

Behavioral consequences of exotic host plant adoption and the differing roles of male harassment on female movement in two checkerspot butterflies

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Abstract Herbivorous insects may evolve convergent behaviors when independent adoption of a shared novel host plant places populations in environmental conditions that diverge from the ancestral state. We investigated the behavioral consequences of adopting *Plantago lanceolata*, an exotic species to North America, in populations of *Euphydryas p. phaeton* and *Euphydryas editha taylori*, on the east and west coasts of North America. Activity budgets and short-term movements suggested that innate species tendencies exert a greater impact on behavior than adoption of the same exotic host plant. However, female *E. phaeton* from a *Plantago*-dependent population spent more time inspecting host plants than females from a population dependent on the native host plant, *Chelone glabra*. Both checkerspot species had similar diffusion coefficients (D) regardless of host plant association. But, *E. e. taylori* step-lengths appeared bimodal and power law distributed while *E. phaeton* step-lengths were unimodal and best fit by an exponential distribution. We attribute this bimodal step-

length distribution to the persistent harassment of courting males which induces a “long-distance” evasive flight in otherwise sedentary *E. e. taylori* females. In contrast, the longer distance step-lengths of *E. phaeton* were associated with the inspection of oviposition plants. These two checkerspot species appear to move greater distances across the reproductive landscape with contrasting motivational states that could lead to either intimate mapping (*E. phaeton*) or coarse mapping (*E. e. taylori*) of host resources.

Keywords Parallel evolution · Host-associated behaviors · Behavioral convergence · Male harassment · Step-length distribution · Checkerspot butterflies

Introduction

Evolution of convergent traits among independent lineages living in shared and similar environments demonstrates how strong environmental pressures can repeatedly select for the same adaptive traits (e.g. Nosil et al. 2002; McBride and Singer 2010; Elmer and Meyer 2011; Andrew et al. 2013; Foster 2013). Studies of parallel and convergent evolution often present the shared changes in morphological traits such as the loss of pigment and eyes when organisms colonize caves (Protas et al. 2006; Gross et al. 2009), ecological niche associated body size and color patterns (Rüber and Adams 2001; Nosil et al. 2002; Boughman et al. 2005; Østbye et al. 2006) and even recurrent species formation (Schwarzbach and Rieseberg 2002). However, behavioral traits may also evolve and converge as a consequence of independent lineages experiencing similar selective environments. For example, divergent *Tetragnatha* spiders on the Hawaiian Islands evolved several types of convergent web construction behaviors (Blackledge and Gillespie 2004), Caribbean *Anolis* lizards independently evolved similar territory sizes and display

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behaviors in association with habitat type (Johnson et al. 2009) and multiple lineages of Mexican cavefish (*Astyanax mexicanus*) have repeatedly lost their ancestral aggressive behavior and gained a new cave-adapted foraging strategy through a conserved serotonin-neurological pathway (Elipot et al. 2013).

Similarly, behavioral convergence could be expressed by native herbivores as an outcome of new interactions to introduced plants. Host switches occur regularly among herbivorous insects (Thompson 1988; Singer and Wee 2005; Fordyce 2010; Jahner et al. 2011) and can constitute as much an environmental conversion as a biochemical one, potentially leading to divergent, host-associated traits and behaviors (Ehrlich and Raven 1964; Wahlberg 2001; Nosil et al. 2002; Feder et al. 2003). Checkerspot butterflies, *Euphydryas* spp. and *Melitaea* spp., have often been employed as model systems for understanding population processes, including host plant switches (Hanski and Singer 2001; Ehrlich and Hanski 2004; Singer and McBride 2012). These butterflies display strong behavioral responses as a consequence of host plant preference evolution. Female checkerspots in northern Europe (*Melitaea cinxia*) and the western US (*Euphydryas editha*) express population-specific oviposition ranking preference for different host plant species (Singer 1982; Kuussaari et al. 2000). Due to this ranking behavior, gravid *E. editha* and *M. cinxia* tend to disperse more readily from habitat patches with lower ranked host plant species than they do from habitat with the preferred host plant (Thomas and Singer 1987; Hanski et al. 2002). For *M. cinxia*, colonization-extinction dynamics are partly influenced by between subpopulation variation in host plant preference (Hanski and Singer 2001). Because checkerspot metapopulation dynamics are strongly tied with both host plant association and oviposition preference, other checkerspots may independently evolve diversifying (with respect to the ancestral state) but convergent behaviors as a consequence of adopting the same novel introduced host plant.

Populations of two North American checkerspot species, *Euphydryas p. phaeton* and *Euphydryas editha taylori*, provide an opportunity to investigate the behavioral consequences of adopting a novel host plant. *E. p. phaeton* is hypothesized to be derived from *E. editha* (Wahlberg 2001; Wahlberg et al. 2005). Through a shared ancestry, these checkerspots should have similar developmental pathways (e.g., canalization; Waddington 1942; Siegal and Bergman 2002; Elipot et al. 2013) that constrain host acquisition to plants with coevolved iridoid glycoside secondary metabolites (Wahlberg 2001), including *P. lanceolata* (Bowers and Stamp 1992). Indeed, populations of *E. phaeton phaeton* and *E. editha taylori*, each on opposite sides of North America, have recently adopted the host plant *Plantago lanceolata* Plantaginaceae (exotic to North America) approximately 40 and 125 years ago, respectively (Danby 1890; Stamp 1979).

Both checkerspots live in grasslands and females are conspicuously sedentary with a labored flight, a characteristic that sharply contrasts with other North American checkerspots (even other subspecies of *E. editha* Scott 1986; Severns pers. obs.). Plant architecture differs between *P. lanceolata* and the ancestral food plants for *E. e. taylori* (*Castilleja hispida* Orobanchaceae) and *E. p. phaeton* (*Chelone glabra* Plantaginaceae), resulting in a marked increase in both inspection and handling times (Breed and Severns unpublished data). Furthermore, *P. lanceolata* is more broadly and abundantly distributed across grass-dominated habitats than either ancestral food plant (*Castilleja* spp. are hemiparasitic and *C. glabra* is wetland restricted) so the frequency with which females encounter *P. lanceolata* is likely to be both greater and more predictable than the ancestral food plant, potentially impacting the landscape movements of gravid females. Although, populations of *E. p. phaeton* and *E. e. taylori* have moved beyond their ancestral niche by adopting *P. lanceolata*, behavioral convergence may have arisen as a consequence of recently adopting the same exotic host plant. We investigated the possibility of convergent behavior by comparing the movements and activity budgets of individuals from *Plantago*-dependent populations of *E. p. phaeton* (Massachusetts, USA), *E. e. taylori* (Oregon, USA) and a population of *E. p. phaeton* (Massachusetts, USA) dependent on its native host plant (*C. glabra*).

Methods

Study species and populations

P. lanceolata is native to Europe and was likely introduced to North America during early European settlement. The earliest documentation of *P. lanceolata* in North America was approximately 200 years ago (Cavers et al. 1980) but it may have been introduced and established as early as the seventeenth century (Wolff and Schaal 2002). *E. e. taylori*, near Victoria, British Columbia, Canada was recorded using *P. lanceolata* as a larval host plant about 120 years ago (120 butterfly generations) (Danby 1890) while *E. p. phaeton* likely adopted *P. lanceolata* more recently, approximately 40 years ago in a New York, US population (Stamp 1979). *P. lanceolata* is widespread throughout North America and is abundant in a broad range of disturbed and semidisturbed landscapes such as roadsides, pastures, upland meadows, the edges of wet meadows and other grass-dominated habitats (Cavers et al. 1980). Compared with *C. glabra*, a patchily distributed wetland species, *P. lanceolata* occurs over a considerably broader range of ecological conditions.

E. p. phaeton (hereafter *E. phaeton*) is typically found in small colonies (several to several hundred adults), flying from late June through July in wet meadows across a broad swath of

temperate mesic eastern North America (Scott 1986; Scholtens 1991; Bowers et al. 1992; Layberry et al. 1998). Females lay 1 to 3 clutches of several hundred eggs on the underside of leaves of *C. glabra* or *P. lanceolata*. Generally, *C. glabra* occurs in areas with one to several centimeters of standing water while *P. lanceolata* grows abundantly in drier graminoid dominated conditions. Populations of *P. lanceolata*-dependent *E. phaeton* oviposit on plants growing in conditions that are divergent from the ancestral state. However, life history and development remains similar. First instar larvae hatch 3 weeks after the eggs are laid in early to mid July. Pre-diapause larvae feed gregariously in silken feeding nests until late August when larval groups construct temporary hibernacula from leaves and silk and overwinter as second or third instar larvae (Stamp 1982a, b). Larvae break diapause the following April or May, feeding alone or in small aggregations until pupation in June (Stamp 1982a; Breed and Severns pers. obs.). *E. phaeton* is common in some parts of its range, but is locally rare and protected in several US states (Durkin 2009).

E. editha taylori is an endangered species endemic to a narrow region of western North America, and is found in dry, south- and west-facing meadows and forest openings from Denman Island, British Columbia, Canada south to Corvallis, OR, USA (Page 2007; Severns and Grosboll 2011). Historically, *E. e. taylori* appeared to use the native *Castilleja hispida* as a primary pre-diapause hostplant although one recently extirpated and two extant populations existed on annual host genera (*Plectritis* now extinct, *Veronica* spp. extant). Approximately half of the extant *E. e. taylori* populations are now dependent on *P. lanceolata* (Severns and Warren 2008; Severns unpublished data). All surviving Oregon populations and two of the four larger surviving Washington populations use *P. lanceolata* as the primary host plant (Severns and Grosboll 2011). Oviposition on *P. lanceolata* occurs on plants that grow in conditions where the surrounding vegetation does not reduce host plant apparency. These conditions include fields with low sward height, sandy soils, and gravel/dirt road margins (Severns and Warren 2008, Severns and Grosboll 2011, Severns, unpublished data). Adults fly in Oregon from late March to early May (up to 2 months later in Washington). Over their reproductive life span, female *E. e. taylori* oviposit multiple clusters of eggs (~20–100/clutch) on the underside of *C. hispida* or *P. lanceolata* leaves and are largely confined to regions of reproductive habitat (Bennett et al 2013, Severns and Breed, unpublished data). Pre-diapause larvae feed gregariously from silken shelters until they enter diapause in June (Severns and Grosboll 2011). Post-diapause larvae begin feeding in early/mid January and are solitary.

In early July of 2011, behavioral observations were made in two Massachusetts *E. phaeton* populations, both actively managed for biodiversity conservation by the Trustees of the

Reservations. The Bullitt Reservation population (*Plantago*-dependent), in Ashfield, MA, is a 4.8-ha old-field and has been annually hayed in the fall for the last several decades. This field is largely upland with *P. lanceolata* of varying density throughout. There are several small (0.10 ha) areas with wet meadow habitat and a seasonal stream along which there is a 10-m segment, containing ~10 *C. glabra* plants. These *C. glabra* plants are used by *E. phaeton*, but >99% of the oviposition occurs on the hundreds of thousands of often densely distributed (>100 plants/m²) *P. lanceolata*. From 2010–2012, this *E. phaeton* population was well in excess of 1,000 adults during peak flight.

The other *E. phaeton* study population, Stevens-Coolidge Place (*Chelone*-dependent), North Andover, MA, (~ 150 km east of the Bullitt Reservation) is a 0.9 ha sedge dominated wet meadow that supports a large population (thousands) of *C. glabra* which occur throughout the field. A small group of ~10 *P. lanceolata* plants grow in the dry periphery but oviposition has never been observed on *P. lanceolata* at this site. Over the last several years, Stevens-Coolidge Place contained between 100 and 300 adults at peak flight.

We selected a *Plantago*-dependent population of *E. e. taylori*, ~ 5 km west of Corvallis, OR (Cardwell Hills) for study (May 2012). Most of this population exists on privately owned lands and butterfly reproductive habitat (areas suitable for oviposition sensu Severns and Warren 2008) consists of three fields bounded by young Douglas fir-forest. We were granted permission to conduct research in two of the three fields where most of the reproducing butterflies are found. One field is ~0.5 ha and the other is ~8.6 ha, both are south- to west-facing dry meadows dominated by short statured native and exotic grasses interspersed with conspicuous clusters and loosely aggregated *P. lanceolata* throughout the study area (Severns and Warren 2008). Annual butterfly counts over the last decade indicate that both fields each support at least 40 and up to several hundred adult butterflies (Dana Ross, Oregon State Arthropod Collection, pers. com.).

Butterfly behavior and movement

We followed opportunistically encountered individuals, recording GPS locations (Garmin eTrex Venture HC) every 15 s for 15 min for *E. phaeton* and every 20 s for 20 min for *E. e. taylori*, to quantify short-term movements in all three study populations. Concerns of detrimental site impacts to *E. e. taylori* habitat from the tracking study resulted in access being granted to only one observer. This 20-s time interval was necessary for the observer to track and record the pertinent movement data. At each timestep, we also made focal behavioral observations (behaviors recorded included flying, perching, basking, courtship, inspecting host plants for oviposition, nectaring, oviposition, and mating) to construct individual activity budgets. Perching was recorded when

individuals were at rest with their wings closed over their thorax, minimizing the exposure of wings to the sun (no lateral basking). Basking occurred when individuals were at rest with their open wings exposed to the sun (dorsal basking). We considered wing exercising equivalent to basking because both activities increase internal thoracic temperatures needed for flight and egg maturation (Berwaerts and Van Dyck 2004; Berger et al. 2008). We recorded host plant inspection when butterflies landed on a potential host plant, walked and dragged the tip of their curled abdomen across leaf and stem surfaces (e.g., Singer and Lee 2001). Courtship occurred when a male interacted with a female in an obvious attempt to mate, which distinctly differs from male-male antagonistic reactions (see Scott 1986, pp. 46–59). We also recorded whether females were receptive to male courtship by noting whether females rapidly fluttered their wings and curled their abdomen away from courting males, the characteristic rebuffing behavior exhibited by female checkerspots (Odendaal et al. 1989). Nectaring was recorded when butterflies were observed to actively probe or insert their proboscis into an open flower. Hiding occurred when individuals crawled beneath vegetation and remained in a perched or lateral position immediately after or during courtship attempts. The behaviors of flying, mating, and oviposition are self-explanatory.

Most of the individuals we followed were previously uniquely marked with metallic ink (Severns 2009) as part of another study. In many cases, we were able to follow individuals for the entire prescribed observation interval of 15 or 20 min but occasionally individuals could not be followed or were otherwise lost, so some tracks were shorter than others. Flight periods of both species are short, usually only several hours each day, and inclement weather can abruptly end an observation period. We implemented rules for aborting behavioral observations in the case of inclement weather or if individuals entered a protracted bout of resting during observation. In these instances, after 5 min of inactivity we terminated the observation and a new individual was selected. Observations were made when weather conditions were ideal for butterfly flight, always under full or near full sun, between 1000 and 1700 hours.

Activity budget analysis

We first used Non-Metric Multi-dimensional Scaling (NMDS, MDS, NMS), a statistically and biologically robust ordination analysis (Clarke 1993), to examine activity budget relationships between populations of *E. phaeon* and *E. e. taylora*. To determine whether populations of *E. phaeon* and *E. e. taylora* had activity budgets that differed from each other we used Multi-Response Permutation Procedure (MRPP) (Mielke and Berry 2001). MRPP is a non-parametric test that assesses whether groups differ from each other by taking all variables into account simultaneously with a distance matrix. The

results of MRPP are a probability value (p), test statistic (T) and chance corrected statistic (A) which describes group similarity. An A value of 1 indicates that all groups are identical and groups become increasingly different as A approaches 0. For NMDS and MRPP (1,000 randomizations for each test) we used the Sørensen (Bray-Curtis) distance in the program PC-ORD (McCune and Mefford 2006) to execute the analyses. No transformations were applied to the activity budget data as the coefficients of variation for both matrix rows and columns were not sufficiently improved by transformations. To determine whether the proportion of time spent flying, nectaring, basking, perching, and courting (the common observed behaviors) differed between populations, we performed an ANOVA with rank-transformed data. Pairwise differences between populations were assessed for statistical significance with a Z test.

Step length and movement analysis

Before analyzing step-lengths, we checked the data quality of the consumer grade GPS unit (Garmin eTrex Venture HC). During data collection at the two *E. phaeon* populations, a GPS location was taken at each 15 s interval regardless of whether the butterfly moved. However, as we independently noted the butterfly's behavior and whether the butterfly had actually moved, we could sort the steps represented by each consecutive GPS location into those representing movement and those representing only GPS error. These data allowed us to compare all the step-lengths in a butterfly pathway where butterflies moved (true steps) to the step-lengths where there was no movement (null steps). We used these errors to estimate the signal-to-noise ratio in the movement pathways we collected with the consumer grade GPS. We found that with respect to the scale of real butterfly step-lengths, the GPS error was very small, with the average null step-length being 0.32 m and a median null step-length of 0.08 m. As the mean step-lengths at all sites were on the order of 3 m, the signal-to-noise ratio in these data was very favorable, on the order to 10:1 if comparing the mean null step to the mean step-length and closer to 40:1 if comparing the median null step to the median step-length.

After determining that the GPS data we had collected was high enough quality for the scale of measured step-lengths, movement pathways were first analyzed by estimating diffusion coefficients, D (Turchin 1998), for each population. The diffusion coefficient is a value indexing the dispersal distance of an animal given the recorded step-lengths and observation time. A larger D value implies more relatively longer distance moves and greater landscape displacement whereas small D values suggest the opposite. Second, we analyzed the step-lengths to determine if they were drawn from the same probability distributions using the maximum likelihood methods described in Edwards et al. (2012), comparing the

exponential, bounded exponential, power law, and bounded power law distributions.

Finally, we fit a series of mixed-effects models to test a series of hypotheses about how movement patterns and step-lengths differ among our three study populations. Mixed-effects models were fit and differences tested using the *nlme* and *lme4* packages in R. These mixed-effects models were primarily used to address the nested random effects of site and the individual so that sample size and effect significance could be appropriately accounted for and estimated. Because step-lengths are positive-real values, models were fit with a log-link. The models took the general form:

$$\mathbf{L}_{i,j} \sim \beta_1 \theta_1 + \beta_2 \theta_2 + \dots + \beta_n \theta_n + \varphi_1(\text{site}_j) + \varphi_2(\text{ind}_{i,j}) \quad (1)$$

where $\mathbf{L}_{i,j}$ is the natural-logged set of step-lengths from the i th individual at the j th study site. $\beta_{1..n}$ is the set of estimated fixed-effects parameters predicting how the variables $\theta_{1..n}$, which include species and the observed behavior at the time the step was made, affect step-length. $\varphi_{1..2}$ is the set of estimated random effects of individual nested within study site.

A mixed-effects model was also used to determine if step-length variances differed among sites, which was accomplished by substituting $\mathbf{L}_{i,j}$ (the step-lengths themselves) with $r_{i,j}$, the step-length residual from the mean step-length. $r_{i,j} = \log(\text{abs}(x_{(i,j)} - \bar{x}_j))$, where $r_{i,j}$ is the natural-logged set of step-length residuals from the i th individual at the j th site, $x_{(ij)}$ is set of steps from the i th individual at the j th study site, and \bar{x}_j is the mean step-length at the j th study site.

Results

Activity budgets

We followed a total of 40 *E. e. taylori*, 25 *Plantago*-dependent and 20 *Chelone*-dependent *E. phaeton* gravid females. Individuals in the NMDS ordination largely grouped in clusters of points according to species with some minimal separation between individuals from the *Plantago*-dependent and *Chelone*-dependent *E. phaeton* populations (Fig. 1). The centroids for perching and basking occurred in the ordination space where individuals of both checkerspot species frequently occurred. However, *E. e. taylori* nectared and courted more often than *E. phaeton* while *E. phaeton* flew and inspected host plants more frequently than *E. e. taylori* (Fig. 1). MRPP indicated that *E. e. taylori* activity budgets strongly differed from the *Plantago*- and *Chelone*-dependent *E. phaeton* populations (MRPP results: *E. e. taylori* vs *Plantago*-dependent *E. phaeton*, $p=0.0000003$, $T=-11.0$, $A=0.076$; *E. e. taylori* vs *Chelone*-dependent *E. phaeton*, $p=0.00014$, $T=-6.59$, $A=0.049$) and the activity budgets of *Plantago*- and *Chelone*-

dependent *E. phaeton* did not form statistically distinct groups (MRPP results: $p=0.13$, $T=-1.04$, $A=0.012$). ANOVA indicated that populations did not statistically differ for perching and basking frequency but all three populations statistically differed for the proportion of time spent inspecting host plants (Table 1). Consistent with the NMDS ordination, *E. e. taylori* differed from the two *E. phaeton* populations in courtship, flying, and nectaring in ANOVA analyses (Table 1).

Movements and step-lengths

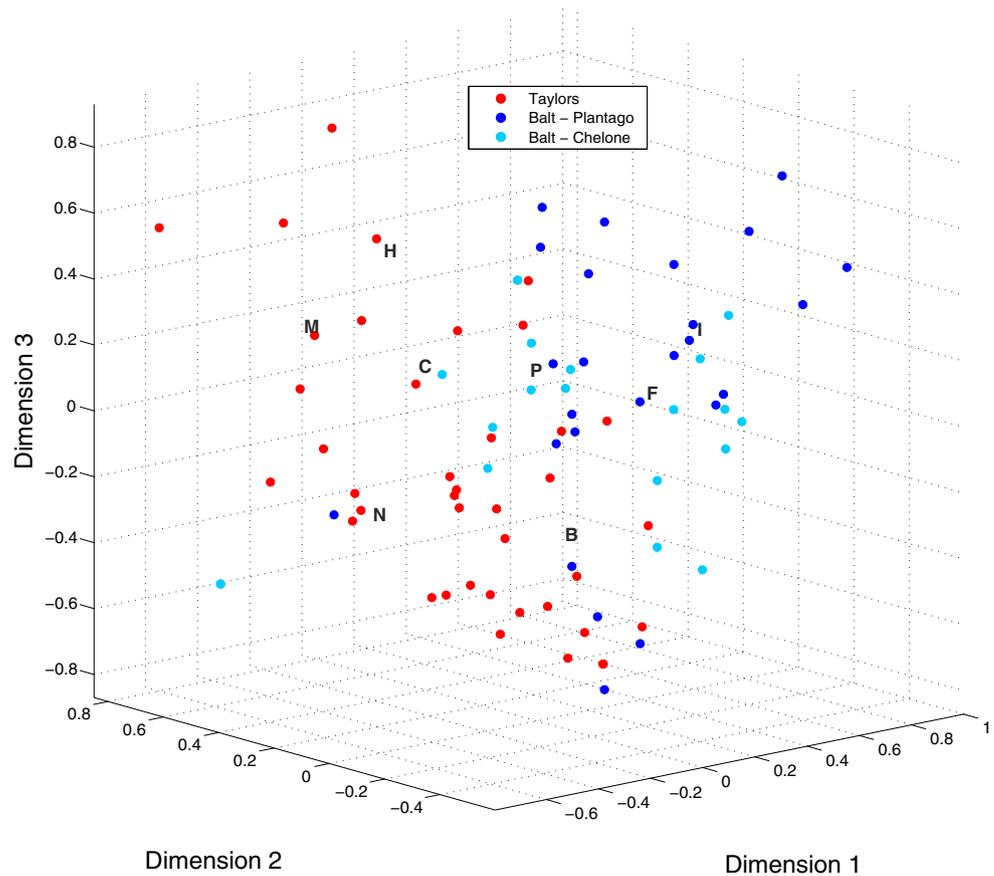
Mean step-lengths at all three study sites were between 2.5 and 3.5 m. Although *E. e. taylori* had the longest mean step-lengths, differences were not statistically significant between populations ($p=0.078$; Table 2). The longer mean step-length in *E. e. taylori* could be due to the 33 % longer duration between fixes in the focal observation periods. In fact, the mean step-length of the *Plantago*-dependent *E. e. taylori* was 33 % longer than the *Chelone*-dependent *E. phaeton* population and 19% longer than the *Plantago*-dependent *E. phaeton* population. However, differences between the step-length medians and means were large in *E. e. taylori* but small in *E. phaeton*, suggesting that the observed differences were due to behavior and not to the minor time difference in the observation intervals.

The median step-length for *E. e. taylori* was 0.72 m, which was much shorter than the mean (3.48 m), while median step-lengths for *E. phaeton* were much closer to their means; 1.59 and 1.70 m for the *Chelone*- and *Plantago*-dependent populations respectively (Table 2). Among all three populations, the *E. e. taylori* population had significantly larger residuals ($p=0.0009$, Table 2) and greater step-length variance than either *E. phaeton* population.

For *E. e. taylori*, the smaller medians and greater variance, but statistically equivalent mean step-lengths, indicate that females make many very short moves with infrequent, more expansive steps compared with *E. phaeton*. *E. phaeton* had step-lengths that were unimodally distributed while *E. e. taylori* step-lengths were long-tailed and appeared to be bimodally distributed (Fig. 2). Maximum likelihood fits to the exponential, bounded exponential, power law, and bounded power law distributions, indicated that *E. e. taylori* step-lengths were strongly favored to be drawn from a power law distribution (Akaike weight=1.000, next best distribution= 1.75×10^{-17}) while both *E. phaeton* produced step-lengths that were best fit by exponential distributions (Akaike weight=1.000 for both populations).

Diffusion coefficients (D) were similar for females in all three populations; *Plantago*-dependent *E. e. taylori*= $0.107 \text{ m}^2 \text{ s}^{-1}$ (CI: 0.054–0.188), *Chelone*-dependent *E. phaeton*= $0.133 \text{ m}^2 \text{ s}^{-1}$ (CI: 0.050 – 0.262), *Plantago*-dependent *E. phaeton*= $0.127 \text{ m}^2 \text{ s}^{-1}$ (CI=0.048 – 0.243). Both *E. phaeton* populations had slightly higher D values than

Fig. 1 Three-dimensional NMDS ordination of *Plantago*-dependent *E. e. taylori*, *Plantago*-dependent *E. phaeton*, and *Chelone*-dependent *E. phaeton* behavioral time budgets. Centroids for butterfly behaviors *H* hiding, *M* mating, *N* nectaring, *C* courting, *P* perching, *B* basking, *F* Flying, *I* Inspecting host plants. NMDS results: 1,000 randomizations, 500 real runs, Sørensen (Bray-Curtis) distance, final ordination stress=9.04, all three axes cumulatively explained 74.7 % of the variation in the 3-D ordination



E. e. taylori, but the 95 % CI overlapped extensively in all populations. The apparent power law step-length distribution in *E. e. taylori* was due, at least in part, to the high rate of male courtship. Mixed-effects models indicated that the proportion of time *E. e. taylori* females were courted during observation was significantly related to step-length ($p=0.031$) and elevated courtship rates were more likely to induce extremely long moves (>20 m) when females attempted to escape harassing males. Such escape attempts were only successful about 30 % of the time as females were again courted by males after making the relatively long evasive flights. When these escape

moves were mixed with the many short steps, normally made by females searching for ovipositing sites and nectar resources, an apparent power law step-length distribution was produced.

By contrast, male *E. phaeton* only occasionally courted gravid females. These courting attempts were much less frequent in *E. phaeton* (1.6 % of time budgets were courting/male harassment in *E. phaeton* vs. 13.7 % in *E. e. taylori*) and rarely escalated to harassment as males typically abandoned mating attempts within several seconds after being rebuffed by females. No other behaviors included in the activity

Table 1 Median activity budget values (with 25th and 75th quartiles) and ANOVA (rank-transformed data) results for *E. e. taylori*, *Plantago*-dependent and *Chelone*-dependent *E. phaeton*

Behavior	<i>Plantago</i> -dependent <i>Euphydryas e. taylori</i> (%) ($n=40$ females)	<i>Plantago</i> -dependent <i>E. phaeton</i> ($n=25$ females)	<i>Chelone</i> -dependent <i>E. phaeton</i> ($n=20$ females)	ANOVA
Perching	6.7 (0, 25.1)a	11.7 (3.3, 27.8)a	11.6 (0, 68.5)a	$\chi=0.67; p=0.72$
Basking	33.3 (9.3, 53.2)a	17 (6.5, 32.5)a	18 (0, 68.5)a	$\chi=5.04; p=0.08$
Flying	3.1 (0, 6.8)a	18.3 (1.7, 25.0)b	10.3 (0, 33.5)b	$\chi=7.95; p=0.019$
Courtship	5.5 (0, 14.3)a	0 (0, 3.1)b	0 (0, 3.1)b	$\chi=15.2; p=0.0005$
Nectaring	9.1 (0, 28.9)a	0 (0, 8.9)b	0 (0, 0)b	$\chi=23.7; p=0.000007$
Host plant inspection	0 (0, 0)a	21.9 (3.3, 41.0)b	0 (0, 25.6)c	$\chi=24.9; p=0.000004$

Lower case letters indicate statistically significant group differences from pairwise Z tests

Table 2 Summary of step-length means, medians, and variance. In spite of different observation periods, mean step-lengths did not differ ($p=0.078$). However, the *E. e. taylori* median step-length is smaller than

E. phaeton, while the variances are greater. Differences between the residuals for fitted models indicate that step-length variance is highly statistically significant between species ($p=0.0009$)

Species (observation freq)	Step-length (meters)			D —diffusion coeff (95% CI) ($\text{m}^2 \text{s}^{-1}$)
	Mean	Median	Variance	
<i>E. phaeton</i> <i>Chelone</i> -dependent (15 s)	2.59	1.58	8.2	0.133 (0.050–0.262)
<i>E. phaeton</i> <i>Plantago</i> -dependent (15 s)	2.92	1.70	14.6	0.127 (0.048–0.243)
<i>E. e. taylori</i> (20 s)	3.48	0.72	30.5	0.107 (0.054–0.188)

budgets significantly increased or decreased step-length in either *E. e. taylori* or *E. phaeton*.

Discussion

Behavioral convergence in stick insects, spiders, lizards, and cavefishes, appears to have arisen through the occupancy of similarly new ecological positions outside of the ancestral state (Nosil et al. 2002; Blackledge and Gillespie 2004; Johnson et al. 2009; Elipot et al. 2013). Through the recent adoption of *P. lanceolata*, the potential for *E. e. taylori* and *E. phaeton* to independently evolve convergent host-associated behaviors seems plausible given that *E. phaeton* is hypothesized to be phylogenetically derived from *E. editha* (Wahlberg et al. 2005), both species likely share an iridoid glycoside pathway for host plant adoption (Wahlberg 2001), and that *P. lanceolata* tends to grow in a wider range of

habitats than either species' native host plant, placing adults and larvae in potentially divergent ecological conditions. By adopting *P. lanceolata*, *E. phaeton* populations are shifted to drier grassland habitats that are similar to the grassland conditions occupied by *E. e. taylori*, suggesting the potential for convergent evolution due to both habitat conditions and shared exotic host plant. However, we found little evidence for convergent behavioral evolution in the patterns of short-term movements and activity budgets. Our study instead suggests that innate species tendencies exert a greater influence on activity budgets and short-term movements than the recent independent adoption of the same novel, exotic host plant.

Ordination of individual activity budgets produced species groups rather than host plant associated groupings; i.e., *Plantago*-dependent *E. phaeton* grouped more closely with *Chelone*-dependent *E. phaeton* than they did with *Plantago*-dependent *E. e. taylori* (Fig. 1). These species groups were

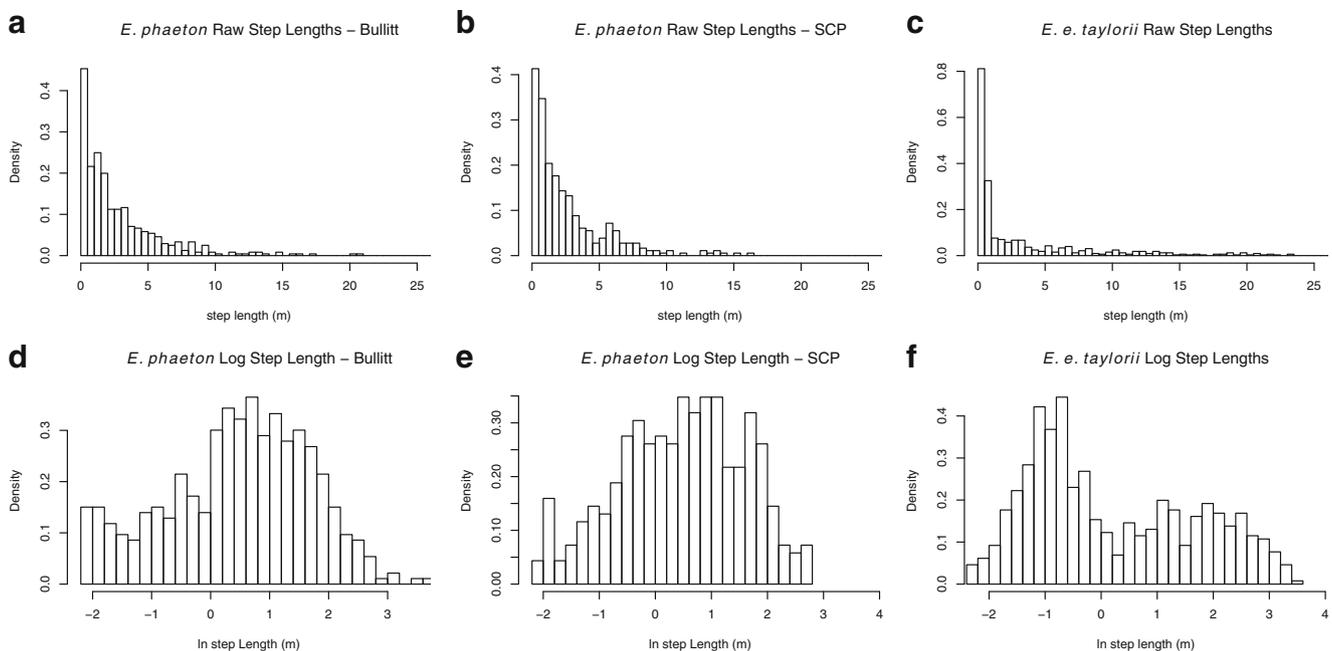


Fig. 2 Step-length (a–c) and natural-logged step-length (d–f) distributions at each study site. The *E. e. taylori* step-lengths (c) have a longer tail which is best fit by a power law distribution (Akaike weight=1.000, next

best distribution= 1.75×10^{-17}) while the *E. phaeton* populations have a short tail best fit by an exponential distribution (Akaike weight=1.000, next best distribution= 5.5×10^{-33})

also supported by the MRPP analysis. ANOVAs on individual behavioral categories revealed that *E. phaeton* females exhibited a greater frequency of time spent flying and host plant inspection, regardless of whether the population was *Plantago*- or *Chelone*-dependent. On the other hand, *E. e. taylori* nectared, courted, and engaged in hiding more frequently than *E. phaeton* (Fig. 1). If the adoption of *P. lanceolata* facilitated convergent behavioral evolution, then both *E. e. taylori* and *E. phaeton* should have similar activity budgets for *Plantago*-dependent populations and the *Chelone*-dependent population should appear distinct in either the NMDS ordination, MRPP, or ANOVA analyses with individual behaviors. Only the frequency of perching and basking was similar between the two species (Fig. 1, Table 1). These behaviors are two of the most common in butterfly activity budgets (Kingsolver 1983; Van Dyck et al. 1997; Grundel et al. 1998; Nielsen and Watt 1998; Severns 2008; Turlure and Van Dyck 2009) and the checkerspot activity budgets would not likely statistically differ if compared to butterflies from different genera and families.

Short-term movements of *E. e. taylori* and *E. phaeton* also grouped by species rather than host plant, echoing the activity budget groupings and providing more evidence that innate species tendency have a greater effect on short-term movements than host plant association. This outcome contrasts with the host preference driven movements of *M. cinxia* (Hanski et al. 2002) and *E. editha* (Thomas and Singer 1987) where, over several days, females were observed to either have a greater emigration rate from habitat with lower ranked host plants or reside for several days in habitats with preferred hosts. We have performed oviposition preference tests (sensu Singer and Lee 2001) with *E. phaeton* and most females expressed a strong ranking preference for the native *C. glabra* plants over *P. lanceolata* (unpublished data). We would therefore expect to observe some similarities in short-term movements of *Plantago*-dependent *E. phaeton* and *E. e. taylori*, but this prediction was not supported by our data. Perhaps, the lack of agreement between our study and movement studies with *M. cinxia* and *E. editha* is due to the time duration over which checkerspots were tracked. Since Hanski et al. (2002) and Thomas and Singer (1987) did not collect data on the short-term movements as we did in this study and we did not collect longer-term dispersal information, only a coarse, qualitative comparison between the three studies can be justified. It is conceivable, however, that the behavioral consequences of switching to an exotic host plant may differ from the adoption of a native host species, on which butterflies would have a longer co-evolutionary history.

Observed animal step-length distributions are typically unimodally distributed with variable long tails (Turchin 1998; Benhamou 2007) and *E. phaeton* step-lengths fit this common pattern (Fig. 2). However, *E. e. taylori* step-lengths appeared to be bimodal with a conspicuously long tail (Fig. 2).

This suggests that, despite the relatively recent adoption of *P. lanceolata* and non-significant diffusion coefficient (*D*) values, the two checkerspot species move across the landscape in fundamentally different ways. In one phase of *E. e. taylori* movement, step-lengths were locally restricted, <2 m, with females commonly perching, basking, nectaring, and occasionally crawling through reproductive habitat, essentially remaining sedentary for a large proportion of the time if not courted. However, aggressive and persistent courtship of unreceptive females (Table 1), frequently by groups of several males, often resulted in relatively long, evasive flights, the second phase of female movement (the second mode in Fig. 2f). We did not observe nor find evidence of courtship-induced long-distance flights in *E. phaeton* nor did the average courtship attempt persist for more than an observation time interval, 15 s (unpublished data). Courtship pressure, and its associated stress on females, can impact the distribution, movement, foraging, and reproductive behaviors for insects and animals across local landscapes (Baguette et al. 1989; Odendaal et al. 1989; Krupa and Sih 1993; Boness et al. 1995; Stone 1995; Gibbs et al. 2005, Kimber et al. 2009; Rossi et al. 2010; Turlure and Van Dyck 2009, Köhler et al. 2011; Tobler et al. 2011; Makowicz and Schlupp 2013). It seems that male harassment influences how female *E. e. taylori* perceives and responds to habitat over the short-term, potentially leading to locally well-explored but patchy landscape knowledge and the suboptimal distribution of oviposition sites with respect to host plant abundance (sensu Odendaal et al. 1989).

Evidence for divergent host-associated behavior

Although there did not appear to be evidence of *Plantago*-associated convergent behavior between *E. e. taylori* and *E. phaeton*, the adoption of *P. lanceolata* may have modified the behavior of *E. phaeton*. *Plantago*-dependent *E. phaeton* females spent a greater proportion of their activity budget inspecting host plants than *Chelone*-dependent females (Table 1). Considering that the adoption of *P. lanceolata* by *E. phaeton* appears to be relatively recent (<40 generations) and that females also prefer to oviposit on *C. glabra* in oviposition preference tests (unpublished data) it is consistent with the slow evolution of fitness related traits (reviewed by Mousseau and Roff 1987; Merilä and Sheldon 1999) that gravid *E. phaeton* would devote more time to investigating and assessing host plants in the *Plantago*-dependent population than they would in the *Chelone*-dependent population. Despite adopting *P. lanceolata*, *E. e. taylori* females spent very little time inspecting host plants, even compared with *Chelone*-dependent *E. phaeton* (Table 1). Unfortunately, we do not have a suitable *C. hispida* dependent population to which we could similarly compare activity budgets within *E. e. taylori* and test for evidence of parallel modifications to

checkerspot activity budgets associated with the adoption of *P. lanceolata*. However, behavior of female checkerspots in our study suggest that innate oviposition behaviors may be slow to evolutionarily converge even though the checkerspots are phylogenetically (Wahlberg 2001) and biochemically predisposed (Bowers 1983; Nieminen et al. 2003) to independently adopt the same novel host plant.

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