

LETTER

Loss of adaptive variation during evolutionary responses to climate change

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Abstract

The changes in species' geographical distribution demanded by climate change are often critically limited by the availability of key interacting species. In such cases, species' persistence will depend on the rapid evolution of biotic interactions. Understanding evolutionary limits to such adaptation is therefore crucial for predicting biological responses to environmental change. The recent poleward range expansion of the UK brown argus butterfly has been associated with a shift in female preference from its main host plant, rockrose (Cistaceae), onto Geraniaceae host plants throughout its new distribution. Using reciprocal transplants onto natural host plants across the UK range, we demonstrate reduced fitness of females from recently colonised Geraniaceae-dominated habitat when moved to ancestral rockrose habitats. By contrast, individuals from ancestral rockrose habitats show no reduction in fitness on Geraniaceae. Climate-driven range expansion in this species is therefore associated with the rapid evolution of biotic interactions and a significant loss of adaptive variation.

Keywords

Butterfly, climate change, host preference, insect, local adaptation, range expansion, rapid evolution, reciprocal transplants.

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INTRODUCTION

To avoid extinction from ongoing climate change, species need to either adapt *in situ* to changing conditions or shift their geographical distribution to colonise newly suitable habitat. Although many animals and plants have shifted their ranges polewards in recent decades (Parmesan & Yohe 2003; Hickling *et al.* 2006), few species show distributional shifts at a rate sufficient to keep pace with climate change (Devictor *et al.* 2012; Corlett & Westcott 2013). The availability of suitable habitat, and the interactions with species that these habitats represent, is an important factor limiting the rate of ongoing range shifts (Hill *et al.* 2001; Warren *et al.* 2001; Laverne *et al.* 2010). Furthermore, variation among species in their response to climate change is altering local biotic interactions and generating novel ecological communities (González-megías *et al.* 2008; Laverne *et al.* 2010) to which organisms must adapt.

Species showing broad habitat requirements that are adapted for diverse interactions with other organisms should therefore dominate communities affected by climate change. This is observed in UK bird communities (Davey *et al.* 2012) and across European Lepidoptera (Warren *et al.* 2001; Pöyry *et al.* 2009; Betzholtz *et al.* 2013). Similarly, broader tolerance to novel enemies has been associated with successful range expansions in plants (Engelkes *et al.* 2008). By contrast, the failure of many specialist species to track climate change suggests that their potential for evolution is limited, either by specialised biotic interactions, or by the small population sizes and low genetic variation associated with their fragmented habitats (Thomas *et al.* 2001; Bridle & Vines 2007; Laverne *et al.* 2010). Measures such as assisted migration, or maximis-

ing local population sizes and connectivity may therefore be necessary to maximise their evolutionary potential (Hill *et al.* 2011; Hoffmann & Sgro 2011; Thomas 2011).

Estimating the potential for adaptive evolution is therefore important when identifying populations and species most at risk of extinction due to climate change (Thuiller *et al.* 2013). The rapid evolution of life history traits, particularly dispersal morphology and physiology, has been frequently observed during recent range shifts (Thomas *et al.* 2001; Feder *et al.* 2010; Hill *et al.* 2011). Rapid adaptation to novel abiotic conditions has also been associated with biological invasions, for example, through evolution of photoperiodic response (Urbanski *et al.* 2012) or trade-offs between flowering time and size (Colautti & Barrett 2013). However, empirical data on how quickly biotic interactions can evolve during range expansion are urgently needed, particularly when such interactions, for example between phytophagous insects and their host plants, are often critical for persistence in new localities (Laverne *et al.* 2010).

The brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae), has rapidly expanded its UK range northwards since 1970–82 (Asher *et al.* 2001), as new habitats have become thermally suitable due to recent climate change (Thomas *et al.* 2001; Pateman *et al.* 2012). In long-established parts of the range, the brown argus is mostly restricted to chalk/limestone grassland dominated by the host plant, rockrose (Cistaceae, *Helianthemum nummularium*). However, some populations persist at locally warm sites dominated by host plants in the Geraniaceae family (*Geranium molle*, *Geranium dissectum* and *Erodium cicutarium*), which are not closely related to rockrose (Soltis *et al.* 2011). By contrast, in recently colonised parts of the UK, brown argus is most

commonly found in habitats dominated by Geraniaceae host plants, which are geographically widespread throughout this area. Population genomic analyses have identified divergent selection associated with rockrose or Geraniaceae habitat use in the established part of the brown argus range, as well as with range expansion (Buckley *et al.* 2012). These signatures of selection and shifts in habitat use associated with the brown argus range expansion provide an opportunity to investigate the evolution of biotic interactions, in this case between insects and their host plants, in response to climate change.

Bridle *et al.* (2014) conducted site-level assays of female egg-laying preference for greenhouse-grown plants of the four host plant species, and revealed variation in female preference among long-established areas, which was associated with the most common host plant at a site. By contrast, recently colonised sites showed consistent preference for *G. molle*, even where this species was locally absent. However, such host choice tests do not reveal the fitness consequences of adaptive divergence in female preference when individuals are presented with different host plant species growing in natural field conditions.

Reciprocal transplant experiments of individuals between habitats are a powerful test for adaptive divergence (Kawecki & Ebert 2004). Reciprocal transplants between central and marginal populations of current distributions have been used to test likely responses of butterflies to climatic variation (e.g. Crozier 2004; Hellmann *et al.* 2008), and for adaptation to host plants (Pelini *et al.* 2009, 2010), but have not previously been used to test for rapid evolutionary shifts in biotic interactions following a climate-driven range shift. Butterfly host preference can rapidly evolve (Singer *et al.* 1993) and shows strong evidence for being genetically determined and heritable (Nylin *et al.* 2005; Klemme & Hanski 2009). However, host use can also be influenced by variation in microclimate and vegetation characteristics, such as sward height (Davies *et al.* 2006). In this study, we conducted reciprocal transplants of female brown argus butterflies onto naturally growing host plants across long-established and recently colonised parts of their UK range, and assayed individual egg-laying behaviour to test experimentally for adaptive divergence in host plant use and the consequences of range expansion for future adaptive potential. These data allow us to determine whether the rapid climate-driven distributional shift observed in this species has been facilitated by the evolution of biotic interactions.

METHODS

Testing for adaptive divergence in the use of different host plants

Details of study sites

Across the UK range of the brown argus, 13 sites (seven rockrose-dominated; six Geraniaceae-dominated) were chosen throughout the long-established (brown argus present since 1970–82) and recently colonised (present since 1995–99) UK range (Asher *et al.* 2001; Table S1). Based on vegetation analysis (see Supporting Information), sites were also divided into habitat types dominated by host plants of the Gerania-

ceae family (*Geranium molle* and *Erodium cicutarium*) or rockrose (*Helianthemum nummularium*) for subsequent analyses (Table S1).

Reciprocal transplant methodology and analysis

Reciprocal transplant experiments were conducted between pairs of sites to test for divergence in egg-laying behaviour with respect to habitat type and colonisation history. For each pair of sites, females were collected and held individually in the same environment for 12–48 h with food provided (sponge soaked in 10% sugar solution). A total of 355 adult female butterflies were collected at 11 of the study sites and used in transplant experiments from 26th July to 14th September in 2008, 2009 and 2010.

Cages (upended metal wire shopping baskets 320 × 450 × 190 mm, covered in 1.35 mm² plastic mesh) were placed over patches of wild host plants distributed across both sites, at locations chosen to reflect the range of microclimates and host plant abundance available to females. Any eggs already laid on these patches by free-flying females were removed, the percentage host plant cover and sward height estimated, and an iButton temperature datalogger (Maxim Integrated Products, San Jose, CA, USA) placed in the centre of each cage.

Females from each site in a pair were randomly split into two treatment groups and either placed in a cage at their home site, or a cage at a new site. The number of females under cages at a site in each treatment ranged from 6 to 24. Females were provided with a sponge soaked in 10% sugar solution and left for 1–3 days, with treatments excluded from the analysis if all females were exposed continual to wet weather, strong winds or average temperatures < 18 °C. The number of eggs laid on host plants was counted and divided by the number of daylight hours (between 8 am and 6 pm) a female spent in a cage to estimate egg-laying rates. To check the sensitivity of transplant effects to different measures of female egg-laying behaviour, the effect of transplant treatment on the proportion of females choosing to lay any eggs was also tested (analysis described in Supporting Information).

Where females were collected over a similar time period from both sites in a transplant pair, the egg-laying rates of transplanted and locally caught females were compared at each site ('local' vs. 'transplanted'). However, where weather conditions prevented the collection of females from one site in a transplant pair, the egg-laying rates of transplanted females were compared to local females collected at a different time period in the same season. Such fully-reciprocated comparisons provide the most reliable evidence for local adaptation (Kawecki & Ebert 2004). Alternatively, when a 'local' vs. 'transplanted' comparison was not possible, the egg-laying rates of a subset of females from a given population at their 'home' site, or at a different site ('away') were compared (termed 'home' vs. 'away' transplants). All statistical analyses were conducted using R statistical software (R Development Core Team 2012).

For each transplant comparison, a Generalised Linear Model with the fixed effect of transplant treatment was used to model variation in egg-laying rates. Likelihood ratio tests

were used to determine whether a model including transplant treatment explained more variance in egg-laying rate than a model excluding this factor. To account for overdispersion in the egg count data, analyses were conducted using GLMs with either negative binomial error (R package: MASS; Venables & Ripley 2002) or zero-inflated negative binomial error (R package: PSCL; Zeileis *et al.* 2008). Vuong's non-nested hypothesis test (R package: PSCL) was used to identify the most appropriate error distribution. An offset variable of log day hours in cage was used to model the egg counts as egg-laying rates. Where no females from a given treatment laid eggs (only observed for KOB females transplanted to SWYN in 2008 and 2009), parameter estimates and standard errors were severely over-inflated, so 0.5 was added to the egg counts to stabilise parameter estimates.

Female survival was recorded ('1' = alive; '0' = dead), with absent females assumed to have been eaten by predators. Where absent females had already laid eggs, these counts were included in the data set, with the female assumed to have been alive in the cage for half the elapsed time. Females moved to a new site rather than returned to their own did not experience consistently increased rates of mortality (GLM with binomial error: transplant effect significant for only 2 of 20 transplants; Table S2), indicating that any observed transplant

effects represent divergence in egg-laying behaviour among the study sites.

The following transplants were conducted:

(1) Thirteen transplant comparisons involving five pairs of sites of *different habitat type*, and either similar or different colonisation histories (Fig. 1a; sample sizes in Fig. 2a).

(2) Seven transplant comparisons involving five pairs of sites of *similar habitat type*, and either similar or different colonisation histories (Fig. 1b; sample sizes in Fig. 2b).

Effect of ecological and thermal variation on the use of different host plants

Variation in temperature, sward height and host cover across study sites

We first tested whether sites grouped by habitat type represented different thermal environments, as estimated using two approaches (details in Supporting Information): (1) To estimate the energy available for larval growth at a site (and the severity of winters), ambient shade temperature 1.5 m above ground level was recorded hourly from 1st October 2008 to 30th September 2010 at the 13 study sites using Tinytag dataloggers (Gemini dataloggers UK Ltd, Chichester, UK); and (2) For three pairs of sites differing in habitat type we also

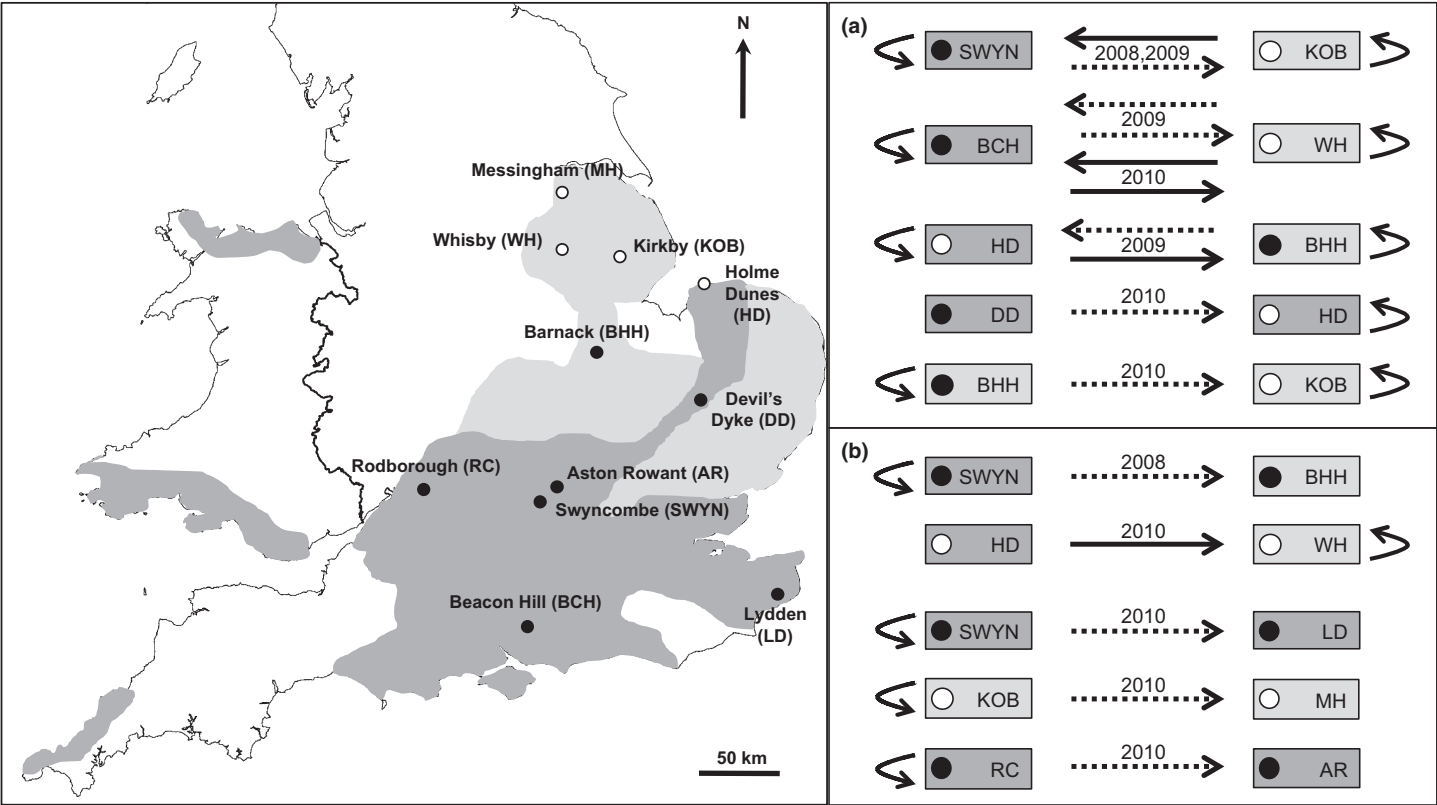


Figure 1 Recent range expansion of *A. agestis* and locations of 11 study sites (with site codes) used in transplants. Darker shading indicates long-established parts of the range; lighter shading indicates recently colonised regions. Open circles indicate sites where Geraniaceae host plants are dominant and filled circles where *H. nummularium* is dominant. (a) and (b) summarise the transplants conducted as defined in the methods. The direction of the arrows represents the site to which females were moved. Solid straight arrows indicate significant changes in egg-laying rates of transplanted/away females relative to local/home females and dashed straight arrows indicate non-significant changes. Curved arrows indicate that female egg-laying rate was assayed at their home site. Year next to arrows indicates the year(s) in which the transplant was conducted.

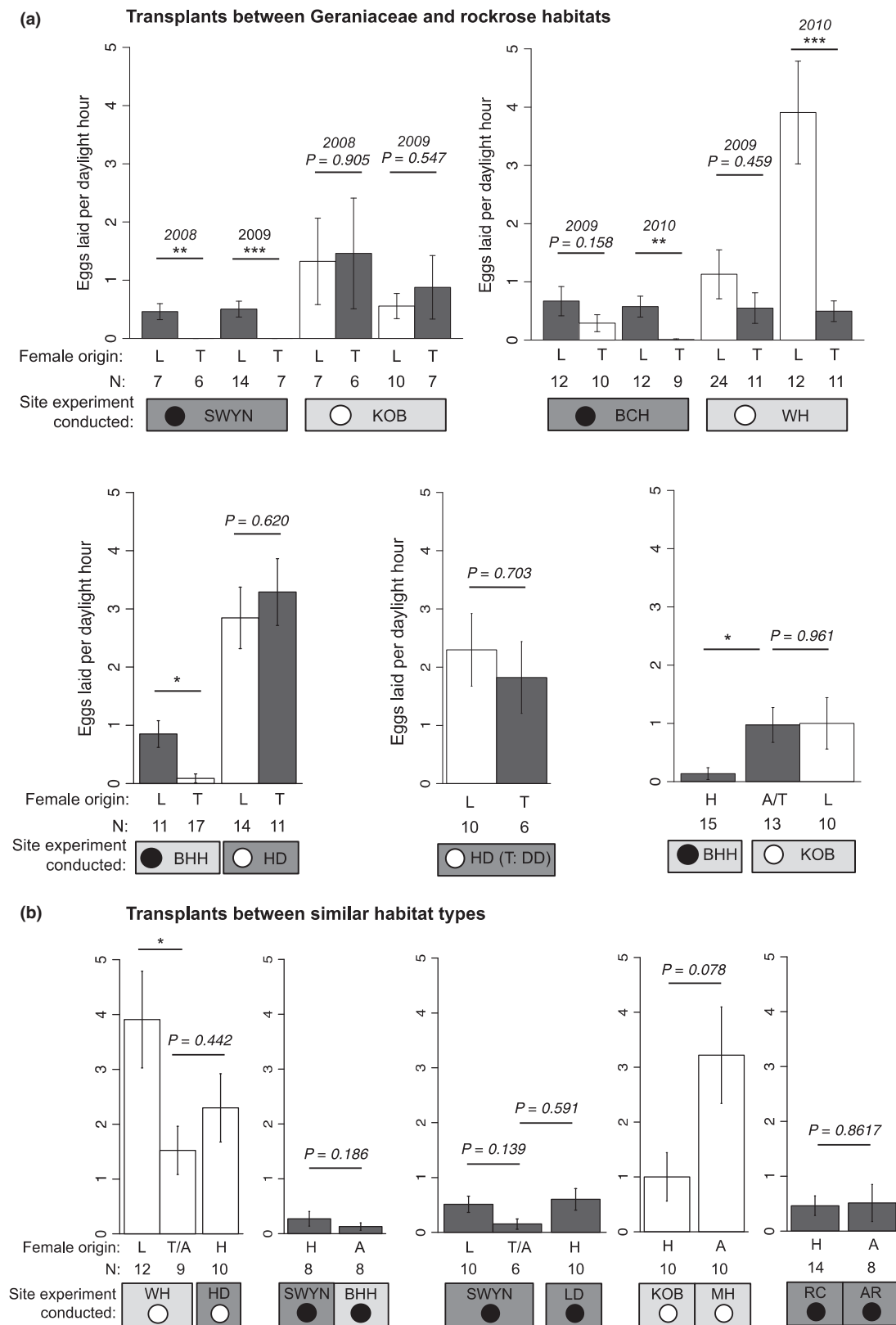


Figure 2 Observed mean egg-laying rates (± 1 SE) of home and transplanted females for the transplants described in Fig. 1. The origin of the females is given as either local ('L')/transplanted ('T') or home ('H')/away ('A'), with the bar colour denoting those females caught at rockrose sites (dark grey) or from Geraniaceae sites (white). The sites at which the experiments were conducted are indicated below the graphs and are coded as for Fig. 1. HD(T:DD) indicates that females from DD were moved to HD, but no reliable data for DD females at DD could be collected. Sample sizes ('N') are also given, along with the year that experiments were conducted (when repeated in consecutive years). The statistics for the difference between home and transplanted females are given (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

recorded variation in temperature during the transplant experiments, at 1–3 cm above the ground adjacent to patches of host plants ('host plant' temperature) using iButton dataloggers.

In addition, vegetation surveys were conducted to estimate variation in percentage cover of the four different host plants and average sward height across the study sites (details in Supporting Information).

The effects of ecological and thermal variation on egg-laying rates at home sites

Variation in egg-laying rates among females tested at their home site (167 females at seven sites) was used to test whether egg-laying rates were consistently associated with temperature, sward height and host cover. First, we tested for differences among study sites and different habitats in average cage temperature, proportion host cover and sward height (details in Supporting Information). We then tested for an interaction between average cage temperature and site of origin, as well as for their separate effects on egg-laying rate, using a zero-inflated negative binomial GLM. This analysis was repeated, grouping sites by habitat type or colonisation history and including study site as a random variable, using zero-inflated negative binomial mixed-effect GLMs as implemented using the R package 'glmmADMB' (Fournier *et al.* 2012). The effects of variation in sward height and host plant cover under cages on egg-laying rates were then tested for Geraniaceae and rockrose sites separately due to confounding effects of habitat type on these variables (Fig. S1a, c).

The effects of thermal variation on egg-laying rates in transplant experiments

Transplant effects may represent a response to differences in temperature experienced under cages by home and transplanted females, particularly when comparing females at a home and an away site, or when local and transplanted females were not tested simultaneously. Differences in mean temperature between cages allocated to different transplant treatments (local/home vs. transplanted/away) were tested for significance using ANOVA. If temperature was significant in explaining variation in egg-laying rate then the direction of any significant differences in temperature (increased or decreased) would be similar to the direction of the observed significant transplant effects (increased or decreased egg-laying rates).

RESULTS

Testing for adaptive divergence in the use of different host plants

Transplants between sites of different habitat

Females moved from recently colonised Geraniaceae-dominated sites to long-established rockrose-dominated sites showed large and significant reductions in the rate of egg-laying relative to local females (WH to BCH and KOB to SWYN; $P < 0.002$ for three of four such transplants; Fig. 2a). Across these four sites, only 5 of 32 females (15.6%) moved from recently colonised Geraniaceae sites laid any eggs on rockrose (compared to 31 of 45, or 68.9%, of local females; Table S3).

By contrast, when females from long-established rockrose sites were moved to recently colonised Geraniaceae sites, egg-laying rates were not different for local and transplanted females (BCH to WH and SWYN to KOB; Fig. 2a; $P > 0.459$ for three of four such transplants), and a similar proportion of local and transplanted females laid eggs (local females: 35 of 53, or 66.0%; transplanted females: 20 of 35, or 57.1%; Table S3).

Interestingly, reduced egg-laying rates were also observed for females moved from a long-established Geraniaceae site to a recently colonised rockrose site (HD to BHH; $P = 0.012$; Fig. 2a), with a significant reduction in the proportion of transplanted females laying any eggs (90.9% local females; 11.8% transplanted females; Table S3). No significant changes in egg-laying rate were observed when moving females from a recently colonised rockrose site to a long-established Geraniaceae site (BHH to HD; Fig. 2a), with 100% of local and transplanted females laying eggs (Table S3).

Sites of differing habitat, but similar colonisation history, showed patterns consistent with the transplants at Geraniaceae sites described above, *i.e.* local Geraniaceae and transplanted rockrose females laid eggs at a similar rate on Geraniaceae (DD to HD and BHH to KOB; Fig. 2a). Insufficient females were collected to test the egg-laying rates of long-established Geraniaceae females at long-established rockrose sites. However, females moved from a recently colonised rockrose site to a recently colonised Geraniaceae site did show increased egg-laying rates on Geraniaceae hosts relative to those females left at home on rockrose (BHH to KOB; $P < 0.05$; Fig. 2a).

Transplants between sites of similar habitat

Transplants between sites of similar habitat type showed no consistent evidence for local adaptation, either in the rate of egg-laying (Fig. 2b), or the proportion of females laying eggs (Table S3). Only one of the six transplants, that of females moved from a long-established Geraniaceae site to a recently colonised Geraniaceae site, showed significantly reduced egg-laying rates relative to local females, suggesting local adaptation (HD females to WH; $P < 0.05$; Fig. 2b). However, a similar proportion of local and transplanted females laid eggs in this transplant comparison (Local = 83.3%; Transplanted = 77.8%; Table S3), and the HD females moved to WH showed no difference in average egg-laying rates relative to HD females left at their home site (Fig. 2b).

Effect of ecological and thermal variation on the use of different host plants

Variation in temperature, sward height and host cover among the study sites

On calcareous grasslands in 2008–2010, only rockrose was found at high abundance (average 13.3%, ranging from 1 to 30% cover at the six sites surveyed; Table S1). At other grassland types, rockrose was not recorded and Geraniaceae host plant cover was low at the four sites surveyed. *G. molle* was found at < 1% cover in all sites and *E. cicutarium* found at < 1% cover in three of four sites surveyed (but 1–5% cover at one coastal dune grassland; Table S1). *Geranium dissectum*

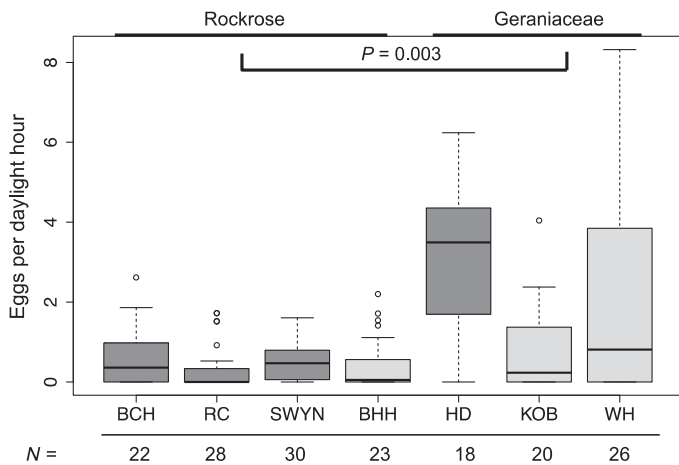


Figure 3 Variation among and within sites in egg-laying rates of females at home and the significance of the difference in egg-laying rates when sites grouped by habitat type. Darker shading indicates long-established sites and lighter shading recently colonised sites.

was not recorded at the study sites. Sites dominated by rockrose had a higher mean sward height than Geraniaceae sites (rockrose = 113.9 mm; Geraniaceae = 48.0 mm). Similar patterns were observed in vegetation surveys conducted in 2005 (Bridle *et al.* 2014; Table S1; results described in Supporting Information). Rockrose and Geraniaceae-dominated sites show no consistent differences in ambient annual temperature measurements (Table S4a, b). However, temperatures adjacent to Geraniaceae host plants monitored during transplant experiments were on average 2.3–4.2 °C warmer than adjacent to rockrose sites in three different pairwise comparisons ($P < 0.0001$; Table S5).

The effects of ecological and thermal variation on egg-laying rates at home sites

Across the seven study sites, there was significant variation in the average egg-laying rate of females at their home site

(Table 1: $P < 0.0001$). Egg-laying rates were 4.5 times higher for females from Geraniaceae sites compared to those from rockrose sites (Geraniaceae average = 2.039; rockrose average = 0.453; $P = 0.003$; Fig. 3), but did not differ for females from sites of differing colonisation history (Table 1; $P = 0.592$).

Across all females assayed at home sites, there was evidence for increased egg-laying rates associated with higher average temperatures under cages ($P = 0.057$; Table 1). However, the amount of variance in egg-laying rates explained by cage temperature in the models was low (5.3%), with site as a factor explaining 18.1% of the variance in egg-laying rate and sites grouped by habitat type explaining 14.4% of the variance in female egg-laying rate (Table 1). Sites also did not differ significantly in the slope of the relationship between average day temperature and female egg-laying rate (site \times temperature interaction; Table 1), suggesting that populations are not locally adapted to varying microclimates.

Given the differences between habitat types in host cover and sward height, but not average temperature under cages (Fig. S1), sites were grouped by habitat type to test the effect of host cover and sward height on egg-laying rates. Across Geraniaceae-dominated sites and across rockrose-dominated sites, there was no association between female egg-laying rate and host cover (Geraniaceae: LR-stat = 0.144, d.f. = 1, $p = 0.704$; rockrose: LR-stat = 0.746, d.f. = 1, $P = 0.388$) or sward height (Geraniaceae: LR-stat = 0.31, d.f. = 1, $P = 0.578$; rockrose: LR-stat = 0.09, d.f. = 1, $P = 0.764$).

The effects of thermal variation on egg-laying rates in transplant experiments

Temperatures experienced by females under cages did vary with respect to transplant treatment in 10 of the 20 comparisons (Table S7). These data show transplanted/away females experienced both increased and decreased temperatures relative to local/home females. However, in only two comparisons was the direction of temperature differences similar to the direction of the change in egg-laying rates when females were

Table 1 Tests for differences in the associations between egg-laying rate and temperature across sites, or across sites grouped by habitat type or colonisation history, for home females

Factor	Statistical model used	Model terms and estimate of explained variance					
		Factor \times Temperature	Exp. Var (%)	Factor	Exp. Var (%)	Temperature	Exp. Var (%)
Effect of site	ZINB	LR = 4.709, d.f. = 6, $P = 0.582$	20.1	LR = 51.60, d.f. = 6, $P < 0.0001$	18.1	Weak association LR = 3.635, d.f. = 1, $P = 0.057$	5.3
Colonisation history	ZINB mixed model (+1 Site)	LR = 1.274, d.f. = 1, $P = 0.259$	7.5	LR = 0.378, d.f. = 1, $P = 0.539$	0.3	Positive association LR = 4.582, d.f. = 1, $P = 0.032$	5.3
Habitat type	ZINB mixed model (+1 Site)	LR = 1.046, d.f. = 1, $P = 0.306$	16.8	LR = 8.91, d.f. = 1, $P = 0.003$	14.4	Positive association LR-stat = 4.906, d.f. = 1, $P = 0.027$	5.3

A Zero-Inflated Negative Binomial (ZINB) GLM was used with an offset variable of log (Day hrs) to model variation in egg-laying rate. A random effect of site (+1|Site) was included in the model when testing the effect of colonisation history or habitat type. Total number of individuals used = 167. The Likelihood-ratio statistic (LR) and significance (P) of removing each factor from the model is given (with values < 0.05 highlighted in bold). Percentage variance explained by each factor (Exp. Var.) was estimated using a negative binomial model without random effects. Parameter estimates for the levels of each factor are given in Table S6.

moved to new sites (Table S7). Under cage temperature does not therefore explain the evolutionary divergence in egg-laying behaviour between females from different habitats observed in the transplant experiments.

DISCUSSION

Shifts in biotic interactions are associated with the colonisation of new habitats during range expansion

The recent range shift of the brown argus butterfly provides a test of how climate-driven range expansion may depend on adaptive shifts in biotic interactions. Previous assays of UK variation in host plant preference using greenhouse-grown host plants revealed significant variation among long-established sites, and a shift to consistent egg-laying preference for *Geranium molle*, independent of the locally dominant host, in recently colonised parts of the range (Thomas *et al.* 2001; Bridle *et al.* 2014). The reciprocal transplant data presented here are the first to address two critical questions: (1) how do these differences in host preference affect the fitness of individual females when presented with natural host plants?; and (2) what are the consequences of such shifts in host preference for the future evolutionary potential of this species?

Our data demonstrate pervasive effects of female egg-laying behaviour on the fitness of females from Geraniaceae habitats when exposed to rockrose host plants. Females from recently colonised Geraniaceae-dominated sites showed strong and consistently reduced egg-laying rates relative to females from rockrose sites when moved to long-established rockrose-dominated sites (KOB to SWYN and WH to BCH; Fig. 2a). In addition, a significantly reduced proportion of females choose to lay any eggs at all when transplanted (15.6% relative to 68.9% local females; Table S3). By contrast, females from long-established rockrose sites that were moved on to Geraniaceae host plants laid eggs at a rate similar to females from recently colonised Geraniaceae sites (SWYN to KOB and BCH to WH; Fig. 2a), with no decline in the proportion of transplanted females choosing to lay eggs (Table S3).

These results indicate that females at Geraniaceae sites show specialisation for the use of Geraniaceae host plants only, whereas females at rockrose sites are able to lay eggs on both Geraniaceae and rockrose plants. Although all females were kept in a common environment on capture, they were collected from the field, meaning that traits measured in our experiments may show maternal or environmental effects, in addition to genetic variation. However, the absence of transplant effects when moving females from rockrose to Geraniaceae sites indicates that adult/larval environment does not determine egg-laying behaviour, as has been observed in other insects (Jaenike 1982). In addition, previous release experiments indicate that female host preference profiles are retained in their offspring following common garden rearing (Thomas *et al.* 2001). It has also been shown in several species that egg-laying behaviour in butterflies is genetically determined and highly heritable (Singer & Lee 2000; Nylin *et al.* 2005; Klemme & Hanski 2009).

Given that the brown argus range expansion has largely involved the colonisation of Geraniaceae-dominated habitats

made suitable by climate warming (Thomas *et al.* 2001; Pateman *et al.* 2012), these transplants confirm that an evolutionary shift to the exclusive use of Geraniaceae host plants has been associated with the colonisation of these new habitats. However, rockrose-dominated habitats are found further north in the UK (Stace 1997), meaning that this loss of ability to use rockrose may limit the continued range expansion of the brown argus. The loss of rockrose use documented here may also make extensive hybridisation with the congeneric northern brown argus species, *Aricia artaxerxes*, less likely during expansion, given that this species only uses rockrose (Mallet *et al.* 2011).

Interestingly, females from the only recently colonised rockrose-dominated study site (BHH) showed egg-laying rates on their local rockrose host plants similar to females from another long-established rockrose-dominated site, SWYN (Fig. 2b), and also laid eggs on Geraniaceae host plants when transplanted to HD, a long-established Geraniaceae site (Fig. 2a). These results suggest local adaptation at BHH for using rockrose, which contrasts with the strong population-level preference for greenhouse-grown *G. molle* observed in 2000, 2001 and 2004 (Bridle *et al.* 2014). Evolution *in situ* since 2004 (10–12 generations), or colonisation by rockrose-using individuals from further south, seems to have rapidly generated the local adaptation observed throughout the established range.

Trade-offs involved in Geraniaceae and rockrose host plant use

Females from rockrose and Geraniaceae-dominated sites across the UK range show consistently high rates of egg laying on Geraniaceae host plants, (Figs 2a and 3). However, only individuals from rockrose-dominated sites consistently lay eggs on rockrose host plants (Fig. 2a). This suggests that at rockrose-dominated sites there is an advantage to females (and a cost to females at Geraniaceae sites) in retaining the ability to lay eggs on both rockrose and Geraniaceae host plants.

Rockrose and Geraniaceae host plants are rarely present in the same local habitat types throughout the brown argus UK range. Rockrose is found at high density locally, but is restricted to chalk grassland habitats (Table S1). In contrast, Geraniaceae host plants (particularly *G. molle* and *E. cicutarium*) are locally scarce (Table S1), but far more geographically widespread throughout the landscape (Stace 1997; figure 3 in Pateman *et al.* 2012), including around the hillsides of chalk grassland where rockrose is dominant. The ability to use Geraniaceae may therefore be favoured in warmer years when individuals colonising new habitats are more likely to encounter Geraniaceae than rockrose. By contrast, the perennial rockrose has supported stable brown argus populations during periods that were too cool for the use of Geraniaceae-dominated habitats (Pateman *et al.* 2012), so adaptation to use this host could provide a more reliable resource across years.

Given the low density of Geraniaceae hosts and the high density of rockrose under cages (Fig. S1), it is surprising that egg-laying rates on Geraniaceae are consistently higher than on rockrose (Geraniaceae average = 2.04 eggs per daylight hour; rockrose average = 0.45; Fig. 3), even for females

moved north from rockrose-dominated sites (Fig. 2a). A high proportion of females also chose to lay eggs on Geraniaceae hosts, independent of their site of origin (Table S3), which suggests that females may be less discriminatory about variation in quality among Geraniaceae plants. Studies of other butterfly-host plant systems show that females can identify variation in host plant quality within species (e.g. Thomas 1987; Singer & Lee 2000), and there is evidence that brown argus females can identify leaves on rockrose plants with higher nitrogen concentration (Bourn & Thomas 1993), which may provide better nutrition for developing larvae.

Strong preference for one host species, but variation in the ability to use other host species, is seen in a range of phytophagous insects (Wee & Singer 2007; Ferrari *et al.* 2008), and may suggest that the preferred host is consistently better for larval growth and survival (Gripenberg *et al.* 2010). Alternatively, higher egg-laying rates may offset losses due to higher herbivory experienced by Geraniaceae plants compared to rockrose (JB and JRB personal observation).

Adaptive divergence in host plant use is not driven by variation in microclimate

Variation in egg-laying rate in relation to microclimate, topography and habitat use revealed strong evidence for temperature-dependent variation in egg-laying rates in another butterfly, *Hesperia comma* (Davies *et al.* 2006; Lawson *et al.* 2014). However, in *A. agestis* egg-laying rates among females placed at their home sites showed only a weak association with temperature (Table 1), with habitat type explaining almost three times more variance in egg-laying rates than temperature alone (Table 1). The absence of strong effects of temperature in these experiments is supported by the lack of variation in ambient temperature associated with different habitat types (Table S4a, b). Temperatures adjacent to Geraniaceae host plants over shorter time periods in summer tended to be significantly warmer than those adjacent to rockrose (Table S5), although this might be predicted given the shorter sward height around Geraniaceae host plants. Associations between reduced sward height and warmer temperatures adjacent to host plants have been previously observed in chalk grassland habitats (e.g. Lawson *et al.* 2014).

Instead, our transplant data show that adaptive divergence in individual host preference, rather than responses to variation in microclimate, underlie the reduced rate of egg-laying when females are moved from Geraniaceae-dominated sites to rockrose-dominated sites. Specifically, temperatures under cages were not consistently lower in treatments associated with strongly reduced egg-laying rates, indicating that temperature is not driving the observed loss of fitness (Table S7).

Implications for predicting and facilitating responses to climate change

We demonstrate a central role for evolutionary shifts in biotic interactions during range expansion, specifically adaptive divergence in the use of different host plant species, and show for the first time that these shifts can involve the rapid loss of variation in ecologically important traits. In the brown argus,

changes in female host plant use associated with range expansion onto new Geraniaceae habitat have been associated with the loss of its ability to use the ancestral rockrose host. Such narrowing of this species' host plant niche may have implications for its further expansion northwards. Limited responses of life history traits to selection have been shown following post-glacial range expansion in the plant, *Mercurialis annua* (Pujol & Pannell 2008). However, our data provide new experimental evidence for a loss of adaptive variation during range expansion, which could itself limit future responses to climate change. Our data also suggest that rockrose host use (and local adaptation) can be established once range expansion slows, as observed at one site, BHH, an observation that demands further investigation. Overall, our data indicate that evolutionary change will be important in facilitating the responses of many species to climate change, particularly those that depend on specialised biotic interactions. In such cases, translocations between populations, or the maintenance of high local population sizes, may be necessary for species to maximise the evolutionary potential to shift their ranges (Thomas 2011). More empirical data on local adaptation and shifts in biotic interactions across the ranges of species with contrasting life histories and habitat requirements are therefore urgently needed to improve our predictions of ecological responses to ongoing climate change (e.g. Hoffmann & Sgro 2011; Thuiller *et al.* 2013).

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AUTHORSHIP

JB and JRB designed the experiments, collected and analysed the data and wrote the manuscript.

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