



The evolutionary history of the ‘alba’ polymorphism in the butterfly subfamily Coliadinae (Lepidoptera: Pieridae)

LISA B. LIMERI* and NATHAN I. MOREHOUSE

Department of Biological Sciences, University of Pittsburgh, 4249 Fifth Ave, Pittsburgh, PA 15232, USA

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Polymorphisms are common in the natural world and have played an important role in our understanding of how selection maintains multiple phenotypes within extant populations. Studying the evolutionary history of polymorphisms has revealed important features of this widespread form of phenotypic diversity, including its role in speciation, niche breadth, and range size. In the present study, we examined the evolutionary history of a ubiquitous colour polymorphism in the sulphur butterflies (subfamily: Coliadinae) termed the ‘alba’ polymorphism. We investigated the origin and stability of the ‘alba’ polymorphism using ancestral state reconstruction analysis. Our results indicate that the ancestor of the Coliadinae was polymorphic and that this polymorphism has undergone repeated transitions to monomorphism. Repeated loss of polymorphism suggests that the ‘alba’ polymorphism may be relatively unstable over evolutionary time. These results provide a framework for future studies on the origin and maintenance of the ‘alba’ polymorphism and guide the direction of future hypotheses. We discuss these results in light of current understandings of how the ‘alba’ polymorphism is maintained in extant populations. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 117, 716–724.

ADDITIONAL KEYWORDS: *Colias* – colour – *Eurema* – molecular phylogeny – polymorphism stability.

INTRODUCTION

Polymorphisms, the presence of multiple distinct, genetically-determined phenotypes within a single interbreeding population, offer a number of key opportunities to study how selection shapes phenotypic diversity (Ford, 1945). Because the fitness of morphs is often interdependent, emergent interactions between selection pressures and phenotypes arise that can deepen our understanding of evolution in complex systems (Gross, 1996). For example, the fitness of each male mate strategy in the trimorphic side-blotched lizard (*Uta stansburiana*) depends on a number of selective pressures, including neighbour identity and male hormone levels, leading to regular temporal oscillations in relative morph frequency (Sinervo & Lively, 1996; Mills *et al.*, 2008). Polymorphisms provide unique insight into how interactions between selective pressures maintain diversity.

One strategy for understanding polymorphisms is to consider them in a comparative framework. This

research perspective allows us to identify both ancestral states and patterns of evolutionary origins or losses. Knowledge of the evolutionary history of polymorphic traits has deepened our understanding of a wide variety of evolutionary processes. For example, researchers have proposed that polymorphisms promote speciation (McLean & Stuart-Fox, 2014) and several phylogenetic studies have supported this claim (Corl *et al.*, 2010; Hugall & Stuart-Fox, 2012). In side-blotched lizards, the repeated loss of one morph is associated with increased rates of phenotypic change and increased divergence between populations (Corl *et al.*, 2010). Corl *et al.* (2010) suggest that this may play a role in speciation. Comparative studies of polymorphic traits have also illuminated the relationship between phenotypic diversity and niche breadth. Other research suggests that disruptive selection plays an important role in maintaining polymorphisms (Galeotti & Rubolini, 2004). A large-scale investigation of owls, nightjars, and raptors supports this hypothesis by revealing that polymorphic species occupy a wider niche breadth than monomorphic species (Galeotti & Rubolini, 2004).

*Corresponding author. E-mail: lisalimeri@gmail.com

We took a comparative approach to understanding the female-limited 'alba' colour polymorphism in the butterfly subfamily Coliadinae. Females of many species may be various shades of yellow/orange/red, caused by different combinations of pterin pigments. Conversely, the 'alba' female morph (named after the Latin word for white) is paler in appearance as a result of reduced and/or altered expression of pterin pigmentation on wing surfaces (Watt, 1973) (Fig. 1). Males of most species are shades of yellows, oranges or reds, similar to the non-'alba' female morph, although they may differ in wing pattern and/or ultraviolet reflectance (Kemp, Rutowski & Mendoza, 2005).

Polymorphic species in this group are found worldwide (Braby, 2000; Grieshuber, Worthy & Lamas, 2012). Despite the prevalence of this polymorphism, little is known about its evolutionary history (Braby, Vila & Pierce, 2006). In addition, the factors contributing to its maintenance within particular species and/or populations remain the subject of experimental inquiry and debate (Graham, Watt & Gall, 1980; Nielsen & Watt, 1998; Kemp & Macedonia, 2006; Limeri & Morehouse, 2014). Polymorphic species have been reported from most of the 18 genera within the Coliadinae, although these data have never before been formally summarized. Questions of current selection on this polymorphism have come from studies of several species in the genus *Colias*

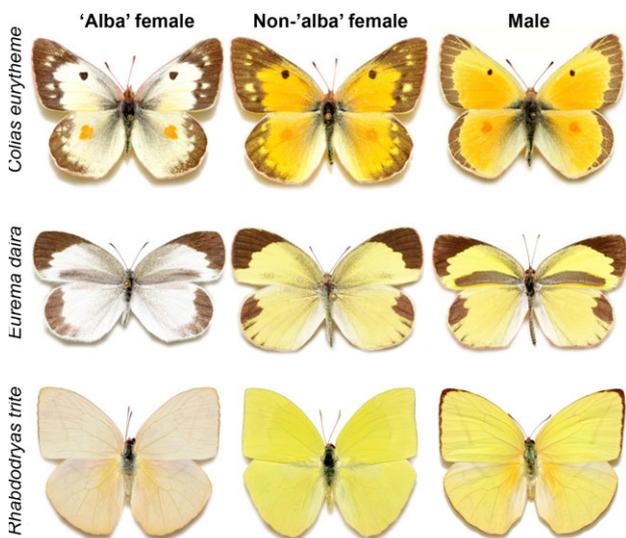


Figure 1. Examples of phenotypic forms of three species in the Coliadinae. The 'alba' female phenotype (left column), the non-'alba' female phenotype (centre column), and the male phenotype (right column) are shown for three different species: *Colias eurytheme* (top row), *Eurema daira* (center row), and *Rhabdodryas trite* (bottom row). Photographs © Kim Davis, Mike Stangeland and Andrew Warren.

(Graham *et al.*, 1980; Nielsen & Watt, 2000). For example, 'alba' females from the species *Colias eurytheme* benefit from a developmental advantage over non-'alba' females by redirecting 0.4 mg of guanosine triphosphate (GTP) from pigment synthesis to other metabolic processes during pupal development (Watt, 1973). This results in 'alba' females developing faster in cold climates, having higher fat body reserves at all ages, and possessing more mature eggs at pupal emergence than non-'alba' females (Graham *et al.*, 1980). Graham *et al.* (1980) have argued that the developmental advantage of 'alba' females is offset by reduced male interest in 'alba' females during mate searching. Subsequent work aiming to test this hypothesis revealed that the selective pressures acting on 'alba' and non-'alba' morphs are strongly context-dependent (Graham *et al.*, 1980; Watt, Kremen & Carter, 1989; Nielsen & Watt, 2000). Fitness advantages associated with the 'alba' morph may differ in strength and direction depending on microhabitat temperature and/or the community of interacting species (Watt *et al.*, 1989; Nielsen & Watt, 2000). For example, co-occurring species that visually resemble the 'alba' phenotype may act as interference competitors during early stages of mate searching (Graham *et al.*, 1980; Limeri & Morehouse, 2014), leading to stronger selection against the 'alba' phenotype in communities with high densities of these interference competitors. The context-dependence of these selective pressures suggests that the 'alba' phenotype may have transient benefits or that the benefits associated with each phenotype may fluctuate dramatically depending on environmental factors, which could lead to sudden fixation of one form or the other. How this environmental context-dependence has influenced the evolutionary trajectories of the 'alba' polymorphism across this clade is not well understood.

Despite numerous studies about the contemporary consequences of the 'alba' polymorphism, we do not know when or how often it arose. A comparative approach can reveal the origin(s) and evolutionary patterns of the 'alba' polymorphism. Because almost all of the currently available information about the 'alba' polymorphism arises from studies of a few species within the *Colias* genus (Graham *et al.*, 1980; Nielsen & Watt, 2000; Kemp & Macedonia, 2006), information about the evolutionary origin and history of the 'alba' polymorphism may be valuable at the level of the *Colias* genus as well as the entire subfamily.

In the present study, we use ancestral state reconstructions on three different phylogenies to discriminate between alternative hypotheses regarding the ancestral state of the Coliadinae: (1) the ancestral state was the 'alba' morph, (2) the non-'alba' morph

or (3) polymorphic for both morphs. In addition, we evaluated these three alternative hypotheses specifically for the genus *Colias*, the most species-rich genus in the Coliadinae. Furthermore, we evaluated the relative stability of the ‘alba’ polymorphism over time by investigating the number of gains and losses across the Coliadinae.

MATERIAL AND METHODS

SPECIES LIST

A comprehensive list of the 18 genera and 203 species encompassed by the subfamily Coliadinae was compiled primarily using three sources: an exhaustive catalogue by Charles Bridges (1988), the Catalogue of Life online database (taxonomic scrutiny of Coliadinae by Haeuser, Holstein and Steiner; Roskov *et al.*, 2015) and an online catalogue of butterflies in North and Central America (Warren *et al.*, 2012). We considered a species name valid if it appeared in one of these three main sources and one additional source (see “species” in Supporting information). Exceptions to this rule and uncertain taxa are outlined below.

The genus *Colias* is particularly well-studied and the taxonomy of *Colias* was extracted directly and exclusively from a recent monograph by Grieshuber *et al.* (2012). Similarly, a recent revision of the genus *Gonepteryx* by Reinhardt (2002) presents a complete description of the genus, and so we adopted this taxonomy in the present study.

The taxonomic status of several species within the Coliadinae remains poorly established. *Sensu* Davis & Barnes (1991), we treated *Catopsilia grandidieri* [Mabille, 1877] as a synonym to *Catopsilia thauruma* [Reakirt, 1866]. We treat *Eurema regularis* [Butler, 1876] as a subspecies of *Eurema desjardinsii* [Boisduval, 1833] in accordance with Yata’s revision of the genus *Eurema* (Yata, 1989) and Larsen (2005). The genus *Teriocolias* [Röber, 1909] is also not well resolved and there is no recent review. In accordance with the most recent sources, we consider *Teriocolias zelia* as a valid species name, with *Teriocolias zelia riojana* and *Teriocolias zelia andina* as subspecies (Vargas, 2012).

DATA COLLECTION

For each species, we recorded the phenotypes present in museum specimens, books, and online databases. We defined the ‘alba’ morph as the female variant in which the ground colour on all wing surfaces is changed from male-like (yellow, orange or red, depending on the species) to white, presumably as a result of a change in pterin pigmentation. Given that it was not

possible to directly assess pterin pigmentation via the images used during data collection, ‘alba’ morphs identified in the present study may be pale or white for a variety of reasons, including complete loss of pterin pigmentation, alteration of pterin profiles or other modifications to the pigmentary basis of female coloration. The non-‘alba’ morph, then, was defined as any phenotype in which the ground colour was not white on all wing surfaces. When a species contained some ‘alba’ females and some non-‘alba’ females, it was considered to be ‘alba’ polymorphic. When all females were the ‘alba’ morph, it was considered monomorphic for the ‘alba’ morph. When all females were characterized by yellow/orange/red pigments on some or all wing surfaces, the species was considered monomorphic for the non-‘alba’ phenotype.

This dataset is prone to a few biases. Morph presence data were collected based on images and reports from a variety of websites, collections, journal articles, and books (see “Female wing phenotypes” in Supporting information). Consequently, our dataset is vulnerable to species identification mistakes, especially for rare species and similarly-appearing species. Furthermore, a morph was considered absent if there were no positive reports, which may lead to an underestimation of the prevalence of rare morphs and artificially inflate the proportion of species that were considered monomorphic. Together, these factors may result in an underestimation of the prevalence of polymorphism.

We were unable to find information on 13 of the 203 species in the Coliadinae and these species were excluded from all analyses.

ANCESTRAL STATE RECONSTRUCTIONS

We performed ancestral state reconstructions on three different phylogenetic trees: a topology resolved to the genus level, a topology including all species, and a phylogeny produced using molecular data from a subset of species for which sequences were available. The genus-level topology and the topology including all species in the Coliadinae were derived from a molecular phylogeny published by Braby *et al.* (2006). Three of the 18 genera were not included in their molecular phylogeny (*Abaeis*, *Prestonia*, and *Rhabdodryas*) and their positions in the final topology are based on morphological characters as they discussed (Braby *et al.*, 2006). This placement yields a polytomy of these three genera. To ensure that this uncertainty did not influence results, we performed ancestral state reconstructions on all three possible resolutions as well as the polytomous arrangement. The arrangement of these genera did not affect the outcome of analyses, and the results are shown with the polytomy.

All ancestral state reconstructions were performed in MESQUITE, version 2.75 (Maddison & Maddison, 2011) using maximum likelihood methods with a general Mk1 model. Each node is estimated independently with a decision threshold of $t = 2.0$.

The goal of the genus-level analysis was to examine the distribution of polymorphic species on a large scale. Accordingly, genera were coded as ‘polymorphic’ if any of the species in the genus exhibited the ‘alba’ polymorphism, and ‘monomorphic’ if all of the species in the genus were monomorphic.

In the topology including all species (species-level analysis), each genus was analyzed as a polytomy of all its species because the species relationships within genera are not resolved. Each species was assigned a character state of ‘polymorphic’, ‘monomorphic “alba”’ or ‘monomorphic non-“alba”’ as described above.

The molecular phylogeny was produced using sequences from one mitochondrial gene (cytochrome *c* oxidase subunit I; *COI*) and one nuclear gene (elongation factor 1a, *ef1a*). Both of these genes exhibit evolutionary rates suitable for examining differences at the genus level (Braby *et al.*, 2006). The molecular phylogeny included all species in the Coliadinae for which the sequences for *COI* and *ef1a* were available: 47 sequences from 35 species encompassing 15 of 18 genera. The sequences were aligned, trimmed, and concatenated in SEAVIEW, version 4.4.2 (Gouy, Guindon & Gascuel, 2010). The resulting sequences consisted of 1128 characters, of which 838 were informative. The sequences were analyzed in MrBayes (Ronquist & Huelsenbeck, 2003) using Markov chain Monte Carlo analysis. We ran two chains using a general time-reversible model with 10 million generations and four gamma categories. The majority rules consensus tree was used for the ancestral trait reconstruction in MESQUITE. Figures were produced using FIGTREE (Rambaut, 2012).

RESULTS

This is the first formal summary of the pervasiveness of the ‘alba’ polymorphism throughout the Coliadinae. The Coliadinae contains 203 species dispersed throughout 18 genera, with relevant colour morph character states available for 190 out of these 203. Seventy-two percent (13/18) of the genera contain polymorphic species. Approximately half of the species in the Coliadinae are polymorphic (88/190; 46%).

TOPOLOGY RESOLVED TO GENUS LEVEL

Ancestral reconstruction of the topology resolved to the genus level indicates overwhelmingly that the

ancestor of the Coliadinae was a polymorphic species (Fig. 2). Only five of the 18 genera (28%) contain no polymorphic species and they are phylogenetically dispersed. These results indicate that polymorphism is likely the ancestral state and that repeated reversions to monomorphism have occurred.

This analysis also reveals that the ancestor of the genus *Colias* is likely polymorphic. Species relationships within *Colias* are not resolved in this analysis and so it was not possible to draw conclusions about the stability of the polymorphism within the genus *Colias*.

TOPOLOGY RESOLVED TO SPECIES LEVEL

Ancestral reconstruction of the topology including all species reveals finer detail (Fig. 3). This analysis indicates equal likelihood that the ancestor of the Coliadinae was polymorphic or monomorphic non-‘alba’ (0.37 each) and a lower probability that the ancestor was monomorphic ‘alba’ (0.26). This analysis also shows numerous transitions between

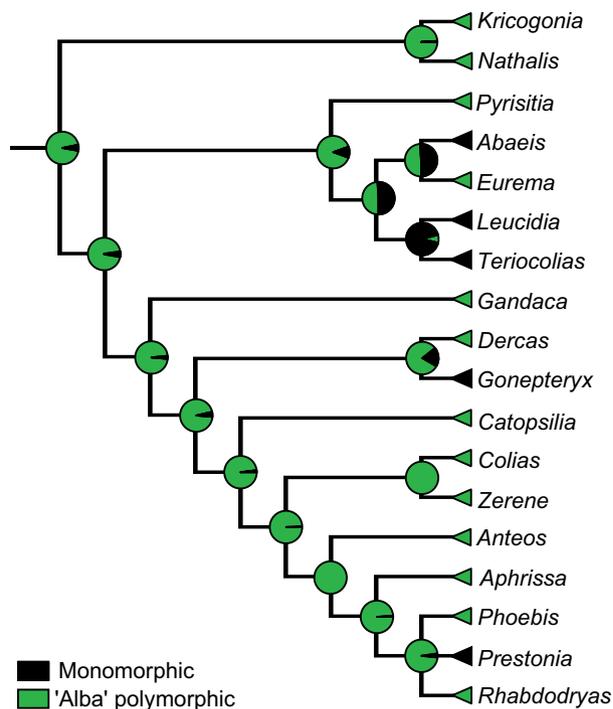


Figure 2. Genus-level ancestral state reconstruction performed on a topology of the 18 genera in the subfamily Coliadinae. Green wedges indicate that at least one polymorphic species is present in the genus, whereas black wedges indicate that all species are fixed. The area of the slices of the pie charts at each internal node represents the probability of that ancestor’s character state.

polymorphism and monomorphism, indicating evolutionary instability.

This analysis reveals with high certainty that the ancestor of the genus *Colias* was polymorphic. The species relationships within the *Colias* are not well resolved. However, the high proportion of monomorphic species (44%) indicates that the polymorphism has likely been lost repeatedly and is unstable. Monomorphic species are relatively evenly split between the two morph types (53% non-‘alba’; 47% ‘alba’).

MOLECULAR PHYLOGENY

The ancestral reconstruction of the molecular phylogeny reveals that the ancestor of the Coliadae was likely a polymorphic species (Fig. 4). The phylogeny also suggests multiple losses of polymorphism within this phylogeny. The topology of this tree is similar to the topology produced by Braby *et al.* (2006), although it differs in its placement of the clade composed of the genera *Aphrissa*, *Phoebis*, *Prestonia*, and *Rhabdodryas*. Braby places these genera as the sister clade to the genus *Anteos*, whereas our phylogeny places them as the sister clade to the

genus *Gandaca* (Braby *et al.*, 2006). Future reconstructions including more sequences would likely provide improved resolution of this topology. However, it is unlikely that this discrepancy affected the results of the present study because our analyses on both our molecular phylogeny and on the topology produced by Braby *et al.* (2006) revealed qualitatively similar results: that the ancestor is polymorphic and there are repeated losses of polymorphism.

This analysis also reveals with high certainty that the ancestor of the *Colias* was polymorphic. However, this analysis only included polymorphic *Colias* species as a result of the availability of genetic sequences. Therefore, any conclusions drawn from this analysis about *Colias* should be interpreted with caution.

DISCUSSION

In the present study, we addressed hypotheses about the origin and evolutionary path of the ‘alba’ polymorphism within the Coliadae. Our analyses revealed that the ancestral state of the Coliadae is likely polymorphic, with two out of three of our

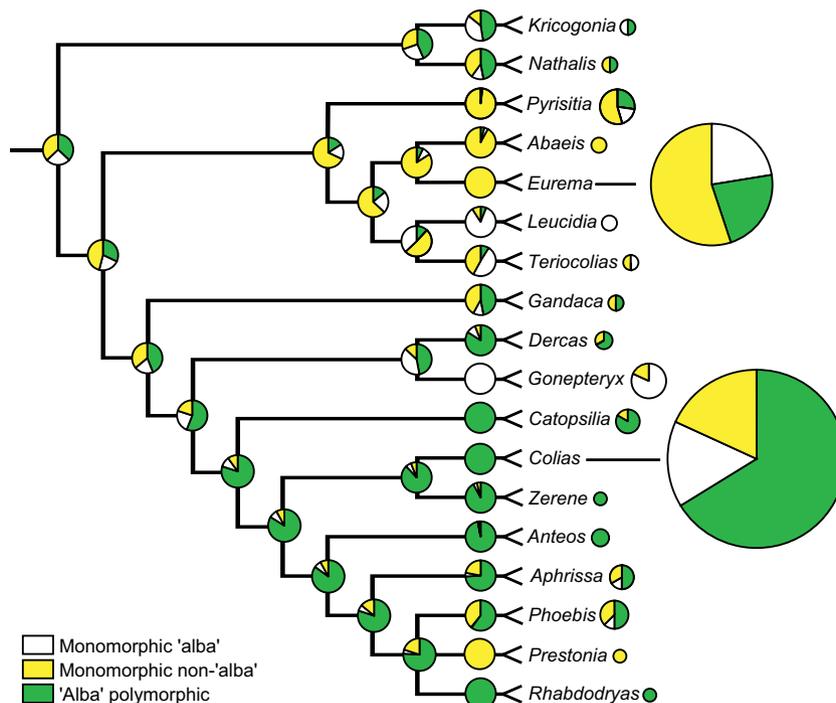


Figure 3. Species-level ancestral state reconstruction performed on a topology including all 190 species in the Coliadae treated as polytomies within each genus (polytomies not shown). Pie charts to the right of the genus names are scaled relative to the number of species within the genus, ranging from 1 (*Prestonia*) to 77 (*Colias*). The slices of the pie represent how many contemporary species in the genus represent each character state. The circles to the left of the genus names at internal nodes on the tree represent the relative probabilities of the character state for that ancestor.

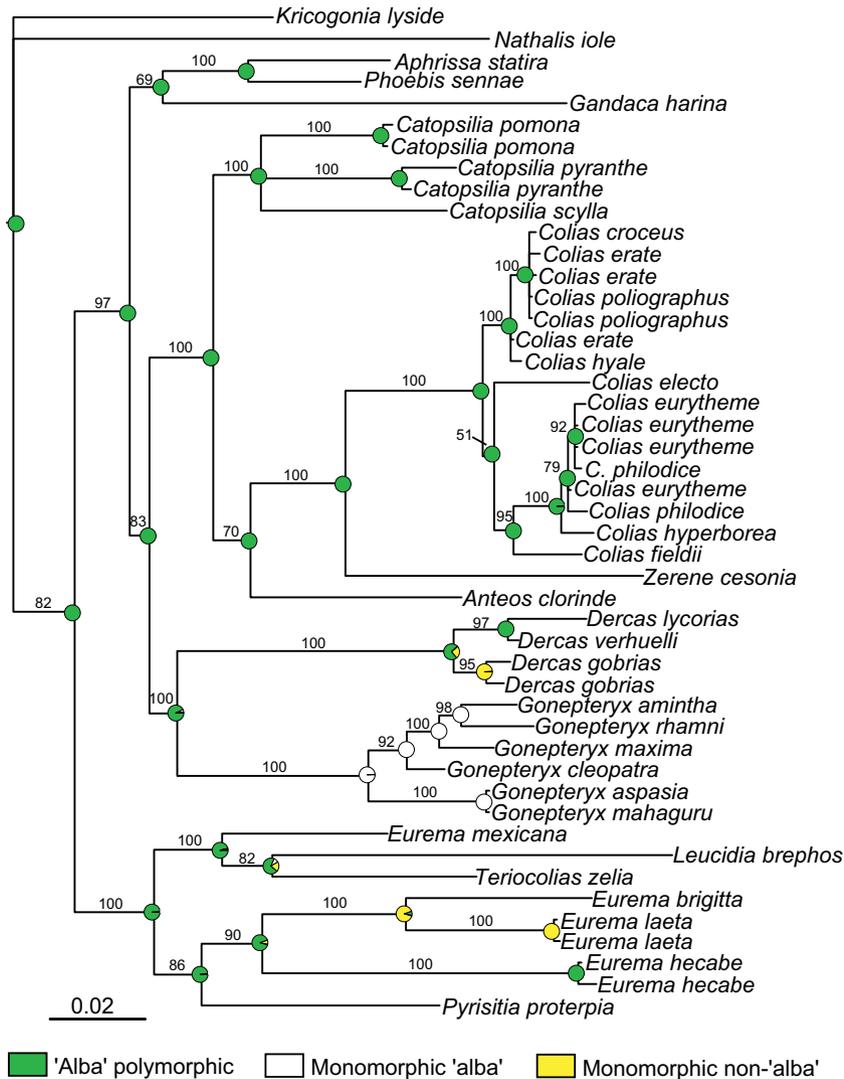


Figure 4. Molecular phylogeny ancestral state reconstruction performed on a phylogeny of 35 species generated by Bayesian analysis in MRBAYES.EXE (Ronquist & Huelsenbeck, 2003). Model parameters are detailed in the Material and methods. Branch lengths are represented by the key at the bottom and posterior probabilities are noted above the branch. The area of the slices of the pie charts at each internal node represents the probability of that ancestor's character state.

phylogenetic approaches providing strong support for a polymorphic ancestral state. The species-level phylogeny yielded inconclusive assignment of the ancestral state. However, we note that this latter analysis was based on the least biologically realistic phylogeny. Because detailed species relationships within genera are not available, this analysis included all species in each genus condensed into a single large polytomy. Better species-level resolution would be valuable in improving both ancestral state estimates, as well as the pattern of gains and losses of polymorphism within genera.

We also conclude that the 'alba' polymorphism is relatively unstable because all three analyses show

numerous monomorphic species that are phylogenetically dispersed. This indicates that the 'alba' polymorphism has been lost repeatedly. Although species relationships within genera are not defined, many genera, especially the large ones (e.g. *Colias* and *Eurema*), have monomorphic species of both forms. This indicates that there could be multiple character state transitions within genera, although a more precise characterization of the evolutionary history of these events cannot be provided without more complete information about species-level phylogenetic relationships.

Colias is the largest genus in the Coliadinae, geographically widespread, and phenotypically diverse

(it contains species of all 3 character states). Presumably, for these reasons, it is the most thoroughly studied genus in the Coliadinae (Graham *et al.*, 1980; Nielsen & Watt, 2000; Grieshuber *et al.*, 2012). All three analyses indicate that the ancestor of the genus *Colias* was polymorphic. The species relationships are not thoroughly resolved, although the high proportion of monomorphic species within the *Colias* suggests repeated losses of polymorphism. Furthermore, reversions to monomorphism involve the loss of both morph types ('alba' and non-'alba') at relatively equal frequencies. This indicates that morph fixation may be a random event, or that selective pressures do not consistently favour one morph over the other. This finding is in contrast to analyses of other groups, such as side-blotched lizards, where it was found that one morph was consistently lost, presumably as a result of consistent selective pressure acting against that morph (Corl *et al.*, 2010).

The finding that the 'alba' polymorphism is likely the result of a single evolutionary origin may have significant implications for understanding its genetic basis. The 'alba' allele is considered to be a single, autosomal locus dictated by Mendelian genetics (Gould, 1923; Komai & Ae, 1953). Future studies could investigate whether genetic regulation of this locus is conserved across the Coliadinae.

Our comparative analysis of the evolutionary history of the 'alba' polymorphism may also help future efforts to parse amongst potential selective mechanisms favouring the maintenance and/or loss of this polymorphism. Numerous different mechanisms may maintain polymorphisms within a population, such as apostatic selection (Bond, 2007), variation over space and/or time (Gray & McKinnon, 2007), and countervailing selection (Nielsen & Watt, 2000). These mechanisms may also vary in their stability over evolutionary time (Haldane & Jayakar, 1963; Gray & McKinnon, 2007). The knowledge that the 'alba' polymorphism is relatively unstable may provide preliminary clues to aid future studies investigating the selective pressures maintaining the polymorphism. For example, future studies could focus on investigating selective pressures that are less stable or consistent over evolutionary time (i.e. environmental factors).

A recent review on polymorphisms suggested that future directions for the field should test the hypothesis that polymorphisms promote speciation (McLean & Stuart-Fox, 2014). Conversely, it is also possible that apostatic selection may constrain population divergence and hence speciation by maintaining polymorphism across multiple populations (Svensson, Abbott & Hardling, 2005). One prediction of the hypothesis that polymorphism promotes speciation is that polymorphic taxa should be ancestral to

monomorphic taxa because speciation of polymorphic ancestors would lead to monomorphic daughter species (McLean & Stuart-Fox, 2014). Phylogenies showing polymorphic ancestors resulting in many monomorphic descendant species would be consistent with this hypothesis, whereas phylogenies showing monomorphic ancestors with polymorphic descendants would refute this hypothesis. Our data are consistent with the former, offering implicit support for the hypothesis that polymorphism may promote speciation.

The present study represents the first evaluation of the origin and history of the ubiquitous 'alba' polymorphism within the butterfly subfamily Coliadinae. The 'alba' polymorphism has been of great interest to researchers in diverse fields such as enzymatics (Watt, 1977), thermoregulation (Kingsolver, 1983), and mate choice (Gilchrist & Rutowski, 1986; Kemp & Macedonia, 2006; Limeri & Morehouse, 2014). Our findings that the 'alba' polymorphism is likely ancestral and unstable is key in enabling future studies to explore the causes and mechanisms contributing to the maintenance of the 'alba' polymorphism. Because the 'alba' polymorphism is likely ancestral to both groups, pertinent questions may focus on why the polymorphism is maintained and what factors lead to the loss of either morph rather than the factors that lead to repeated origins of the polymorphism. Understanding the environmental drivers of the polymorphism is important, although more natural history information is a prerequisite. With improved natural history information, future studies could search for correlations between environmental factors (i.e. climate, host plant, co-occurring species) and the maintenance or loss of polymorphism.

CONCLUSIONS

Understanding the evolutionary history of this trait is an important first step in characterizing the unique selective pressures acting to maintain a widespread colour polymorphism. We conclude that the 'alba' polymorphism is ancestral to the Coliadinae and evolutionarily unstable. Further hypotheses should address questions related to the maintenance and repeated loss of this polymorphism.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. The complete list of female wing phenotypes for Coliadinae species.