

## SHORT COMMUNICATION

# Seasonal wing colour plasticity varies dramatically between buckeye butterfly populations in different climatic zones

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**Abstract.** 1. In the present study, how seasonal wing colour plasticity in buckeye butterflies (*Junonia coenia* Hübner) differs between populations in different climatic regions, and whether these differences are explained by regional environmental differences or reflect genetic divergences in plasticity is addressed.

2. Using museum specimen data, it is shown that buckeye wing colour variation is much greater in populations from the humid and subtropical climates of the eastern U.S.A. than in populations from the desert and mediterranean climates of the western U.S.A.

3. Museum specimen data further show that wing colour in eastern populations is strongly correlated with seasonal variation in day length and temperature, whereas wing colour in western populations is only weakly associated with these cues.

4. Controlled incubator experiments comparing Southern California and North Carolina populations suggest that regional differences in wing pattern variation are attributable to dramatically increased robustness to environmental variation in western populations.

5. Together these experiments show that while phenotypic variation can be influenced by environmental cues, the range of this variation can genetically diverge between populations in different regions.

**Key words.** *Junonia coenia*, plasticity, polyphenism, robustness, variation, wing patterns.

## Introduction

In spite of the importance of phenotypic plasticity in ecological adaptation, we are just beginning to understand how plasticity itself evolves, especially below the species level (Nijhout, 2003; Pfennig *et al.*, 2010; Beldade *et al.*, 2011; Moczek *et al.*, 2011). Here we explore regional variation in seasonal plasticity in buckeye butterflies (*Junonia coenia* Hübner), a model system for studies of seasonal plasticity (Nijhout & Koch, 1991; Smith, 1991; Rountree & Nijhout, 1995a,b). Most previous work on *J. coenia* has been done on animals originating from North Carolina where there are two primary adult colour morphs determined by photoperiod and temperature cues experienced during development (Smith, 1991; Rountree & Nijhout, 1995a). Cold temperatures and short days produce red ventral

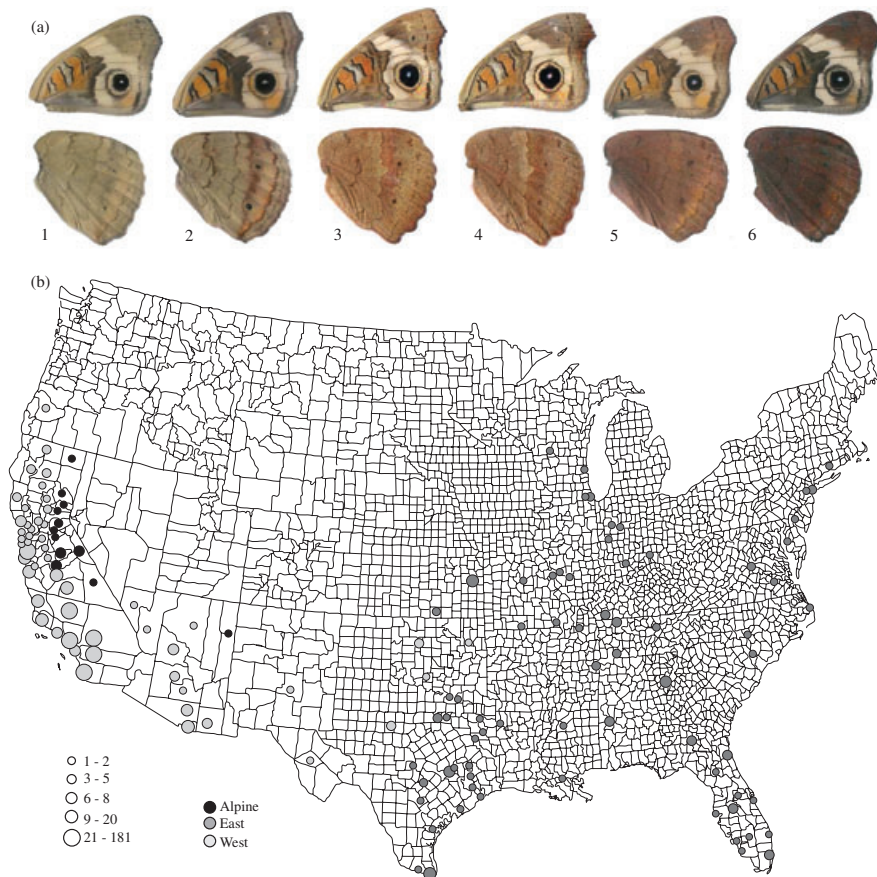
wing surfaces, whereas warm, long days produce pale brown ventral wing surfaces. Red wings are thought to aid in crypsis when butterflies perch amongst autumn leaves; a hypothesis strengthened because red forms of many butterfly species are also associated with leaf-like wing shape (Brakefield & Larsen, 1984). In the present study we address two questions. First, how does the distribution of *J. coenia* wing colour phenotypes differ between populations in different climatic regions across the United States? Second, can some of these differences be explained by a response to environmental differences, or do they reflect genetic divergence of plastic response?

## Materials and methods

### Scoring wing phenotypes

Our wing phenotype scoring scale, expanded from Smith (1991), defines a range from one to six (Fig. 1a). (1) pure pale

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**Fig. 1.** (a) A scale for scoring wing colour morphs of *Junonia coenia*. Specific descriptions of each score may be found in the text. (b) Geographical distribution of museum specimens sampled in the present study. Point size indicates the number of samples from a given county and point shading indicates region.

brown colour on the variable regions of ventral wing surfaces; (2) only a narrow band of red present parallel to the wing margin; (3) more than a narrow band of red but less than 50% of the total surface covered in red scales; (4) more than 50% but less than 100% red coverage; (5) solid red scale coverage; and (6) solid dark red scales in the variable ventral regions. All wing scores used in the present study were estimated by the same person (E.V.D.).

#### Analysis of museum specimens

Wing colour was examined for all *J. coenia* specimens at the Natural History Museum of Los Angeles and the UC Riverside Entomology Research Museum (Fig. 1b). Historical climate records were accessed online from the PRISM research group (<http://www.prism.oregonstate.edu/>) and average maximum and minimum temperatures were found for the month that each butterfly would have been experiencing as their critical environmental induction period. This date was estimated to be 2 weeks before the recorded capture of each butterfly, when they would have been undergoing pupal or late larval development. Specimens specifically selected for inclusion

in the present study had a high standard of wing condition which usually indicates that the adult butterfly emerged less than 1 week before capture (Lehnert, 2010). Only specimens that had county-level locality information and the exact day of collection recorded were included in the dataset. Day lengths experienced by these butterflies during their critical exposure period of development were retrieved from the NOAA Solar Calculator (<http://www.esrl.noaa.gov/gmd/grad/solcalc/>) based upon latitude and date of collection.

A total of 856 museum specimens were analysed in the present study. Each was classified as belonging to one of the following three climatic regions: 'west' (localities west of 100°W longitude and less than 1000 m in elevation), 'alpine' (localities west of 100°W longitude and greater than 1000 m in elevation), and 'east' (localities east of 100°W longitude) (Fig. 1b). These data approximated a Poisson distribution, with many individuals being of pale brown colour (low score) and very few individuals being dark red (high score), such that the distribution of specimens among categories 1 through to 6 was 341, 202, 167, 95, 56, and 4 (respectively), and the data had a ratio of mean to variance of 1.3. Accordingly, all analyses were conducted using Poisson's regression with the GENMOD procedure in SAS 9.2. (SAS Institute, 2008). Parallel

analyses (not shown) using both the general linear model and log-transformed data (GLM procedure) and an ordinal logistic regression (LOGISTIC procedure) produced qualitatively identical conclusions as those obtained through Poisson regression. The score was modeled as dependent on day length, average minimum temperature, region, and the two-way interactions between day length and region and between average minimum temperature and region. Where significant interactions occurred between region and either day length or minimum temperature, it was necessary to determine among which pairs of regions these differences resided. Accordingly, three sets of *post hoc* analyses were conducted in which the effects of day length or average minimum temperature were compared between each pair of regions (i.e. west vs. alpine, west vs. east, and alpine vs. east) with Bonferroni-adjusted *P*-values (Garcia, 2004). Finally, multiple regressions were performed separately for each region using the GLM procedure in SAS 9.2 to determine the proportion of variance in score (i.e.  $R^2$ , the coefficient of determination) explained by temperature and day length.

#### Controlled rearing experiment

Two populations of *J. coenia* were used for the controlled rearing experiment, one from Durham, North Carolina (35°59'19"N, 78°54'26"W, provided by Fred Nijhout) and one from Audubon Starr Ranch in Trabuco Canyon, Southern California (33°37'46.18"N, 117°33'15.12"W). Lab colonies were reared in incubators, fed a standard artificial diet (Nijhout, 1980), and regularly supplemented with new individuals to prevent inbreeding. Two sets of conditions known to induce alternative colour morphs in North Carolina populations were applied (Smith, 1991): 21 °C with 8 h light for short/cool day conditions and 27 °C with 16 h light for long/warm day conditions. Within 24 h of emergence each adult was scored for wing colour.

Incubator trials were conducted in multiple iterations (i.e. generations of adults emerging from a specific environmental condition) with groups of larvae from each geographical region run in parallel with each other in either short/cool or long/warm conditions. For North Carolina larvae 4 iterations (213 larvae) were reared under short/cool day conditions and 10 iterations (607 larvae) were reared under long/warm day conditions. For California larvae three iterations (41 larvae) were reared under short/cool day conditions and eight iterations (310 larvae) were reared under long/warm day conditions. Wing score was modelled as dependent upon population, growth conditions, and their interaction with the MIXED procedure in SAS 9.2, with experimental iteration included as a random effect.

## Results and discussion

#### *Eastern populations exhibit a wider range of seasonal wing colour variation*

Analysis of museum specimens showed that the range of wing colours observed in the different geographical regions varied dramatically. Butterflies from the west and alpine

regions had a narrower range of wing colour phenotypes than butterflies from the east, with a maximum colour score of five rather than six as observed in the east (Fig. 2a). Overall, wing score was significantly influenced by day length ( $F_{1,856} = 50.78$ ,  $P < 0.0001$ ), temperature ( $F_{1,856} = 5.27$ ,  $P = 0.02$ ), and region ( $F_{2,856} = 16.62$ ,  $P < 0.0001$ ). The regions also differed in the influence of day length (region  $\times$  day length interaction:  $F_{2,856} = 8.09$ ,  $P = 0.0003$ ) and temperature (region  $\times$  temperature interaction:  $F_{2,856} = 5.77$ ,  $P = 0.0032$ ).

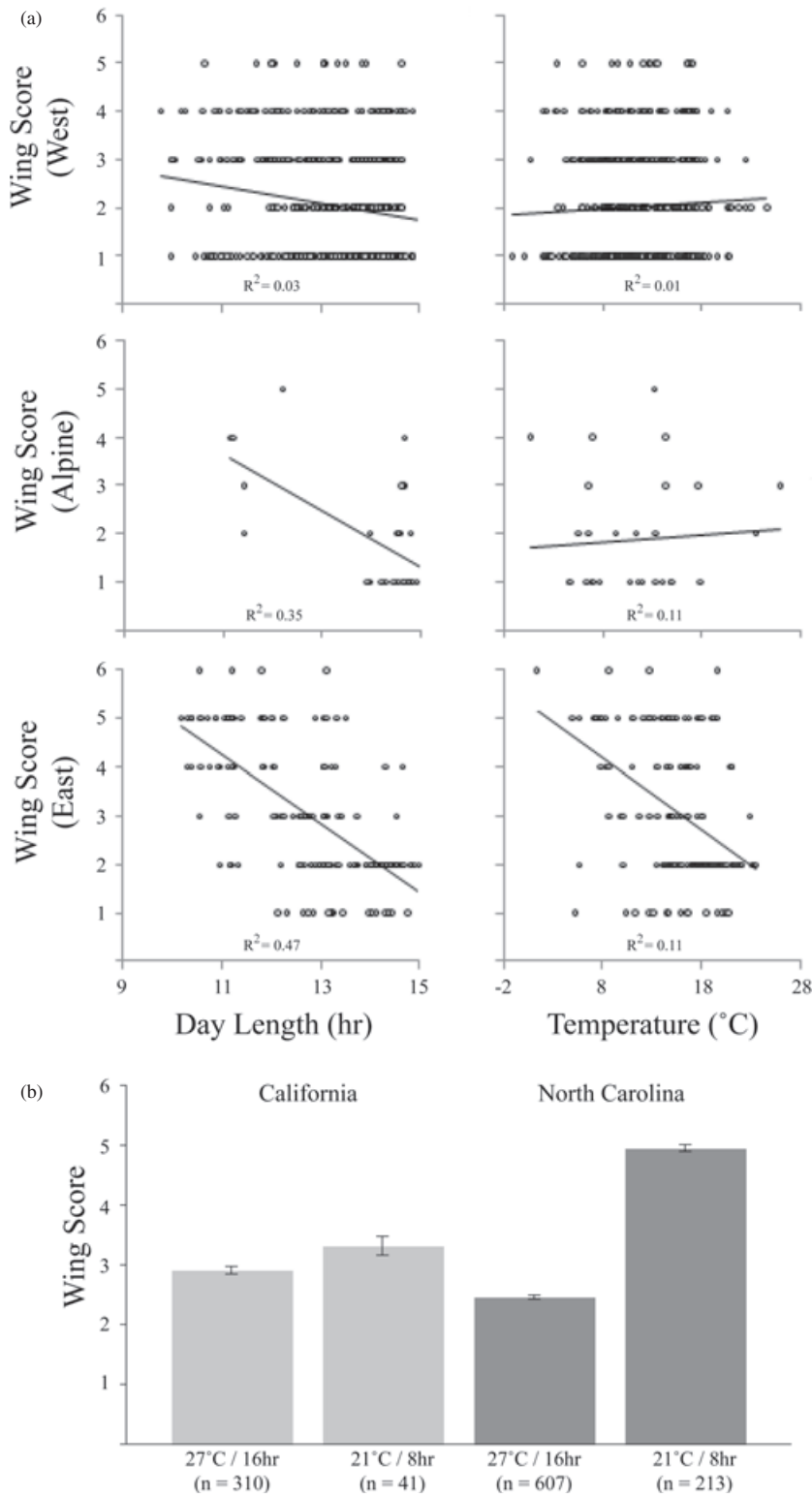
*Post hoc* comparisons investigating the source of regional differences for day length effects showed a similar influence of day length between the east and alpine regions ( $P = 0.17$ ), which together differed from the west (alpine vs. west:  $P = 0.0225$ ; east vs. west:  $P = 0.0039$ ). Day length had a significant negative effect on score for all regions, with the effect being stronger for east and alpine regions as compared with the west. In the multiple regression analyses including both day length and temperature, the partial  $R^2$  for day length was relatively low for the west (0.03), but high for both the east (0.47) and alpine regions (0.35) (Fig. 2a).

*Post hoc* comparisons investigating the source of regional differences for temperature effects showed a similar influence of temperature between the west and alpine regions ( $P = 0.57$ ), which together differed from the east (alpine vs. east:  $P = 0.0039$ ; west vs. east:  $P = 0.0114$ ). Temperature had a significantly positive effect on score in the west and alpine regions, but had a significantly negative effect in the east (Fig. 2a), although multiple regression analyses including both day length and temperature showed low partial  $R^2$  values for temperature (0.01, 0.11, and 0.11 for west, alpine, and east regions respectively).

Most cases of seasonal plasticity in temperate species involve both photoperiod and temperature cues (Shapiro, 1984). Our analysis of museum specimens supports this pattern for the eastern buckeye populations (Fig. 2a), as have extensive incubator experiments with North Carolina populations (Smith, 1991). We were thus surprised to find that variation in western populations is only weakly predicted by either of these variables. Even more surprising is that in both west and alpine regions the direction of the temperature correlation is the opposite of eastern populations and is directly opposed to the photoperiod response (Fig. 2a). These observations show that there has been major regional divergence in how different environmental cues predict buckeye wing colour.

#### *Western buckeyes are developmentally robust to environmental variation*

California and North Carolina buckeyes reared under controlled conditions showed significant differences in wing colour response to environmental cues (Fig. 2b). Tukey's *post hoc* tests showed significant pair-wise differences ( $P < 0.01$ ) among all four population-by-environment treatment combinations. There were significant effects of population ( $F_{1,1146} = 38.24$ ,  $P < 0.0001$ ), growth conditions ( $F_{1,1146} = 241.76$ ,  $P < 0.0001$ ), and their interaction ( $F_{1,1146} = 129.45$ ,  $P =$



**Fig. 2.** (a) Region-specific regression plots of *Junonia coenia* wing colour score versus temperature and day length during the estimated larval critical period. All depicted relationships are significant ( $P < 0.05$ ). For day length, the slopes of alpine and east do not differ significantly from each other ( $P > 0.05$ ), but each differs from the west ( $P < 0.05$ ). For temperature, the slopes of west and alpine do not differ from each other ( $P > 0.05$ ), but each differs from the east ( $P < 0.05$ ). (b) Mean wing scores from California and North Carolina *J. coenia* populations reared under different temperature and day length conditions. Pair-wise *post hoc* tests show that all four means are significantly different from each other ( $P < 0.05$ ). Bars represent SE.



<0.0001). North Carolina butterflies displayed extreme shifts in wing colour in response to environmental stimuli with a mean increase of 2.5 wing score units with the transition from long/warm days ( $2.45 \pm 0.05$ , mean  $\pm$  1SE) to short/cool days ( $4.96 \pm 0.08$ ), as compared with California butterflies that showed a mean increase of only 0.4 units between long/warm days ( $2.94 \pm 0.06$ ) and short/cool days ( $3.33 \pm 0.15$ ).

Our experimental results were consistent with the museum observations in showing dramatically reduced plasticity-associated wing colour variation in western populations relative to eastern populations. Further, they allow us to deduce that, for the sampled populations, the regional differences in wing pattern variation can be attributed to genetic factors and not simply environmental differences between regions. This genetic variation in plasticity between western and eastern populations was quite pronounced; short/cool day conditions consistently induce dark red phenotypes in *J. coenia* from North Carolina, however, across all of our trials, California butterflies raised under identical conditions never produced this phenotype. The difference in plasticity between California and North Carolina populations was an issue of magnitude; indeed, the mean difference in wing colour response in the California population we studied was so minimal as to be barely perceptible. This is in marked contrast to a recent study in *Bicyclus anynana* Butler butterflies showing that the magnitude of plastic response in Malawi and South Africa populations was similar, with the populations simply having different baseline phenotypes (deJong *et al.*, 2010). Seasonal wing colour plasticity is prevalent throughout *Junonia* and related genera and is therefore likely to be the ancestral state. We speculate that the California population's relatively stable wing coloration represents an evolutionary reduction of plasticity, where wing patterns have become more robust to the effects of environmental variation. Although we cannot yet determine whether this change in plasticity was as a result of drift or selection, it is possible that the dark red autumn phenotypes common in eastern populations may be selected against in western habitats owing to a paucity of dense and/or colored autumn foliage.

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