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Gene expression in closely related species mirrors local adaptation: consequences for responses to a warming world

SHAWN T. O'NEIL,*[1] JASON D. K. DZURISIN,† CAROLINE M. WILLIAMS,‡ NEIL F. LOBO,† JESSICA K. HIGGINS,†[2] JILLIAN M. DEINES,† RORY D. CARMICHAEL,* ERLIANG ZENG,* JOHN C. TAN,† GRACE C. WU,†[3] SCOTT J. EMRICH* and JESSICA J. HELLMANN†

*Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN 46556, USA, †Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA, ‡Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA

Abstract

Local adaptation of populations could preclude or slow range expansions in response to changing climate, particularly when dispersal is limited. To investigate the differential responses of populations to changing climatic conditions, we exposed poleward peripheral and central populations of two Lepidoptera to reciprocal, common-garden climatic conditions and compared their whole-transcriptome expression. We found evidence of simple population differentiation in both species, and in the species with previously identified population structure and phenotypic local adaptation, we found several hundred genes that responded in a synchronized and localized fashion. These genes were primarily involved in energy metabolism and oxidative stress, and expression levels were most divergent between populations in the same environment in which we previously detected divergence for metabolism. We found no localized genes in the species with less population structure and for which no local adaptation was previously detected. These results challenge the assumption that species are functionally similar across their ranges and poleward peripheral populations are preadapted to warmer conditions. Rather, some taxa deserve population-level consideration when predicting the effects of climate change because they respond in genetically based, distinctive ways to changing conditions.

Keywords: climate, diapause, energy metabolism, Lepidoptera, local adaptation, oxidative stress, range shift

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Introduction

Ecologists often assume that widespread geographic range shifts will occur in response to modern climate change based on recent and historic changes in the geography of some species (Davis & Shaw 2001; Parmesan 2006). These shifts probably occur when populations increase near the poleward range boundary as climate change brings a species' fitness optimum closer to peripheral populations (Hoffmann & Blows 1994; Davis & Shaw 2001). On the other hand, physiological data suggest that populations within many species will respond to climate change in complicated or localized ways (Rehfeldt *et al.* 2002; Deutsch *et al.* 2008; Leimu & Fischer 2008) and that processes of population structure and adaptive evolution produce complicated patterns of functional genetic variation across a species' range (Mitchell-Olds & Schmitt 2006; Rodríguez *et al.* 2007; Fournier-Level *et al.* 2011; Hancock *et al.* 2011). Whether a species is relatively uniform across its range (such

Correspondence: Jessica J. Hellmann, Fax: (541) 631 7413; Email: hellmann.3@nd.edu

[1]Present address: Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97333, USA

[2]Present address: Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

[3]Present address: Energy and Resources Group, University of California, Berkeley, CA 94720, USA

that populations react similarly to climatic change) or is composed of distinct climatic ecotypes can strongly influence a species' future distribution, abundance and genetic diversity.

Temperature is one of the primary determinants of ectotherm performance. Many traits respond strongly to temperature, including gene expression and enzyme activity, growth and development rates, and fitness correlates such as fecundity. Individual genotypes influence the responses of such traits to temperature, and thermal reaction norms describe the relationship between environmental temperature and the value of a trait (Angilletta *et al.* 2006). The reaction norms of individual genotypes can be sampled and averaged to yield population-level estimates of reaction norms for a given trait (Fig. 1). These reaction norms can then be compared among populations to determine whether there are localized responses to temperature and whether they are genetically based (genotype × environment interactions; Kawecki & Ebert 2004).

If reaction norms indicate that populations at the edge of a species' range are similar to their central counterparts, warming can increase performance as peripheral populations move closer to their optimum temperature (Fig. 1a, b). If the trait in question is positively correlated with fitness, these increases can increase peripheral population size and increase the likelihood of poleward colonization. This type of response is assumed in statistical niche models (Wiens *et al.* 2009). If, however, the thermal optimum has diverged among populations, then warming may reduce performance and perturb populations from their local fitness optimums (Fig. 1c, d; Vergeer & Kunin 2013). If the trait is a fitness correlate, then this will translate into fitness being higher in natal compared to non-natal environments, leading to the populations being locally adapted (Savolainen *et al.* 2004). Local adaptation is the result of divergent selection pressures experienced by individual populations within a species and is most likely to develop when gene flow is restricted, although it can occur under moderate to high gene flow if local selection is strong (Sultan & Spencer 2002; Garant *et al.* 2007). This perturbation effect assumes that local fitness and population responses dominate over dispersal and that *in situ* reaction to climate change is the primary determinant of a species' response to climate change. It also assumes that genes for nonlocally adapted phenotypes are infrequent in the population such that simple changes in gene frequency cannot provide evolutionary rescue.

Were dispersal or evolution to take place, however, local adaptation could be advantageous to a species

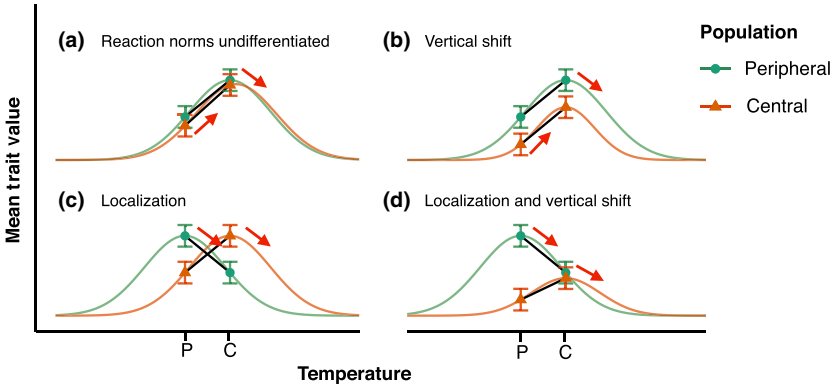

Fig. 1 Hypothetical mean thermal reaction norms (i.e. average response of phenotype or trait to temperature) of individuals from central and peripheral populations (Kingsolver 2009). Orange and green dots indicate the mean trait measurements of central and northern-peripheral populations, respectively, under current central (C) or peripheral (P) conditions, and connecting lines estimate the slope of the reaction norm in a common-garden translocation experiment. Red arrows indicate mean trait value in the current climate (base of arrow) and how the trait might change under warming. (a) Reaction norms are undifferentiated, and central populations have a higher trait value in their natal environment. With climate change, central populations move down their reaction norm while peripheral populations move up. (b) Reaction norms have diverged between populations such that the central population expresses higher trait values across all temperatures, but the peak trait value occurs at the same temperature for both populations. Climate change will decrease trait values for central and increase for peripheral populations. (c) Peak temperature of reaction norms has diverged between populations, leading to equal trait values under natal conditions. (d) Peak temperatures and overall traits have diverged between populations, leading to trait peaks of different heights under natal conditions. We refer to patterns (c) and (d) as localization (as identified by an interaction term in an ANOVA test); both result in differential adjustment of reaction norms in response to climate change. If the trait of interest is adaptive, (c) and (d) suggest local adaptation such that both populations will probably decline under climate change (Kawecki & Ebert 2004; Hellmann *et al.* 2012).

under climate change. If multiple ecotypes within a species' range can each track changing conditions through dispersal, for example, a species may be able to persist and maintain genetic diversity that is useful for future adaptive evolution (Hamrick *et al.* 1992; Savolainen *et al.* 2004). Population differentiation within a species' range also can provide genetic diversity that could be useful in coping with changing conditions (Bridle & Vines 2007).

With so many complicating factors, it is unsurprising that a wide variety of reactions to recent climate change have been noted, including range adjustments, predator/prey and insect/host-plant asynchrony, and population extinctions (Parmesan 2006; Menéndez 2007). Poleward range expansion has been detected in butterfly species in England as early as 1945 (Ford 1945). Similar shifts have been detected in other European locales, sometimes in concordance with equatorial contraction (Kaisila 1962; Saarinen *et al.* 2003). In some cases, species have retracted at their equatorial range edge without corresponding poleward expansion (Parmesan *et al.* 1999). While responses to warming have been considered by many observational studies of natural populations, fewer studies have identified phenotypic effects and their origins in controlled experiments.

We have previously studied how populations at the northern range edge of two co-occurring butterfly species respond in fitness-related traits to climate warming. We showed that the weak-flying Propertius duskywing (*Erynnis propertius*; Lepidoptera, Hesperiidae) appears to be locally adapted in the winter months such that overwintering metabolism is tuned to local conditions. In the strong-flying and less genetically structured Anise swallowtail (*Papilio zelicaon*; Lepidoptera: Papilionidae), we found an overall lack of responsiveness to warming in peripheral populations, but no evidence for local adaptation (Zakharov & Hellmann 2008; Pelini *et al.* 2009; Williams *et al.* 2012).

Whether a species is ecologically uniform, shows strong population divergence, or somewhere in between, quantifying the extent and nature of local adaptation is a crucial part of understanding how a species will react to climatic change. In particular, relating local adaptation to genetics will help in identifying mechanisms and separating genetic versus developmental effects (Hoffmann & Willi 2008). With knowledge about functional variation in climatic response across a species' range, management interventions can be designed so that local adaptation can enable and reinforce range change rather than be a hindrance (Richardson *et al.* 2009; Schwartz *et al.* 2012; Aitken & Whitlock 2013).

As adaptive differences are frequently (although not exclusively) determined by gene expression, we hypothesize that investigating whole-transcriptome expression will reveal many candidate genes and functional categories involved in local adaptation. In this case, we consider the expression of each gene itself as a phenotype of interest. If expression differences between populations and climate regimes reflect the known adaptive differences in a common-garden design, then we can relate localized genes (with expression patterns similar to Fig. 1c, d) to the species' ecology. For example, based on previously observed local adaptation in the duskywing but not the swallowtail (Pelini *et al.* 2009), we hypothesize a larger signature of localized gene expression in the former than the latter (see, e.g. similar work in *Arabidopsis*: Fournier-Level *et al.* 2011; Hancock *et al.* 2011). Alternatively, given that swallowtail populations are genetically different (Zakharov & Hellmann 2008), the swallowtail may nevertheless show localization at the genetic level in response to temperature or other factors (Fong *et al.* 2005).

Materials and methods

Study species

Both the Propertius duskywing and Anise swallowtail are native to western North America, spanning latitudinally from Baja California, Mexico, to Vancouver Island, British Columbia, Canada (Fig. S1, Supporting information). Both species are univoltine in northern and northern/central locales, but multivoltine in southern locales (Pelini *et al.* 2010).

The duskywing lives in oak-savanna and oak-grassland systems throughout the coastal, western states and provinces of Mexico, the United States, and Canada (Hellmann *et al.* 2008). In univoltine populations, larvae feed during the spring and summer months until they drop to the ground as diapausing late-instar caterpillars (Prior *et al.* 2012). Microsatellite and mitochondrial markers reveal restricted gene flow across the species' range with northern populations in mainland Washington and British Columbia and island populations in British Columbia, as well as southern populations in southern California (and presumably Baja California, Mexico) being differentiated from more central populations (Zakharov & Hellmann 2008; Zakharov *et al.* 2009). The duskywing feeds on the genus *Quercus* (Fagaceae), and in the northern third of the species range feeds only on *Quercus garryana*, a host plant that is patchily distributed towards its own (and the butterfly's) northern range limit.

The swallowtail feeds on plants in the Apiaceae family, including desert parsley, sweet fennel and numerous other native and non-native species (Wehling & Thompson 1997; Shapiro 2002). These plants occur in native habitats including oak-savanna and oak-grassland

habitats as well as roadsides, residential areas and disturbed habitats. This swallowtail occurs throughout western North America with similar northern and southern coastal boundaries as the duskywing (Scott 1992). Individuals overwinter as pupae. Mitochondrial and microsatellite markers suggest that Vancouver Island populations in British Columbia are differentiated from the rest of the species' range in Washington, Oregon and California, but much of the mainland range is undifferentiated genetically (Tong & Shapiro 1989; Zakharov & Hellmann 2008).

Corresponding to their ecological and genetic differences, reciprocal transplant experiments in both the field and the laboratory have indicated that duskywing populations are locally adapted to thermal conditions, while the swallowtail shows no signs of local adaptation (Pelini *et al.* 2009; Williams *et al.* 2012). Duskywing individuals from both northern-peripheral and central duskywing populations attain larger body size and increased survivorship during summer months under central summer conditions. However, central winters impose an energetic cost on northern-peripheral populations through increased rates of metabolism, which may cancel out fitness gains resulting from warming over summer (Pelini *et al.* 2009). Conversely, central populations appear to have decreased metabolic costs at central relative to northern-peripheral temperatures. The relatively thermally variable central conditions induce a decrease in the thermal sensitivity of metabolism of overwintering duskywing larvae originating from or raised in these conditions, although this decrease in thermal sensitivity is not sufficient to fully compensate for the increased energetic demands of the more variable central environment (Williams *et al.* 2012). The corresponding results for the swallowtail revealed no species-wide response to climate, except as moderated by host plant: warmer summer conditions result in lower pupal mass when individuals are fed the relatively furanocoumarin-rich *Petroselinum crispum* (Pelini *et al.* 2009). While swallowtail overwinter metabolic rates were not responsive to climate, northern-peripheral populations displayed an increased overwinter metabolic rate in both central and peripheral conditions. Thus, there is little evidence for the possibility of peripheral population enhancement (i.e. population increases at the poleward range margin) due to warming for either species.

Microarray construction

The transcriptomes of both study species were previously described by O'Neil *et al.* (2010) based on genetically diverse samples from central Oregon. In O'Neil *et al.* (2010), assembled transcripts were clustered based on BLAST sequence similarity, indicating the difficulty in assembling population-level transcriptome data confused by allelic variation. Thus, for microarray creation, the reads and quality scores contributing to each cluster were reassembled separately with the Celera Assembler using assembly parameters given in O'Neil *et al.* (2010), following a strategy suggested by Surget-Groba & Montoya-Burgos (2010). This process increased average transcript coverage relative to the original assemblies described by O'Neil *et al.* (2010) (10.05 × to 10.19 × for the duskywing and 9.63 × to 9.77 × for the swallowtail) and reduced the number of reads not assembled with other reads (10 934 to 8910 for the duskywing and 18 847 to 15 743 for the swallowtail. The Celera Assembler identifies variant regions in transcripts – polymorphic regions that may span more than one nucleotide but are generally only a few tens of nucleotides long (Denisov *et al.* 2008; O'Neil *et al.* 2010). Because allelic variation may confuse expression analysis (Walter *et al.* 2007), before microarray creation, variant positions identified in variant regions containing more than one polymorphic locus were masked so that microarray oligonucleotides would not cover those positions.

In total, 25 734 sequences for the duskywing and 34 609 sequences for the swallowtail were sent to Nimblegen Inc. for probe selection. The selection process aimed for five 60-mer representative probes per transcript for the duskywing and four 60-mer probes per transcript for the swallowtail. Each representative probe was printed to a 385K microarray in triplicate. For the duskywing, 23 928 transcripts were represented by at least one unique probe (average, 4.54). In the swallowtail, 25 734 transcripts were represented by at least one unique probe (average, 3.70). Transcripts that were not represented by any probe unique to the transcript but that were represented by one or more 'exemplar' probes numbered 623 for the duskywing and 1087 for the swallowtail. Transcripts not represented on the microarrays numbered 1183 for the duskywing and 7788 for the swallowtail.

Temperature treatment and microarray hybridization

Adult females of both species were captured from four locations in southern Oregon (central source) and four locations in Vancouver Island, British Columbia (peripheral source), and housed in oviposition cages in portable greenhouses in each region (Fig. S1, Table S1, Supporting information). Because cage space was limited and more duskywings were sampled than swallowtails, between one and five duskywing mothers were housed per cage while exactly one swallowtail mother was housed per cage. Nevertheless, overall

maternal diversity contributing to samples was similar for both species (Tables S1 and S2, Supporting information). Eggs produced were transported to greenhouses at the University of Notre Dame and allowed to hatch under temperature conditions representative of their source regions. Larvae from each mother were divided equally between temperature treatments representative of the source conditions in a fully crossed design: peripherally sourced individuals in a peripheral temperature treatment, peripherally sourced in a central treatment, centrally sourced in a peripheral treatment and centrally sourced in a central treatment (see Tables S1 and S2, Supporting information). Temperature treatments were carried out in Conviron MTR30 growth chambers throughout the larval growth period. As in Pelini *et al.* (2009), these treatment conditions were based on historical temperature data (1997–2006) collected at the Rogue Valley International-Medford Airport in Oregon and the Victoria International Airport in Victoria, British Columbia (Fig. S1, Supporting information). Experimental temperatures cycled diurnally between the average long-term minimum and maximum and were adjusted at 2-week intervals to reflect seasonal changes. Average summer temperatures were 15 °C in peripheral chambers and 22 °C in central chambers. These temperature treatments are ~5 °C different on average, which also simulates a moderate to high amount of warming over the next 85 years for peripherally sourced individuals (Pelini *et al.* 2009). Both species are univoltine under these conditions, and these samples provide a snapshot of gene regulation at the threshold of a developmental stage that has high mortality and determines future fecundity (Hahn & Denlinger 2011). All temperature treatments were started just after hatching and were held at 12 h:12 h (light/dark; Pelini *et al.* 2009). Duskywing larvae were fed native host plant (*Q. garryana*) shipped from field sites, while swallowtails were fed *Petroselinum crispum*, a suitable non-native host, propagated in the greenhouse.

Duskywings were sampled as mature prediapause sixth-instar larvae. Because swallowtails pupate before diapause, swallowtail larvae were sampled as late instars in their final stage before showing evidence of initiating pupation to facilitate comparison between species. Sampled larvae were frozen in liquid nitrogen and stored at −80 °C. Whole-body RNA from these frozen individuals was extracted using RNeasy kits (QIAGEN Inc.) over a period of 2 months. Equal quantities (Nanodrop 2000, Agilent 2100) of extracted RNA from each experimental individual (12 or 13 duskywing individuals and 12–18 swallowtail individuals per experimental group; Table S2, Supporting information) contributed to 10-microgram subpools as biological replicates (4–6 individuals per replicate; Table S1, Supporting information), transcribed into cDNA (Invitrogen Superscript Kits), were fluorescently labelled (NimbleGen One-Color DNA Labeling Kits) and were run on custom microarrays at the University of Notre Dame.

Microarray analysis

Array image data were processed using NIMBLESCAN v2.6. For each species, probes identified as having expression higher than background (as identified by NimbleScan) in more than half of the 12 microarrays were kept for statistical test and constituted the set of 'expressed genes' (Wheat *et al.* 2011). NimbleScan data were $\log_2$ transformed and normalized using a quantile normalization method (Bolstad *et al.* 2003). For each species, multiply corrected two-way ANOVAs identified transcripts showing up- or down-regulation by source population (peripheral relative to central), those showing up- or down-regulation by temperature treatment (peripheral relative to central) and those showing source-by-treatment interactions ('localized' genes). *P*-values were adjusted for false discovery rate (FDR) using Storey's *Q*-value method within each species (Storey & Tibshirani 2003). Although including deme as a factor is suggested for tests of local adaptation based on adaptive traits alone due to the possibility of deme-level effects (Kawecki & Ebert 2004), here we focus on gene expression as related to previous work on fitness-based local adaptation for these populations (Pelini *et al.* 2009). Given limited microarray resources and deme sampling, but known genetic similarities across locations/demes within populations (Zakharov & Hellmann 2008), we thus opted to pool demes and focus on biological replication across them (Tables S1 and S2, Supporting information).

As described in O'Neil *et al.*, gene ontology (GO) terms and Blast2GO gene family annotation were mapped to transcripts using the BLAST2GO tool, using as input BLASTX results against the NR protein database (Ashburner *et al.* 2000; Conesa *et al.* 2005). Further annotation was performed via best BLASTX hit (*e*-value cut-off 1e-5) against SWISSPROT (O'Neil *et al.* 2010).

Gene ontology terms were mapped to GO-Slim categorical terms (Ashburner *et al.* 2000) obtained on 20 August 2012 using BLAST2GO (version 2.5.0) and Map2Slim (GO-PERL package release 0.13). Transcripts that were annotated with one or more GO-Slim terms numbered 4793 for the duskywing and 6299 for the swallowtail. Subsets of up- or down-regulated genes were tested for enrichment of GO-Slim terms using two-tailed Fisher's exact tests, comparing GO-Slim counts in subsets to annotation background counts among expressed genes. For example, of the 4178 expressed

and GO-Slim annotated duskywing transcripts, 147 were annotated as 'Generation of precursor metabolites and energy' and 4031 were not. Of the same 4178 expressed and annotated genes, 48 were identified as down-regulated by peripheral populations, eight of which were annotated as 'Generation of precursor metabolites and energy'. Thus, a two-tailed Fisher's exact test indicates that 'Generation of precursor metabolites and energy' is over-represented in transcripts down-regulated by peripheral source populations. These *P*-values were multiply corrected on a per-subset basis to account for the 143 GO-Slim terms represented and tested (Storey & Tibshirani 2003) before being assessed at a significance threshold of $\alpha = 0.05$.

Possibility of confounding factors for expression measurement

Because the swallowtail is a more genetically diverse species than the duskywing (Zakharov & Hellmann 2008), assembly of population-sourced data from the swallowtail was more difficult, possibly resulting in microarray probes competing for hybridization (O'Neil *et al.* 2010). While the assembled swallowtail transcriptome is 34% larger, possibly due to haplotype separation during the assembly process, highly increased haplotype separation is unlikely because BLAST results suggest that the swallowtail transcriptome is actually larger: the number of annotated genes is larger for the swallowtail than that of the duskywing (7588 vs. 5550, representing 21.9 and 21.6% of unigenes, respectively), as is the number of unique annotations used (4655 vs. 3671), and we do not suspect annotation bias between species (Table S3, Supporting information). Greater hybridization inefficiency in the swallowtail also would predict relatively fewer informative microarray values. However, we gained similar expression information for each species: of the 25 734 duskywing genes, expression was computed for 16 613 (64.5%), whereas of the 34 609 swallowtail genes, expression was computed for 18 118 (52.3%).

Second, because of these differences in transcriptome size and limited microarray space, there were different representative probe counts for each species on the arrays (five probes/gene for the duskywing; four probes/gene for the swallowtail). Thus, duskywing expression readings may include more single-spot data points, reducing within-replicate variance and increasing power. Such biases are difficult to separate from biological effects; nevertheless, the majority of significant differences were found in the swallowtail and were consistent with previous results (see Discussion). Overall, while these species are unique in their transcriptomic profiles, we do not suspect that this resulted in biases in the gene expression results presented here.

Third, sequences for microarray design were sourced from central populations. Although these populations harbour most of the genetic diversity of both species (Zakharov & Hellmann 2008) and we masked variant loci for microarray construction, the microarrays may nevertheless be specific to central populations, particularly for the more population-structured duskywing. We expect such effects to be minimized by the normalization of microarray data, but if only a subset of probes is population specific, this adjustment will be less effective. When only significant fold changes are considered, 494 (79.0%) of duskywing genes were up-regulated by central populations, and 1848 (42.1%) swallowtail genes were (Table S4, Supporting information). When considering all fold changes, 8052 duskywing genes (48.4%) and 8421 swallowtail genes (46.4%) were up-regulated by central populations. Thus, we cannot rule out such specificity, at least for the duskywing. Again, this is difficult to separate from biological effects without a genomic reference and only affects our study of localization insofar as localized genes were also identified as being source-differentiated.

Results

Differential expression by source populations

In the duskywing, 3.7% of expressed genes were differentially expressed between central and peripheral populations (a significant main effect of source population from ANOVAS; $Q < 0.05$; Table S4, Supporting information), with $\log_2$ fold changes ranging from -3.03 to 3.83 (peripheral/central). Figure 2 shows expression profiles for these duskywing genes as well as those differentiated by source population for the swallowtail. Conversely, no genes were differentially expressed between individuals in warm- versus cool-temperature treatments (no main effects of temperature treatment from ANOVAS at $Q < 0.05$). Comparison with protein databases (SWISSPROT and NR) identified homologues for 21% and assigned functional categorization for 18% of genes for the duskywing (GO-Slim GO terms, Table S3, Supporting information). Of those genes for which we could infer functional information, those up-regulated by peripheral populations did not show enrichment for any functional categories, but genes that were down-regulated by peripheral populations were over-represented in three GO-Slim functional categories: oxidoreductase activity (catalysis of an oxidation–reduction reaction); generation of precursor metabolites and energy (the chemical reactions and pathways resulting in the formation of precursor metabolites, substances from which energy is derived and any process involved in the liberation of energy from these substances); and

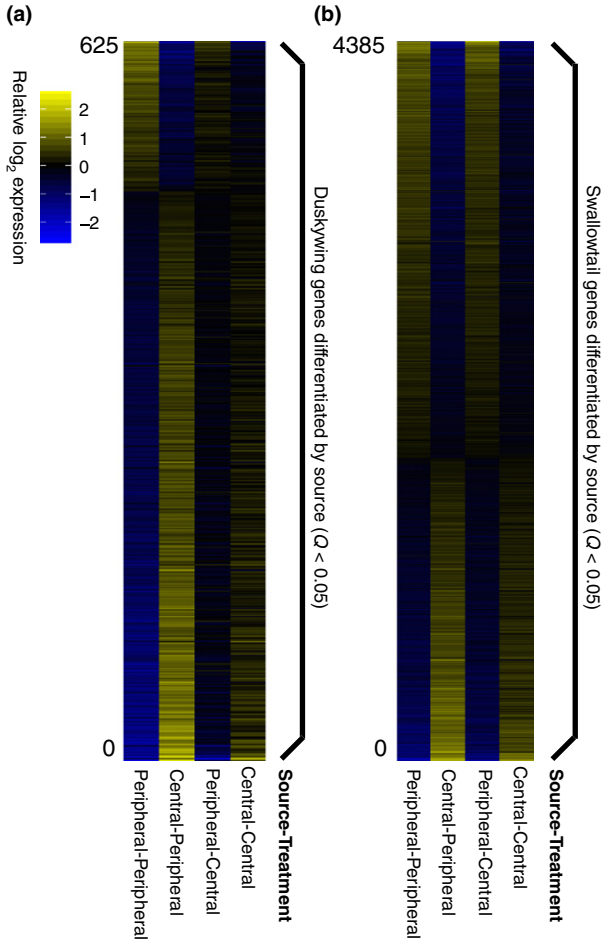

Fig. 2 Log_2-adjusted and mean-normalized expression profiles. Profiles are for genes differentially expressed by source populations ($Q < 0.05$) for the duskywing (a) and swallowtail (b) sorted by fold change of source differentiation.

transferase activity, transferring alkyl or aryl (other than methyl) groups (catalysis of the transfer of an alkyl or aryl group from one compound to another, e.g. cysteine synthase). This suggests that redox activity, energy metabolism and transferase activity may be down-regulated in peripheral populations.

In swallowtail pupae, approximately 24% of genes were differentially expressed between central and peripheral populations (a significant main effect of source population from ANOVAs, $Q < 0.05$; Table S4, Supporting information), with fold changes ranging from −5.8 to 5.9. These fold changes were large relative to the duskywing (duskywing mean absolute value log_2 fold: 0.397, swallowtail: 0.493). Similar to the duskywing, no genes were significantly differentiated by temperature treatment. Swallowtail genes that were up-regulated by peripheral source populations were enriched for a number of GO-Slim functional categories, as were genes that were down-regulated by peripheral source populations (Fig. 3). Overall, peripheral populations of the swallowtail up-regulate processes related to transcription and translation (mRNA binding, structural constituent of ribosome, ribosome, translation), while central populations up-regulate processes related to detoxification and oxidative stress (oxidoreductase, transferase and hydrolase activity).

Similarly annotated genes were frequently differentially expressed between source populations in the same ways for both species (Fig. S2, Supporting information). For example, genes involved in detoxification (cytochrome P450 4 g15) and energy mobilization (lipase member I) were up-regulated in peripheral sources of both species, while an enzyme involved in the breakdown of neuroactive fatty acids (fatty-acid amide hydrolase 2; Cravatt *et al.* 1996) and a contractile protein (tropomyosin 1) were down-regulated in peripheral sources of both species. There were also a number of similarly annotated genes with alternate expression profiles for the swallowtail (i.e. versions down- and up-regulated by peripheral sources), where a single duskywing gene followed one of these patterns or was localized (Fig. S2, Supporting information) – these genes were involved in detoxification (myrosinase 1 and glutathione S-transferase 2) and immune function (ecdysteroid-regulated 16-kDa proteins; Shi *et al.* 2012). Finally, a few similarly annotated genes showed different patterns of regulation between the two species (Fig. S2, Supporting information). These genes were involved in oxidative stress (cytochrome b5-related proteins) synthesis of larval cuticle (larval cuticle proteins) and the cytoskeleton (tubulin β-1 chains) and were up-regulated by duskywing peripheral populations (and localized in the case of larval cuticle proteins) but were down-regulated by swallowtail peripheral populations. Soluble carrier proteins were down-regulated by duskywing peripheral populations and up-regulated by swallowtail peripheral populations.

Localized expression

We found much stronger evidence for expression localization (an interaction between source population and rearing environment) in the duskywing than in the swallowtail. After adjusting the ANOVA results for FDR, there were no localized duskywing genes with $Q < 0.05$ and only 16 with $Q < 0.10$. As higher Q-value cut-offs are appropriate for exploratory studies (Storey & Tibshirani 2003; Subramanian *et al.* 2005), we also considered an FDR cut-off of $Q < 0.15$ that returned 300 localized duskywing genes (0.2: 828, 0.25: 2,462) with an estimated false-positive count of 45. At the 0.15 level, these localized duskywing genes were not enriched for any

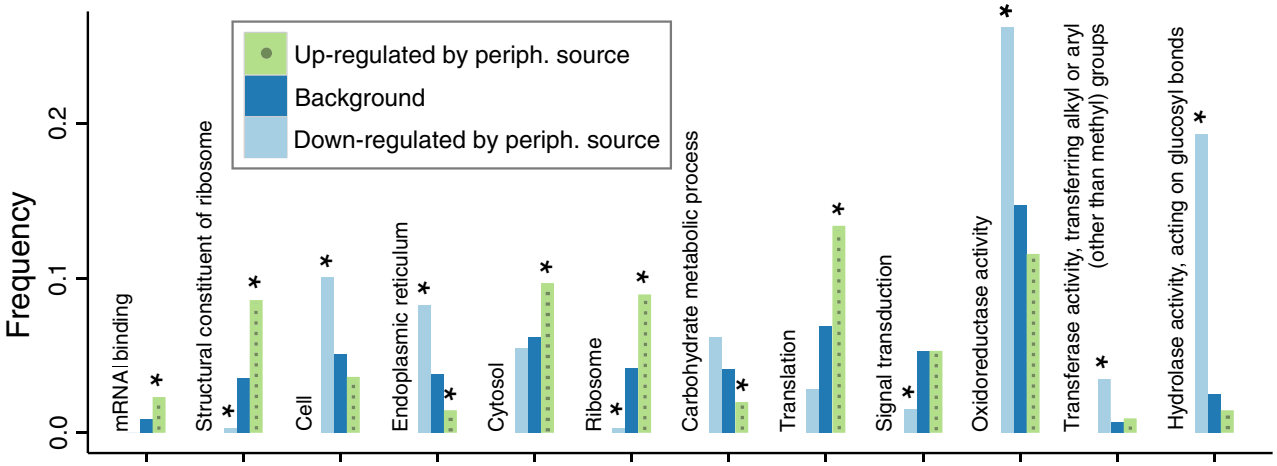

Fig. 3 Enrichment of GO-Slim functional terms. Terms shown for swallowtail genes up- and down-regulated by peripheral source population, as compared to background frequencies for expressed genes. Stars indicate significant differences from background frequencies (see Materials and Methods).

GO-Slim functional terms. By contrast, no localized genes were found for any cut-off <0.5 in the swallowtail (Fig. S3, Supporting information).

Figure 4b shows mean-normalized and $\log_2$-adjusted expression profiles for localized duskywing genes ($Q < 0.15$), grouped by whether they were also identified as differentiated by source ($Q < 0.05$) and within these groupings sorted by the fold change of the localization. This figure reveals a consistent pattern of localization: while populations show strong expression differences in peripheral conditions (columns 1 and 2), expression is similar in central conditions (columns 3 and 4, also see Fig. S4, Supporting information).

Several transcripts stand out in the pattern of localization. First, genes involved in energy metabolism showed a consistent signature of localization (Fig. 4a, Fig. S5 and Table S5, Supporting information). For example, transcripts up-regulated by the peripheral (relative to the central) population in peripheral conditions included a lipase, a glucose transporter, a catalytic protein kinase that regulates growth via interactions with mTOR and insulin signalling pathways, and glutamine synthase that is involved in cellular proliferation. The central population in central conditions up-regulated three transcripts related to ATP synthesis and translocation: an NADH-ubiquinone oxidoreductase that is part of the mitochondrial electron transport chain; isocitrate dehydrogenase, a TCA cycle enzyme; and succinate semialdehyde dehydrogenase that regenerates succinate, a key TCA cycle intermediate. Second, we found that the environmental responsiveness of oxidative stress pathways was modified between populations. Transcripts coding for key antioxidant enzymes were up-regulated in the peripheral population under peripheral conditions (superoxide dismutase and glutathione synthetase). Two members of the glutathione S-transferase superfamily were up-regulated by the central population under central conditions. Third, genes involved in cuticle synthesis pathways were up-regulated in the central population under peripheral conditions: two chitin deacetylases and two chitin-binding proteins followed this pattern and are probably related to growth and feeding (Zhao *et al.* 2010).

Discussion

Consistent with previously detected local adaptation in the duskywing using fitness-related measures, we found a number of genes showing a distinct pattern of localized expression, reinforcing that in this organism populations react differently to climate. The duskywing genes showing a pattern of localization (as in Fig. 1c, d, as well as many of those identified as differentiated by source population only, Fig. 1b) were expressed similarly between populations in central conditions and differently between populations in peripheral conditions. Further, average expressions between populations in peripheral treatments tended on balance to equal central expressions such that we saw no main effects of temperature treatment. Thus, it appears that in central conditions, peripheral populations retain the capacity to express a 'central' response, whereas in peripheral conditions the expression repertoire of these populations has diverged. Such a response pattern could be interpreted as local adaptation in the presence of overall differences between the populations driven by other factors (Fig. 1d; Kawecki & Ebert 2004). While this set is based on an FDR cut-off of $Q < 0.15$, 97% of these

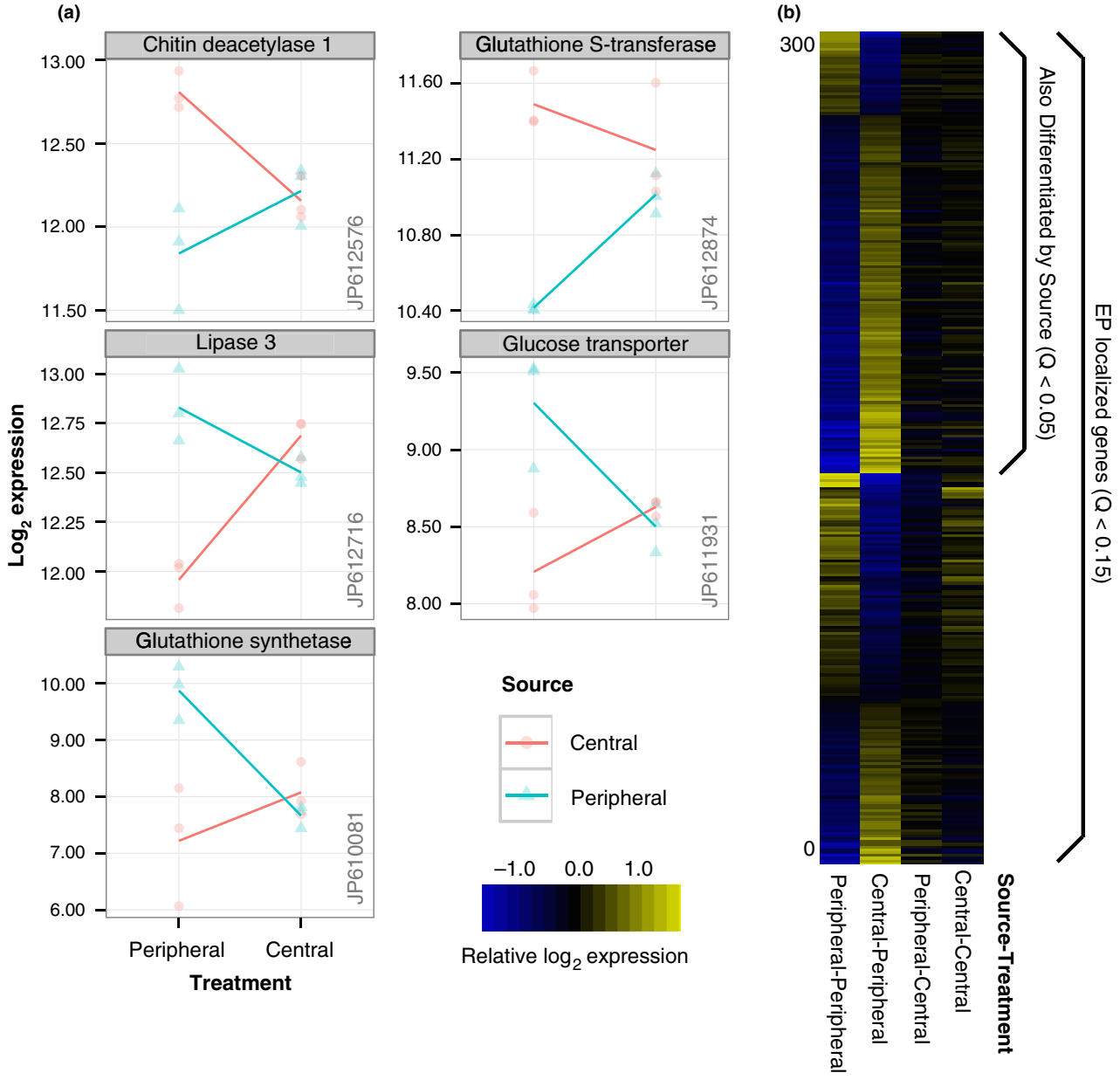

Fig. 4 Localized ($Q < 0.15$) duskywing mean-normalized and $\log_2$-adjusted expression profiles. Expression profiles (b) are grouped by those also showing differentiation by source population ($Q < 0.05$) and sorted within groups by the fold change of localization. Select annotated localized responses are also shown (a), annotated with Blast2GO functions based on BLAST results against NR (see Materials and Methods). Points represent individual replicate expression values, and lines indicate averages; GenBank accessions are given in lower-right corners of panels. These selected transcripts are involved in oxidative stress (glutathione synthetase and S-transferase), energy metabolism (glucose transporter and lipase 3) and growth/feeding (chitin deacetylase 1). Localization may reflect population-specific differences in thermal reaction norms for antioxidant production, metabolism, growth and development. No swallowtail results are illustrated here, as we found no swallowtail genes localized up to an FDR cut-off of $Q < 0.5$ (Fig. S3, Supporting information).

300 localized transcripts showed this pattern of larger peripheral treatment divergence, compared to 66.7% of remaining 16K expressed genes, highlighting the unique and consistent response of localized genes (χ^2 $P < 0.0001$).

By contrast, we found no swallowtail genes localized up to an FDR cut-off of $Q < 0.5$ (whereas 11K duskywing genes showed Q-values below this very liberal cut-off; Fig. S3, Supporting information). Given the significant difference between swallowtail source populations,

it is nevertheless possible that this species is locally adapted to factors not addressed in our design such as extreme or winter temperatures, or host-plant availability and toxicity. Indeed, previous results suggest that swallowtail populations may be locally adapted to host plant, and had we included host-plant differences in these tests, we might have seen gene expression localization patterns reminiscent of Fig. 1c or d, rather than population-only differences such as displayed in Fig. 1b (Pelini *et al.* 2009). Similarly, this experiment focused on the phenotype of gene expression; local adaptation resulting from structural modifications of proteins would not be found even if present.

Although duskywing larvae do not show local adaptation in growth rates in either the field or the laboratory, our expression results correspond with previous observations that individuals from peripheral populations adjust their overwinter metabolic rate in response to climate, while individuals from central populations do not (Pelini *et al.* 2009; Williams *et al.* 2012). As such, we suspect that these localization effects are not the result of drift, although it may be that some expression localizations are the result of linkage disequilibrium or shared *cis*-regulatory elements with genes undergoing putative selection (Mezey *et al.* 2008), such as those involved in metabolism. As we sampled at the end of the growing season, gene expression may reflect preparations for winter diapause. We found differences in expression in many genes involved in metabolism and oxidative stress. These processes are intimately coupled through generation of reactive oxygen species by the electron transport chain during energy generation, and oxidative stress is a key integrator of environmental stress into life histories (Dowling & Simmons 2009). Presumably, central populations are ancestral as northern populations occur in regions that were glaciated ~15 000 years ago (Marsico *et al.* 2009), and mtDNA data indicate that haplotypes corresponding to the most likely ancestor for the duskywing are widely distributed throughout the core of the species' range and genetic diversity is reduced in the periphery (Zakharov & Hellmann 2008). Thus, the differences in gene expression in the peripheral environment may reflect the selection pressures involved in colonizing northern latitudes: if the observed localization in gene expression translates to increased fitness over the whole life cycle, then this could partially represent the functional basis for local adaptation.

Our study also reveals a number of expression differences between source populations for both the duskywing (3.7% of expressed genes) and the swallowtail (24% of expressed genes). Although the two species appear to be quite different in their amounts of by-source differentiation, care should be taken when comparing these percentages given the difficulties of *de novo* transcriptome assembly and the possibility of differential transcriptome sizes (see Materials and Methods). Genes differentially expressed by swallowtail populations were enriched for a variety of functional categories, and genes up-regulated by central populations for both species were enriched for oxidoreductase and transferase activity, functions implicated in host-plant detoxification in other insects (Cohen *et al.* 1992; Snyder *et al.* 1995). For the swallowtail, this mirrors previous results suggesting that host-plant availability and furanocoumarin toxicity (and local adaptation to these factors) may play an important role for this species under climate change (Pelini *et al.* 2009). Duskywing populations are similarly sensitive to host-plant availability and quality (Pelini *et al.* 2010). While this study kept host plant constant while varying rearing temperature, additional studies investigating genetic localization to host plant in addition to climate may thus be informative.

We have previously demonstrated strong effects of environmental temperature on the phenotypes of both species, both in the laboratory and in the field (Pelini *et al.* 2009), whereas in this study we found no genes differentially expressed in the main effect of temperature treatment. The transcriptional changes underlying these phenotypic differences were thus probably either transient (and finished by the time of sampling) or of insufficient magnitude to be statistically detectable. An extension to this study thus would be to evaluate expression as well as fitness-related phenotypes such as growth rate throughout the larval season. Further, we note that these results are most reflective of earlier results for overwinter metabolic rate (Pelini *et al.* 2009), wherein metabolic rates of dormant swallowtail pupae showed strong population differentiation while those of dormant duskywing larvae showed an interaction between population and environmental temperature (suggesting local adaptation to winter conditions). Thus, patterns of transcriptional regulation during the immediate prediapause phase may be more similar to overwintering physiology than larval growth.

We considered ways that species differences and experimental design could confound expression measurement and found biases between species and populations to be minimal (see Materials and Methods for details). Nevertheless, care should be taken in the interpretation of these results. This study does not capture the expression of individuals that perished as early instars and therefore does not examine expression profiles of fit versus unfit phenotypes at those stages. Our experiment also analyses expression at the end of larval development – a key life stage – but previous results suggest that the overwintering or other life stages may

play even more important roles for these species. Direct comparisons between the duskywing and swallowtail are complicated by their differing life histories (e.g. perhaps the swallowtail is localized to temperature, but not at the prediapause stage) and potential susceptibility to maternal effects that we could not alleviate via multiple generations of laboratory rearing. (Though, we sampled treated individuals as similarly as possible in their prediapause stages, and parental lineages were sourced at similar locations and times.) Finally, the common-garden design utilized here only represented warming for the peripheral populations; studies of warming on central or even equatorial populations may be informative as well.

Our findings have implications for predictions about the effects of climate change on species and populations within them. Although we found abundant population-specific expression for the swallowtail, neither population responded to the temperature treatments. For the duskywing, in central conditions peripherally sourced individuals responded like centrally sourced individuals, although these populations diverged in peripheral conditions [mirroring results for overwintering metabolic rate (Pelini *et al.* 2009)]. Absent from other information, this result would support peripheral enhancement for the duskywing under warming. However, this adjustment is coupled to an overbearing overwinter metabolic cost affecting survivorship (Pelini *et al.* 2009) and is likely to be compounded by an increase in thermal variability as climate warms (Williams *et al.* 2012). Indeed, our results indicate that many localized genes are involved in energy metabolism, stress and downstream pathways such as those related to host-plant detoxification and growth.

These results are specific to our particular study organisms, but their implications for climate change biology may be quite general. Species with relatively high gene flow such as the swallowtail may at best be indifferent to climate change: the current study found no evidence for localized gene expression for this species, and previous studies showed little evidence for peripheral enhancement under moderate warming (Pelini *et al.* 2009). While widely available host plants generally facilitate gene flow, hindering local adaptation (Sultan & Spencer 2002), previous studies suggest that the choice of host plant itself may influence responses to climate (Pelini *et al.* 2009). On the other hand, although effects of climate change on more structured species such as the duskywing are also likely to be complex, they are also more likely population specific. As this study shows, the specific effects of climate change may not generalize even among related species. By measuring and integrating responses across multiple levels of biological organization, we can begin to explore the significance of population-specific responses to environmental change in many nonmodel organisms. Finally, the existence of local adaptation influenced by gene expression challenges a central assumption in global change biology: that species are functionally similar across their range and that poleward peripheral populations are preadapted to warmer conditions. It may frequently be the case that populations, rather than species, are adapted to climate and may thus respond individually to climate change.

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Conflicts of interest

The authors declare no conflict of interests.

References

Aitken SN, Whitlock MC (2013) Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 367–388.

Angilletta MJ, Bennett AF, Guderley H *et al.* (2006) Coadaptation: a unifying principle in evolutionary thermal biology. *Physiological and Biochemical Zoology*, **79**, 282–294.

Ashburner M, Ball CA, Blake JA *et al.* (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics*, **25**, 25–29.

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, **19**, 185–193.

Bridle JR, Vines TH (2007) Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution*, **22**, 140–147.

Cohen MB, Schuler MA, Berenbaum MR (1992) A host-inducible cytochrome P-450 from a host-specific caterpillar: molecular cloning and evolution. *Proceedings of the National Academy of Sciences, USA*, **89**, 10920–10924.

Conesa A, Götz S, García-Gómez JM *et al.* (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, **21**, 3674–3676.

Cravatt BF, Giang DK, Mayfield SP *et al.* (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature*, **384**, 83–87.

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. *Science*, **292**, 673–679.

Denisov G, Walenz B, Halpern AL *et al.* (2008) Consensus generation and variant detection by Celera Assembler. *Bioinformatics*, **24**, 1035–1040.

Deutsch CA, Tewksbury JJ, Huey RB *et al.* (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences, USA*, **105**, 6668–6672.

Dowling DK, Simmons LW (2009) Reactive oxygen species as universal constraints in life-history evolution. *Proceedings Biological Sciences/The Royal Society*, **276**, 1737–1745.

Fong SS, Joyce AR, Palsson BØ (2005) Parallel adaptive evolution cultures of *Escherichia coli* lead to convergent growth phenotypes with different gene expression states. *Genome Research*, **15**, 1365–1372.

Ford EB (1945) *Butterflies*. Collins, London.

Fournier-Level A, Korte A, Cooper MD *et al.* (2011) A map of local adaptation in *Arabidopsis thaliana*. *Science*, **334**, 86–89.

Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, **21**, 434–443.

Hahn DA, Denlinger DL (2011) Energetics of insect diapause. *Annual Review of Entomology*, **56**, 103–121.

Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New Forests*, **6**, 95–124.

Hancock AM, Brachi B, Faure N *et al.* (2011) Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, **334**, 83–86.

Hellmann JJ, Pelini SL, Prior KM, Dzurisin JDK (2008) The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. *Oecologia*, **157**, 583–592.

Hellmann JJ, Prior KM, Pelini SL (2012) The influence of species interactions on geographic range change under climate change. *Annals of the New York Academy of Sciences*, **1249**, 18–28.

Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives. *Trends in Ecology & Evolution*, **9**, 223–227.

Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. *Nature Reviews Genetics*, **9**, 421–432.

Kaisila J (1962) *Immigration und Expansion der Lepidopteren in Finnland in den Jahren 1869–1960*. Entomological Society of Finland, Helsinki.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Kingsolver JG (2009) The well-temperatured biologist. *The American Naturalist*, **174**, 755–768.

Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants (A Buckling, Ed.). *PLoS One*, **3**, e4010.

Marsico TD, Hellmann JJ, Romero-Severson J (2009) Patterns of seed dispersal and pollen flow in *Quercus garryana* (Fagaceae) following post-glacial climatic changes. *Journal of Biogeography*, **36**, 929–941.

Menéndez R (2007) How are insects responding to global warming? *Tijdschrift voor Entomologie*, **150**, 355–365.

Mezey JG, Nuzhdin SV, Ye F, Jones CD (2008) Coordinated evolution of co-expressed gene clusters in the *Drosophila transcriptome*. *BMC Evolutionary Biology*, **8**, 2.

Mitchell-Olds T, Schmitt J (2006) Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature*, **441**, 947–952.

O'Neil ST, Dzurisin JDK, Carmichael RD *et al.* (2010) Population-level transcriptome sequencing of nonmodel organisms *Erynnis propertius* and *Papilio zelicaon*. *BMC Genomics*, **11**, 310.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.

Parmesan C, Ryrholm N, Stefanescu C *et al.* (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, **399**, 579–583.

Pelini SL, Dzurisin JDK, Prior KM *et al.* (2009) Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. *Proceedings of the National Academy of Sciences, USA*, **106**, 11160–11165.

Pelini SL, Keppel JA, Kelley AE, Hellmann JJ (2010) Adaptation to host plants may prevent rapid insect responses to climate change. *Global Change Biology*, **16**, 2923–2929.

Prior KM, Dzurisin JDK, Pelini SL, Hellmann JJ (2012) Biology of larvae and adults of *Erynnis propertius* at the northern edge of its range. *The Canadian Entomologist*, **141**, 161–171.

Rehfeldt GE, Tchebakova NM, Parfenova YI *et al.* (2002) Intraspecific responses to climate in *Pinus sylvestris*. *Global Change Biology*, **8**, 912–929.

Richardson DM, Hellmann JJ, McLachlan JS *et al.* (2009) Multidimensional evaluation of managed relocation. *Proceedings of the National Academy of Sciences, USA*, **106**, 9721–9724.

Rodríguez A, Villuendas R, Yáñez L *et al.* (2007) Molecular heterogeneity in chronic lymphocytic leukemia is dependent on BCR signaling: clinical correlation. *Leukemia*, **21**, 1984–1991.

Saarinen K, Lahti T, Marttila O (2003) Population trends of Finnish butterflies (Lepidoptera: Hesperioidea, Papilionoidea) in 1991–2000. *Biodiversity & Conservation*, **12**, 2147–2159.

Savolainen O, Bokma F, García-Gil R, Komulainen P, Repo T (2004) Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management*, **197**, 79–89.

Schwartz MW, Hellmann JJ, Mclachlan JM *et al.* (2012) Managed relocation: integrating the scientific, regulatory, and ethical challenges. *BioScience*, **62**, 732–743.

Scott J (1992) *The Butterflies of North America: A Natural History and Field Guide*. Stanford University Press, Palo Alto, California.

Shapiro AM (2002) The Californian urban butterfly fauna is dependent on alien plants. *Diversity & Distributions*, **8**, 31–40.

Shi X-Z, Zhong X, Yu X-Q (2012) *Drosophila melanogaster* NPC2 proteins bind bacterial cell wall components and may function in immune signal pathways. *Insect Biochemistry and Molecular Biology*, **42**, 545–556.

Snyder MJ, Walding JK, Feyereisen R (1995) Glutathione S-transferases from larval *Manduca sexta* midgut: sequence of two cDNAs and enzyme induction. *Insect Biochemistry and Molecular Biology*, **25**, 455–465.

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences, USA*, **100**, 9440–9445.

Subramanian A, Tamayo P, Mootha VK *et al.* (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences, USA*, **102**, 15545–15550.

Sultan SE, Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. *The American Naturalist*, **160**, 271–283.

Surget-Groba Y, Montoya-Burgos JI (2010) Optimization of de novo transcriptome assembly from next-generation sequencing data. *Genome Research*, **20**, 1432–1440.

Tong ML, Shapiro AM (1989) Genetic differentiation among California populations of the anise swallowtail butterfly, *Papilio zelicaon* Lucas. *Journal of the Lepidopterist's Society*, **43**, 217–228.

Vergeer P, Kunin WE (2013) Adaptation at range margins: common garden trials and the performance of *Arabidopsis lyrata* across its northwestern European range. *The New Phytologist*, **197**, 989–1001.

Walter NAR, McWeeney SK, Peters ST *et al.* (2007) SNPs matter: impact on detection of differential expression. *Nature Methods*, **4**, 679–680.

Wehling WF, Thompson JN (1997) Evolutionary conservatism of oviposition preference in a widespread polyphagous insect herbivore, *Papilio zelicaon*. *Oecologia*, **111**, 209–215.

Wheat CW, Fescemyer HW, Kvist J *et al.* (2011) Functional genomics of life history variation in a butterfly metapopulation. *Molecular Ecology*, **20**, 1813–1828.

Wiens JA, Stralberg D, Jongsomjit D, Howell CA, Snyder MA (2009) Niches, models, and climate change: assessing the assumptions and uncertainties. *Proceedings of the National Academy of Sciences, USA*, **106**(Suppl), 19729–19736.

Williams CM, Marshall KE, MacMillan HA *et al.* (2012) Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. (CA Navas, Ed,). *PLoS One*, **7**, e34470.

Zakharov EV, Hellmann JJ (2008) Genetic differentiation across a latitudinal gradient in two co-occurring butterfly species: revealing population differences in a context of climate change. *Molecular Ecology*, **17**, 189–208.

Zakharov EV, Lobo NF, Nowak C, Hellmann JJ (2009) Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). *Heredity*, **102**, 590–599.

Zhao Y, Park R-D, Muzzarelli RAA (2010) Chitin deacetylases: properties and applications. *Marine Drugs*, **8**, 24–46.

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Data accessibility

The expression data reported in this paper are accessible in the Gene Expression Omnibus (GEO) database under accession no GSE56405; microarray designs are based on sequences deposited in GenBank under accession nos JP593164–JP616577 (*Erynnis propertius*), JP616578–JP631634 and JP709351–JP722269 (*Papilio zelicaon*).

Supporting information

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

Fig. S1 Swallowtail and duskywing species' ranges and sampling locations.

Fig. S2 Similarly annotated genes differentially expressed between source populations for duskywing and swallowtail (FDR value < 0.05), grouped by annotation and sorted by source fold change.

Fig. S3 Distribution of FDR values for interaction terms (localized genes) in the duskywing (EP, top) and swallowtail (PZ, bottom).

Fig. S4 Duskywing gene plasticity between treatment conditions (left) and source populations (right).

Fig. S5 Expression profiles for select localized genes, annotated with Blast2GO functions based on BLAST results against NR (see Materials and Methods).

Table S1 Duskywing (E.p.) and swallowtail (P.z.) sample sites, regions, cages per site, and the number of individuals contributing to experimental groups.

Table S2 Duskywing (E.p.) and swallowtail (P.z.) total individual counts and number of mothers producing these individuals.

Table S3 Summary statistics for annotation based on best BLASTX results against SWISSPROT (*e*-value cutoff 1e-5).

Table S4 Summary statistics for fold changes in main effect of source population, main effect of treatment condition, and interaction term.

Table S5 Localized duskywing genes (FDR value < 0.15) also showing either an annotation against SWISSPROT (1e-6 cutoff) or assigned a functional annotation using BLAST2GO as annotated against NR (see Materials and Methods).