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Prehistorical climate change increased diversification of a group of butterflies

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Satyrinae butterflies (Lepidoptera: Nymphalidae) and grasses (Poaceae) are very diverse and distributed worldwide. Most Satyrinae use grasses as host plants, but the temporal scale of this tight association is not known. Here, we present a phylogenetic study of Satyrinae butterflies and related groups, based on 5.1 kilobases from six gene regions and 238 morphological characters for all major lineages in the ‘satyrine clade’. Estimates of divergence times calibrated using a fossil from the Late Oligocene indicate that the species-rich tribe Satyrini diversified to its current 2200 species simultaneously with the expansion and radiation of grasses during the dramatic cooling and drying up of the Earth in the Oligocene. We suggest that the adaptive radiation of grass feeders in Satyrini has been facilitated by the ubiquitousness of grasses since 25 Myr ago, which was triggered by a change in global climate.

Keywords: climate change; host plants; butterfly; evolutionary history

1. INTRODUCTION

The highly diverse butterfly subfamily Satyrinae (Nymphalidae) comprises approximately 2500 species that are distributed worldwide and dominate butterfly communities in several habitats (Pyrz & Wojtusiak 2002). The evolutionary history of Satyrinae is closely tied to the evolutionary history of grasses (Poaceae) on which the majority of species are specialized. Grasses are globally distributed with more than 10 000 species and are important components of ecosystems providing livelihood for a variety of organisms including humans (e.g. cereals and sugarcane; Osborne & Beerling 2006). However, the investigation of the origin and times of diversification of butterflies, such as Satyrinae, is hindered by the lack of higher level phylogenies and scarce fossil record (Braby *et al.* 2006). These issues have prevented the study of key chronological events in the evolutionary history and biogeography of the group (Wheat *et al.* 2007). Recent studies suggest that the origin of butterflies dates back to beyond the Cretaceous–Tertiary boundary (Braby *et al.* 2006; Wahlberg 2006;

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Wheat *et al.* 2007), but the broader implications of this to the evolutionary history of butterflies are just beginning to be explored (Wheat *et al.* 2007).

Butterflies in Satyrinae feed on monocotyledonous plants (Ackery 1988) and the bulk of species (tribe Satyrini, approx. 2200 species) almost exclusively use grasses (Peña *et al.* 2006). Grasses are known to have been involved in coevolutionary relationships with grazing vertebrates (MacFadden 2005; Prasad *et al.* 2005) that have developed adaptations for coping with the high levels of silica in grass leaves, which increases their abrasiveness. However, the interactions between grasses and the megadiverse insects are not well studied. Although some groups of insects that feed on grasses are diverse, these are mainly sap sucking (Dietrich *et al.* 1997), and there are very few cases of insects grazing on grasses. It is known that silica content wears out the mandibles of lepidopteran larvae (Dravé & Laugé 1978) and silica ingestion impairs absorption of nitrogen, affecting growth and fitness (Van Soest & Jones 1968; Smith *et al.* 1971; Massey *et al.* 2006). Larvae of satyrine butterflies are all external grazers of their host plants, and most species feed exclusively on grasses. Any link between satyrine butterflies and grasses can only be understood by placing a robust phylogenetic hypothesis of the butterfly subfamily in a temporal framework and comparing this with the time frame of the diversification of grasses. Dating butterfly lineages has been recently attempted for some groups with the aid of molecular techniques (Braby *et al.* 2006; Wahlberg 2006; Wheat *et al.* 2007), but nothing is known about ages of diversification in Satyrinae. Here, we present insights into the diversification of Satyrinae butterflies by employing an estimate of divergence times of Satyrinae butterflies based on a robust phylogeny calibrated using a fossil from the Late Oligocene.

2. MATERIAL AND METHODS

In order to obtain a phylogenetic hypothesis for Satyrinae butterflies and related subfamilies, estimate the dates of diversification and explore the role of grasses in its patterns of evolution, we analysed data from 238 morphological characters and 5143 base pairs of DNA sequences from five nuclear genes and one mitochondrial gene, for 79 Satyrinae taxa and out-groups representing all three extant subfamilies and all 15 extant tribes of the ‘satyrine clade’ (Wahlberg *et al.* 2003; Peña *et al.* 2006; Wahlberg & Wheat *in press*; see electronic supplementary material). Within the most diverse tribe Satyrini, all 13 subtribes are represented. We performed a maximum-parsimony analysis of the complete dataset using unordered and equally weighted characters. We also analysed the combined dataset using Bayesian inference (see electronic supplementary material). We used the resulting phylogenetic hypotheses to estimate divergence times using the rate smoothing method of penalized likelihood (PL; Sanderson 2002), with a fossil from the Late Oligocene (Nel *et al.* 1993) as a fixed calibration point of 25 Myr ago (figure 1).

3. RESULTS

Phylogenetic analyses using diverse methods (see electronic supplementary material) resulted in a well-resolved phylogeny, in which the three subfamilies are strongly supported as independent lineages, with Calinaginae being sister to Charaxinae + Satyrinae (figure 1). Within Satyrinae, our current results largely corroborate those of a previous study based on three gene sequences (Peña *et al.* 2006), i.e. the traditional concept of Satyrinae is polyphyletic without the

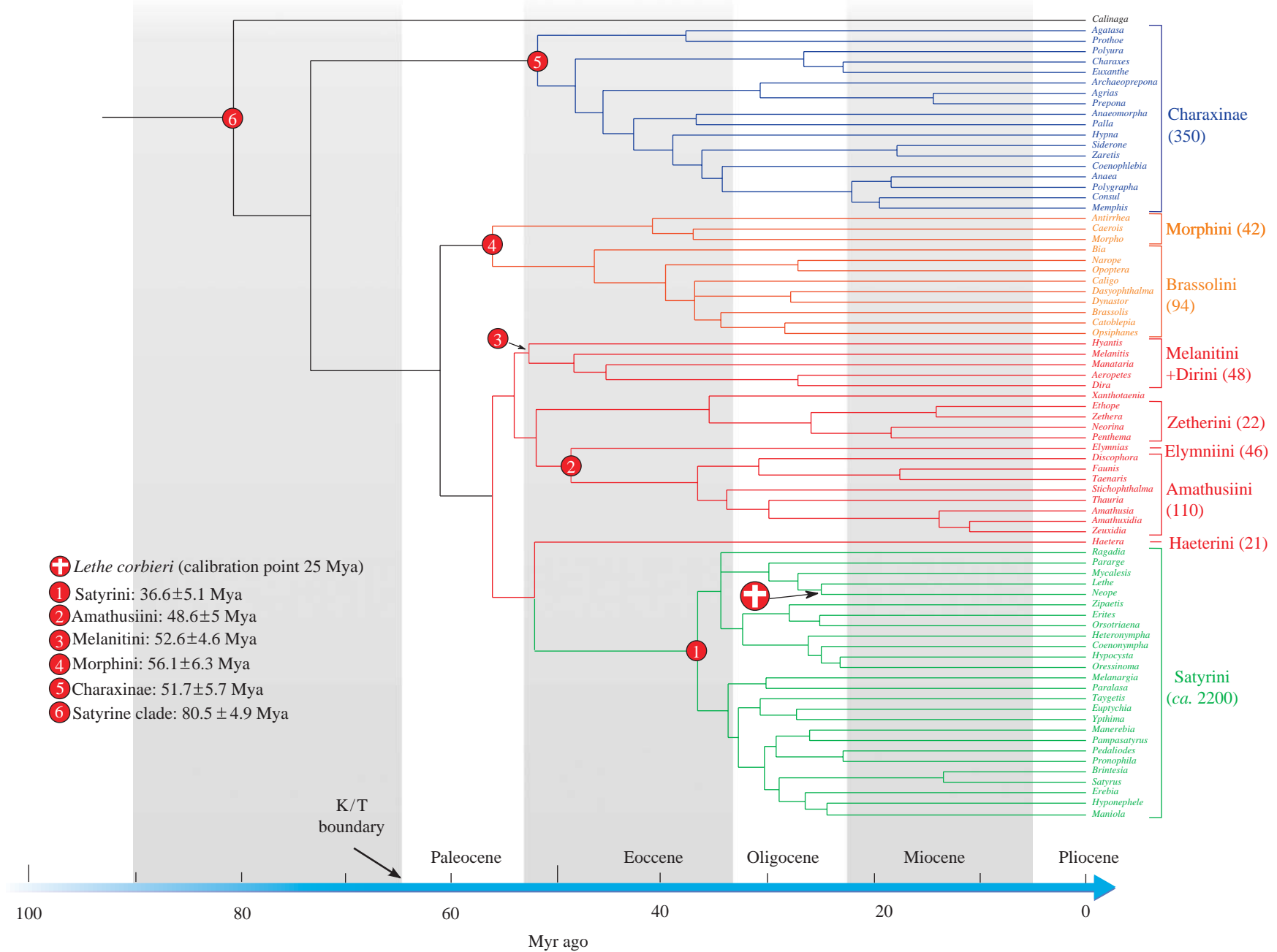


Figure 1. (Caption opposite.)

Figure 1. (*Opposite.*) Estimated times of divergence by PL using the topology of the Bayesian tree. Relative ages were calibrated using the age of *Lethe corbieri* (25 Myr ago as minimum age) as the split between *Lethe* and *Neope*. Numbers in parentheses indicate the number of extant species for the higher level taxa.

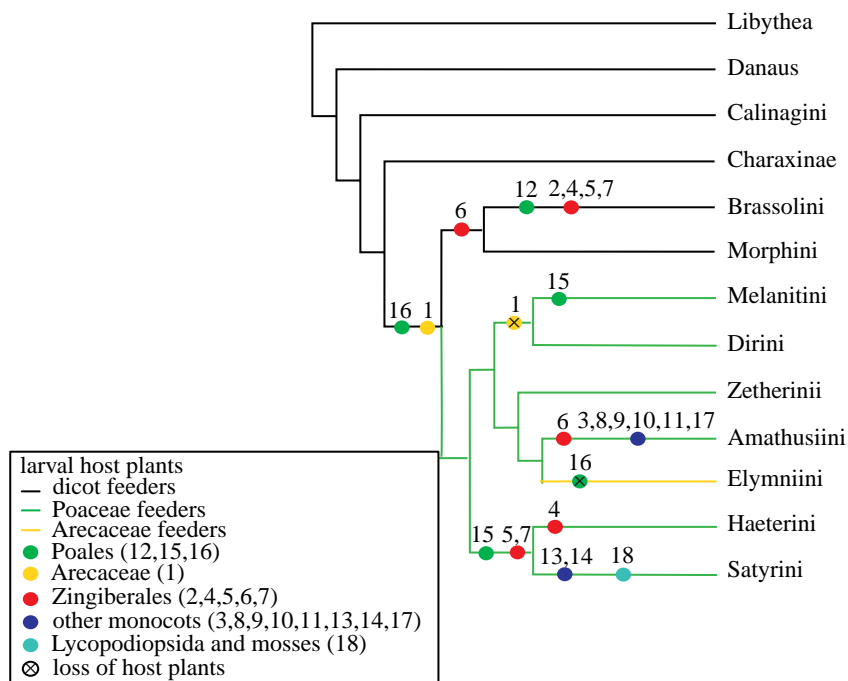


Figure 2. Optimization of larval host plant clades mapped onto 'satyrine' tribal level phylogeny reduced from the Bayesian tree. Host plant characters: 1, Arecaceae; 2, Cannaceae; 3, Smilacaceae; 4, Heliconiaceae; 5, Marantaceae; 6, Musaceae; 7, Zingiberaceae; 8, Agavaceae; 9, Liliaceae; 10, Orchidaceae; 11, Pandanaceae; 12, Bromeliaceae; 13, Restionaceae; 14, Xyridaceae; 15, Cyperaceae; 16, Poaceae; 17, Flagellariaceae; 18, lower plants (Lycopodiopsida and mosses).

inclusion of the tribes Morphini, Brassolini and Amathusiini. The clade that comprises the species-rich tribe Satyrini diversified after a relatively long branch (figure 1).

Our results provide evidence for an age of origin of butterflies older than the 70 Myr ago frontier (Vane-Wright 2004), in agreement with an implied age obtained from molecular dating of butterflies in the subfamily Nymphalinae (Wahlberg 2006) and in the family Pieridae (Wheat *et al.* 2007). Extant lineages in the satyrine clade diversified only after the big impact on the composition and organization of plant–insect associations caused by the K/T extinctions (Labandeira *et al.* 2002). The similar ages of origin and diversification of the major lineages in the satyrine clade indicate a near simultaneous origin and radiation of lineages that took separate evolutionary paths by colonizing different groups of angiosperms.

By mapping host plant use by the satyrine clade from the literature (table S3, electronic supplementary material) onto our phylogenetic hypothesis (figure 2), and taking into account the dates of diversification as estimated by molecular clock techniques, we found that the satyrine clade originated in the Late Cretaceous (*ca* 80 Myr ago), significantly after the estimated origin of angiosperms (Mesozoic, between 180 and 140 Myr ago; Bell *et al.* 2005). The four main clades diversified almost simultaneously, between 50 and 56 Myr ago. Strikingly enough, extant Satyrini went through a notable delay before

diversification, taking place as recently as 36 Myr ago, after the appearance of grasses, which took place between the Late Cretaceous and the Early Tertiary (65 and 55 Myr ago). We also identified five major plant colonization events by the major lineages of butterflies in the satyrine clade, which took place considerably after the main diversification and radiation of angiosperms (*ca* 100 Myr ago), an average delay of 48 ± 11 Myr ago. Charaxinae diversified in the Tertiary, feeding on the ancestral dicotyledonous plants. Monocotyledonous plants were colonized early on by the ancestor of Satyrinae, which shifted to Arecaceae and/or Poaceae (figure 2).

4. DISCUSSION

At the time of this early stage of Satyrinae evolution (*ca* 60–50 Myr ago), dicotyledonous plants were dominant in the vast forests that covered the planet (Willis & McElwain 2002) and the only readily available monocots were forest-dwelling early lineages such as Arecales, Liliales, Zingiberales and basal Poales (Bromeliaceae). Early diverging Satyrinae lineages (Morphini, Brassolini and Amathusiini) expanded their host ranges to include young lineages of Poales and other monocotyledonous plants from forested habitats (i.e. Bromeliaceae and Zingiberales families, respectively). Extant Satyrinae in the early diverging lineages continue to be mainly forest dwellers.

At the same time, graminoid Poales (Poaceae) were poor in species and restricted to marshy and nutrient-poor habitats (Linder & Rudall 2005), making them ineffectual host plants for driving diversification (Janz *et al.* 2006). During the Tertiary, dramatic global climate changes, such as lower levels of CO₂, decreased temperature and increased aridity, transformed ecosystems. During the Oligocene (33–26 Myr ago), these changes paved the way for the expansion and radiation of grasses (Willis & McElwain 2002), which replaced the former forested land with grasslands by developing innovations for coping with these harsh conditions (i.e. appearance of the C₄ photosynthetic pathway several times). Grasses expanded globally and by 25 Myr ago were ubiquitous, forming extensive grasslands and savannahs (Willis & McElwain 2002).

According to our age estimates, even though the tribe Satyrini was already present before grasses spread, the main lineages diversified during *ca* 36–23 Myr ago (figure 1) simultaneously with the spread of grasses, radiating spectacularly into approximately 2200 species (one-third of all species in the family Nymphalidae), forming the bulk of the subfamily Satyrinae and spreading all over the world. Even though other Satyrinae lineages feed also on grasses (figure 2), these inhabit forested areas that are not optimal habitats for sun-demanding grasses. Hence, a crucial adaptation for the spread of early lineages of Satyrini throughout the globe was being able to inhabit open areas dominated by grasses such as the extensive grasslands of the Oligocene. In this way, the conditions were set for local speciation by ecological and biogeographic events that resulted in the current species-rich clades and genera in the Satyrini.

We infer that the rise and expansion of grasses was a determinant factor in the evolutionary history of Satyrini, which allowed different evolutionary processes to drive the explosive diversification of this group. According to our host plant optimizations (figure 2), it is probable that adaptations to cope with silica appeared early in the evolution of Satyrinae and proved invaluable for Satyrini when grasses became an abundant and probably under-exploited resource. Mechanistic studies on the dynamics of butterflies and grasses associations will be needed for testing a coevolutionary scenario that may have increased speciation of these organisms (Wheat *et al.* 2007). However, it is plausible that the dispersal of grasses permitted geographical expansions of Satyrini species, which probably promoted diversification by geographical isolation (Janz *et al.* 2006) and vicariant events. A more detailed study of Satyrini will be needed to elucidate which factors were important in the spectacular diversification of these butterflies.

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