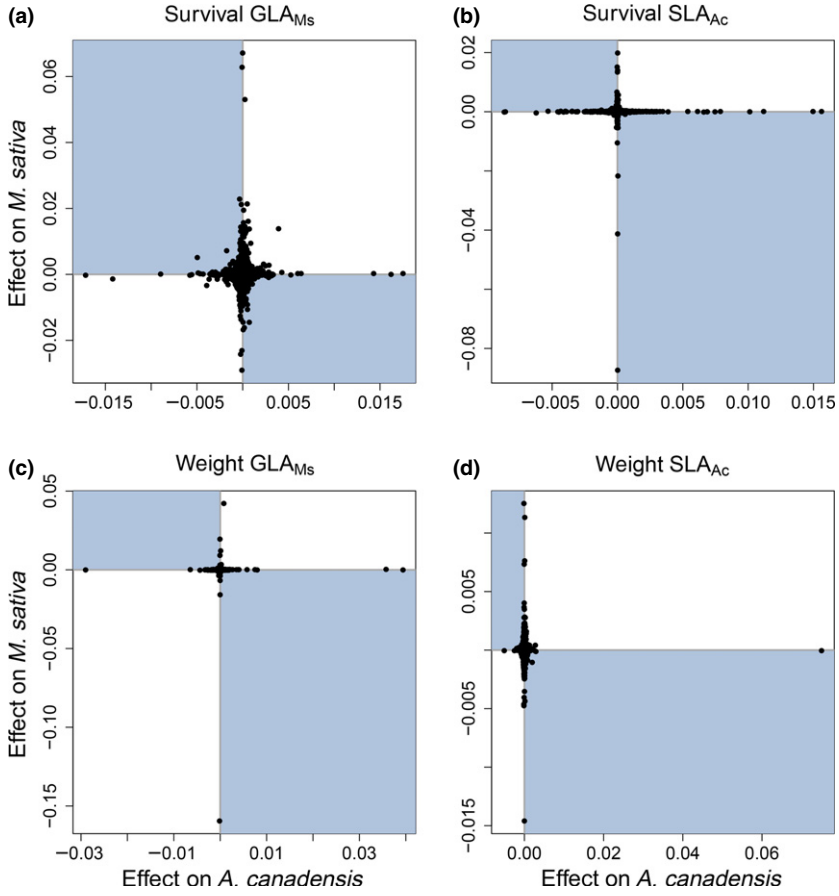

Fig. 5 Scatterplots show the relationship between the effects of single nucleotide variants (SNVs) on performance for larvae reared on different host plants. Results are shown for survival (a, b) and adult weight (c, d). In each pane, points denote model-averaged locus effect estimates, and lines indicate the *x*- and *y*-intercepts. Antagonistically pleiotropic SNVs occupy the coloured region of the plot, whereas conditionally neutral SNVs should fall along the *x*- or *y*-axis.

Table 1 Classification of locus effects on performance (the number of single nucleotide variants in each category is given)

Set	Population	Trait	Antagonistic pleiotropy	Conditional neutrality	Common effects
Top 0.1%	GLA_{Ms}	Survival	2	317	1
	GLA_{Ms}	Weight	0	306	1
	SLA_{Ac}	Survival	0	298	0
	SLA_{Ac}	Weight	1	296	1
	Combined	Survival	0	319	0
	Combined	Weight	0	309	1
Top 0.01%	GLA_{Ms}	Survival	0	36	0
	GLA_{Ms}	Weight	0	32	1
	SLA_{Ac}	Survival	0	31	0
	SLA_{Ac}	Weight	0	31	0
	Combined	Survival	0	34	0
	Combined	Weight	0	31	0
Top 0.5%	GLA_{Ms}	Survival	8	1558	12
	GLA_{Ms}	Weight	4	1490	12
	SLA_{Ac}	Survival	4	1487	5
	SLA_{Ac}	Weight	11	1458	11
	Combined	Survival	9	1535	16
	Combined	Weight	6	1489	19

survival on its natal host and thus that ongoing adaptation to this resource is not limited by a lack of genetic variation. Indeed, GLA_{Ms} harboured more variation for survival on alfalfa than on the native host *A. canadensis* (Fig. 4a). This contrasts with the expectation that natural selection should erode additive genetic variation for

Table 2 Summary of binomial probability tests of over-representation of top performance-associated single nucleotide variants (SNVs) in repeat regions

Treatment	Trait	Top SNVs (%)	No. SNVs	× enrichment	*P*-value
Ac	Survival	0.1	5	2.9	0.008
Ac	Survival	0.05	4	4.7	0.002
SLA_{Ac}	Survival	0.1	5	3.0	0.006
All	Survival	0.1	4	2.3	0.029
GLA_{Ms} × Ac	Weight	0.1	4	2.4	0.027
GLA_{Ms} × Ac	Weight	0.05	4	4.7	0.002
GLA_{Ms} × Ms	Weight	0.05	2	2.5	0.045
Ms	Weight	0.1	4	2.4	0.027
Ms	Weight	0.05	3	3.6	0.010
SLA_{Ac}	Weight	0.05	2	2.4	0.048
All	Weight	0.05	2	2.4	0.048

fitness (discussed in, e.g. Jones 1987; Merila & Sheldon 1999). Yet this standing genetic variation could be readily explained if variation in survival on alfalfa is one of several factors that contribute to variation in total fitness. Alternatively, selection might have favoured alleles that were initially rare but that now segregate at intermediate frequencies, thereby temporarily increasing genetic variation (relative to phenotypic variation) for performance on alfalfa.

We identified a few SNVs with substantial phenotypic effects on alfalfa or *A. canadensis* (Table S2, Figs S5 and S6, Supporting information), but more variation in performance was explained by a multitude of SNVs with uncertain, small or near-infinitesimal effects (i.e. via overall genetic relatedness and SNVs with small main effects; Table S2, Figs 4 and S3, Supporting information). A polygenic basis for performance is not surprising given the multifaceted (survival) and quantitative (adult weight) nature of this trait, and polygenic inheritance of host use has been documented in other systems using experimental crosses (e.g. Jeanike 1987; Scriber *et al.* 1989; Sheck & Gould 1993; Schoonhoven *et al.* 2010). Our results are novel, however, because the analytical approach we used allowed us to quantify the contribution of many DNA sequence variants to variation in performance in outbred experimental populations (rather than crosses) that were indicative of the genomic composition of wild populations.

We used BSLMMs to estimate parameters describing the genetic basis of performance in a way that integrated over uncertainty in individual SNV-by-phenotype associations (Meuwissen *et al.* 2001; Guan & Stephens 2011; Zhou *et al.* 2013) and thus partially overcame some of the limitations of traditional genomewide association studies (Beavis 1998; McCarthy *et al.* 2008; Rockman 2012). However, even with 1200 experimental larvae, estimates of genetic architecture parameters, such as the proportion of phenotypic variance explained, were characterized by high levels of uncertainty (Figs 4 and S3, Supporting information), and, because LD was low and decayed rapidly in the *L. melissa* populations (Fig. 3), we expect that some causal variants were not effectively tagged by our 206 047 SNV data set. Moreover, while we were able to explain a sizable proportion of the variation in performance with the polygenic models, these models had little (but some) predictive power (Fig. S4, Supporting information). Genomic prediction is difficult, and low predictive performance would be expected if a multitude of rare variants affect survival.

Genetic variants affecting performance on different hosts

We found little to no evidence of genetic trade-offs for performance on alfalfa vs. *A. canadensis* and consequently no support for the hypothesis that genetic trade-offs limit adaptation to alfalfa in *L. melissa*, at least for the GLA_{Ms} population we analysed. Instead, cross-host genetic correlations among families were near-zero (Fig. 1), and nearly all SNVs affecting performance on one host had little to no effect on the other host, such that locus effects were uncorrelated or weakly positively correlated across hosts (Table 1, Figs 5 and S7, Supporting information). However, this lack of evidence for genetic trade-offs does not necessarily mean that genetic trade-offs do not exist in this system. In particular, we cannot rule out genetic trade-offs associated with host preference or other life history stages, or trade-offs that depend on aspects of the environment not captured by the laboratory (e.g. Scheirs *et al.* 2005). Nonetheless, we think that in the light of these results, alternatives to the trade-off hypothesis for host range constraint in herbivorous insects warrant more attention (discussed further below).

Our findings fit well with results from classic quantitative genetic studies, which have generally found positive rather than negative cross-host genetic correlations among families (Joshi & Thompson 1995; Fry 1996; Scriber 2005; Agosta & Klemens 2009), and are also consis-

tent with the study of fitness components in other organisms, which are most often positively correlated rather than constrained by trade-offs (Kingsolver & Diamond 2011). In the past, it has been difficult to distinguish between two explanations for the absence of negative cross-host genetic correlations in herbivorous insects: (i) genetic variants that increase performance on one host but decrease performance on another host could be very rare or nonexistent, or (ii) genetic variants that increase performance on one host and decrease it on another could be somewhat common, but still cause a smaller proportion of the phenotypic variation than those variants that increase performance across hosts or only affect performance on a single host (Houle 1991; Agrawal *et al.* 2010). The current results constitute a stronger test and rejection of the genetic trade-off hypothesis, at least in *L. melissa*, as we were able to determine the number and relative abundance of SNVs exhibiting antagonistic pleiotropy (very rare) vs. conditional neutrality (very common) or those with common effects across plants (also quite rare) (Table 1). With that said, there are a few known cases where antagonistically pleiotropic quantitative trait loci contribute substantially to local adaptation, and thus, the genetic trade-off hypothesis remains viable in other systems and for other components of fitness in *L. melissa* (Hawthorne & Via 2001; Anderson *et al.* 2013).

Molecular and functional genetics of larval performance

A potential strength of the genomic approach adopted in this study is that we were able to ask whether specific structural or functional genetic elements contributed disproportionately to variation in larval performance and thereby generate hypotheses to further advance our understanding of the biology of host plant adaptation. Along these lines, we found that genetic variants most associated with larval performance reside in repeat regions more often than expected by chance. Most of these were in transposable elements, with the greatest proportion in the Zenon family of non-LTR retrotransposons (Zenon elements are known from other butterflies and account for 0.32% of the *Heliconius melpomene* genome; Lavoie *et al.* 2013). Transposable elements are known to contribute to adaptation, particularly to stresses (including insecticides) or novel environments, in part because their mobilization in response to stress generates genetic variation (Schmidt *et al.* 2010; Chénais *et al.* 2012). Based on our results, the hypothesis that transposable elements have been important for host plant adaptation in *L. melissa* deserves further investigation.

We detected an excess of performance-associated SNVs in or near annotated genes with a variety of molecular functions (Tables S3 and S4, Supporting information). Several of these functions were statistically over-represented among the top performance-associated SNVs in multiple treatments (e.g. catalytic activity and G-protein-coupled receptor activity). However, in most cases, the same SNVs were responsible for the enrichment in each treatment, and often these SNVs were on the same genome scaffold and associated with the same annotated gene. Although this replication of pattern supports the hypothesis of a true association between these variants and performance, it provides little support for the hypothesis that genes with these molecular functions are particularly important. Indeed, in all cases where we detected a significant enrichment, it involved only two or three SNVs; in other words, enrichments were caused by a few SNVs in or near one or a few genes with molecular functions that were very rare at the genome level. This means that we did not identify one or a few functional classes that represent particularly good candidates for further study, but instead we hypothesize that diet evolution in *L. melissa* involves a functionally diverse set of genes.

Conclusions

In summary, the results reported here suggest that the genetic architecture of variation in *Lycaeides melissa* larval performance on a native and novel host consists of a number of loci of mostly small and independent effects across host plants. Antagonistically pleiotropic loci that would inherently constrain diet breadth by preventing the optimization of performance across hosts were very rare or absent, at least in the simplified laboratory environment. If limited genetic variation or genetic trade-offs do not constrain insect herbivores from utilizing additional host plants, then why are so many insect herbivores so highly specialized? We can posit a simple model of diet evolution in which the colonization of a novel host is facilitated by some level of pre-adaptation or 'ecological fitting' (Agosta 2006), followed by selection on standing genetic variation that improves performance on the novel host. Whether that expansion results in a wider diet breadth or a true host switch would then presumably depend on population structure and gene flow among ancestral and novel-associated populations. In the absence of gene flow, alleles associated with performance on the ancestral host, but not the novel host, could be lost in small, isolated populations through drift, the effect of which would potentially be enhanced by small population sizes on novel hosts (if they are relatively inferior hosts). Validation of this model will wait on further studies such as the one reported here, and in particular on studies that link experimental molecular work with

surveys of genetic variation in the wild to test the hypothesis that host range evolution in herbivorous insects is determined by the rise and fall of a multitude of loci with independent, small effects across hosts (such work is ongoing in *L. melissa*).

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Z.G., M.F. and C.A.B. designed the study. M.F., J.P.J., C.F.S., J.S.W., J.A.F. and C.C.N. conducted the rearing experiment. L.K.L., C.C.N. and Z.G. generated the DNA sequence data. Z.G., C.A.B. and V.S. analysed the data. Z.G. and M.F. wrote the study. All authors edited and helped revise the study.

Data accessibility

DNA sequence data have been archived in the NCBI SRA (SUB889871; SRX978153). The experimental data, reference genome sequence, annotations, sequence alignments, variant data, GEMMA infiles, and polygenic model (association mapping) results and computer scripts have been archived in DRYAD (doi: 10.5061/dryad.7b5m7).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Proportion of larvae in each family that survived to the adult stage.

Fig. S2 Boxplots show the variability of adult weight within and among butterfly families.

Fig. S3 Violin plot of the posterior probability distributions of the proportion of phenotypic variance explained by the polygenic model (PVE; a, b), and the number of SNVs affecting phenotypic variation (c, d). Estimates from combined treatments are shown for survival (a, c) and adult weight (b, d).

Fig. S4 Polygenic model goodness of prediction based on 20% repeated random subsampling cross-validation.

Fig. S5 Manhattan plots from single SNP linear mixed model analyses of host plant by population treatments.

Fig. S6 Manhattan plots from single SNP linear mixed model analyses of combined treatments.

Fig. S7 Scatterplots show the relationship between the effects of SNVs on performance for larvae reared on different host plants.

Table S1 Parameter estimates [median and 95% equal tail probability intervals (ETPIs)] from the Bayesian analysis of larval performance.

Table S2 PVE in the null linear mixed model and the number of SNVs with genomewide significant effects on survival or adult weight.

Table S3 Summary of randomization tests for the top (0.1%) survival-associated SNVs.

Table S4 Summary of randomization tests for the top (0.1%) weight-associated SNVs.

Appendix S1 Single locus genomewide association analysis.

Appendix S2 Molecular functions of the top (0.1%) performance-associated SNVs.