Habitat opening fostered diversity: impact of dispersal and habitat-shifts in the evolutionary history of a speciose afrotropical insect group

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Research article

Habitat opening fostered diversity: impact of dispersal and habitat-shifts in the evolutionary history of a speciose afrotropical insect group

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The opening of habitats associated with the emergence of C_4 grasslands during the Neogene had a massive influence on the evolution of plant and animal communities. Strikingly, the impacts of grassland expansion on species diversification in Africa, where the largest surface of grasslands and savannas in the world is located, are not well understood. To explore the impact of habitat opening, we investigate the evolution of noctuid stemborers, a group of moths mostly associated with open habitats, and whose diversity is centered in the Afrotropics. We generate a dated molecular phylogeny for ca 80% of the known stemborer species, and assess the role of habitat opening on the evolutionary trajectory of the group through a combination of parametric historical biogeography, ancestral character state estimation, life history traits and habitat-dependent diversification analyses. Our results support an origin of stemborers in Southern and East Africa ca 20 million years ago (Ma), with range expansions linked to the increased availability of open habitats to act as dispersal corridors, and closed habitats acting as potent barriers to dispersal. Early specialization on open habitats was maintained over time, with shifts towards closed habitats being rare and invariably unidirectional. Analyses of life history traits showed that habitat changes involved specific features likely associated with grassland adaptations, such as variations in larval behavior and color. We compare these findings to those previously inferred for an Afrotropical butterfly group that diversified roughly in parallel with the stemborers but distributed predominantly in closed habitats. Remarkably, these two

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groups show nearly opposite responses in relation to habitat specialization, whether in terms of biogeographical patterns, or in terms of rates of transition between open and closed habitats. We conclude that habitat opening played a major role in the evolutionary history of Afrotropical lineages through dispersal and adaptation linked to habitat shifts.

Introduction

The opening of habitats associated with the emergence of C_4 grasslands during the Neogene was a major event in the evolution of biomes, transforming the biosphere (Edwards et al. 2010, Strömberg 2011). The impact of these major habitat changes on the diversity and evolution of lineages has been mainly studied in herbivorous mammals (Bobe and Behrensmeyer 2004, Uno et al. 2011, Cantalapiedra et al. 2017). Our knowledge of this subject in arthropods is more limited, save for a few studies on butterflies (Peña and Wahlberg 2008, Sahoo et al. 2017), ants (Nelsen et al. 2023), flies (Safonkin et al. 2022) and spiders (Ceccarelli et al. 2019). Africa, which has the largest area of open grassland in the world (Dixon et al. 2014), presents an even greater knowledge gap. The Late Cenozoic climatic and geological history of Africa has been highly dynamic. At the beginning of the Early Miocene (ca 23 Ma, million years ago), a humid and warm climate set in, allowing the development of rainforests (Couvreur et al. 2021). This period was followed by a decline of global temperatures during the Middle Miocene Climatic Optimum (MMCO; 17–15 Ma; Zachos et al. 2001) and a drop in atmospheric CO_2 that favored C_4 grasslands (Palazzesi et al. 2022). These climate changes drove the expansion of savannas (Bobe 2006) that split the rainforest belt. By the Late Miocene (10–6 Ma), C_4 grasslands further spread across Northwestern and East Africa (Polissar et al. 2019). Following the Late Miocene Cooling (LMC; 7–5.4 Ma; Herbert et al. 2016), numerous phases of savanna expansion and retreat occurred, resulting in cycles of reconnection and fragmentation of the tropical rainforest (Bonnefille 2010). Today, the African rainforest consists of two main blocks: the first from Guinea to the Congo Basin, and the second in East Africa where patches of tropical rainforest exist in mosaic environments dominated by savannas and woodlands (Couvreur et al. 2021). Extensive savannas are distributed around West and Central Africa and extend beyond, forming a belt from West Africa to Southern Africa (Fuchs et al. 2011). Allopatric speciation associated with vicariance events resulting from cycles of fragmentation and reconnection of African biomes played a major role in driving species richness in the Afrotropics, especially around the MMCO for forest-adapted species (Aduse-Poku et al. 2022, Dagallier et al. 2023). Dispersal events played a major role in the evolutionary history of species adapted to open habitats, particularly through the opening of the eastern arid corridor following the MMCO (Sanmartín et al. 2010, Kamiński et al. 2022).

Open and closed habitats represent opposite extremes in several respects. Rainforests consist of an evergreen canopy (Turner 2001) which acts as a climatic buffer, whereas grasslands and savannas are highly exposed with less vertical structure and face strong climatic fluctuations (Knapp and Smith 2001). The direct transition from a stable closed habitat such as a tropical rainforest to a more dynamic disturbance-dependent structure such as an open grassland is expected to be rare as it requires pre-adaptations in several key traits (Donoghue and Edwards 2014). In this context intermediate or transitional habitats such as forest margins and woodlands play a potentially important role (Halali et al. 2020, 2021, Aduse-Poku et al. 2022, Espeland et al. 2023). One focal trait is the feeding strategy of herbivores, and more particularly endophagy, which has been acquired and lost repeatedly in the evolution of herbivorous insects (Tooker and Giron 2020), and may be impacted by habitat shifts. These shifts can be a potential driver of diversification, as demonstrated by Aduse-Poku et al. (2022), who highlighted the importance of habitat-dependent diversification by means of shifts among open (grasslands/savannas), intermediate (forest fringes, woodlands), and closed (rainforests) habitats in the evolution of a speciose (ca 100 species) forest-adapted Afrotropical butterfly genus *Bicyclus*. Lastly, extinction likely played a major role for lineages that had trouble adapting to drier and more open environments, as in the case of polyommatine butterflies in the Euchrysops section (Espeland et al. 2023).

To develop our understanding of how habitat opening may have fostered diversity over time, studies on various groups of organisms are needed, in particular insects which represent the bulk of biodiversity (Foottit and Adler 2009). Old World stemborer moths (Noctuidae: Apameini: Sesamiina) consist of about 300 species that are mostly distributed in the Afrotropics and have been recently introduced as a model system for studying plant–herbivore interactions in Neogene grasslands (Kergoat et al. 2018). Stemborers offer a stark contrast to the genus *Bicyclus* investigated by Aduse-Poku et al. (2022), as they have diametrically opposite ecological preferences in terms of both habitat (open habitats instead of forested habitats of *Bicyclus*) and host-use (specialization on C_4 grasses rather than C_3 grasses). The Sesamiina are ancestrally endophagous, while *Bicyclus* are exclusively external feeders. Both groups originated in the Afrotropics ca 20 Ma and share a common peak of diversification ca 15 Ma followed by a decrease in diversification rates as a function of time and temperature (Kergoat et al. 2018, Aduse-Poku et al. 2022). Therefore, comparing their respective diversification patterns might not only highlight distinct evolutionary responses to the past environmental changes associated with habitat opening but also perhaps more general trends that apply to other insect radiations in Africa. Moreover, the knowledge accumulated on the ecology of this group through years of fieldwork

(Kergoat et al. 2018) enables us to explore the potential impact of habitat shifts on feeding behavior as well as on larval morphology.

The stemborer model thus constitutes an excellent system to study the role of habitat opening in generating species richness in the Afrotropics. To do that, this study aims 1) to investigate the potential role of dispersal in the evolutionary history of Sesamiina following habitat opening, and specifically test the presence of an eastern dispersal corridor between 16 and 7 Ma, and 2) to explore whether shifts in habitats are associated with changes in stemborer larval behavior, color and host-plant use, and to assess the extent of habitat-dependent diversification.

Material and methods

Sampling of specimens and ecological data

Sampling of visually damaged grasses and sedges was conducted to collect larval stages of noctuid stemborers on their wild host-plants in hundreds of localities from 13 sub-Saharan countries. Larvae were then reared on artificial diet until pupation and emergence of adults. Light traps were also used in almost every locality to sample adults. Supplementary specimens were also obtained through collaborations or by loan from museum collections (Acknowledgments). All collected Sesamiina species were identified by one of us, an expert of the group (some of the newly collected specimens are new to science, pending their description and that of new genera they are listed using the abbreviations (NG) for new genus and (n.sp.) for new species).

Molecular dataset

A molecular dataset of four mitochondrial (cytochrome *c* oxidase subunit I (COI), cytochrome b (Cytb), 12S ribosomal RNA (12S), 16S ribosomal RNA (16S)) and three nuclear (28S ribosomal DNA (28S), elongation factor-1a (EF1a) and dopa decarboxylase (DDC)) gene fragments was assembled. This dataset was generated for the most part by our research group, with additional sequences generated for 32 species (Supporting information). In addition, seven species were also recovered from other studies and downloaded from GenBank (Supporting information). All mitochondrial and nuclear sequences were aligned using MAFFT ver. 7 (Katoh et al. 2019) with default settings, then merged using Mesquite ver. 3.70 (Maddison and Maddison 2021), and finally the quality of alignment was checked manually (more particularly poorly aligned regions and presence of stop codons) resulting in a concatenated dataset of 248 species (including 229 Sesamiina, of which 219 are exclusively Afrotropical) and 6039 aligned characters (Supporting information).

Phylogenetic and dating analyses

Phylogenetic analyses were carried out under maximum likelihood (ML), as implemented in IQ-TREE ver. 2.1.3

(Minh et al. 2020; see the Supporting information for setting details). The concatenated dataset was divided a priori into 15 partitions, with three partitions (one per codon position) defined for each coding gene fragment (COI, Cytb, EF1, DDC) and one partition defined for each non-coding gene fragment (12S, 16S, 28S). Best-fit substitution models and partition schemes were selected using the Bayesian information criterion (BIC) through ModelFinder (Kalyaanamoorthy et al. 2017; Supporting information). Clade support for all analyses was assessed using 1000 replicates for both SH-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and ultrafast bootstraps (uBV; Minh et al. 2013). Nodes corresponding to SH-aLRT values ≥ 80% and uBV ≥ 95% were considered strongly supported following authors' recommendations.

Divergence times were estimated using Bayesian relaxed clocks as implemented in BEAST ver. 1.10.4 (Suchard et al. 2018) through the CIPRES Science Gateway ver. 3.3 (Miller et al. 2010; www.phylo.org). Dating analyses relied on the same secondary calibrations used in the study of Kergoat et al. (2018) and based on Wahlberg et al. (2013) (Supporting information). The best-scoring tree from the phylogenetic analyses was used, with two distinct uncorrelated lognormal clocks for the mitochondrial and the nuclear genes, and the tree model set to a birth-death speciation process (Supporting information).

Historical biogeography analyses

Ancestral area reconstructions were inferred using the Dispersal–Extinction–Cladogenesis (DEC) of Ree and Smith (2008), as implemented in BioGeoBEARS (Matzke 2014). For all biogeographic analyses the dated phylogeny obtained with BEAST was used as an input without considering outgroups. Seven areas were defined (Fig. 1, Supporting information) and a maximum number of seven areas was allowed to account for the extant distributional ranges of the most widespread species. A matrix of scaling factors for dispersal rates (DR) between areas (from 0 to 1) was defined to account for the respective positions of the geographic areas through time and for potential geographical barriers (e.g. deserts, forests; Supporting information). To accommodate changes through time, three distinct slices were used (23–16 Ma, 16–7 Ma, 7 Ma-present), each representing a period of major changes in forest connections/fragmentations and desert originations (Supporting information). Five distinct analyses were carried out: an unconstrained analysis (M0; null model; DR of 1.0 between all areas); a constrained analysis (M1; reference model) implementing the rates presented in the Supporting information; and three additional analyses to test specifically for the potential role of the savanna Belt as a major dispersal corridor (see the Supporting information for more details). Furthermore, the number and type of biogeographic events were estimated with a biogeographical stochastic mapping (BSM) analysis (Dupin et al. 2017) using 50 stochastic replicates on 50 post-burnin trees randomly sampled from BEAST analysis (script available at
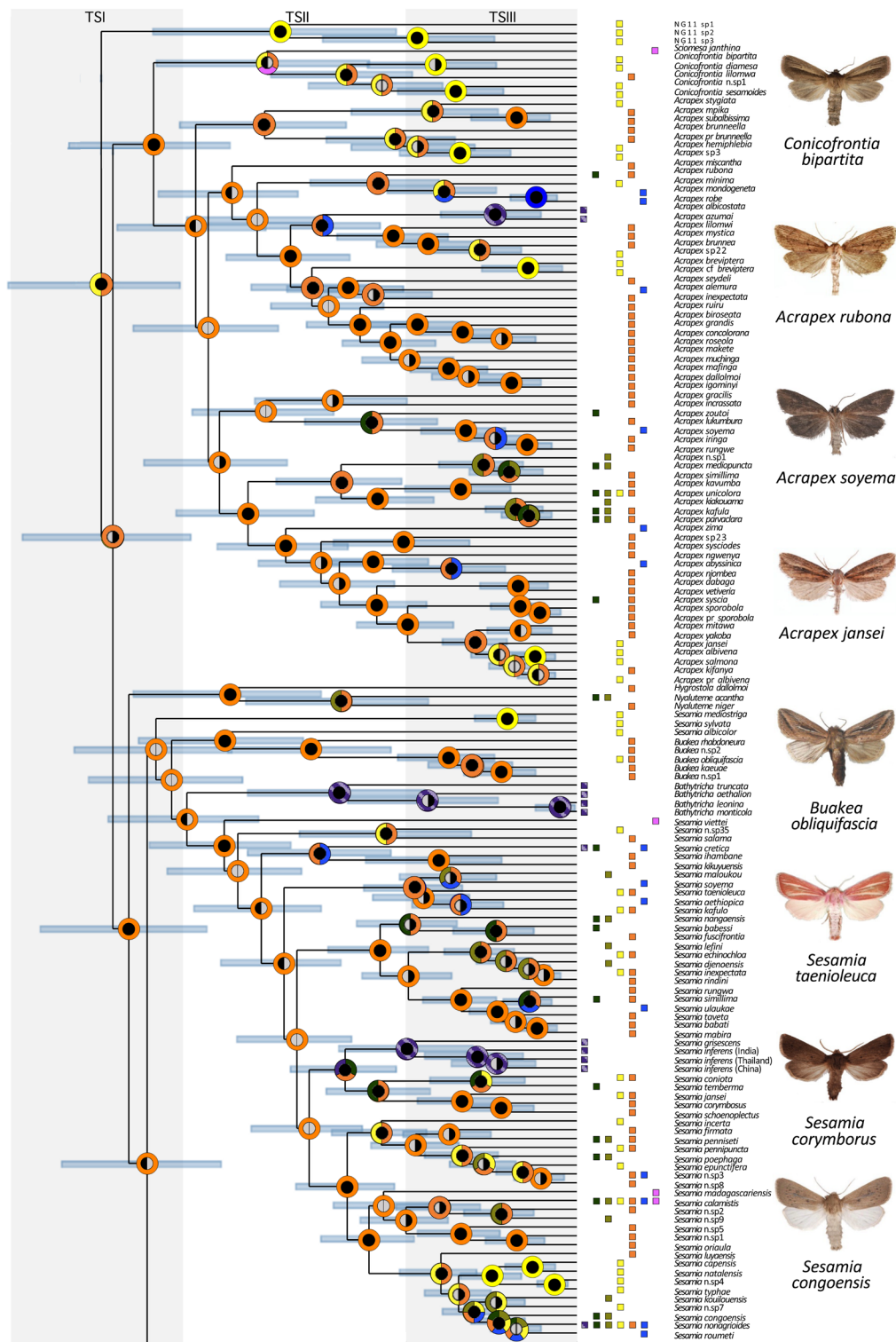
Figure 1. Dated phylogeny and historical biogeography of Sesamiina. Support values from phylogenetic analyses (SH-aLRT and uBV) are represented by circles on nodes, and 95% HPD of ages from BEAST analyses by blue bars. Only the most likely ancestral states inferred from the reference model M1 (DEC model) are presented. The three time slices (TSI, TSII and TSIII) are represented in the background using grey bands. On the right, the habitus of several Sesamiina species are represented. On the bottom left, main geological and climatic events are represented along with the progressive rise of C_4 grasslands (Couvreur et al. 2021, Palazzesi et al. 2022), as well as the dispersal event counts under DEC model. On the bottom right, the extent of the bioregions used is figured: [A] outside the Afrotropics, [B] north-western sub-Saharan Africa, [C] Central Africa, [D] Southern Africa, [E] Eastern region, [F] Horn of Africa, and [G] Madagascar.
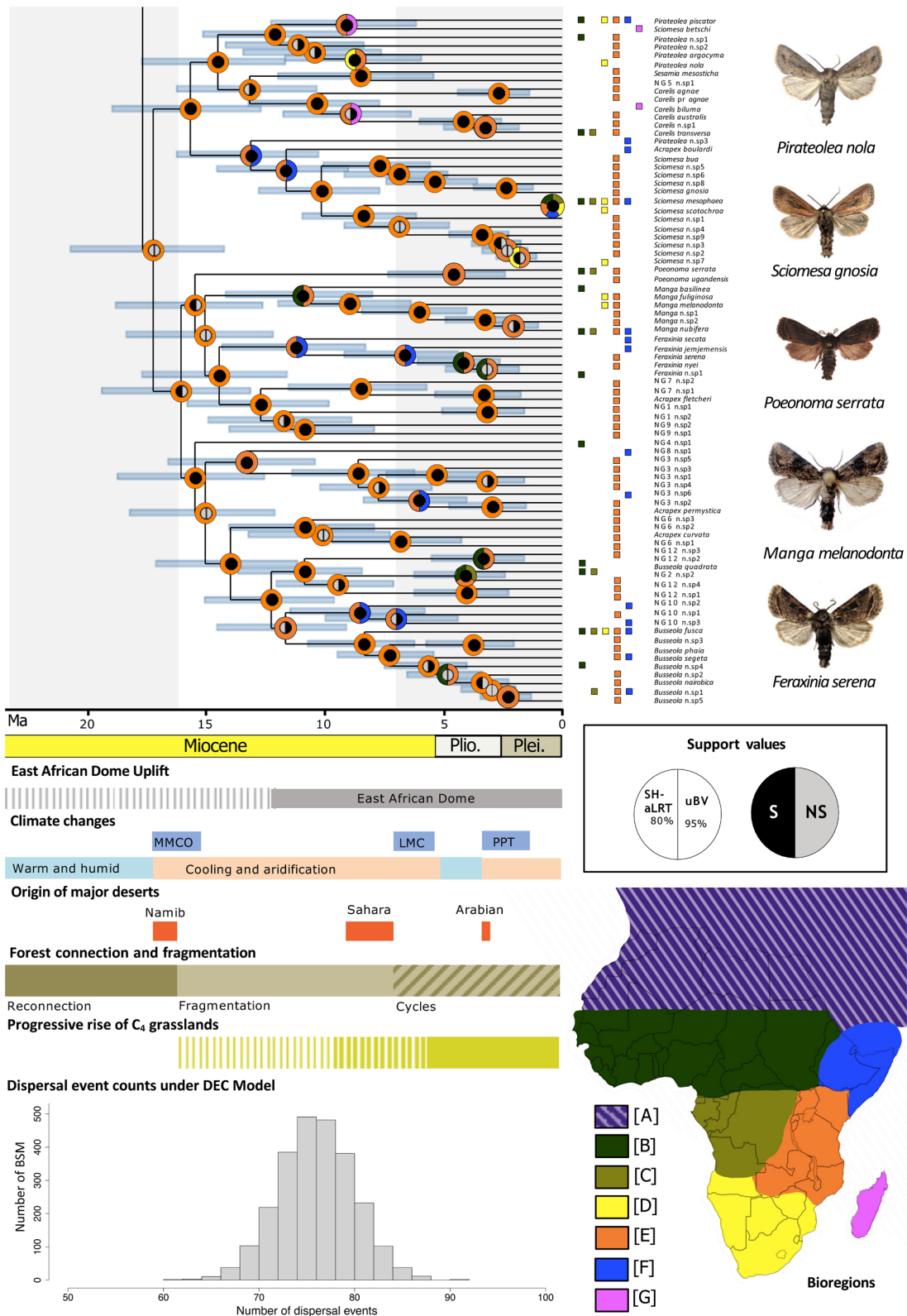
Figure 1. Continued.

https://github.com/ivanlfm/BGB_BSM_multiple_trees, Magalhaes et al. 2021).

Phylogenetic signal and ancestral state reconstruction of life history traits

Four life history traits were chosen and characterized based on field observations and data from the literature (Supporting information): habitat preference (open, closed and intermediate), feeding behavior (strict stemborers and partial leaf feeders), larval color (pink, buff and white), and host-plant preference (C_3, C_4 and grass generalist) (Supporting information). For each life history trait, species for which states cannot be determined were removed from the dataset by pruning the reference tree resulting in a 215-species dataset with habitat preferences, a 145-species dataset with feeding behavior, a 149-species dataset with larval color, and a 217-species dataset with host-plant preferences.

To test for a phylogenetic signal, the D-statistic (Fritz and Purvis 2010) was estimated for each character (habitat preference, feeding behavior, larval color, host-plant preferences) using the script of Miller and Stroud (2021) (Supporting information).

Ancestral character state estimations (ASE) were conducted on all aforementioned characters (habitat preference, feeding behavior, larval color, host-plant preferences). Three transition models were tested using the *fitMk* function in the R package 'phytools' (www.r-project.org, Revell 2012): a symmetric model (SYM), an equal-rates model (ER) and an all-rates-different model (ARD). The transition models allow us to estimate transition rates between discrete character states that are expressed as substitutions per unit of time, here per million years. The best-fitting model was determined using AICω. Then, ancestral state reconstructions were estimated using stochastic character mapping implemented in the *make.simmap* function in the R package 'phytools' with 1000 simulations.

Correlated evolution and assessment of associations of life history traits

To explore the extent of correlated evolution among the four studied life history traits, Pagel tests (Pagel 1994) were conducted for every possible pairwise combination, using the *fitPagel* function implemented in 'phytools' and the script of Miller and Stroud (2021). Pagel's test allows investigating whether two binary traits evolved independently by examining rates of evolutionary transition. For each pairwise combination, two transition models (ARD, ER) as well as four dependency models ('dependent *x*', 'dependent *y*', 'interdependent' and 'independent' models) were tested and compared using AICω, resulting in a total of 48 models for all six pairwise comparisons of the four life history traits. To that end, a binary subsampling of 145 species for which all four life history traits were available was used (see the Supporting information for binary coding). To assess the association of life history traits further, clustering analyses were also performed to group the four life history traits using non-binary

subsampling, and thereby ascertain whether species with similar habitat preferences also share similar behavior, color or plant preferences (Supporting information).

Habitat-dependent diversification

To test for the direct impact of habitat shifts in the diversification of Sesamiina, we used the several examined and concealed states-dependent speciation and extinction (SecSSE) model (Herrera-Alsina et al. 2019), which is a ML approach implementing hidden states. Here, for comparative purposes, we relied on the methods, models and scripts used in the study of Aduse-Poku et al. (2022). A total of 36 models that combine trait dependence (examined trait-dependent 'ETD', concealed trait-dependent 'CTD', constant rate 'CR'), speciation (dual and single inheritance), and habitat evolution (unconstrained single rate, unconstrained six rates, constrained single rate, constrained four rates, constrained specialist, and constrained openness) were run (Supporting information). To validate the SecSSE analyses, 30 datasets of phylogenetic trees were also simulated (Supporting information).

Results

Phylogenetic and dating analyses

The best-scoring tree from the ML analyses of the concatenated dataset (Supporting information) is well supported overall with 70% of the SH-aLRT values > 80% and 79% of the uBV > 95% (Fig. 1). Sesamiina are recovered as monophyletic with a high support (SH-aLRT value > 80% and uBV value > 95%). Out of the 14 extant Sesamiina genera, five are not monophyletic in our analyses (most conspicuously in *Acrapex* and *Sesamia*). The results of the phylogenetic analyses reveal nine distinct lineages with specific combinations of morphological characters, which could warrant the future description of new genera.

The post-burn-in parameters of the BEAST analyses show ESS > 200 for all relevant parameters (Supporting information). The subtribe Sesamiina is estimated to have originated during the Miocene ca 19.36 Ma (95% HPD: 23.20–16.19 Ma) (Fig. 1). The two most speciose genera *Acrapex* and *Sesamia* are inferred to have originated ca 15.52 Ma (95% HPD: 18.75–12.72 Ma) and ca 14.35 Ma (95% HPD: 16.71–11.24 Ma), respectively.

Historical biogeography analyses

The origin of Sesamiina in Southern and East Africa is recovered as most likely by the DEC analyses with (M1, maximum likelihood marginal probability = 0.33; Supporting information) and without (M0, maximum likelihood marginal probability = 0.28; Supporting information) constraints, and by the BSM analyses (all 50 replications recovered DE as the most likely origin; Supporting information). Overall, the

BSM analyses inferred from 61 to 91 dispersal events, from 50 to 96 allopatry events, from 42 to 120 subset sympatry events, from 262 to 342 in situ speciation events, and from zero to 13 extinction events (Fig. 1, Supporting information). Until 16 Ma (TSI), ancestral areas are only found in Southern and/or East Africa (Fig. 1). Most of the inferred Sesamiina diversification occurred within East Africa (average of 264 in situ speciation events) and Southern Africa (average of 24 in situ speciation events, Supporting information). East Africa is also recovered as the only bioregion that acted more as a source than a sink (average of 69 'dispersal from' events versus average of less than one 'dispersal into' events, Supporting information). The biogeographic analyses inferred no reverse colonization events from regions outside the Afrotropics. For terminal clades encompassing species distributed in more than four bioregions, there is significant uncertainty in the inference of ancestral ranges associated with high hidden probabilities (sums of all ranges that have probabilities lower than 5%). Regarding the additional analyses conducted to test for specific scenarios, the model that favors a Late Miocene Eastern dispersal corridor is recovered as having the greatest likelihood and the lowest AICc (M4; Supporting information).

Phylogenetic signal and ancestral character state estimations of life history traits

D-statistic scores indicate a highly conservative phylogenetic signal in the evolution of all four life history traits explored across the Sesamiina phylogeny (D = −0.34 for habitat preferences, D = −1.24 for behavior, D = −1.27 for color, and D = −0.38 for host-plant preferences; Supporting information).

Our results suggest that the MRCA for Sesamiina had pink, stem-boring larvae associated with C_3 grasses in open habitats (Supporting information). The *fitMk* model comparisons support the ARD model for habitat preferences (AICω = 0.61), behavior (AICω = 0.70), and host-plant preferences (AICω = 1.00), and the ER model for color (AICω = 0.70). The highest transition rates for habitat preferences inferred by ASE (Fig. 2, Supporting information) are from intermediate to open habitat (0.047) and to closed habitat (0.036); low transition rates are inferred from open to closed habitats (0.005) and to intermediate habitats (0.013), and no transitions are inferred from closed habitats. The highest transition rate for behavior recorded by ASE is from external feeding to stem-boring (0.016), with a lower transition rate in the opposite direction (0.004; Supporting information). Concerning the color of larvae, the estimated transition rate between the pink, buff and white larvae is low (0.005; Supporting information). The highest transition rate for host-plant preferences is from [C_3 and C_4] to C_3 (0.146), followed by the transition rate from C_3 to C_4 (0.074), low transition rates are inferred from C_4 to C_3 (0.002) and to [C_3 and C_4] (0.007), and no transitions are inferred from C_3 to [C_3 and C_4] or from [C_3 and C_4] to C_4 (Supporting information).

Detection of potential correlated evolution and assessment of associations of life history traits

Pagel tests for correlated evolution among all life history traits (Supporting information) favored an ARD model where habitat preference and feeding behavior coevolved (AICω = 0.57) or where the evolution of behavior depended on habitat preferences (AICω = 0.37), larvae color evolved depending on habitat (AICω = 0.66), host-plant evolved depending on habitat (AICω = 0.50) or the contrary (AICω = 0.35), larvae behavior and color coevolved (AICω = 0.73), host plant preference evolved depending on larvae behavior (AICω = 0.99), and larvae color evolved depending on host plant preference (AICω = 1.00).

The dendrogram obtained with the clustering analyses recovered two main clusters (Supporting information). The first cluster groups species primarily found in open habitats, with a strict stemborer behavior and pink larvae developing on C_4 grasses; the second cluster groups species found in closed and intermediate habitats, partially feeding on leaves and with buff or white striped larvae developing on C_4 grasses.

Habitat-dependent diversification

Overall, CTD models are best supported with the best model selected being a CTD model with dual inheritance and unconstrained six rates (AICω = 0.99; Supporting information). Dual inheritance is strongly supported among all comparable scenarios (Supporting information). In the best recovered model (Fig. 2), the highest transition rates are found from intermediate habitats (0.059 to open habitats and 0.048 to closed habitats), and the next highest are from open to intermediate habitats (0.013). A low transition rate is found from open to closed habitats (0.005) and almost zero transitions are found from closed to other habitats (of the order of 10^{-8}). Model validation analyses correctly selected the CTD model in 18 of 30 cases, and erroneously selected it in only one of these (Supporting information), supporting the choice of the CTD model. The main results of the analyses are summarized in Table 1.

Discussion

Biogeographic patterns

The historical biogeographic analyses support the hypothesis that Sesamiina stemborers originated in Southern and East Africa during the Early Miocene (ca 19 Ma; Fig. 1), and that until 16 Ma, Sesamiina were confined to that region (Fig. 3). This is consistent with the potential presence of open habitats there (Burke and Gunnell 2008, Couvreur et al. 2021), contrary to the well-documented presence of closed habitats (rainforests) in Central and northwestern sub-Saharan Africa at that time (Jacobs 2004, Plana 2004).

The opening of habitats following the MMCO (17–15 Ma) likely facilitated the colonization of Central and
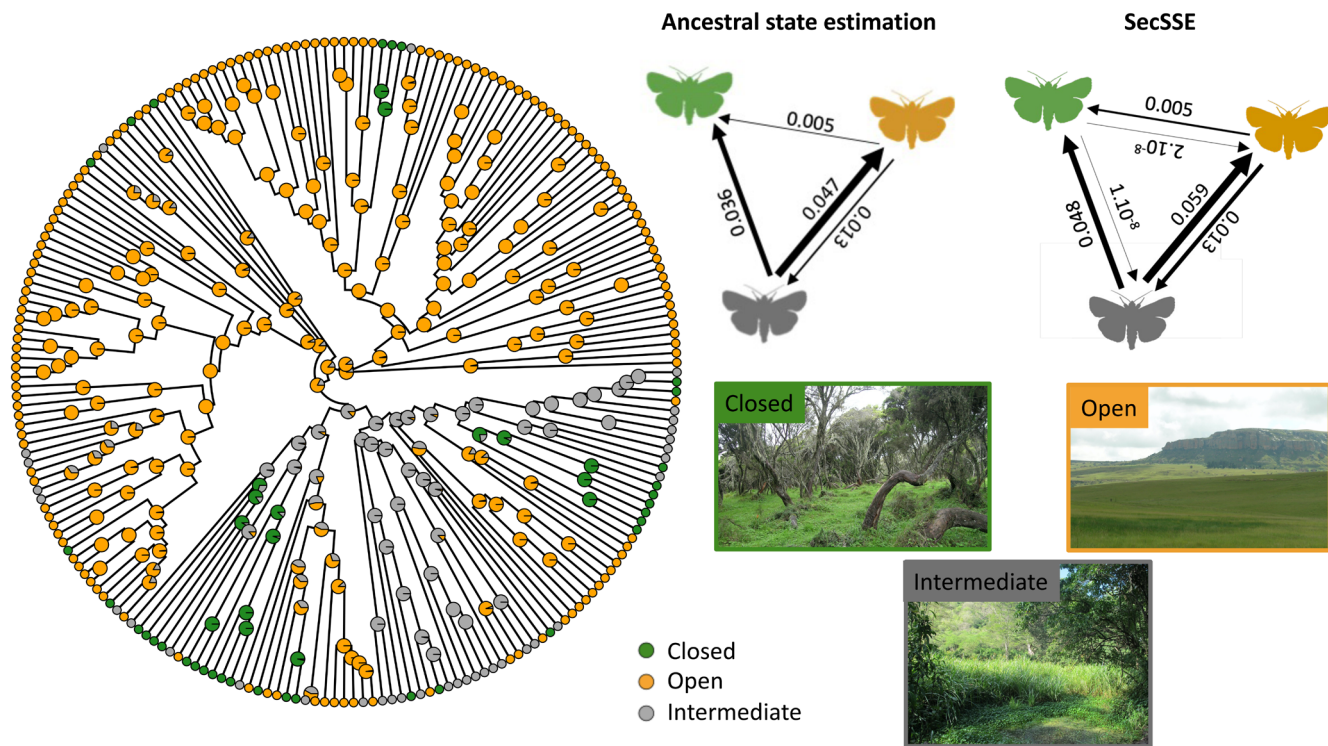
Figure 2. Ancestral state estimation for habitat preferences of Sesamiina. The arrows between the colored moths indicate transition rates between different habitats depending on ancestral state estimation analyses and SecSSE analyses.

Table 1. Summary of the analyses performed and main results.

Analyses	Methods used	Main results
Historical biogeography analyses	BioGeoBEARS	The origin of Sesamiina is inferred at ca 20 Ma in Southern and Eastern Africa.
		Dispersal events occurred through the eastern corridor after the opening of habitats ca 16 Ma.
		Most of the speciation events occurred within Southern and Eastern Africa.
Phylogenetic signal	D-statistic	$D < 0$ are recovered for all four life history traits, reflecting a high phylogenetic signal.
Ancestral state reconstruction	Phytools	The ancestral states inferred are the following: open habitats, strict stemborer, pink larvae, and feeding on C_3.
		Successful shifts were more likely from open habitats, the higher rates are recovered from intermediate habitats, and null rates are recovered from closed habitats.
Correlated evolution of life history traits	Pagel's test	Habitat preferences and feeding behaviors coevolved or the evolution of feeding behaviors depended on habitat preferences.
		Larval colors evolved depending on habitat preferences.
		Host-plant preferences evolved depending on habitat preferences or the contrary.
		Larval behaviors and colors coevolved.
Clustering analyses	Clustering	Two clusters are recovered: 1) open habitats, strict stemborers, pink larvae, mostly on C_4;
		2) Intermediate/closed habitats, partial leaf feeders, buff and white larvae, mostly on C_4.
Habitat-dependent diversification	SecSSE	Dual inheritance is strongly supported.
		Habitat changes do not appear to constitute the main driver of diversification.
		Successful shifts were more likely from open habitats, the highest rates are recovered from intermediate habitats, and rates close to zero are recovered from closed habitats.

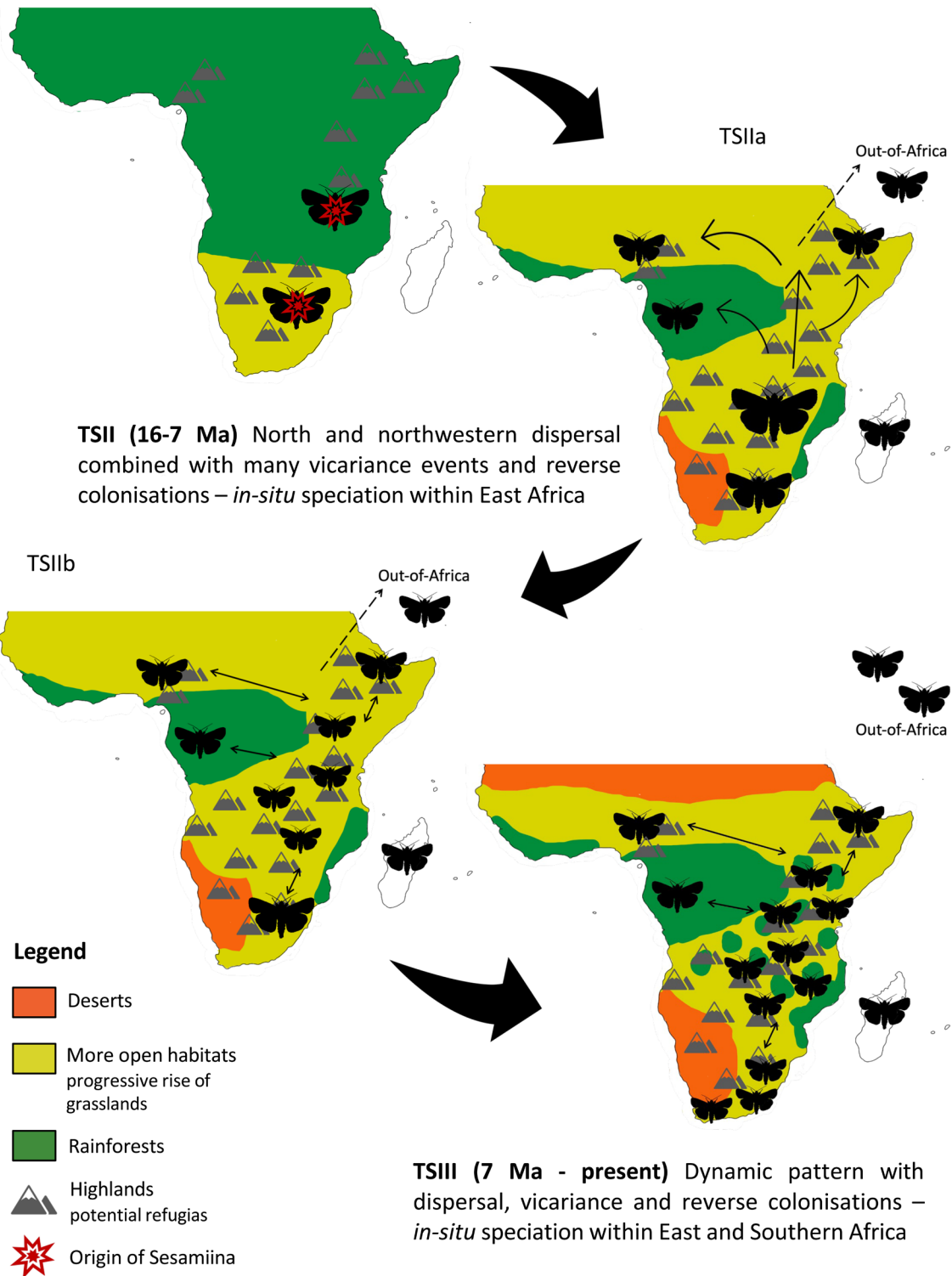**TSI (23-16 Ma)** Origin of the Sesamiina in East and Southern Africa

TSIIa

Out-of-Africa

TSII (16-7 Ma) North and northwestern dispersal combined with many vicariance events and reverse colonisations – *in-situ* speciation within East Africa

TSIIb

Out-of-Africa

Out-of-Africa

Legend

Deserts

More open habitats progressive rise of grasslands

Rainforests

Highlands potential refugias

Origin of Sesamiina

TSIII (7 Ma - present) Dynamic pattern with dispersal, vicariance and reverse colonisations – *in-situ* speciation within East and Southern Africa

Figure 3. Schematic representation of the biogeographic history of Sesamiina with respect to the three defined time slices (TSI, TSII and TSIII).

northwestern sub-Saharan Africa by the Sesamiina through the Eastern corridor/Savanna Belt (Fig. 1, 3; M4 considered as the best of the three alternative models), a pattern that has been found in other studies (Fuchs et al. 2011, Menegon et al. 2014, Kamiński et al. 2022). Our results also highlight the role of the Namib Desert and the Central African rainforest as barriers to Sesamiina dispersal (average of less than one inferred dispersal event from Southern to Central Africa). Many vicariance and reverse colonization events reflect a highly dynamic pattern from, to, and within East and Southern Africa between the MMCO and the LMC 7 Ma (Fig. 1, 3). During this period, the orogenesis of several mountain ranges was likely instrumental in spurring allopatric speciation due to a combination of growing environmental gradients and geographic barriers (Supporting information). This hypothesis is supported by the high proportion of in situ speciation events (average of 264 in situ speciation events) in East Africa, given that this bioregion contains several important African Highlands (East African Dome, Zambia Highlands) hosting many endemic lineages in other groups (Küper et al. 2004, Burgess et al. 2007, Plumptre et al. 2007, Ayebare et al. 2018, Dagallier et al. 2020). The opening of the pathway between the African and Eurasian continents associated with the progressive rise of grasslands in northern Africa ca 18–15 Ma (Tejero-Cicuéndez et al. 2022) and in Pakistan ca 10 Ma (Edwards et al. 2010) likely facilitated the out-of-Africa dispersal of Sesamiina species during the Middle-Late Miocene (ca 9.6–6.9 Ma). Conversely, it may be hypothesized that the aridification of the Sahara desert ca 9–7 Ma (Senut et al. 2009) and of the Arabian desert ca 3 Ma (Vaks et al. 2013) probably acted as barriers to more recent dispersal events.

After the beginning of the LMC (7–5.4 Ma), cycles of fragmentation and expansion of the former rainforest belt led to a mosaic environment in East Africa, with patchy forested refugia separated by savannas (Jacobs 2004, Plana 2004). Within this heterogeneous environment, an array of dispersal, vicariance, reverse colonization events, and in situ speciation events within East Africa are inferred during the last 7 million years (Fig. 1, 3). African highlands (Supporting information for a detailed map) along the Eastern corridor probably acted as altitudinal refugia during the cycles of expansion and contraction of the savannas (Plana 2004, Couvreur et al. 2021), resulting in the connection and isolation of related species in highland refugia among both forest- and arid-adapted lineages (Menegon et al. 2014, Huntley and Voelker 2017, Portillo et al. 2018, Mulvaney et al. 2022, Dagallier et al. 2023). This hypothesis is supported by: 1) the fact that Sesamiina species endemic to the African highlands belong to distinct lineages (e.g. *Se. babessi* in the Cameroon Highlands; *A. abyssinica*, *F. secata* and *Se. ulaukae* in the Ethiopian Dome; *A. incrassata*, *Sc. gnosia* and *Se. mesosticha* in the East African Dome; *A. mpika* in the Zambia Highlands), associated with either closed or open habitats, and 2) the low number of in situ speciation events inferred for northwestern sub-Saharan Africa, Central Africa and the Horn of Africa (respectively average of less

than one, one and three) compared to the inferred numbers of dispersal events from and into these three bioregions (respectively average of three and two, four and less than one, and one and three). Interestingly, almost all inferred speciation events within Southern Africa occurred during the last 7 million years (Fig. 1). This result is consistent with those found in other insect groups that are either endemic to or have radiated within Southern Africa (Hernández-Vera et al. 2013, Price et al. 2019, Talavera et al. 2020, Hévin et al. 2022). Finally, it may be hypothesized that the aridification that occurred during the Pliocene–Pleistocene Transition (ca 3.6–1.4 Ma; Bobe 2006), the frequent reconnection of forest patches at that time, and the associated rise of C_4 grasslands favored the dispersal and speciation of Sesamiina.

Habitat-dependent diversification and association with life history traits

Facing habitat changes, species can either go extinct, undergo range shifts (niche conservatism) or adapt to new environments (niche evolution) (Donoghue and Edwards 2014). The affinity of Sesamiina stemborers for open environments is an inferred ancestral condition for the group, retained throughout their diversification as shown by the high level of phylogenetic niche conservatism (D = −0.34, Fig. 2). Accordingly, habitat-dependent diversification analyses indicate that speciation events mostly took place in open habitats (dual inheritance strongly supported). However, a few stemborer lineages have recently colonized novel environments successfully (e.g. *Feraxinia* species in intermediate habitats and genus *Carelis* in closed habitats). The highest inferred transition rates across habitats were from intermediate habitats (Fig. 2), this trend is not surprising as the importance of intermediate habitats has been described for a variety of other taxa (Halali et al. 2021, Aduse-Poku et al. 2022, Espeland et al. 2023), implying that direct transitions between radically distinct habitats are quite rare (Donoghue and Edwards 2014). Finally, both ASE and SecSSE analyses found out that lineages shifting to closed habitats never reversed to intermediate or open habitats (transition rates either null or close to zero, Fig. 2), supporting the hypothesis that shifting towards closed habitats could have been as an evolutionary dead-end for the corresponding Sesamiina lineages.

Adaptations are often prerequisites to surviving habitat changes, although this is not always the case (Donoghue and Edwards 2014). The ancestral association with C_3 grasses highlighted by the corresponding ASE analysis (Supporting information) contrasts with the results of Kergoat et al. (2018), which were based on a smaller taxon sample and the DEC model. These new results are consistent with the fact that ca 20 Ma, C_3 grasses were widespread and dominant in Afrotropics (Sage et al. 1999) until the Late Miocene (Polissar et al. 2019). The ancestral association with C_3 grasses also reflects those of their putative sister tribe (the Apameina), which are mostly associated with C_3 grasses (Goldstein and Fibiger 2005). As C_4 grasses grew dominant, Sesamiina

stemborers successfully shifted to this resource, developing special adaptations for stemborers such as enlarged cephalic muscles (even more enlarged than in Apameina; Goldstein and Fibiger 2005), since the tissues from C_4 grasses are often more fibrous than those from C_3 grasses due to the higher density of fiber bundles, veins, and silica phytoliths (reviewed by Nokelainen et al. 2016). This adaptive shift is also recovered in the clustering analyses, where both clusters support an association with C_4 grasses (Supporting information).

Open and closed habitats differ in terms of the selection pressures they impose, including competition, desiccation, and a unique suite of well-adapted natural enemies, all of which are believed to mediate internal feeding strategy (Tooker and Giron 2020). These differences in selection pressures appear to be reflected in the results of the clustering analyses, where species in open habitats are mostly strict stemborers, while species in closed and intermediate habitats are mostly partial leaf feeders (Supporting information), as well as in the results of the Pagel tests, which either suggest the co-evolution of habitat preferences and feeding behavior or the evolution of behavior with respect to habitat (Supporting information). External feeding behavior incurs unique selection pressures, especially in terms of predators and pathogens. The literature on Lepidoptera indicates that predation by visually hunting predators such as birds or wasps is often limited in external feeders by protective camouflage combining specific colors and striped patterns (Stamp and Wilkens 1993, Robinson et al. 2023). In contrast, species whose larvae are endophagous tend to be pale and without discernible pattern (Aiello and Solis 2003). This may explain the co-evolution between larval behavior and color patterns evidenced by Pagel tests (Supporting information). In Sesamiina, larvae found in closed habitats (partial leaf feeder cluster) generally have more contrasting patterns (often striped) than larvae found in open habitat (strict stemborer cluster), which have bland pink colors. Interestingly, similar camouflage-related adaptations have been found in other open-adapted arthropod lineages; a striking example of this can be found in the convergent morphological evolution displayed by three lineages of anyphaenid sac spiders, where the anterior narrowing of the prosoma allows them to extend their first two pairs of legs to better blend with grass blades (Ceccarelli et al. 2019).

Adaptation to a new habitat may or may not also influence subsequent diversification rates (Donoghue and Edwards 2014), depending on the nature of the resource driving the adaptation. As stemborers have an ancestral association with open habitats (Fig. 2), the origin of Sesamiina does not appear to be a case of habitat-dependent evolution in response to a shift from closed to open habitats. Changes in habitat preferences are also not identified as a primary driver of diversification by the SecSSE analyses (since CTD models are considered as best models; Supporting information). Therefore, habitat shifts played a major role, but are not the only driver of diversification for the Sesamiina subtribe. Host diversity may have played a role, as it is known that in phytophagous insect groups with a high level of specialization,

an increase in host diversity should lead to an increase in the diversity of associated phytophagous insects (Lind et al. 2015). In other arthropod groups, habitat shifts and adaptation to C_4 grasses have been proposed as main drivers of diversification. This is the case for the spectacular (over 2200 known species) radiation of Satyrini butterflies (Peña and Wahlberg 2008) or in skipper butterflies where increased diversification rates have been found in a grass-feeding lineage of Hesperiinae (Sahoo et al. 2017).

Comparison with a forest-adapted clade

Bicyclus originated in Central Africa and most of their diversification occurred in Central African rainforests even if, interestingly, the second most speciose clade of the genus, which encompasses species adapted to forest, intermediate and open habitats, diversified in East Africa (Aduse-Poku et al. 2022). By contrast, Sesamiina have an origin in Southern and East Africa where most of their diversification occurred (Fig. 1, 3). Since the Middle Miocene, ancestral populations of *Bicyclus* were fragmented within isolated forest refugia (Aduse-Poku et al. 2022), whereas in Sesamiina there appear to have been many dispersal events associated with the rise of C_4 grasslands (Fig. 1, 3). These results support the hypothesis that the cycles of fragmentation and expansion of savannas and rainforests had opposite effects on the evolutionary trajectories of each of these groups. Furthermore, Aduse-Poku et al. (2022) showed that habitat-specialization is phylogenetically constrained in species from all habitat types (open, closed and intermediate) and played an important role in *Bicyclus* diversification. They found a higher transition rate from intermediate to closed habitats (0.101) than to open habitats (0.026) whereas all other transition rates are very low (< 4.03×10^{-8}), and null between open and closed habitats. Interestingly, the opposite pattern in the transition rates is inferred by ASE and SecSSE analyses for the Sesamiina (Fig. 2, Supporting information), with a higher transition rate associated with the shift from intermediate to open habitats (0.047 by ASE, 0.059 by SecSSE) than with the shift from intermediate to closed habitats (0.036 by ASE, 0.048 by SecSSE), all other transition rates are low (< 0.013), and near zero from closed habitats (null by ASE, by the order of 10^{-8} for SecSSE). Whereas Aduse-Poku et al. (2022) found support for habitat-dependent diversification, this is not the case for Sesamiina as CTD models are mostly supported by SecSSE analyses (Supporting information). Otherwise, both studies found dual inheritance as the main speciation model, suggesting that speciation is more likely to occur without being associated with habitat shift.

Conclusion

With 229 Sesamiina species, this study is the largest of its kind to date on the biogeographic history of an insect group in Africa. Biogeographic analyses support a Miocene origin in Southern and East Africa followed by range expansions

linked to the opening of an Eastern arid corridor, and speciation events often associated with highlands that probably acted as climate refugia during cycles of forest fragmentation and reconnection. Ancestral state estimation analyses reveal an initial adaptation to open habitats, maintained over time, with transitions to closed habitats being rare and invariably unidirectional. Life history trait analyses showed that habitat changes led to specific adaptations, such as variations in larval behavior and color. Comparing these findings with those previously inferred for *Bicyclus* butterflies is particularly interesting as these two groups show almost opposite responses with respect to the opening of habitats in the Afrotropics. Overall, these results support the hypothesis that adaptations to open and closed habitats have played a key role in generating biodiversity in the Afrotropics.

Author contributions

Noémie M.-C. Hévin: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Paul Z. Goldstein**: Conceptualization (equal); Investigation (equal); Writing – review and editing (lead). **Kwaku Aduse-Poku**: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). **Jérôme Barbut**: Investigation (supporting); Resources (equal); Writing – review and editing (supporting). **Andrew Mitchell**: Investigation (supporting); Resources (supporting); Writing – review and editing (supporting). **Alberto Zilli**: Investigation (supporting); Resources (supporting); Writing – review and editing (supporting). **Anne Laure-Clamens**: Data curation (equal); Investigation (supporting); Writing – review and editing (supporting). **Claire Capdevielle-Dulac**: Data curation (equal); Investigation (supporting); Writing – review and editing (supporting). **Niklas Wahlberg**: Investigation (supporting); Writing – review and editing (supporting). **Bruno P. Le Ru**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Investigation (equal); Resources (lead); Supervision (equal); Writing – review and editing (lead). **Gael J. Kergoat**: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (lead); Investigation (lead); Methodology (equal); Project administration (lead); Resources (supporting); Supervision (lead); Writing – original draft (supporting); Writing – review and editing (lead).

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Data availability statement

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/

m9.figshare.22263175 (Hévin et al. 2024). Accession numbers for all gene fragments are listed in the Supporting information.

Supporting information

The Supporting information associated with this article is available with the online version.

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