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# To mate, or not to mate: The evolution of reproductive diapause facilitates insect radiation into African savannahs in the Late Miocene

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### Abstract

- Many tropical environments experience cyclical seasonal changes, frequently with pronounced wet and dry seasons, leading to a highly uneven temporal distribution of resources. Short-lived animals inhabiting such environments often show season-specific adaptations to cope with alternating selection pressures.
- African *Bicyclus* butterflies show strong seasonal polyphenism in a suite of phenotypic and life-history traits, and their adults are thought to undergo reproductive diapause associated with the lack of available larval host plants during the dry season.
- 3. Using 3 years of longitudinal field data for three species in Malawi, dissections demonstrated that one forest species reproduces continuously, whereas two savannah species undergo reproductive diapause in the dry season, either with or without pre-diapause mating. Using additional data from field-collected and museum samples, we then documented the same three mating strategies for a further 37 species.
- Phylogenetic analyses indicated that the ancestral state was a non-diapausing forest species, and that habitat preference and mating strategy evolved in a correlated fashion.
- 5. *Bicyclus* butterflies underwent rapid diversification during the Late Miocene, coinciding with expansions into more open savannah habitat. We conclude that the ability to undergo reproductive diapause was a key trait that facilitated colonization and eventual radiation into savannahs in the Late Miocene.

#### KEYWORDS

*Bicyclus* butterflies, correlated evolution, diapause, key traits, Late Miocene, mating strategy, savannah, seasonality

## 1 | INTRODUCTION

Many tropical environments experience cyclical seasonal changes. Alternating wet and dry seasons are especially widespread, the former with abundant resources (e.g. host plants for phytophagous insects), whereas the latter are characterized by minimal resources (Denlinger, 1986; Jones, 1987; Tauber, Tauber, & Masaki, 1986). Thus, animals inhabiting such environments face dramatic changes in selection pressures with selection typically favouring traits that increase reproduction in the wet season and survival in the dry

season. Insects in seasonal environments often synchronize their reproduction with the phenology of associated plants to optimize the abundance and quality of resources available both for larvae and adults (Denlinger, 1986; Tauber et al., 1986).

Many tropical insects enter diapause at a particular life stage (egg, larval, pupal or adult) or move to more favourable habitats to escape extreme environments of the dry season (Denlinger, 1986; Tauber et al., 1986). Diapause is an arrest of development or ontogeny coupled with coordinated changes involving numerous physiological and life-history traits such as low metabolic activity, increased fat deposits and longevity, and altered or reduced behavioral acitivity (Hahn & Denlinger, 2007, 2010; Tatar & Yin, 2001; Tauber et al., 1986). Together, these traits are considered to facilitate survival in the resource-depleted environment (Tauber et al., 1986). Although diapause can be a highly effective strategy allowing temporal escape of unfavourable conditons, it is likely to come with costs including a higher mortality and a significant reduction in post-diapause fecundity (Ellers & van Alphen, 2002; Hahn & Denlinger, 2007). From a macroevolutionary perspective, strategies such as diapause can be a key life-history trait which can enable colonization of environments where habitat quality fluctuates temporally (Furness, Reznick, Springer, & Meredith, 2015; Panov & Caceres, 2004).

Mycalesina butterflies (Nymphalidae: Satyrinae: Satyrini) are distributed throughout the Old-World tropics and have undergone parallel radiations in Southeast Asia, Sub-Saharan mainland Africa and Madagascar (Aduse-Poku et al., 2015; Kodandaramaiah et al., 2010). The adults of many Mycalesina butterflies show seasonal polyphenism in wing pattern with alternative wet and dry season forms produced through developmental phenotypic plasticity (Brakefield & Larsen, 1984; Brakefield & Reitsma, 1991). The wet season form has characteristic conspicuous eyespots with concentric coloured rings positioned along the wing margins. These have been shown to function in deflecting predator attacks to the wing margins and away from more vulnerable body parts (Lyytinen, Brakefield, Lindström, & Mappes, 2004; Lyytinen, Brakefield, & Mappes, 2003; Olofsson, Vallin, Jakobsson, & Wiklund, 2010; Prudic, Stoehr, Wasik, & Monteiro, 2015). In contrast, the dry season form generally lack these eyespots, and they rely mainly on crypsis when at rest on dead leaf litter for survival against predators hunting by sight (Lyytinen et al., 2003, 2004; Prudic et al., 2015). Along with the wing pattern changes, both forms differ in a suite of other morphological, behavioural, physiological and life-history traits, as an integrated response which is linked by a common hormonal switch as has been extensively explored in laboratory settings in a single species Bicyclus anynana (Oostra et al., 2010, 2014; van Bergen & Beldade, 2019; van Bergen et al., 2017). Such an integrated system helps to maintain a season-specific adaptive phenotype by making use of environmental cues to predict approaching seasonal shifts (Brakefield & Reitsma, 1991; Kooi & Brakefield, 1999).

Species of the African Mycalesina genus *Bicyclus* inhabit both forests and open savannah habitats with forest species probably reflecting the ancestral state (van Bergen, 2016). They mainly use grasses (Poaceae) as their larval host plants with a few records of utilizing plants belonging to Zingiberaceae and Marantaceae family (Lasen, 2005). Stable isotope analysis has also shown that forest and savannah species of Bicyclus prefer C<sub>3</sub> or C<sub>4</sub> grasses, respectively, as larval host plants (van Bergen et al., 2016). C<sub>3</sub> grasses are usually forest-linked, while C<sub>4</sub> grasses predominate in savannah grasslands, the former being the ancestral state (Edwards, Osborne, Stromberg, Smith, & C4 Grasses Consortium, 2010). Savannah grasslands began to open up from forest-dominated habitats in Africa during the Late Miocene and Pliocene (3-8 Ma) (Cerling et al., 1997; Edwards et al., 2010; Jacobs, 2004; Osborne, 2008). Interestingly, Bicyclus (and the Satyrini tribe in general) showed a burst of radiation at this time suggesting that expansion of savannahs opened up new niches and subsequent colonization of these habitats may have resulted in the rapid radiation of these butterflies (Aduse-Poku et al., 2015; Brakefield, 2010; Kodandaramaiah et al., 2010; Peña & Wahlberg, 2008). However, colonizing highly seasonal savannahs from more stable forest habitats may only have been possible through the evolution of key adaptations (Brakefield, 2010).

One such potential key trait, that has been hypothesized to be used by Bicyclus, is the capacity of adults to undergo reproductive diapause during the dry season. During reproductive diapause, the development of oogenesis, vitellogenesis, accessory glands, and mating behaviour is arrested (Tatar & Yin, 2001; Tauber et al., 1986). In Bicyclus butterflies, B. anynana in particular, when entering reproductive diapause, females arrest reproduction coupled with changes in other traits such as cryptic colour pattern, large body size, increased fat deposits, increased longevity, and reduced behavioural activity for the entire dry season until the onset of the rains (Brakefield & Reitsma, 1991; Oostra et al., 2014; van Bergen et al., 2017). The induction of diapause and correlated expression of relevant traits is dependent on the availability of environmental cues, mainly temperature in the case of B. anynana, that reliably predict the future adult environment (Brakefield & Reitsma, 1991; for temperate insects, see Nylin, 2013). This would enable a suspension of reproductive effort, while still allowing opportunistic feeding and dispersal behaviour by adults and allowing a rapid switch to reproduction as the rains arrive and larval host plants become available again. However, this hypothesis has rarely been tested in the field. A survey on three Australian Mycalesina butterflies showed that species exhibit different mating strategies in the dry season in a habitat-dependent manner. Thus, the savannah species undergo reproductive diapause, whereas forest species reproduce continuously in line with constant availability of host plants throughout the year (Braby, 1995; Moore, 1986). Another study in Zomba, Malawi, examined 49 females of B. safitza from the peak of the dry season and found that 36% of these carried spermatophores, a clear evidence of having mated. They also had mature eggs in the dry season, apparently indicating that not all adults of this species undergo reproductive diapause (Brakefield & Reitsma, 1991).

In this study, we explore these patterns further. First, we use 3 years of continuously collected field samples from Zomba, Malawi, and document three different mating strategies that females use in the dry season: no reproductive diapause or reproductive diapause, either with mating before diapause (pre-diapause mating) or with mating only after diapause (complete reproductive diapause). Second, by examining museum samples for an additional 37 species, we demonstrate similar three mating strategies across all the species. Furthermore, phylogenetic analyses show that habitat preference and mating strategy evolve in a correlated fashion with nondipausing forest species as the ancestral state. We also construct an evolutionary pathway which suggests that the gain of the ability to show reproductive diapause is a key trait in facilitating colonization of savannahs in these butterflies in the Late Miocene and Pliocene.

### 2 | MATERIALS AND METHODS

# 2.1 | Using long-term field samples to investigate mating strategies in the dry season

We used field-collected samples to explore the potential mating and reproductive strategies used by *Bicyclus* butterflies in the dry season. Butterflies were collected daily using three fruit-baited traps (fermented banana) operated over 3 years from the beginning of the dry season in 1995 until the end of the wet season in 1998 in Zomba, Malawi, and all the collected samples were dried after collection and stored in glassine envelopes. These samples have previously been used in another study (van Bergen et al., 2016) where the specimens we now refer to as *B. campina* were treated as *B. vansoni*. Our reassignment of their species identity was based on the geographical location and the wing morphology, as well as some doubts about the validity of the species *B. vansoni* (O. Brattström, personal communication, January 21, 2019).

Three species, *B. campina*, *B. safitza* and *B. ena*, were abundant in the collections and well represented across all the 3 years of sampling. *B. campina* mainly prefer forest habitats, including forest edges (Brakefield & Reitsma, 1991; van Bergen et al., 2016; Windig, Brakefield, Reitsma, & Wilson, 1994). *B. safitza* is one of the most abundant and widely distributed species of *Bicyclus* which mainly occurs in open savannah and degraded forests (Brakefield & Reitsma, 1991; van Bergen et al., 2016; Windig et al., 1994). *B. ena* mainly occurs in the open savannahs with a preference for dry rocky habitats (Brakefield & Reitsma, 1991; van Bergen et al., 2016; Windig et al., 1994).

In butterflies, the male transfers sperm along with some nutrients in a spermatophore which is stored in the *bursa copulatrix* of the female (Burns, 1968). The presence/absence and the number of spermatophores has been widely used as a good proxy to infer mating status and mating rate from field caught samples (Braby, 1995; Burns, 1968; Larsdotter Mellström & Wiklund, 2010). Although spermatophores can become degraded upon utilization by the female, some remnants almost always remain making it possible to accurately count them (Burns, 1968; but see Walters, Stafford, Hardcastle, & Jiggins, 2012).

We aimed to dissect at least 10 females of each species per month throughout the whole sample period, but enough individuals were not always available in December to January. For carrying out dissections, we first soaked the abdomens in 0.4 ml of 5% trisodium phosphate and Ilfotol solution (master mix of 50 ml containing 48 ml 5% of trisodium phosphate in water and 2 ml Ilfotol, modified from Burns, 1968), and kept them at room temperature overnight (c. 12–15 hr). During the dissections, we counted spermatophores and noted the presence/absence of mature (yolk filled) eggs.

To classify butterflies into seasonal forms, we photographed all the individuals and measured the relative area of the CuA<sub>1</sub> eyespot and hindwing wing area using a custom written macro in the IMAGEJ 1.8.0 software (Schneider, Rasband, & Eliceiri, 2012; Figure S1). We plotted histograms to explore the distribution of eyespot areas, and butterflies were then classified as the wet or dry form by setting a threshold (see van Bergen et al., 2016; Figure S2). We estimated repeatabilities by re-measuring a sub-sample of 60 butterflies on different dates giving  $R^2$  values of .98 and .96 for eyespot area and wing area, respectively.

# 2.2 | Sampling populations across habitats to test variation in the mating strategy

Insect mating strategies can change across populations inhabiting different habitats (Denlinger, 1986; Dingle, 1968; Tauber et al., 1986). To test whether this happens in *Bicyclus*, we used samples collected from a field survey in Ghana conducted in the early dry season from 5 to 19 December 2018. We sampled two selected species, *B. funebris* and *B. vulgaris*, from multiple populations in Ghana across different habitats ranging from wet rainforest to dry forest edges. Similarly, we checked three populations of *B. anynana* from personal collections and museum samples from South Africa, Tanzania and Zimbabwe to test whether there is geographical variation in mating strategy. Samples from South Africa and Zimbabwe were sampled from a single dry season but from different years, while samples from Tanzania were combined across different years.

### 2.3 | Sampling of species across the phylogeny

In addition to the three *Bicyclus* species from Zomba, we surveyed smaller numbers of samples of 37 additional *Bicyclus* species collected in the middle of the dry season. We primarily used material from the collections of the African Butterfly Research Institute (ABRI) in Nairobi, Kenya, and from field surveys across several sites in Ghana (December 2018) and Kakamega forest in Kenya (February 2019), and from the personal collection of Oskar Brattström. We aimed to sample 10 specimens per species from museum samples and up to 25 samples per site in the field. For sampling the museum specimens from the middle of the dry season, we first checked annual climate graphs for the specific locality to identify a suitable time period as distant as possible from any substantial rainfall. Analysis of the long-term samples from the peak dry

season (July–October) is sufficient to identify the mating strategy (see Section 3) and all our additional species could be fitted into the same classifications. For some of the additional species, it was not possible to analyse only individuals from a single location, and therefore we occasionally combined samples from multiple sites and sometimes countries. This did not affect assigning species to specific mating strategies as populations exhibited the same strategy across populations (see Section 3).

We sampled a total of 228 specimens from 23 species from the collections at ABRI (mean per species = 9.9; range = 6–19; Table S1). The field sampling in Ghana provided 334 specimens from 11 species (M = 30.7; range = 7–100; Table S2) and the Kenyan sampling provided 73 specimens from 5 species (M = 14.6; range = 7–37; Table S1). We also included 25 Nigerian and South-African samples from personal collections of two species (M = 12.5; range = 9–16; Table S1). Combining these data with the three species from our long-term collection, our final dataset consisted of 40 species of *Bicyclus*.

# 2.4 | Classification of mating and reproductive strategies, and habitat preferences

Following examination of the total dataset for 40 species, we used the percentage of females with presence of spermatophores and mature eggs to categorize species into one of three dry season mating and reproductive strategies: (a) non-diapausing with continuous reproduction (henceforth non-diapausing) where females have spermatophores and mature eggs in the dry season, (b) completely diapausing with no spermatophores or eggs in the middle of the dry season and mating occurs only at the end (in a few cases a small proportion of mid-season females have spermatophores) and (c) prediapause mating where females mate in the early dry season but do not develop eggs until the end of the dry season (see Kato, 1986). In this strategy, females may mate again in the late dry season (Kato, 1986).

For categorizing species into one of the three mating strategies, we considered a threshold of 50%. That is, if  $\geq$ 50% of females had spermatophores and mature eggs, then the species was classified as non-diapausing. Similarly, if <50% of females had spermatophores and eggs it was considered as a diapausing species. In cases where  $\geq$ 50% of females had spermatophores but less than half of those had eggs, it was considered as a pre-diapause mating species. Most of the species exhibited clear mating strategies except for two forest species, *B. dorothea* and *B. procora*, which had 50% of specimens containing spermatophores and eggs. There is evidence that time to reproductive maturity is longer in forest than open habitat species and hence it is possible that these species may take a longer time to mate and develop eggs (Braby, 2002). Based on this reasoning, we classified both these species as non-diapausing.

In the case of one species *B. larseni*, one of the smallest of all *Bicyclus* species, it was difficult to find spermatophores in 78% of specimens, but the majority had mature eggs. We classified this

species as non-diapausing. This is justified by the observation that it is costly for the female to have mature eggs without mating in the dry season, as heavy abdomens can affect flight and hence increase predation (Marden & Chai, 1991).

Habitat preference was classified for each species as one of three categories: forest-restricted, forest-dependent or savannah. This was based on literature (Condamin, 1973; Kielland, 1990; Lasen, 2005; van Bergen, 2016), and our own extensive experience from field work. Forest-dependent species usually occur in forest fringes and can tolerate some degree of habitat degradation but are generally not found in true savannah habitats. While, forest-restricted species are generally found in rainforests.

#### 2.5 | Ancestral state reconstruction

We used an updated recent ultrametric phylogeny of Mycalesina (O. Brattström, K. Aduse-Poku, E. van Bergen, V. French, & P.M. Brakefield, Unpublished data, February 12, 2019) which is built on previous phylogenies (Aduse-Poku, Brakefield, Wahlberg, & Brattström, 2017; Aduse-Poku et al., 2015) for our phylogenetic analyses after trimming it down to those 40 species surveyed here. We performed ancestral state reconstruction to visualize the evolution of mating strategies and habitat preference applying Bayesian stochastic mapping of ancestral states (Bollback, 2006; Huelsenbeck, Nielsen, & Bollback, 2003) by simulating characters 1,000 times on maximum clade credibility tree using make.simmap function implemented in Phytools Ver.0.6.60 (Revell, 2012). For both investigated traits, we fitted three models of character evolution: equal rates, symmetric and all rates different model. The models were fitted using the fitMk function in PHYTOOLS and the best fitting model was chosen based on the Akaike information criteria (AIC) weights and was then used for the ancestral state reconstruction. Unless otherwise stated, we performed all the analyses in RSTUDIO Ver.3.4.4. 'Kite-Eating Tree' (R Development Core Team, 2017).

# 2.6 | Testing for correlated evolution of habitat preference and mating strategies

We used DISCRETE model in BAYESTRAITS V.3.0.1 (Meade & Pagel, 2016) to test for evidence of correlated evolution between habitat preference and mating strategy which implements Reversible Jump Markov Chain Monte Carlo (RJ MCMC) to model all the possible forward and backward transitions but does not allow simultaneous transitions in both the states (Pagel, 1994; Pagel & Meade, 2006). For the analysis of correlated evolution of discrete traits, this is currently the only available method and the DISCRETE model is limited to binary traits. While both the habitat preference and mating strategy data had three states. Therefore, we merged the diapausing and pre-diapause mating strategies into a single category. This is biologically justified because in both strategies females have immature oocytes and do not undergo active reproduction even if they mate and store spermatophores, such as in pre-diapause mating (see Kato, 1986, 1989). Similarly, for habitat preference, the forest-dependent state was merged with forest-restricted to form a single forestlinked state. Forest-dependent species usually occur in the forest fringes or edges but never in true savannah habitats suggesting that forest cover is essential. Hence, merging this category with forest-restricted species is more appropriate than into savannahs.

The test for correlated evolution was performed by evaluating support for the independent and dependent model. In the dependent model, the rate of change of one trait depends on the other trait, while the independent model considers no such association. We ran RJ MCMC chains for 10 million iterations with a burnin period of one million after which the chain was sampled every 500th iteration. The chains were visually inspected for convergence. We used exponential hyperprior 0-10 and placed 1,000 stepping stones each iterating 10,000 times to obtain the marginal likelihood value for both the independent and dependent models. We ran the analysis three times to check for the stability of the likelihood values. We then calculated Bayes Factor which is a measure of the strength of correlated evolution using the marginal likelihood value for both models. A value between 2 and 6 is considered as positive evidence, whereas the value of 6-10 is considered strong evidence for correlated evolution (Currie & Meade, 2014).

Furthermore, using the values of transition parameters resulting from RJ MCMC, we calculated *Z* values for the transitions which is the proportion of times a certain transition parameter is set to zero among all sampled models (Pagel & Meade, 2006). A high proportion of zeros, and hence a high *Z* value, suggests that the transition between states is unlikely. Using the *Z* values, we constructed a pathway for habitat-dependent evolution of mating strategies.

#### 2.7 | Validation of co-evolutionary models

We used five approaches to validate our co-evolutionary models. (a) We tested whether our analysis is sensitive to the choice of prior using two more priors, uniform 0-100 and exponential hyperprior 0-100. (b) We tested the effect of using different thresholds for classification of mating strategies using an additional threshold of 10% and 20%. That is, species having spermatophores and eggs greater and lower than the threshold were classified as nondiapausing and diapausing species, respectively. And, pre-diapause mating if presence of spermatophores were greater and eggs less than the threshold. (c) Two species, B. dorothea and B. procora, in which females had 50% of spermatophores and eggs, were reclassified as diapausing species (contrary to non-diapausing in the original dataset) to test whether our analysis is sensitive to this classification. (d) We tested if merging forest-dependent state with savannahs affects our conclusion regarding correlated evolution compared to the original dataset where forest-dependent

state was merged with forest-restricted. (e) We also tested the effect of sample size on the co-evolutionary models by randomly removing 30% of species from the dataset and repeated this with 10 different subsets (for similar analysis, see Watts, Sheehan, Atkinson, Bulbulia, & Gray, 2016).

#### 3 | RESULTS

# 3.1 | Seasonality and mating patterns in the long-term data

A total of 232 B. campina, 373 B. safitza and 206 B. ena females from Zomba, Malawi were dissected. Over the whole series of samples from the peak dry season (July-October) over three annual cycles, 61% of *B. camping* females had spermatophores and eggs, while in B. safitza 22% and 20% of females had spermatophore and eggs, respectively. Interestingly, in B. ena, 88% of the females had spermatophores, but only 28% of all females had eggs (Figure 1). Reproductive activity appeared to increase at the end of the dry season just before the onset of the rain in November (Figure 1). Furthermore, in all the three species, there was no significant yearly variation in the proportion of females that had spermatophores and eggs in the middle of the dry season ( $\chi^2$  test, p > .05). Combining all seasons for all three species, the presence of mature eggs was correlated with the presence of spermatophores in 98% of the females, but not vice versa (i.e. spermatophores did not predict egg presence). Based on the percentage of females having spermatophores and eggs in the dry season, B. campina was classified as a non-diapausing species. In B. ena, females had spermatophores but generally lacked mature eggs, and so it was classified as a pre-diapause mating species. In B. safitza, only about a quarter of the females were mated and had eggs, and it was classified as a diapausing species.

#### 3.2 | Mating strategies across populations

In the two species, *B. funebris* and *B. vulgaris*, with samples from five and four localities, respectively, the mating strategy (diapausing) was consistent across all populations (Table 1). In addition, *B. anynana* from South Africa, Tanzania and Zimbabwe exhibited the same mating strategy (pre-diapause mating; Table 2).

### 3.3 | Ancestral state reconstruction

For ancestral state reconstruction, the equal rates model showed the highest AIC weight for both habitat preferences and mating strategies (Table S2). Ancestral state reconstruction of mating strategies suggested that non-diapausing was the ancestral state. Similarly, for habitat preference, forest-restricted was indicated as the ancestral state (Figure 2).



**FIGURE 1** Mating strategies of females for three *Bicyclus* species, *B. safitza, B. campina* and *B. ena* across alternating wet and dry seasons in Zomba, Malawi, from July 1995 to May 1998. Circles in the top three left panels represent individual butterflies (brown, dry season form; green, wet season form; open circles, mature eggs absent; filled circles, mature eggs present) with variation in temperature (red line) and rainfall (blue bars) shown in the lower panel. The seasons are marked with brown (dry) and green (wet) overlay across all panels. Note that the data points of top three left panels are jittered for clarity. The dry season is characterized by lower temperature and usually lasts from June to mid-November. In contrast, the wet season is characterized by a higher temperature and abundant rain and usually lasts from late November until late May. Proportion plots besides each species depict the percentage of females carrying spermatophores and eggs in the peak dry season (July–October)

TABLE 1	Populations of	of two species of	Bicyclus s	sampled across	different habitat	s in Ghana
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	Amedzofe		Atewa		Bonkro		Bobiri		Oporo	
Species	N	% mated	N	% mated	N	% mated	N	% mated	N	% mated
B. funebris	13	0	26	0	15	0	19	0	21	9.52
B. vulgaris	13	0	20	5	-	_	15	0	20	5

*Note*: In both the species, the presence of spermatophores was always associated with the presence of mature eggs, and hence only the percentage of females having spermatophores is provided in the table (Amedzofe, slightly degraded forest; Atewa, forest; Bonkro, forest; Oporo, forest-savannah ecotone; Bobiri, forest).

#### HALALI ET AL.

# 3.4 | Correlated evolution and the evolutionary pathway

Using exponential hyperprior 0–10, the dependent model of evolution provided a better fit (average marginal likelihood: –40.48) than

**TABLE 2** Mating strategies across three populations of *Bicyclus anynana*, a species which exhibits the pre-diapause mating strategy

	N	% mated	% presence of eggs
South Africa	16	100	0
Tanzania	9	77.77	22.22
Zimbabwe	10	100	0

the independent model (average marginal likelihood: -47.49), and the values were similar for three independent runs (Table S3). The marginal likelihood value for both the models resulted in an average Bayes Factor of 14.01. The likelihood values and Bayes factor were similar for the other two priors, uniform 0-100 and exponential hyperprior 0-100, albeit slightly lower for the former prior (Table S3). Furthermore, the analyses conducted to test the sensitivity of co-evolutionary models to classification of habitat preference, mating strategies and sample size, all provided consistent positive support for correlated evolution (Tables S4–S7).

The evolutionary pathway (Figure 3) shows that the transition from the ancestral state (Forest, Non-diapausing) to the derived state (Savannah, Diapausing) was generally reached through one of the two possible intermediate states (Forest, Diapausing). The



FIGURE 2 Ancestral state reconstruction for habitat preferences (left) and mating strategies (right) for species of Bicyclus butterfly

**FIGURE 3** The evolutionary pathway depicting the habitat-dependent evolution of the dry season mating strategies using 50% threshold and exponential hyperprior 0–10. The arrows represent three rate classes: thick black arrows are highly likely transitions between the states (Z < 0.20); thin black arrows are the transitions with low confidence (Z value from 0.20 to 0.80); dashed red arrows represent unlikely transitions (Z > 0.80). The mean of posterior distribution of transition parameters are provided in the parentheses



transition rates leading up to the other alternative intermediate state (Savannah, Non-diapausing) were assigned to zero on most occasions and none of our sampled species show this combination of traits.

### 4 | DISCUSSION

Seasonal cycles in the tropics result in a highly uneven distribution of resources, including a minimal availability of resources in the dry season. Also, the extent of seasonality can vary across habitats, and forests may buffer extreme climatic variation across seasons while savannahs are highly seasonal (Archibald & Scholes, 2007). These types of dynamics can affect reproductive strategies in insects (Braby, 1995; Jones, 1987; Moore, 1986; Tauber et al., 1986). For example, populations or species inhabiting areas where larval hostplants are present throughout the year reproduce continuously, while they diapause where hostplant availability is seasonal (Braby, 1995; Dingle, 1968; Jones & Rienks, 1987). Such differential distribution of larval host plants in both habitats likely to have led to the evolution of habitat-dependent mating strategies in Mycalesina butterflies. Adults of Bicyclus butterflies show three different dry season reproductive strategies: non-diapausing, diapausing and prediapause mating. The incidence of these strategies varies across habitats. In general, the majority of forest-restricted species do not diapause, while all the savannah species show one of the two strategies involving some sort of reproductive diapause. Differences in strategies among species can be attributed to the phenology of host plants.

Some Bicyclus species of savannah habitats exhibit a prediapause mating strategy where females typically mate at the start of the dry season, store and perhaps utilize spermatophores, and then produce eggs at the onset of the rains. Two adaptive hypotheses have been proposed for this type of strategy. First, spermatophores could provide nutrients to the female which may increase survival in the harsh dry season (Kato, 1986, 1989; Konagaya & Watanabe, 2015). This is especially relevant for savannah species as nutrients provided by spermatophores may favour female longevity until the end of the dry season (Svärd & Wiklund, 1988). Second, if the probability of encounters with potential mates after diapause is low due to high dry season mortality of males, mating before the onset of diapause would reduce the risk of being unfertilized when larval host plant conditions are favourable for oviposition. With respect to Bicyclus, this enables females to lay eggs immediately at the end of the dry season without any time lag in finding mates. Pre-diapause mating has been shown to occur in the Japanese population of a butterfly Eurema mandarina which shows a diapausing autumn-morph and a non-diapausing summer-morph (Kato, 1986, 1989; Konagaya & Watanabe, 2015). In this species, autumn-morph females mate with the summer-morph males at the start of the winter. In contrast, autumn-morph males diapause and show reproductive activity only at the end of the winters, and hence females may mate again with the same morph males. Whether this scenario is similar in Bicyclus butterflies is unclear, but it is possible that the dry season form females may mate with the wet season form males as there can be some overlap in flight between both forms. However, complete reproductive diapause could still be beneficial for females as mating at the end of the dry season means that their mate has expressed a strong dry season survival ability. Such a high mate quality might be more important than the potential benefits of the pre-diapause mating in some habitats, explaining why both strategies appear throughout *Bicyclus*.

Furthermore, the type of female reproductive strategy may also determine the male reproductive strategy (Gotthard, Nylin, & Wiklund, 2000; Wiklund, Gotthard, & Nylin, 2003). For example, given that females reproduce in the dry season in non-diapausing species, males are also expected to be reproductively active. In contrast, in completely diapausing species where females arrest their reproduction, males are also expected to enter reproductive diapause as females are inactive. Also, active reproduction trades-off with longevity (Ellers & van Alphen, 2002) and hence not entering diapause when females are inactive may decrease the probability of survival of males until the end of the dry season. For pre-diapause mating species, two scenarios are possible. First, when the dry season males are mating with the dry season females, then early mortality of males is expected due to the reproduction-longevity trade-off (for a similar scenario, see Wiklund et al., 2003). Second, if the wet season males are mating with the dry season females in a similar way to E. mandarina (Kato, 1986, 1989) as discussed above, then the dry season males are expected to undergo complete reproductive diapause.

In our long-term field samples, B. safitza exhibited an interesting pattern in which about one-quarter of the females had mature eggs in the peak dry season. The data show that the dry season form females tend to mate more often and have mature eggs in the early part, rather than in the middle of the dry season. This could be a bet-hedging strategy where a small proportion of females are reproductively active, while others undergo reproductive diapause (e.g. Bradford & Roff, 1993). B. safitza predominantly prefers C<sub>4</sub> grasses but in the dry season some individuals shift their preference towards utilization of C<sub>3</sub> grasses in the forest (Nokelainen, Ripley, van Bergen, Osborne, & Brakefield, 2016; van Bergen et al., 2016). Hence, it is possible that a few reproductively active females found in the dry season might have shifted their preference to C<sub>3</sub> grasses. Such mixed strategies can only be fully identified properly by longitudinal analysis of large number of samples in multiple dry seasons which is lacking in our museum samples. With few exceptions, a majority of our sampled species from the museum and smaller field collections showed a much clearer pattern suggesting that potential mixed strategies are rare. Two forest species, B. dorothea and B. procora, had 50% of spermatophores and eggs. There is evidence that time to reproductive maturity is longer in forest species (Braby, 2002) and hence detecting reproductive activity in 50% of specimens in these species may be due to delayed mating and slower maturation of eggs. On the contrary, if the species undergoes complete reproductive diapause, it is expected that all the females should be reproductively inactive. Our samples showed that a small percentage of females were mated with mature eggs but testing the robustness of our co-evolutionary models by setting narrow thresholds of 10% and 20% to classify species as diapausing consistently provided support for correlated evolution between habitat preference and mating strategies (Table S6).

Ancestral state reconstructions for habitat preference show that the ancestral *Bicyclus* were most likely forest-restricted species. This is in accordance with other studies demonstrating that forest habitats were ancestral followed by a more recent expansion of savannahs in the Late Miocene and Pliocene (Cerling et al., 1997; Edwards et al., 2010). In our analyses, the more ancestral clades are largely dominated by forest-restricted species, but there have been multiple independent colonization events into drier and more seasonal habitats during the Late Miocene and Pliocene. Similarly, for mating strategies, two diapause-related strategies evolved during a similar time frame to that of habitat preferences. This congruent pattern suggests that the Late Miocene–Pliocene expansion of savannahs led to a rapid turnover in habitat preference and mating strategies in *Bicyclus* butterflies.

The evolution of key traits or innovations allows species to exploit novel niches and can also facilitate diversification (Hunter, 1998). It has been hypothesized that expansion of savannahs during the Late Miocene-Pliocene opened up new niches and their eventual colonization triggered diversification in the Mycalesina butterflies (Brakefield, 2010). Such savanna-linked diversification dynamics has been documented in several taxonomic groups (Aduse-Poku, Vingerhoedt, & Wahlberg, 2009; Cerling et al., 1997; Davis, Bakewell, Hill, Song, & Mayhew, 2018; De Silva, Peterson, Bates, Fernando, & Girard, 2017; Fuchs, Johnson, & Mindell, 2015; Kergoat et al., 2018; MacFadden & Hulbert, 1988; van Velzen, Wahlberg, Sosef, & Bakker, 2013; Zarlenga, Rosenthal, La Rosa, Pozio & Hoberg, 2006; Toussaint, et al., 2012). Bicyclus and other butterfly genera (e.g. Cymothoe and Charaxes, as well as the Satyrinae tribe in general) show a comparable burst of diversification during the Late Miocene which coincides with the expansion of savannahs (Aduse-Poku et al., 2009; Peña & Wahlberg, 2008; van Velzen et al., 2013). Cymothoe and Charaxes are generally forest species and diversification in these genera is hypothesized to be due to allopatric speciation driven by extensive forest fragmentation caused by savannah expansion (Aduse-Poku et al., 2009; van Velzen et al., 2013). However, in Bicyclus, the forest species have radiated into savannahs and colonizing these habitats may have required novel adaptations, especially to survive in the resource-depleted dry season environment compared to the stable ancestral forest habitat. Though many studies have explored the link between the expansion of savannahs and diversification dynamics, potential key trait(s) that may have facilitated initial colonization of these habitats have seldom been explored. For example, the comparative studies of tooth morphology from fossil horses show that the grazing species from the Late Miocene had higher crown length than ancestral browsing species suggesting such adaptations may have helped in efficient grazing of tough C<sub>4</sub> grasses (MacFadden, 2005; MacFadden & Hulbert, 1988). Similarly, grasshoppers feeding on  $C_3$  and  $C_4$  grasses show different mandibular morphology (Patterson, 1984), but this study lacked a phylogenetic approach. These studies reveal probable adaptations in herbivores linked to change in the vegetation structure during the Late Miocene.

The evolutionary pathway for Bicyclus species suggests that the gain of a capacity to undergo reproductive diapause in forest species preceded the habitat shift to savannahs (Figure 3). It is therefore possible that the initial gain of diapause in forest species evolved to utilize drier forest edges which likely became a more common habitat as a result of extensive fragmentation during the savannah expansions (Jacobs, 2004). However, ancestral state reconstructions suggest that around 10 Ma the ancestors of the species that later colonized savannahs were generally forestdependent species, but without a capacity to diapause (Figure 2). Taking this into consideration, it appears that diapause was not strictly necessary to use forest edge habitats, but this trait evolved quickly multiple times when Bicyclus began to inhabit more open and seasonal habitats 5-10 Ma. Given the multitude of current forest-dependent species that follow a diapausing strategy, it is likely that a continuing drying out of forest edge habitats played a key part in the evolution of reproductive diapause, and that having this ability further enabled Bicyclus species to eventually colonize novel savannah habitats.

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#### AUTHORS' CONTRIBUTIONS

S.H., P.M.B. and O.B. designed the study. S.H., O.B. and S.C.C. carried out the field work and collected samples from the museum. S.H. carried out all the analyses and wrote the manuscript with inputs from all the authors.

#### DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi. org/10.5061/dryad.h44j0zpfv (Halali, Brakefield, Collins, & Brattström, 2019).

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#### REFERENCES

Aduse-Poku, K., Brakefield, P. M., Wahlberg, N., & Brattström, O. (2017). Expanded molecular phylogeny of the genus *Bicyclus* (Lepidoptera: Nymphalidae) shows the importance of increased sampling for detecting semi-cryptic species and highlights potentials for future studies. Systematics and Biodiversity, 15, 115–130. https://doi.org/10.1080/ 14772000.2016.1226979

- Aduse-Poku, K., Brattström, O., Kodandaramaiah, U., Lees, D. C., Brakefield, P. M., & Wahlberg, N. (2015). Systematics and historical biogeography of the old world butterfly subtribe Mycalesina (Lepidoptera: Nymphalidae: Satyrinae). BMC Evolutionary Biolology, 15, 167. https://doi.org/10.1186/s12862-015-0449-3
- Aduse-Poku, K., Vingerhoedt, E., & Wahlberg, N. (2009). Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetic* and Evolution, 53, 463–478. https://doi.org/10.1016/j.ympev.2009. 06.021
- Archibald, S., & Scholes, R. J. (2007). Leaf green-up in a semi-arid African savanna-separating tree and grass responses to environmental cues. *Journal of Vegetation Science*, 18, 583–594.
- Bollback, J. P. (2006). SIMMAP: Stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics, 7, 88. https://doi. org/10.1186/1471-2105-7-88
- Braby, M. F. (1995). Reproductive seasonality in tropical satyrine butterflies: Strategies for the dry season. *Ecological Entomology*, 20, 5–17. https://doi.org/10.1111/j.1365-2311.1995.tb00423.x
- Braby, M. F. (2002). Life-history strategies and habitat templets of tropical butterflies in north-eastern Australia. Evolutionary Ecology, 16, 399–413. https://doi.org/10.1023/A:1020226426031
- Bradford, M. J., & Roff, D. A. (1993). Bet hedging and the diapause strategies of the cricket Allonemobius fasciatus. Ecology, 74, 1129-1135. https://doi.org/10.2307/1940482
- Brakefield, P. M. (2010). Radiations of mycalesine butterflies and opening up their exploration of morphospace. *The American Naturalist*, 176(Suppl. 1), S77-S87. https://doi.org/10.1086/657059
- Brakefield, P. M., & Larsen, T. B. (1984). The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biological Journal of Linnean Society*, 22(1), 1–12. https://doi.org/10.1111/j.1095-8312.1984.tb00795.x
- Brakefield, P. M., & Reitsma, N. (1991). Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. *Ecological Entomology*, *16*, 291–303. https://doi.org/10.1111/ j.1365-2311.1991.tb00220.x
- Burns, J. M. (1968). Mating frequency in natural populations of skippers and butterflies as determined by spermatophore counts. Proceedings of the National Academy of Sciences of the United States of America, 61, 852–859.
- Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., & Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature*, 389, 153–158.
- Condamin, M. (1973). Monographie du genre Bicyclus (Lepidoptera: Satyridae). Dakar, Senegal: IFAN-Dakar.
- Currie, T. E., & Meade, A. (2014). Keeping yourself updated: Bayesian approaches in phylogentic comparative methods with a focus on Markov chain models of discrete character evolution. In L. Z. Garamszegi (Ed.), Modern phylogenetic comparative methods and their applications in evolutionary biology (pp. 263–286). Berlin, Germany: Springer.
- Davis, K. E., Bakewell, A. T., Hill, J., Song, H., & Mayhew, P. (2018). Global cooling & the rise of modern grasslands: Revealing cause & effect of environmental change on insect diversification dynamics. *BioRxiv*, 392712. https://doi.org/10.1101/392712
- De Silva, T. N., Peterson, A. T., Bates, J. M., Fernando, S. W., & Girard, M. G. (2017). Phylogenetic relationships of weaverbirds (Aves: Ploceidae): A first robust phylogeny based on mitochondrial and nuclear markers. *Molecular Phylogenetics and Evolution*, 109, 21-32. https://doi.org/10.1016/j.ympev.2016.12.013
- Denlinger, D. L. (1986). Dormancy in tropical insects. Annual Review of Entomology, 31, 239–264. https://doi.org/10.1146/annurev.en.31. 010186.001323

- Dingle, H. (1968). Migration and diapause in tropical, temperate, and island milkweed bugs. In H. Dingle (Ed.), Evolution of insect migration and diapause (pp. 254–276). New York, NY: Springer.
- Edwards, E. J., Osborne, C. P., Stromberg, C. A. E., Smith, S. A.; C4 Grasses Consortium. (2010). The origins of C4 Grasslands: Integrating evolutionary and ecosystem science. *Science*, *328*, 587-591.
- Ellers, J., & Van Alphen, J. J. (2002). A trade-off between diapause duration and fitness in female parasitoids. *Ecological Entomology*, 27, 279–284. https://doi.org/10.1046/j.1365-2311.2002.00421.x
- Fuchs, J., Johnson, J. A., & Mindell, D. P. (2015). Rapid diversification of falcons (Aves: Falconidae) due to expansion of open habitats in the Late Miocene. *Molecular Phylogenetics and Evolution*, 82, 166–182. https://doi.org/10.1016/j.ympev.2014.08.010
- Furness, A. I., Reznick, D. N., Springer, M. S., & Meredith, R. W. (2015). Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. *Proceedings of Royal Society B: Biological Sciences*, 282, 1–9. https:// doi.org/10.1098/rspb.2014.2189
- Gotthard, K., Nylin, S., & Wiklund, C. (2000). Mating opportunity and the evolution of sex-specific mortality rates in a butterfly. *Oecologia*, 122(1), 36–43. https://doi.org/10.1007/PL00008833
- Hahn, D. A., & Denlinger, D. L. (2007). Meeting the energetic demands of insect diapause: Nutrient storage and utilization. *Journal of Insect Physiology*, 53, 760–773. https://doi.org/10.1016/j.jinsphys.2007. 03.018
- Hahn, D. A., & Denlinger, D. L. (2010). Energetics of insect diapause. Annual Review of Entomology, 56, 103–121. https://doi.org/10.1146/ annurev-ento-112408-085436
- Halali, S., Brakefield, P. M., Collins, S. C., & Brattström, O. (2019). Data from: To mate, or not to mate: The evolution of reproductive diapause facilitates insect radiation into African savannahs in the Late Miocene. Dryad Digital Repository, https://doi.org/10.5061/dryad.h44j02pfv
- Huelsenbeck, J. P., Nielsen, R., & Bollback, J. P. (2003). Stochastic mapping of morphological characters. Systematic Biology, 52, 131–158. https://doi.org/10.1080/10635150390192780
- Hunter, J. P. (1998). Key innovations and the ecology of macroevolution. Trends in Ecology and Evolution, 13(1), 31–36. https://doi.org/10.1016/ S0169-5347(97)01273-1
- Jacobs, B. F. (2004). Palaeobotanical studies from tropical Africa: Relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of Royal Society B: Biological Sciences*, 359, 1573–1583.
- Jones, R. E. (1987). Reproductive strategies for the seasonal tropics. International Journal of Tropical Insect Science, 8, 515–521. https://doi. org/10.1017/S1742758400022566
- Jones, R. E., & Rienks, J. (1987). Reproductive seasonality in the tropical genus Eurema (Lepidoptera: Pieridae). Biotropica, 1, 7–16. https://doi. org/10.2307/2388454
- Kato, Y. (1986). The prediapause copulation and its significance in the butterfly Eurema hecabe. Journal of Ethology, 4(2), 81–90. https://doi. org/10.1007/BF02348109
- Kato, Y. (1989). Differences in reproductive behavior among seasonal wing morphs of the butterfly Eurema hecabe. Journal of Insect Behaviour, 2, 419–429. https://doi.org/10.1007/BF01068066
- Kergoat, G. J., Condamine, F. L., Toussaint, E. F., Capdevielle-Dulac, C., Clamens, A. L., Barbut, J., ... Le Ru, B. (2018). Opposite macroevolutionary responses to environmental changes in grasses and insects during the Neogene grassland expansion. *Nature Communications*, 9, 5089. https://doi.org/10.1038/s41467-018-07537-8
- Kielland, J. (1990). Butterflies of Tanzania. Melboune, Australia: Hill House.
- Kodandaramaiah, U., Lees, D. C., Müller, C. J., Torres, E., Karanth, K. P., & Wahlberg, N. (2010). Phylogenetics and biogeography of a spectacular Old World radiation of butterflies: The subtribe Mycalesina

(Lepidoptera: Nymphalidae: Satyrini). BMC Evolutionary Biology, 10, 172. https://doi.org/10.1186/1471-2148-10-172

- Konagaya, T., & Watanabe, M. (2015). Adaptive significance of the mating of autumn-morph females with non-overwintering summer-morph males in the Japanese common grass yellow, *Eurema mandarina* (Lepidoptera: Pieridae). *Applied Entomology and Zoology*, 50(1), 41–47. https://doi.org/10.1007/s13355-014-0300-0
- Kooi, R. E., & Brakefield, P. M. (1999). The critical period for wing pattern induction in the polyphenic tropical butterfly *Bicyclus anynana* (Satyrinae). *Journal of Insect Physiology*, 45, 201–212. https://doi. org/10.1016/S0022-1910(98)00093-6
- Larsdotter Mellström, H., & Wiklund, C. (2010). What affects mating rate? Polyandry is higher in the directly developing generation of the butterfly *Pieris napi*. Animal Behaviour, 80, 413–418. https://doi. org/10.1016/j.anbehav.2010.05.025
- Lasen, T. (2005). Butterflies of West Africa. Stenstrup, Denmark: Apollo Books.
- Lyytinen, A., Brakefield, P. M., Lindström, L., & Mappes, J. (2004). Does predation maintain eyespot plasticity in *Bicyclus anynana? Proceedings of Royal Society B*, 271, 279–283. https://doi.org/10.1098/ rspb.2003.2571
- Lyytinen, A., Brakefield, P. M., & Mappes, J. (2003). Significance of butterfly eyespots as an anti-predator device in ground-based and aerial attacks. Oikos, 100, 373–379. https://doi.org/10.1034/j.1600-0706.2003.11935.x
- MacFadden, B. J. (2005). Fossil horses Evidence for evolution. *Science*, 307, 1728–1730.
- MacFadden, B. J., & Hulbert, R. C. (1988). Explosive speciation at the base of the adaptive radiation of Miocene grazing horses. *Nature*, 336, 466–468.
- Marden, J. H., & Chai, P. (1991). Aerial predation and butterfly design: How palatability, mimicry, and the need for evasive flight constrain mass allocation. *The American Naturalist*, 138, 15–36. https://doi. org/10.1086/285202
- Meade, A., & Pagel, M. (2016). *Bayes traits*. Retrieved from http://www. evolution.rdg.ac.uk/BayesTraitsV3.0.1/BayesTraitsV3.0.1.html
- Moore, G. J. (1986). Host plant discrimination in tropical satyrine butterflies. *Oecologia*, 70, 592–595. https://doi.org/10.1007/BF00379910
- Nokelainen, O., Ripley, B. S., van Bergen, E., Osborne, C. P., & Brakefield, P. M. (2016). Preference for C4 shade grasses increases hatchling performance in the butterfly, *Bicyclus safitza*. *Ecology and Evolution*, *6*, 5246–5255. https://doi.org/10.1002/ece3.2235
- Nylin, S. (2013). Induction of diapause and seasonal morphs in butterflies and other insects: Knowns, unknowns and the challenge of integration. *Physiological Entomology*, 38, 96–104. https://doi.org/10.1111/phen.12014
- Olofsson, M., Vallin, A., Jakobsson, S., & Wiklund, C. (2010). Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. *PLoS ONE*, *5*, e10798. https://doi. org/10.1371/journal.pone.0010798
- Oostra, V., de Jong, M. A., Invergo, B. M., Kesbeke, F., Wende, F., Brakefield, P. M., & Zwaan, B. J. (2010). Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. *Proceedings of Royal Society B: Biological Sciences*, 278, 789–797. https://doi.org/10.1098/rspb.2010.1560
- Oostra, V., Mateus, A. R. A., van der Burg, K. R. L., Piessens, T., van Eijk, M., Brakefield, P. M., ... Zwaan, B. J. (2014). Ecdysteroid hormones link the juvenile environment to alternative adult life histories in a seasonal insect. *The American Naturalist*, 184, e79–e92. https://doi. org/10.1086/677260
- Osborne, C. P. (2008). Atmosphere, ecology and evolution: What drove the Miocene expansion of C4 grasslands? *Journal of Ecology*, *96*(1), 35–45. https://doi.org/10.1111/j.1365-2745.2007.01323.x
- Pagel, M. (1994). Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. *Proceedings of Royal Society B: Biological Sciences*, 255, 37–45.

- Pagel, M., & Meade, A. (2006). Bayesian analysis of correlated evolution of discrete characters by Reversible-Jump Markov Chain Monte Carlo. *The American Naturalist*, 167, 808–825. https://doi. org/10.1086/503444
- Panov, V. E., & Caceres, C. (2004). Role of diapause in dispersal of aquatic Invertebrates. *Journal of Limnology*, 63, 56–69.
- Patterson, B. D. (1984). Correlation between mandibular morphology and specific diet of some desert grassland Acrididae (Orthoptera). The American Midland Naturalist, 1, 296–303. https://doi.org/10.2307/ 2425324
- Peña, C., & Wahlberg, N. (2008). Prehistorical climate change increased diversification of a group of butterflies. *Biology Letters*, 4, 274–278. https://doi.org/10.1098/rsbl.2008.0062
- Prudic, K. L., Stoehr, A. M., Wasik, B. R., & Monteiro, A. (2015). Eyespots deflect predator attack increasing fitness and promoting the evolution of phenotypic plasticity. *Proceedings of Royal Society B: Biological Sciences*, 282, 20141531. https://doi.org/10.1098/rspb.2014.1531
- R Development Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675. https:// doi.org/10.1038/nmeth.2089
- Svärd, L., & Wiklund, C. (1988). Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly. *Behavioral Ecology Sociobiology*, 23(1), 39–43. https://doi.org/10.1007/ BF00303056
- Tatar, M., & Yin, C. M. (2001). Slow aging during insect reproductive diapause: Why butterflies, grasshoppers and flies are like worms. *Experimental Gerontology*, 36, 723–738. https://doi.org/10.1016/S0531-5565(00)00238-2
- Tauber, M. J., Tauber, C. A., & Masaki, S. (1986). Seasonal adaptations of insects. New York, NY: Oxford University Press.
- Toussaint, E. F. A., Condamine, F. L., Kergoat, G. J., Capdevielle-Dulac, C., Barbut, J., Silvain, J. F., & Le Ru, B. P. (2012). Palaeoenvironmental shifts drove the adaptive radiation of a noctuid stemborer tribe (Lepidoptera, Noctuidae, Apameini) in the Miocene. *PLoS ONE*, 7(7), e41377. https://doi.org/10.1371/journal.pone.0041377
- van Bergen, E. (2016). Into the great wide open: Exploring patterns of ecological diversification in mycalesine butterflies. PhD thesis, University of Cambridge.
- van Bergen, E., Barlow, H. S., Brattström, O., Griffiths, H., Kodandaramaiah, U., Osborne, C. P., & Brakefield, P. M. (2016). The stable isotope ecology of mycalesine butterflies: Implications for plant-insect co-evolution. *Functional Ecology*, 30, 1936–1946. https:// doi.org/10.1111/1365-2435.12673

- van Bergen, E., & Beldade, P. (2019). Seasonal plasticity for anti-predatory strategies: Matching color and color preference for effective crypsis. *Evolution Letters*. https://doi.org/10.1002/evl3.113
- van Bergen, E., Osbaldeston, D., Kodandaramaiah, U., Brattström, O., Aduse-poku, K., & Brakefield, P. M. (2017). Conserved patterns of integrated developmental plasticity in a group of polyphenic tropical butterflies. BMC Evolutionary Biology, 17, 59. https://doi.org/10.1186/ s12862-017-0907-1
- Van Velzen, R., Wahlberg, N., Sosef, M. S. M., & Bakker, F. T. (2013). Effects of changing climate on species diversification in tropical forest butterflies of the genus *Cymothoe* (Lepidoptera: Nymphalidae). *Biological Journal of Linnean Society*, 108, 546–564.
- Walters, J. R., Stafford, C., Hardcastle, T. J., & Jiggins, C. D. (2012). Evaluating female remating rates in light of spermatophore degradation in *Heliconius* butterflies: Pupal-mating monandry versus adult-mating polyandry. *Ecological Entomology*, 37, 257–268. https:// doi.org/10.1111/j.1365-2311.2012.01360.x
- Watts, J., Sheehan, O., Atkinson, Q. D., Bulbulia, J., & Gray, R. D. (2016). Ritual human sacrifice promoted and sustained the evolution of stratified societies. *Nature*, 532, 228–231. https://doi.org/10.1038/ nature17159
- Wiklund, C., Gotthard, K., & Nylin, S. (2003). Mating system and the evolution of sex-specific mortality rates in two nymphalid butterflies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 1823–1828. https://doi.org/10.1098/rspb.2003.2437
- Windig, J. J., Brakefield, P. M., Reitsma, N., & Wilson, J. G. M. (1994). Seasonal polyphenism in the wild: Survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecological Entomology*, 19, 285–298. https://doi.org/10.1111/j.1365-2311.1994.tb00420.x
- Zarlenga, D. S., Rosenthal, B. M., La Rosa, G., Pozio, E., & Hoberg, E. P. (2006). Post-Miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 7354–7359. https://doi.org/10.1073/pnas.0602466103

### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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