Novel host plant use by a specialist insect depends on geographic variation in both the host and herbivore species

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Abstract

Understanding the circumstances under which insect herbivores will adopt a novel host plant is a longstanding question in basic and applied ecology. While geographic variation in host use can arise through both differences in herbivore preference and in plant quality, there is a tendency to attribute geographic variation to regional differences in herbivore preference alone. This is especially true for herbivores specialized on one or a few plant species. We compared the contribution of variation in herbivore preference versus host plant quality to regional differences in adoption of a non-native host by a highly specialized herbivore, *Euphydryas phaeton*. In parts of its range, *E. phaeton* uses only a native host, *Chelone glabra*, while in others it also uses an introduced host, *Plantago lanceolata*. We offered female butterflies from each region the non-native host plant sourced from both regions and compared their oviposition behavior. The non-native host was almost universally rejected by butterflies in the region where only the native plant is used. In the region where butterflies use both hosts, females accepted non-native plants from their natal region twice as often as non-native plants from the other region where they are not used. Acceptance differed substantially among individual butterflies within regions but not among plants within regions. Thus, both individual preference and regional differences in both the insect and non-native host contributed to the geographic variation in different ways. These results highlight that, in addition to herbivore preference, regional differences in plant quality may be important drivers of diet breadth.

Introduction

Plant-herbivore interactions are ubiquitous across terrestrial ecosystems and many herbivores are highly specialized to one or a few hosts (Bernays and Chapman 1994, Forister et al. 2015). Despite having access to fewer resources relative to generalists, specialist herbivores persist due to their efficiency in host searching, consumption, and acquired defenses (Fox and Morrow 1981, Bernays 1992b, Renwick 2001). These efficiencies enable specialists to dominate communities at local scales (Sudta et al. 2022). Most specialist herbivores exhibit geographic variation in host use throughout their range but may specialize on a single host plant species at local scales (Fox and Morrow 1981). Ecologists and evolutionary biologists have long been interested in the mechanisms that drive variation in host diet breadth because of its relationship to diversification in both plants and insects (Kuussaari et al. 2000, Gompert et al. 2019, Mason 2016). Understanding how patterns of specialist herbivory vary in space and time is essential for understanding the evolution of plants and animals (Agrawal et al. 2012), predicting future host shifts (Forister et al. 2013), and effectively managing both plant and animal species in changing environments (Braga and Janz, 2021).

Geographic variation in diet breadth can depend on geographic variation in insect preferences, plant quality, or both. Although numerous studies have documented variation in insect host use (Jaenike, 1990) and plant quality (Renwick 2001, Harrison et al. 2016), few have analyzed the relative contributions of both insect preference and plant quality to geographic variation in host use. Three decades ago, Singer & Parmesan (1993) evaluated geographic variation in oviposition preference by an oligotrophic herbivore,

Edith's checkerspot, *Euphydryas editha*, which oviposits on a range of plants in the order Lamiales and has substantial among-population variation in host preference. They found that host plant preference depends on the origin of both the butterfly and plant, i.e., butterflies were more likely to oviposit on some plant species if the plants were from sites where the butterflies used that plant species. More recent studies showed that the Melissa blue butterfly, *Lycaeides melissa*, oviposits on a range of species in the legume family (Fabaceae), with geographic variation in host use due to among-population differences in both insect preferences and host plants (Harrison et al. 2016, Gompert et al. 2019). Yet, studies such as these that explicitly examine variation in both herbivores and host plants remain rare. Far more studies have examined differences in host plant use among herbivore populations without regard to the origin of plant species. In doing so there is an implicit suggestion that geographic variation in host plant use reflects geographic differences within herbivore species, as opposed to geographic differences within their host plant species.

In this study, we evaluate contrasting patterns in the use of a non-native (novel) host plant by the Baltimore checkerspot, *Euphydryas phaeton*, and the extent to which this phenomenon can be explained by geographic differences within both the butterfly species and its non-native host plant. Unlike Edith's checkerspot and the Melissa blue, the Baltimore checkerspot is a strict specialist in parts of its range, with oviposition and pre-diapause feeding almost exclusively restricted to its native host plant, white turtlehead *Chelone glabra*. Half a century ago, Baltimore checkerspots in Eastern North America were first recorded ovipositing on a second, non-native plant, English plantain *Plantago lanceolata* (Stamp 1979). Some populations of Baltimore checkerspots in the northern part of their range now oviposit on plantain and use it as a pre-diapause host instead of turtlehead (Bowers et al. 1992b). During post-diapause development, Baltimore checkerspots will feed on English plantain throughout their range (Bowers et al. 1992b, Arriens et al. 2021), though it may be a lower quality food plant in some respects (Abarca et al. 2019, Arriens et al. 2021; but see Brown et al. 2017, Muchoney et al. 2022). In the southern part of their range, no population of Baltimore checkerspots have been observed ovipositing on English plantain or using it as a pre-diapause host, despite the ubiquity of plantain as a common lawn weed. Many traits of English plantain are known to differ among populations (Marshall et al. 2019), but there is relatively little spatial genetic structure in its introduced range (Smith et al. 2020). Given that the Baltimore checkerspot is a strict specialist and that English plantain has low population structure in its introduced range, it is credible that patterns of host use in the Baltimore checkerspot are driven solely by differences in insect preferences across its range.

To quantify the relative contributions of host plants versus insect preference to geographic variation in host plant use, we compared oviposition preference of butterflies from two regions for plants from each of the same two regions. We compared oviposition preference of female Baltimore checkerspots from Massachusetts, where English plantain and turtlehead are both used, with oviposition preference of checkerspots from Maryland, where there are no reports of Baltimore checkerspots butterflies ever ovipositing on plantain or feeding on it in the pre-diapause stage. In both regions, we offered English plantain from each region to gravid Baltimore checkerspot females to test whether they accepted each plant and attempted to oviposit. Specifically, we asked: (1) Do rates of oviposition acceptance of English

plantain differ between butterflies in different regions? And (2) Do rates of oviposition acceptance of English plantain differ between plants from different regions? We also used these results to quantify the extent of phenotypic variation among individual insects and host plants within regions. Our results provide strong evidence that geographic variation in both the herbivore and the host plant contributes to geographic variation in oviposition behavior, even in this highly specialized system.

Methods

Study system:

Baltimore checkerspots are a Nymphalid butterfly native to wetlands and wet meadows of Eastern North America. The Baltimore checkerspot is univoltine and lays eggs in clusters of several hundred in mid-summer. Caterpillars form silk nests after hatching and overwinter as fourth instar caterpillars, emerging in the subsequent spring (Bowers, 1978). Pre-diapause caterpillars are highly specialized in their diet, feeding solely on the host plant on which they emerged (Bowers et al. 1992b). In contrast, post-diapause caterpillars become less specialized and can venture long distances from their original nest location, especially if their original plant source has been depleted (Bowers et al. 1992b).

English plantain, *Plantago lanceolata*, is a short-lived perennial forb, native to Eurasia, but naturalized in many locations worldwide (Smith et al. 2020). It is common in urban lawns and frequently mowed old fields, and often occurs on the margins of wet meadows in which the Baltimore checkerspots' native host is found. English plantain is a native host of many European checkerspot butterflies and is commonly adopted as a host of North American checkerspot species (Stamp 1979). In the 1970s, Baltimore checkerspots in the northeastern US (Massachusetts, New York and Rhode Island) were frequently reported ovipositing on English plantain, using it as a primary pre-diapause food source (Stamp 1979, Bowers et al. 1992b). To our knowledge, this behavior is not present in populations south of New York.

English plantain is known to have considerable intraspecific variation and is an excellent candidate to explore how variation in plants may affect the species' interactions. Intraspecific differences of English plantain have been extensively studied in a phytochemical, structural, and population genetic context. English plantain has extensive within-population variation in defense-related traits such as the concentration and absolute amount of iridoid glycosides (Darrow & Bowers, 1997; Bowers, 1992a). Other traits, such as thermal tolerance, vary geographically across the native range of English plantain with corresponding genetic variation (Marshall et al. 2019). Populations in the native European range of English plantain also have strong spatial genetic structure associated with geographic distance and precipitation seasonality, although this variation tends to be lower in the nonnative ranges (Smith et al. 2020).

Experimental design

We evaluated oviposition preference of female checkerspot butterflies at one field site in Massachusetts and two in Maryland in the summer of 2018. Experiments were conducted in the field because Baltimore

checkerspots in Maryland have small populations that may be in decline (Frye et al. 2013), and we did not want to remove individuals from these sensitive populations. Each butterfly was given a unique, four-color identification code using metallic gel pens to ensure they were not tested twice.

Unlike many butterflies which lay eggs singly on host plants, Baltimore checkerspots lay egg clusters of up to hundreds of eggs. This trait introduces experimental challenges in terms of evaluating preference and willingness to use a host plant. Our experimental setup was based on Singer's study of oviposition preference in Edith's checkerspot *Euphydryas editha* (1982). Mated females were caught and placed on the native host plant, white turtlehead, and observed for three minutes to assess their willingness to oviposit (Fig. 1). Those that visually appeared to be mated and performed typical oviposition behavior, tapping the underside of a leaf using their forelimbs and curling their abdomen, were considered gravid and used for English plantain preference tests while those that did not were released. During preference trials, female butterflies were rotated among single English plantain plants from three sources (potted plants from Maryland, potted plants from Massachusetts, and naturally growing plants). For each trial, butterflies were placed on an English plantain plant within a net cage and monitored for up to three minutes to see if they began to exhibit oviposition behavior. If the female did begin to exhibit oviposition behavior on the plant, the butterfly was removed before laying any eggs. Between trials, butterflies were allowed to rest in a cage with no host plants for at least five minutes. The sequence of the individual plants that were used in trials was selected from a randomly generated list. Butterflies were released after completing five cycles of trials wherein three new randomly selected plants, one from each of the three sources, were used (Fig. 1). Butterflies were tested for a total of approximately 2.5 hours across all trials including time while they were at rest. On occasion, trials would not be fully completed due to weather or time constraints.

Local lineages of turtlehead plants were used in both Maryland and Massachusetts as a control to test for gravidity of females. Turtlehead plants were obtained from two nurseries, Chesapeake Natives in Maryland and Garden in the Woods in Massachusetts, which carry local genotypes. English plantain, *Plantago lanceolata*, was sourced from three sites in Massachusetts: Sharon (lat., lon. = 42.1, -71.2), Harvard (42.5, -71.6), and Upton (42.2, -71.7) and from three sites in Maryland: Alesia (39.7, -76.5), Norrisville (39.7, -76.5), and White Hall (39.7, -76.6). These plants were transplanted into pots early in the growing season and transported to the field populations in which oviposition trials took place. Potted plants were watered daily and were kept outside, exposed to natural sunlight. As an experimental control for effects of potting, English plantain growing naturally in the soil at each site was also included in oviposition trials; hereafter we refer to these experimental controls as "ground" plants.

Data analysis

All analyses were carried out in R (version 4.1.3, R Core Team 2022). Generalized linear models (GLMs) were implemented with the glm() function, and generalized linear mixed models (GLMMs) were implemented with the lme4::glmer function (Bates et al. 2015) For all models, we evaluated statistical significance of terms using type II marginal likelihood ratio tests, implemented with the car::Anova function (Fox and Weisberg 2019).

Butterfly response to any plant was recorded as either an acceptance (oviposition behavior), or rejection (no oviposition behavior). We tested the effects of geographic differences between plants and butterflies using a binomial family, logit link, GLMM with a fixed effect of butterfly state origin (MA or MD), plant state origin (MA or MD), and their interaction, as well as random effects of butterfly individual and plant individual. Butterfly individual and plant individual were included as random effects to account for individual variation since each butterfly was offered multiple plants, and potted plants were used in with different butterflies. In addition to statistically accounting for repeated measures of individuals, these random effects have an ecological interpretation; they are estimates of how much individuals vary within populations of each species (e.g., see Brown & Crone 2016). However, because including plant ID did not explain any of the variation in oviposition preference (see *Results*) and occasionally prevented model convergence, we excluded this term from models used for inference about geographic differences in host quality and oviposition preference.

We also compared rates of acceptance for our two procedural controls: (1) oviposition acceptance of turtlehead in Maryland versus Massachusetts trials (as a coarse measure of regional differences in preference for turtlehead), and (2) oviposition preference of potted versus ground English plantain plants (as an experimental control for effects of using potted plants). We compared rates of acceptance of turtlehead using simple binomial family, logit link GLMs, with region (Massachusetts versus Maryland) as a fixed effect. We compared acceptance of potted plants from each region to “ground” plants at each site (i.e., we compared potted Maryland plants tested in Maryland versus plants in the ground in Maryland, and potted Massachusetts plants tested in Massachusetts versus plants in the ground in Massachusetts). These comparisons used a binomial family, logit link, GLM with fixed effects of state (Maryland versus Massachusetts), plant type (potted or natural), and their interaction. Random effects of plant ID were removed due to convergence issues.

As a second metric of acceptance, we analyzed time until oviposition for butterflies willing to oviposit. This analysis included only trials in which butterflies demonstrated oviposition behavior on the non-native host, and therefore included only butterflies from Massachusetts (see *Results*). Time to oviposition was analyzed using a Gaussian (normal) GLMM with log-transformed time to oviposition behavior as the response variable, a fixed effect of plant state of origin as the predictor variable, and random effects of plant individual, butterfly individual, and plant site of origin.

Results

Overall, acceptance of English plantain differed significantly with butterfly state of origin ($\chi^2 = 9.4$, $df = 1$, $P = 0.002$) and plant state of origin ($\chi^2 = 7.0$, $df = 1$, $P = 0.008$; Fig. 2a). Gravid females from Massachusetts were more likely to accept English plantain (acceptance probability 0.3, CI = 0.12–0.62), than gravid females from Maryland (acceptance probability 0.002, CI = 0.0001-0.08). Gravid females from Massachusetts oviposited on Massachusetts plantain about half the time (acceptance probability 0.49, CI = 0.15–0.84) and on Maryland plantain about one fifth the time (acceptance probability 0.19, CI = 0.083-0.40), reflecting the main effects of plant state of origin. Only one of the nine gravid females from

Maryland attempted to oviposit on English plantain; out of ten trials on potted English plantain plants, this butterfly attempted to oviposit twice, once on a plant from each state. There was no significant interaction between butterfly and plant origin ($\chi^2 = 0.56$, $df = 1$, $P = 0.45$) on patterns of oviposition. Overall, random effects of butterfly identity explained substantial variation among female butterflies (standard deviation (SD) = 2.31) while random effects of plant identity did not explain substantial variation (SD < 0.001).

Time to oviposition was only analyzed for butterflies from Massachusetts (Fig. 2b), since only one butterfly from Maryland ever attempted to oviposit on English plantain. For trials where the butterfly did exhibit oviposition behavior, time until oviposition when placed on English plantain did not differ as a function of plant state of origin ($\chi^2 = 0.082$, $df = 1$, $P = 0.77$). As in the analyses of oviposition acceptance, oviposition time differed somewhat among individual butterflies (random effect SD = 33.88). Unlike the analysis of oviposition acceptance, there was also some difference among individual plants (random effect SD = 34.78).

Our experimental controls supported inference from these experiments. We evaluated 17 Baltimore checkerspot butterflies in Maryland and 20 Baltimore checkerspot butterflies in Massachusetts on the native host plant, turtlehead. In both states, nine of the butterflies exhibited oviposition behavior on turtlehead (Fig. 3a). The proportion of butterflies willing to oviposit on turtlehead did not differ between Maryland and Massachusetts ($\chi^2 = 0.23$, $df = 1$, $P = 0.63$; estimated probabilities of turtlehead acceptance in Maryland: 0.58, CI: 0.4–0.84; and Massachusetts: 0.45, CI: 0.2-0.638). In other words, there were no significant differences in oviposition acceptance of the native turtlehead between the two states.

In the comparison of potted plants versus plants in the ground in each state, butterflies had no significant preference for either potted or naturally growing English plantain ($\chi^2 = 1.4$, df = 1, $P = 0.24$) and there was no interaction between state and potting status ($\chi^2 < 0.001$, $df = 1$, $P = 1$). The nonsignificant effect was in the direction of slight preference for natural over ground plants (Fig. 3b). As in the main analysis of data from potted plants only, this analysis showed strong differences among states ($\chi^2 = 8.25$, $df = 1$, $P = 0.004$). The same seven butterflies that attempted to oviposit at least once on potted plants in Massachusetts also attempted to oviposit on plants in the ground. The butterfly from Maryland that did attempt to oviposit did so only on potted plants (one from Massachusetts and one from Maryland).

Discussion

The geographic disparity in the interaction between Baltimore checkerspots and English plantain reflects variation in both interacting species. Butterflies from Massachusetts were more likely than butterflies from Maryland to accept English plantain, and plants from Massachusetts were preferred over plants from Maryland. However, the patterns of regional variation differed qualitatively between both members of the interaction with butterfly origin having a larger effect than plant origin (See Fig. 1 and Results). The patterns found for the specialist Baltimore checkerspot broadly resemble geographic variation in the oligotrophic Edith's checkerspot (Singer, 1982) in two ways. First, populations of Edith's checkerspots

which did not use one host plant species in the field nearly always rejected it in oviposition trials (similar to Baltimore checkerspots and English plantain in Maryland). Second, another Edith's checkerspot population preferred plants from a site where they were used in the field over plants from sites where they were not used (similar to Baltimore checkerspots and English plantain in Massachusetts). Our results differ from populations of the Melissa blue, in which variation in oviposition preference for alfalfa (*Medicago sativa*) depended on both plant and butterfly population of origin but did not align with host use in the field (Harrison et al. 2016).

The significant effect of plant origin on oviposition acceptance and minimal effect of plant identity on oviposition acceptance suggest that plant quality is determined regionally. Given the limited genetic population structure observed in its introduced range (Smith et al. 2020), it is possible that plastic differences driven by climate or phenology drive the regional discrepancy in perceived plant quality. One possible explanation for geographic variation in host plant acceptance is climate-induced variation in phytochemistry. Baltimore checkerspot feeding is known to respond to variation in two iridoid glycosides: catalpol and aucubin (Bowers et al. 1992a). Both chemicals act as feeding stimulants, and Baltimore checkerspots sequester them as chemical defenses (Bowers et al. 1986). Baltimore checkerspots have higher concentrations of catalpol and almost no aucubin when reared on turtlehead after diapause and have higher concentrations of aucubin when reared on English plantain after diapause (Muchoney et al. 2022). In English plantain, both of these secondary chemicals are also known to vary with climate. For example, Darrow & Bowers (1997) reported an elevational gradient in catalpol concentrations of English plantain, with more catalpol in plants from higher-elevation sites. Orians et al. (2019) exposed English plantain plants to drought and heat treatments, with warmer, drier conditions resulting in more aucubin and less catalpol. From these studies, is tempting to speculate that English plantain from the cooler northern location, Massachusetts, have a higher concentration of catalpol, and chemical profiles more similar to turtlehead than English plantain plants from Maryland. Potential differences in phytochemistry and effects on oviposition preference between these regions merit further investigation.

More generally, English plantain has considerable within- and among-population genetic and plastic variation (Marshall et al. 2019; Smith et al. 2020). In English plantain, secondary chemistry has a genetic basis that can interact with environment to affect iridoid glycoside content (Bowers et al. 1992b). Concentrations of both catalpol and aucubin were higher in family lines collected from semi-natural field than a mowed lawn (Adler et al. 1995). Darrow and Bowers (1997) also reported lower aucubin and catalpol concentrations in English plantain from mowed sites, though they did not evaluate whether this difference was due to heritable variation. In our study regions, geographic variation in land management might mimic some of the differences between mowed lawns and more natural areas. Sites in Massachusetts were typically small wet meadows in natural areas managed for conservation and light recreation. Sites in Maryland were typically small meadows in the corners of farm fields that were too wet to farm. We used sites with different land use patterns because, to our knowledge, there were no natural areas managed for Baltimore checkerspots in our study region in Maryland that were occupied by Baltimore checkerspots at the time of our study.

Adoption of novel hosts has been observed multiple times in butterflies (Yoon & Read, 2016) and especially in the Meliteaini tribe to which checkerspots belong (Thomas et al. 1987; Singer & Wee, 2005). In some cases, novel host plants are ultimately preferred over native hosts. We did not explicitly test for geographic variation in preference for the native host plant, and our trials on English plantain included only butterflies who were willing to oviposit on the native turtlehead. In part, this design was because no previous study of Baltimore checkerspots specifically have revealed a preference for exotic English plantain over native turtlehead when turtlehead is available (Bowers, 1992b). However, some populations of Baltimore checkerspots in Massachusetts and adjacent states appear to exclusively use English plantain (Bowers and Richardson 2013). Towards the end of our study, there were three Massachusetts butterflies that did not accept turtlehead but were still offered English plantain. These butterflies were from a site at which turtlehead is rare and where we have only observed Baltimore checkerspot nests on English plantain. These butterflies were not included in our main analyses, but two of these three individuals demonstrated oviposition acceptance behavior when placed on English plantain. To further explore possible preference for English plantain, we conducted a *post hoc* analysis comparing time to acceptance of turtlehead versus English plantain, using female Massachusetts butterflies that were willing to oviposit on both hostplant species (Supplemental Fig. 1). In this analysis, time to oviposition acceptance did not differ between turtlehead and English plantain (post hoc GLMM of time to acceptance, with a main effect of plant species and random effect of butterfly ID: $\chi^2 = 0$, $df = 1$, $p = 0.99$). Nevertheless, it would be interesting to more explicitly test whether some northern populations of Baltimore checkerspot butterflies have evolved preference for English plantain over turtlehead.

Baltimore checkerspot populations seem to be declining in Maryland (Frye et al. 2013) but are stable in more northern parts of their range, including Massachusetts (Breed et al. 2013, Michielini et al. 2021). It is interesting to contemplate whether population viability would increase or decrease in Maryland if Baltimore c heckerspots were willing to oviposit on English plantain in Maryland. In Maryland, post-diapause larvae of Baltimore checkerspots prefer turtlehead and grow faster on turtlehead (Arriens et al. 2021). In addition, Abarca et al. (2019) reported that Baltimore checkerspot butterflies collected in Massachusetts were less likely to survive heatwaves if reared to diapause on English plantain sourced from Washington D.C. than if reared on turtlehead. It would be interesting to repeat Abarca et al.'s experiment using English plantain from both regions, given the differences in preference and potential chemical differences between English plantain from Maryland versus Massachusetts. Even if larval survival is lower on English plantain at some life stages, however, English plantain seems to be a suitable host plant for Baltimore checkerspots in Massachusetts in terms of population persistence (Brown et al. 2017). This may be because the spatial structure and arrangement of English plantain on the landscape supports substantially larger pre-diapause nest sizes that does turtlehead (Brown et al. 2017; L. M. Brown, *pers. obs.*). This difference is challenging to account for in laboratory-based studies, and the larger per nest number of individuals supported by English plantain in the field could counter lower survival at the individual level seen in the laboratory.

Studies comparing butterfly host plant preferences among host plant species often use plants sampled from a single source population (Wehling & Thomspon, 1997; Ladner & Altizer, 2005; Haan et al. 2021; McCarty & Sotka, 2013). In some cases, the results of these studies might change if there were substantial within-species variation among host plant populations. For example, Wehling & Thompson (1997) evaluated Anise swallowtail (*Papilio zelicaon*) preference by offering plants sourced from a single area to butterflies sourced from populations across their geographic range and found that rank order preference for hosts was largely conserved across different sites. If apparent host quality differed among populations at levels comparable to those we observed between plants from different regions, however, the rank order of Anise swallowtail hosts may not be as widely conserved. For example, a doubling of preference (similar to the difference in acceptance of English plantain between states by Massachusetts' Baltimore checkerspots) for a lesser-used plant *Foeniculum*, could have changed the rank order preference of some Anise swallowtail populations. Ladner & Altizer (2005) found similar results in monarch butterflies: both eastern and western monarchs (*Danaus plexippus*) preferred swamp milkweed (*Asclepias incarnata*) over other milkweed species. In this case, monarch butterflies from western and eastern North America both laid the majority of eggs on swamp milkweed when presented with an array of milkweed species (Ladner & Altizer, 2005); a doubling of preference for any other milkweed species would not change this rank order. In general, however, it would be useful to know more about how geographic variation in host plants affects butterfly oviposition preference.

Our results emphasize the importance of variation in both insects and host plants in determining intraspecific variation in host plant preference, even in a narrowly specialized species like the Baltimore checkerspot butterfly. In our system, these differences in oviposition acceptance of a non-native host plant appear to reflect different causal factors. High among-individual variation in butterflies is broadly consistent with a genetic basis for variation in preference among individuals. Heritable differences in host plant preference are common in other checkerspot species (Kuussaari et al. 2000, Singer et al. 1994), and early studies of Baltimore checkerspots (Brussard and Vawter 1975) suggested high within-population genetic diversity. Revisiting host plant preference with modern genomic methods could be an interesting next step in understanding this system. In contrast, low among-individual variation in plant quality, as measured by oviposition acceptance, seems more consistent with responses to regional climate differences, though there could of course be fixed genetic differences among regions. It is tempting to speculate that, if English plantain plants in Maryland were more appealing to Baltimore checkerspots, a host range expansion to this widespread weedy plant could help declining checkerspot populations persist in this part of their range. On the other hand, it may be that English plantain is a lower-quality host plant in Maryland than Massachusetts in terms of larval performance (e.g., Abarca et al. 2019), and a shift in preference could negatively impact population viability. In either case, understanding the future dynamics of this system will depend on understanding the basis of variation in both players in the interaction.

Declarations

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Consent for Publication: Not applicable

Availability of data and material: Should the manuscript be accepted, the data used for these analyses will be archived in a public repository and the data DOI will be included in the article

Code availability: Should the manuscript be accepted, the data used for these analyses will be archived in a public repository and the data DOI will be included in the article

Authors' contributions: EC, LB, CO and XY conceived of the study. LB, EC, SG and XY designed the study. XY and SG carried out the experiment and collected the data with minor assistance from LB, EC, and JM. JM, SG, and EC analyzed and interpreted the data. JM and EC led the draft writing, with LB and CO providing editorial advice. All authors have reviewed and approved the manuscript.

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Figures

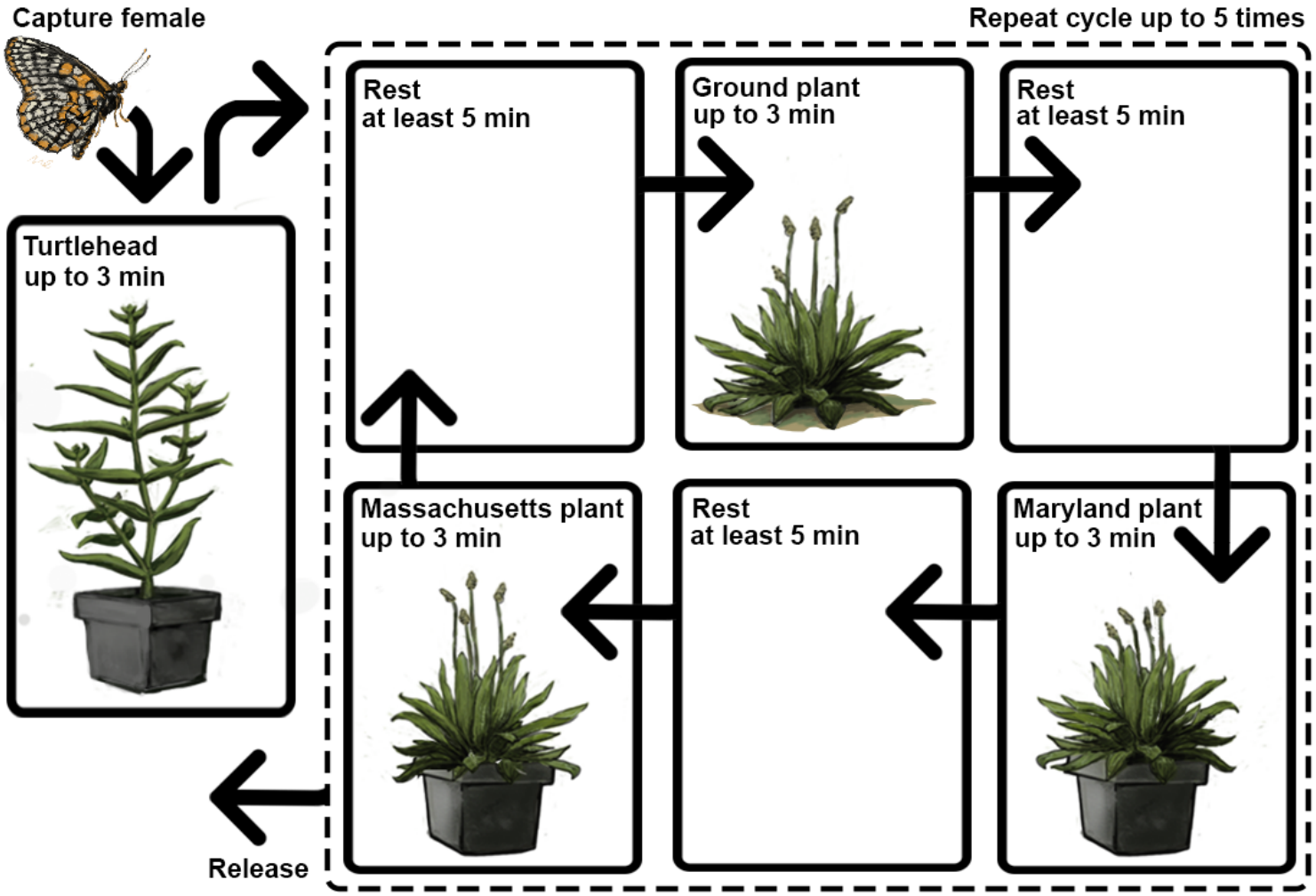

Figure 1

Experimental design to measuring Baltimore checkerspots host plant acceptance (adapted from Singer, 1982). Captured females were marked and placed in a tent on a turtlehead plant. If a female attempted to oviposit within the first five minutes, it was then used in trials with plantain. After resting for five minutes the female was then placed on a randomly selected English plantain and observed until they attempted to oviposit or until three minutes passed, which we considered a rejection of the plant. A single cycle of trials included three English plantain plants: one potted plant from Maryland, one potted plant from Massachusetts, and one “ground” plant growing naturally on site. Females were allowed to rest in between offerings with the plant at least five minutes. This cycle was repeated up to five times in total using randomly selected plants from different sites when offering potted plants, after which females were

released. Each female willing to oviposit on turtlehead was therefore tested on 15 different plantain plants altogether and females were not tested more than once.

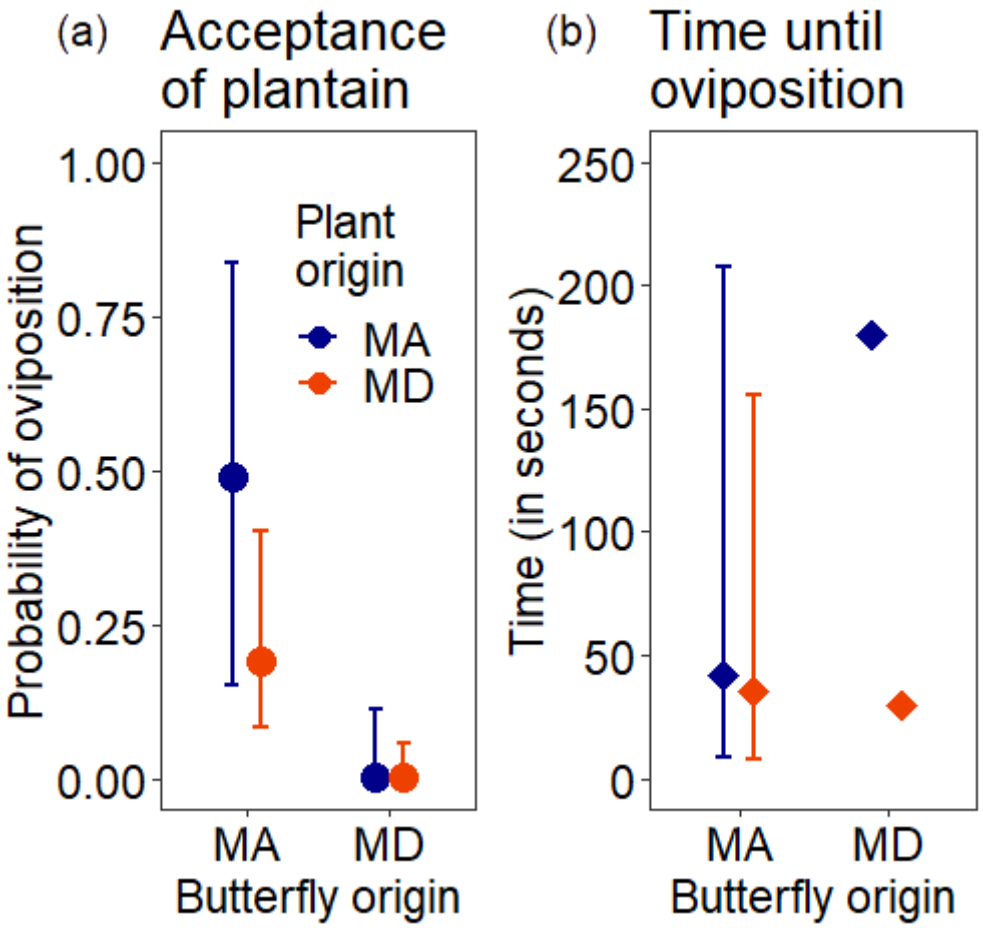

Figure 2

a. Baltimore checkerspot female estimated willingness to oviposit on English plantain given the state origin of both the butterfly and the plant (MA = Massachusetts, MD = Maryland), as measured by oviposition acceptance behavior. Error bars represent 95% confidence intervals. N = 248 across 18 butterflies. b. Estimated time (in seconds) until female Baltimore checkerspots began to exhibit oviposition behavior on plantain for Massachusetts butterflies. Error bars represent 95% confidence intervals. N = 40 across 9 butterflies. Single points and no confidence intervals given for the single Maryland butterfly which accepted one plantain from Massachusetts and another from Maryland.

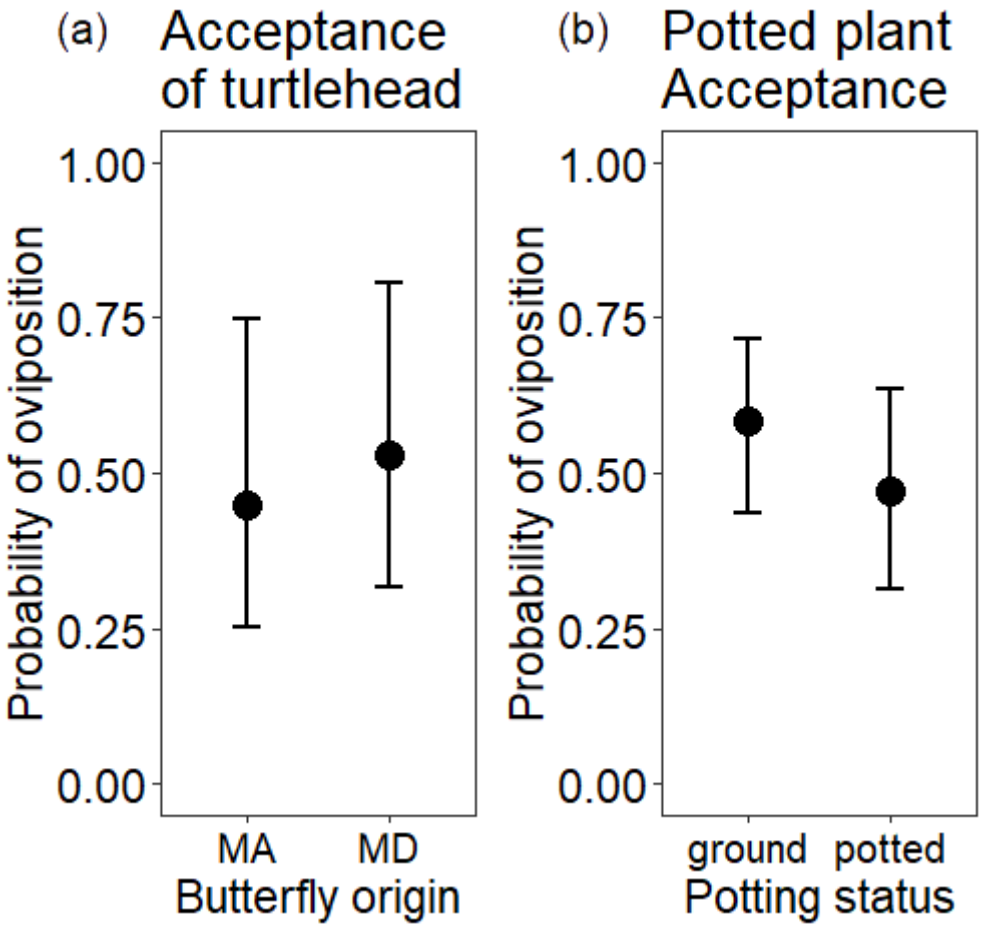

Figure 3

a. Baltimore checkerspot female estimated willingness to oviposit on white turtlehead given the butterfly state of origin in preliminary control trials for gravidity. Error bars show 95% confidence intervals. N = 37 (20 butterflies in Massachusetts and 17 butterflies in Maryland). **b.** Baltimore checkerspot female estimated willingness to oviposit on English plantain if potted in control trials. Points represent the probability a female would accept the plant if it were potted or growing naturally in soil at the site where the trail was conducted and where the female was captured. Error bars show 95% confidence intervals. N = 159 across 18 butterflies.

Supplementary Files

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