Coevolution of Lepidoptera and their host plants: The Salient Aroma Hypothesis

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Article

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Abstract

Host plant specialization by moths and butterflies has been a primary research focus in the field of coevolution and ecological specialization. However, factors underlying the evolution of host specificity remain largely unclear despite decades of research. Here, we demonstrate that host plant specialization is closely linked to diurnality in the Lepidoptera: diurnal butterflies and moths tend to specialize in their range of host choices, whereas nocturnal moths tend to be relative generalists. We further show that plants, on average, release larger amounts of volatile organic compounds (VOC) during the day than at night. Given that plant VOCs are important chemical cues for host searching in herbivores, we propose the 'Salient Aroma Hypothesis (SAH)'. Under SAH, herbivores that are active during the period when plant VOCs are more available are better able to discriminate between potential host species and therefore have more specialized diet breadth. Conversely, those active at night, when VOCs are not released as much, are more likely to have wider diet breadth. Antennae are the primary organs used to perceive VOCs. Differences in their morphologies also support predictions from the SAH: Diurnal females have larger antennae relative to their body sizes than nocturnal females. Our study underscores the importance of plant VOCs in Lepidoptera evolution, suggesting a possible evolutionary pathway for host specialization in herbivorous insects.

Introduction

Species constantly adapt to their biotic and abiotic environments[1, 2]. Ecological specialization is linked to processes such as competition [3], predation and parasitism [4], and factors such as habitat change and distribution of resources. Host plant specialization in moths and butterflies has been the focus of much research in the context of coevolutionary arms races or patterns of escape-and-radiate evolution that might help to explain patterns of diversification in the Lepidoptera [5, 6]. Still, despite several decades of research [6], many factors that influence the evolution of host specificity remain unclear.

The host specificity of insect herbivores is determined physiologically and behaviorally by the abilities of adult females to identify and lay eggs on appropriate host plants, as well as the ability of larvae to overcome plant defenses ranging from mechanical and constitutive defenses through to toxic chemicals and a variety of induced defenses [5, 7]. Female Lepidoptera are known to utilize a hierarchy of cues when deciding to lay eggs, and plant volatile organic compounds (VOCs) are among the most important cues for host recognition [8]. The evolution of host specificity in Lepidoptera is therefore likely to be influenced by plant VOCs.

The release and synthesis of plant VOCs are tightly linked to photosynthesis and stomatal opening [9]. Due to the associations between sunlight, photosynthesis, and almost all aspects of plant physiology, plants exhibit cyclical diurnal activities in processes including carbon assimilation [10], growth [11], and defense [12]. It seems likely that VOC emission follows a similar circadian rhythm, and it is generally known that plants emit more VOCs during the day than at night [13], this phenomenon has not been systemically investigated across a broad range of plant taxa. In terms of the impact of VOC circadian rhythm on plant-insect interactions, several well-known studies have demonstrated that pollinators such as foraging hawk moths tightly synchronize their activities to times when floral VOCs are available [14, 15]. However, the extent to which the activities of insect herbivores are coordinated with diurnal cycles of plant VOC release has not been fully explored.

Like plants, most animals are specialized to be more active during different times of the daily cycle [16–19]. Differences in daily activities are critical in distinguishing the respective ecological niches of different but closely related animal species [20]. Lepidoptera demonstrate strong diel activity preference potentially due to predation pressure [20], and this is most dramatically illustrated by butterflies as a group, whose some 18,000 species are almost entirely diurnal, and by day-flying, night-flying, and crepuscular moths (see [20]). Since plant VOC emission follows a daily cycle and plant VOCs are crucial for host plant searching, it seems likely that the diel activity preferences of different species of Lepidoptera influences their host searching efficiency and therefore their host breadth.

Herbivorous insects are notable for specializing on particular host plants, with less than 10% of herbivores feeding on more than 3 host plant families [21]. Most herbivores specialize on a specific plant species [22–24] and often on particular parts of plants [25, 26]. However, exceptional cases of diet generalists also occur [6] and are especially well studied in crop pests such as *Helicoverpa armigera* and *H. zea* [27] or whiteflies that have acquired detoxification genes via horizontal gene transfer from plants [28]. Why certain herbivores deviate from the general tendency of host specialization will depend upon their specific ecology and evolutionary history and remains largely unclear.

Studies of the evolution of host specificity have focused disproportionately on insect physiological adaptations and non-VOC plant metabolites [29, 30]. Plant VOCs, which critically influence host searching are seldom investigated (but see Wang et al. 2020 [31]). We hypothesize that the availability of plant VOCs correlates with the degree of host plant specialization and insect diel activity preference. Lepidoptera species that are active during the daylight hours may have more specialized diets because of the greater abundance of plant VOCs that can be used to facilitate discovery and identification. In contrast, species that are active at night would not have the same cues available for fine-scale navigation and thus might tend towards more generalized diets. Olfactory traits such as antennal size might be expected to evolve in parallel with foraging patterns, and to be more strongly selected in females since they must identify and lay eggs on suitable host plants. Males and females are likely differ in their olfactory motivations since males typically search for mates and only secondarily for host plants as locations where mates might be more likely to be found.

We expect to see these differences in ecological priorities reflected in the physiology of the two sexes. Using meta-analysis, phylogenetic, and morphological comparative approaches, we here investigate the diel pattern of plant VOC emission and the links between two forms of specialization by herbivores: diel activity and dietary breadth. We focus on Lepidoptera, whose some 160,000 species comprise one of the largest groups of herbivorous insects. Recent advances in resolving the higher-level phylogeny of the

Lepidoptera in addition to the availability of ecological data make this an ideal group to investigate the role of circadian activity in influencing the evolution of diet specialization.

Materials And Methods

Literature-based characterization of plant volatile emission pattern

We searched on Google Scholar for publications that reported diel emission patterns of plant VOCs using combinations of the following keywords: “plant”, “biogenic”, “volatile”, “diel”, “circadian”, “rhythms”, “light or dark”, “day or night”. We used a suite of criteria to ensure the quality of selected references (see Note S1) and eventually obtained 115 references (describing 149 cases of distinct plant species or landscapes) for further analysis (Table S1). Data from each reference were categorized based on different attributes such as (1) the collection location (e.g., lab or landscape), (2) plant habit (e.g., conifer, broadleaf tree, shrub, herbaceous plant), (3) plant family, (4) plant species, (5) physical damage type (e.g., undamaged, herbivory), (6) stress type (e.g., non-stressed, biotic, abiotic stress), (6) VOC source (e.g., leaf, floral, landscape). We also recorded flowering time (day or night). We extracted the means, standard deviations (SD), or standard errors (SE) of total VOC emission during the daytime and the nighttime from each reference.

Meta-analysis of diel VOC emission pattern

We calculated effect sizes (Hedges’ *g*) to evaluate the differences between daytime VOC emission and nighttime VOC emission by plants [32] using the function *metacont* in the package *meta* [33] in R [34]. Hedges’ *g* is calculated from the standardized mean differences between daytime and nighttime VOC emission divided by the variance. A random-effect model approach (inverse variance method) was used to evaluate the overall effect of day and night on VOC emission.

To evaluate the influence of different attributes (see above) on day-night VOC differences, we performed a subgroup analysis of VOCs collected from flowers, leaves, and landscapes. Similar subgroup analyses were also performed on different plant habits (e.g., conifers, cycad, broadleaf trees, shrubs, and herbaceous plants) and plant families. To evaluate small study effects [32], we performed trim-and-fill analysis using the function *funnel* and *trimfill* [33], and the Begg and Mazumdar Test using the function *metabias* in package *meta* [33] (see Note S2).

Lepidoptera phylogeny and diel activity preference

We used a comprehensive phylogeny of Lepidoptera covering all major lineages as the reference tree for our study (197 species of Lepidoptera) [35]. For each species, we recorded diel preference based on primarily peer-reviewed publications and occasionally on online sources when literature records were limited (Table S2). Species that lacked records were omitted from the downstream analysis. Although diel activity is a continuous trait [16], it is usually scored as categorical due to the relative lack of detailed behavioral data. How diel activity is categorized might critically influence how we interpret the results. To investigate this potential caveat, the diel activity preference was scored as one of four states: all-day, crepuscular, diurnal, nocturnal (as described [20]) or two states: active during light periods (daytime, light-active), active during dark periods (nighttime, dark-active).

Lepidoptera host plant diversity

For each species in the Lepidoptera phylogeny, we determined its host plant(s) based on peer-reviewed publications, field guides, and the HOSTS database from the British Natural History Museum [36]. We then used phylogenetic metrics to quantify the host richness and divergence in species that feed on seed plants (Spermatophytes). Prior to this analysis, we removed species without genus-level host identification.

First, we used a calibrated phylogeny of seed plants as the reference for phylogenetic quantification [37]. We pruned the tree to include two species per plant genus whose divergence time represented the crown group age of the genus. For paraphyletic genera, we used a custom python script to identify the earliest diverging monophyletic clade to set the crown group. Second, we used the functions *pd*, *ses.mpd*, and *ses.mntd* from the R package *picante* to calculate six phylogenetic metrics [38]. For host plant richness, the raw number of host plant genera (Table S3) and Faith's Phylogenetic Diversity (PD) were used. For host plant divergence, the mean pairwise distance (MPD), the mean nearest taxon distance (MNTD), and the distance-based speciation index (DSI) were used [39–41]. Specifically, DSI is a Z-score of MPD or MNTD that measures specialization as a deviation from a random expectation [41]. The R script is available on GitHub (https://github.com/lmcai/day_and_night_moth). Finally, to evaluate the significance of differences in host diversity between species exhibiting different diel activity preferences, we conducted the Wilcoxon rank sum test using the function *wilcox.test* in R package *stats*.

Correlated evolution of diel preference and host specificity

To statistically evaluate the correlation between Lepidoptera diel preference and host specificity, we applied the binary phylogenetic generalized linear mixed model (PGLMM) to our data. For host specificity, species were scored as either specialist (feed on one plant family) or generalist (feed on multiple families) [22]. The adult diel activity was scored as described in the previous section (i.e., 2-state and 4-state). We then used the function *binaryPGLMM* from the R package *ape* [42] to test for the significance of trait correlation under the PGLMM model.

Correlated evolution between traits also sets a unique expectation on how easily a species can transit from one state to another for a particular trait combination. To explore this principle, we reconstructed the ancestral state of diel activity and host specificity under the all-rates-different model using the *ace* function in the R package *ace* [42]. We then performed stochastic mapping from *phytools* [43] to summarize the frequency of transitions between the following four states: (1) light-active specialists, (2) dark-active specialists, (3) light-active generalists, (4) dark-active generalists.

Antennal size quantification

Antennal size was quantified based on the specimen images from the Lepidoptera collection of the Museum of Comparative Zoology at Harvard were used in the analysis, and we included six male and six female specimens for each species. Species without adequate replicates were not included. The dorsal and ventral sides were imaged using a high-resolution SLR camera (Nikon D800) for each specimen. After background removal and image segmentation in MATLAB R2019b, the estimated area of the antenna was then used as a proxy for its actual size. To mitigate the impact of body size variation, we measured the length and width of the body and then normalized the antennal size by the body size, which were estimated as an ellipsoid. Both left and right antennal sizes were measured. When boundaries between body parts could not be confidently discriminated from images, the samples were not included. Finally, the antennal size of each species was determined and scored as the median across replicates for each of the two sexes (Table S4). The Wilcoxon sum rank test was applied to compare differences in host specificity and diel activity preference. Crepuscular species were not included in the statistical analysis due to the small sample size.

Results

Diel emission pattern of plant VOCs

Regardless of variation in growth form and evolutionary history, plants emit significantly higher amounts of VOCs during the day than at night (Fig. 1a, Hedges' $g = 1.66$, $Z = 8.77$, $P < 0.0001$). Here, positive Hedges' g values indicate a higher daytime VOC emission than nighttime emission. We found a significant difference in effect size between VOC types (Fig. 1b, $Q = 120.06$, $P < 0.0001$). Studies that report landscape-level VOC emission demonstrate the highest day-night VOC differences (Hedges' g = 3.40). Vegetative VOC emission showed more day-night VOC differences than floral VOCs (Hedges' g = 2.29 versus Hedges' g = 0.06). We also detected large variation in emission patterns among plants with different habit types (Fig. 1c, $Q = 94.58$, $P < 0.0001$). Woody plants such as gymnosperms and broadleaf trees showed the highest day-night VOC differences (Hedges' g = 3.38 and 2.30, respectively), compared to shrubs (Hedges' g = 2.10), grasses (Hedges' g = 1.24), and herbaceous plants (Hedges' g = 0.09). We also found significant day-night VOC differences between plant families (Fig. 1d, $Q = 90.39$, $P < 0.0001$). While most plant families have positive Hedges' g (higher VOC emission during the day), Apocynaceae and Solanaceae have higher VOC emission at night (Hedges' g = -1.83 and -0.94, respectively). This difference may be generated in part by the disproportionate number of investigations of floral VOC in these families since they both often recruit nocturnal pollinators.

Associations between adult diel activity and host specificity of Lepidoptera

We incorporated data for 145 out of the 197 (73.6%) species from the reference Lepidoptera phylogeny to investigate the association between diel activity and host specificity. When the diel activity is scored as four states (i.e., diurnal, nocturnal, crepuscular, and all), we found that nocturnal species are significantly more likely to be generalists (Table 1, PGLMM, nocturnal: estimate = -2.77, Z = -2.47, P = 0.014). When diel activity preference was scored as two states (i.e., light-active, dark-active), we again found that light-active species are significantly more likely to be specialists (Table 1, PGLMM, light: estimate = 2.08, Z = 4.52, P < 0.0001), and diel activity preference exhibits significant phylogenetic signal (s^2 = 2.38, P < 0.0001). Overall, the results support the hypothesis that nocturnal lepidopterans are more likely to have generalist diets and that different ways of categorizing diel activity do not significantly change this correlation.

To investigate the link between adult diel activity and host plant diversity of Lepidoptera, we collected genus-level host plant data for 136 out of the 197 (69.0%) species in the higher-level Lepidoptera phylogeny. The median number of host plant genera for these species is 3.5, and 29.4% of the sampled Lepidoptera only feed on one genus. For species with different diel preferences, nocturnal species have significantly higher host plant richness compared to diurnal species. They, on average, feed on 14.8 plant genera compared to 8.7 in diurnal species (Wilcoxon test, P = 0.30). The average PD is 600.0 in nocturnal species versus 405.5 in diurnal species (Wilcoxon test P = 0.004). When quantifying host divergence with pairwise phylogenetic distances, we found that nocturnal species also have higher host plant divergence compared to diurnal species (mean MPD = 155.2, mean MNTD = 99.7, mean DSI_{MPD} = -1.53, mean DSI_{MNTD} = -1.91). The results are highly consistent among the four metrics used, including the normalized pairwise distances (DSI_{MPD} and DSI_{MNTD}). Here, the negative values of DSI_{MPD} and DSI_{MNTD} suggest that host plants are phylogenetically clustered (DSI < 0) rather than randomly distributed (DSI = 0).

Our ancestral state reconstruction indicates that the diet breadth of the ancestral lepidopteran was more likely specialist (53.13%) than generalist (46.87%), and adult diel activity was more likely light-active (54.27%) than dark-active (45.73%). Interestingly, generalist diet breadth shows a somewhat “tippy” distribution, with most internal nodes throughout the phylogeny being very likely specialist (average = 67.33%)—the exception being several early-diverging “micromoth” lineages (e.g. Nepticuloidea), which are more equivocal (50.12-65.27% specialist) (Fig. 3). The very few clades that are ancestrally generalist are also likely ancestrally night-active, i.e., the *Actias-Attacus*, *Biston-Idaea*, *Agrotis-Amphipyra*, and *Arctia-Amata* clades (Fig. 3, Fig. S1 with species name listed).

The transition frequency matrix of diel preference and host diversity showed that light-active specialist and dark-active generalist seem to be the two most stable endpoints of all the possible evolutionary pathways, with longer estimated time spent in either of these states than the two alternatives (Fig. 4). Light-active generalist is the least stable endpoint by this criterion. Light-active specialists are more likely to transition to becoming dark-active generalists than either possible alternative where diet breadth and diel cycle do not shift in tandem. On the other hand, the most likely shift when starting with a dark-active generalist is a shift to a specialist diet, with or without also shifting diel cycle (roughly equal rates for shift to dark-active specialist and light-active specialist at 17.02 and 16.75, respectively). Light-active generalists are most likely to transition to becoming specialists, retaining their diel cycle. Dark-active specialists are most likely to transition to becoming light-active, retaining their diet breadth. Thus, it appears that overall, the pathway of least resistance is in the direction of specialist over generalist states, and light-active over dark-active states. The shift to generalist or to dark-active states is only likely when concurrent, i.e., becoming generalist necessitates becoming dark-active and vice versa.

Morphological traits and plant VOC availability

We quantified antennal and body size for 582 specimens out of 94 (47.7%) species (Table S4). The antennal size of adult Lepidoptera covaries with sex, diel activity (4-state category), and host diversity (Fig. 5). Females of diurnal species have significantly larger antennae relative to body size than do nocturnal species (Fig. 5a, Wilcoxon test, W = 406, $P < 0.0001$). In contrast, males of diurnal species have significantly smaller relative antennal size compared to nocturnal species (Fig. 5a, W = 167, P = 0.032). Females of species that are active during both day and night appear to have smaller relative antennal size compared to purely diurnal species, and larger relative antennal size compared to nocturnal species, but these differences are not statistically significant (Fig. 5a; comparison to diurnal females: W = 54, P = 0.46; comparison to nocturnal females: W = 31, P = 0.62) Males of species that are both day and night active seem to have larger relative antennal size compared to males of either diurnally or nocturnally active species (Fig. 5a; comparison to diurnal males: W = 21, P = 0.61; comparison to nocturnal males: W = 22, P = 0.77), but again, these differences are not statistically significant. Females of specialist species have significantly larger relative antennal size compared to generalist species (Fig. 5b, W = 305, P = 0.006) In contrast, males of specialist species have significantly smaller relative antennal size compared with generalist species (W = 117, P = 0.006).

Discussion

Studies of host plant specialization in the Lepidoptera have contributed much to our current understanding of coevolution and ecological specialization. While many attempts have been made to explain specialization patterns in Lepidoptera [6], the primary factors shaping the evolution of their host breadth remains unclear. We identified a significant correlation between Lepidoptera host plant specificity and adult diel activity with three lines of evidence: (1) trait correlation, (2) host phylogenetic divergence quantification, and (3) trait transition matrix inference. Such correlation corresponds closely with the circadian rhythm of plant volatile emission. More specifically, light-active moths and butterflies forage and mate when host plant-related VOCs are more readily available, and these diurnal species have more specialized diets. In contrast, dark-active species are active when host-related VOCs are less available, and these nocturnal species are more likely to have generalized diets. Based on the strong links between diel plant volatile emission, adult diel activity, and host specificity of Lepidoptera, we propose that the Salient Aroma Hypothesis provides a mechanistic explanation for the evolution of host specialization by highlighting a striking ecological constraint [6] – the lack of available plant VOCs to use as cues in host-finding at night – that seems likely to affect the potential for diet specialization in nocturnal Lepidoptera. It rests partly on the assumption that at least for those species where it has been tested [44–46], plant VOCs play an essential role in helping egg-laying females locate and identify specific host plants. Thus, even if specialization is the preferred state, other factors, such as the availability (i.e., quantity and diversity) of VOCs as cues, might constrain the evolution of specialization by nocturnal Lepidoptera to some degree.

Ecological constraints have seldom been evoked as a mechanism for the evolution of specialization [6]. The Escape and Radiate Hypothesis model of coevolution proposed by Ehrlich and Raven [47] has profoundly influenced our views regarding ecological specialization and its effect on the generation of biodiversity. In their model, the benefit of specialization lies in the metabolic trade-offs between the need to cope with specific plant toxins as a plant generalist and the adaptive advantages of being able to detoxify those toxins as a plant specialist. This model has contributed significantly to our understanding of the evolution of specific systems [48–50] and the role of natural selection and coevolutionary arm races in generating convergent phenotypes more broadly [51]. However, beyond a handful of well-worked cases, the model's explanatory power has had limited success, and many alternative models have been proposed [48, 52–54]. As Hardy, Kaczvinsky [6] pointed out, alternative explanations for the evolution of specialization are needed, and ecological constraints are an important area to consider. The observation that diurnal Lepidoptera species are more likely to be specialists and vice versa, suggests that VOC availability may be one crucial ecological constraint for Lepidoptera host specialization. The strong links between light availability [55], photosynthesis [9], stomatal behavior [56], temperature [57] and VOC synthesis/release are among the main factors underlying general diel patterns of plant VOC emission that result in lower VOC emission during the night than during the day in most land plants, and lower plant VOC availability at night is a common attribute of terrestrial ecosystems (Fig. 1b). It likely serves as an ecological constraint contributing to the strong association between adult diel activity and host specificity in Lepidoptera.

While most Lepidoptera species follow the light-active specialist and dark-active generalist pattern, several nocturnal specialist species do not initially seem to fit well into the Salient Aroma Hypothesis. These exceptions commonly feed on nocturnal plants that produce flowers that blossom at night or release VOCs nocturnally to attract nighttime pollinators [58]. This pattern is also more commonly observed in herbaceous plants (Fig. 1c). Although mutualism with nocturnal pollinators may be more common in herbaceous plants than other plant types, another possible explanation is that the result of this meta-analysis is biased by the numerous studies focusing on specific herbaceous model systems. For instance, *Nicotiana spp*. and *Petunia spp.* are known to have flowers that attract nocturnal pollinators [59, 60] that are also specialist herbivores (e.g., tobacco hornworm, *Manduca sexta*) [15]. Nocturnal herbivores specializing on plants that produce strong VOC emission at night are the kinds of exceptions that help prove the rule and add support to the Salient Aroma Hypothesis. Another, non-mutually exclusive explanation relates to the likelihood that specialization is ancestral, and possibly the favored state in Lepidoptera. It is conceivable that present-day nocturnal specialists could be a result of some evolutionary lag in a previously nocturnal generalist lineage wherein host breadth has shifted before diel cycle; thus, these lineages would be on the path of eventually becoming diurnal as well (Fig. 4).

Switches in diel activity preference occur quite often across the phylogeny of Lepidoptera (Fig. 4), and switches from ancestral diurnality to other diel states, such as nocturnality, and subsequently back to diurnality may have occurred in some lineages (see [20]). Switches to nocturnality in animals have been shown to result sometimes from predator-prey interactions [17, 61, 62], and predation-driven diel activity changes have also been proposed as central explanations for shifts in diel activity in Lepidoptera [20, 35]. Although the factors that drive the switches in lepidopteran diel activity may differ in different lineages, they often accompany switches in host specificity in the direction predicted by the Salient Aroma Hypothesis (Fig. 4). It has been assumed that specialization comes with the cost of losing the genetic variation to use alternative plants and, therefore, can only give rise to additional specialized lineages. Although this has led to speculation that specialized lineages should be prone to extinction and distributed mainly at the tips of phylogenies, little evidence has supported the idea of specialization as an evolutionary dead end [1, 63], and we, like others, find evidence that host specificity is relatively labile and can switch between specialist and generalist states [64], as predicted by the Oscillation Hypothesis [54], where the oscillation of host range is one of the key drivers promoting the diversity of insect herbivores. Whether switches in diel activity promote host range oscillation and biodiversity, and whether VOC availability plays a part in these changes remain unknown and are promising areas for future discovery.

Associations between relative antennal size, host specificity, and diel activity of Lepidoptera provide additional evidence showing the importance of VOCs to herbivore trait evolution. In support of our prediction, relative antennal size is bigger in females of diurnal specialist species than females of nocturnal species, likely due to the central role of olfaction in host searching [65]. However, relative antennal size is smaller in males of diurnal species than males of nocturnal generalist species. We speculate that this may be due to mating system of nocturnal species more frequently involving females releasing pheromones to call males than those of diurnal species [66, 67].

Our study demonstrates strong links between diel activity preference of Lepidoptera and their host specificity. We show that temporal variation in plant VOC availability (i.e., quantity and diversity) between day and night may exert constraints on the host breadth of insect herbivores. The Salient Aroma Hypothesis highlights the key role that plant VOCs might play as signals that have shaped the evolution of herbivory.

Declarations

Declaration of interests

The authors declare no competing interests.

Data availability statement

All data and R codes associated with this manuscript will be made available as supplementary information upon acceptance.

Tables

Table 1 PGLMM model estimates: associations between host specificity and diel activity preference

	Estimate	SE	Z score	P-value	s^2
Two-level diel activity					2.38 ($P < 0.0001$)
Intercept	0.31	0.82	0.38	0.7	
Light active	2.08	0.26	4.52	**<0.0001**	
Four-level diel activity					2.85 ($P < 0.0001$)
Intercept	3.34	1.4	2.38	0.017	
Crepuscular	-2.14	1.83	-1.17	0.242	
Diurnal	-0.72	1.18	-0.61	0.541	
Nocturnal	-2.77	1.12	-2.47	**0.014**	

s^2 = Phylogenetic signal

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Figures

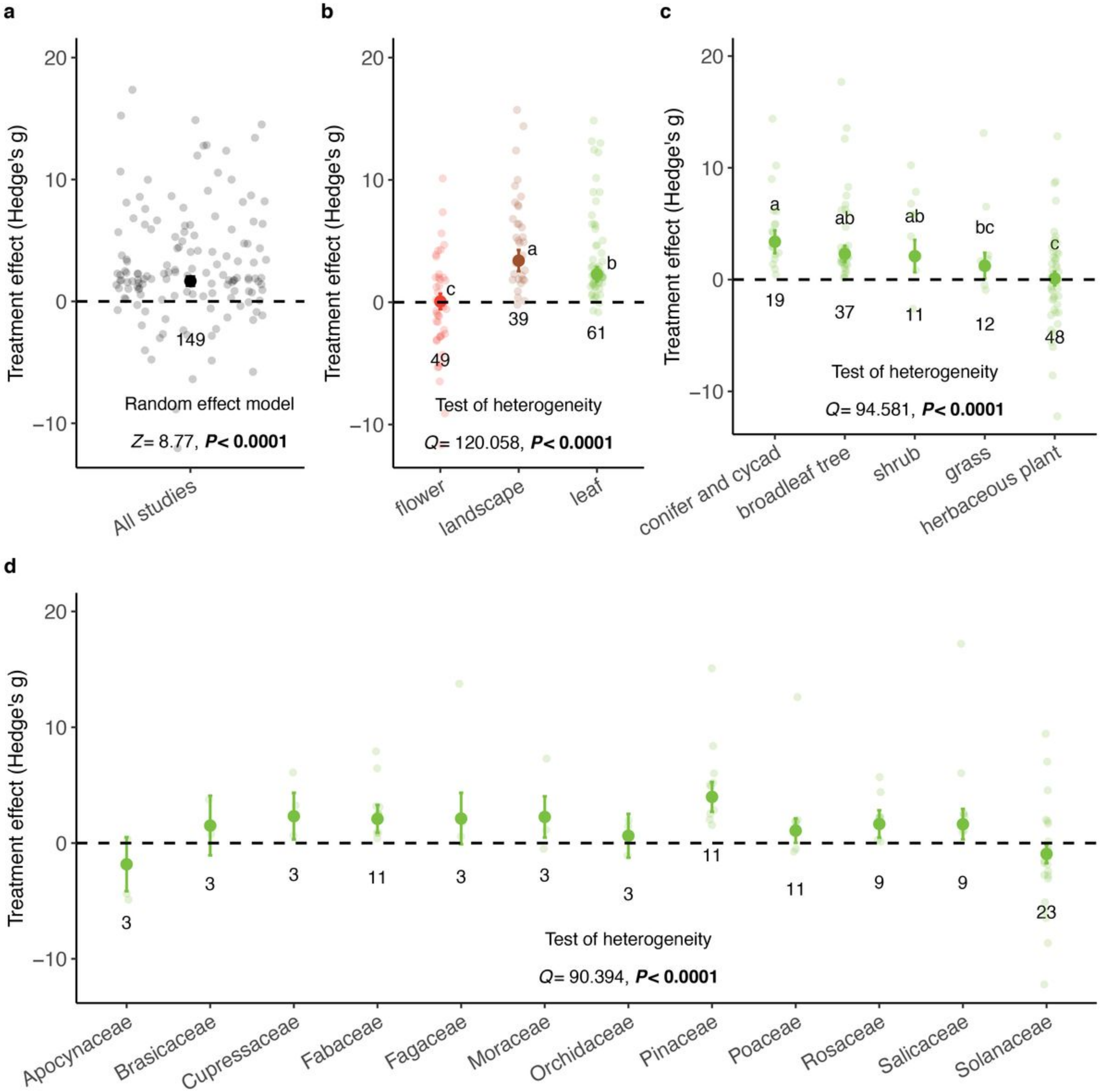

Figure 1

Diel emission pattern of plant VOCs

Diel emission patterns of plant VOCs. (a) result of meta-analysis on diel VOC emission patterns from all studies. (b-d) results of subgroup analyses on (b) VOC type, (c) plant habit, and (d) plant family. Hedges' *g* indicates the standardized mean differences between daytime and nighttime VOC emission. Numbers below the circles indicate sample size. Letters above the circles indicate significant differences between subgroups.

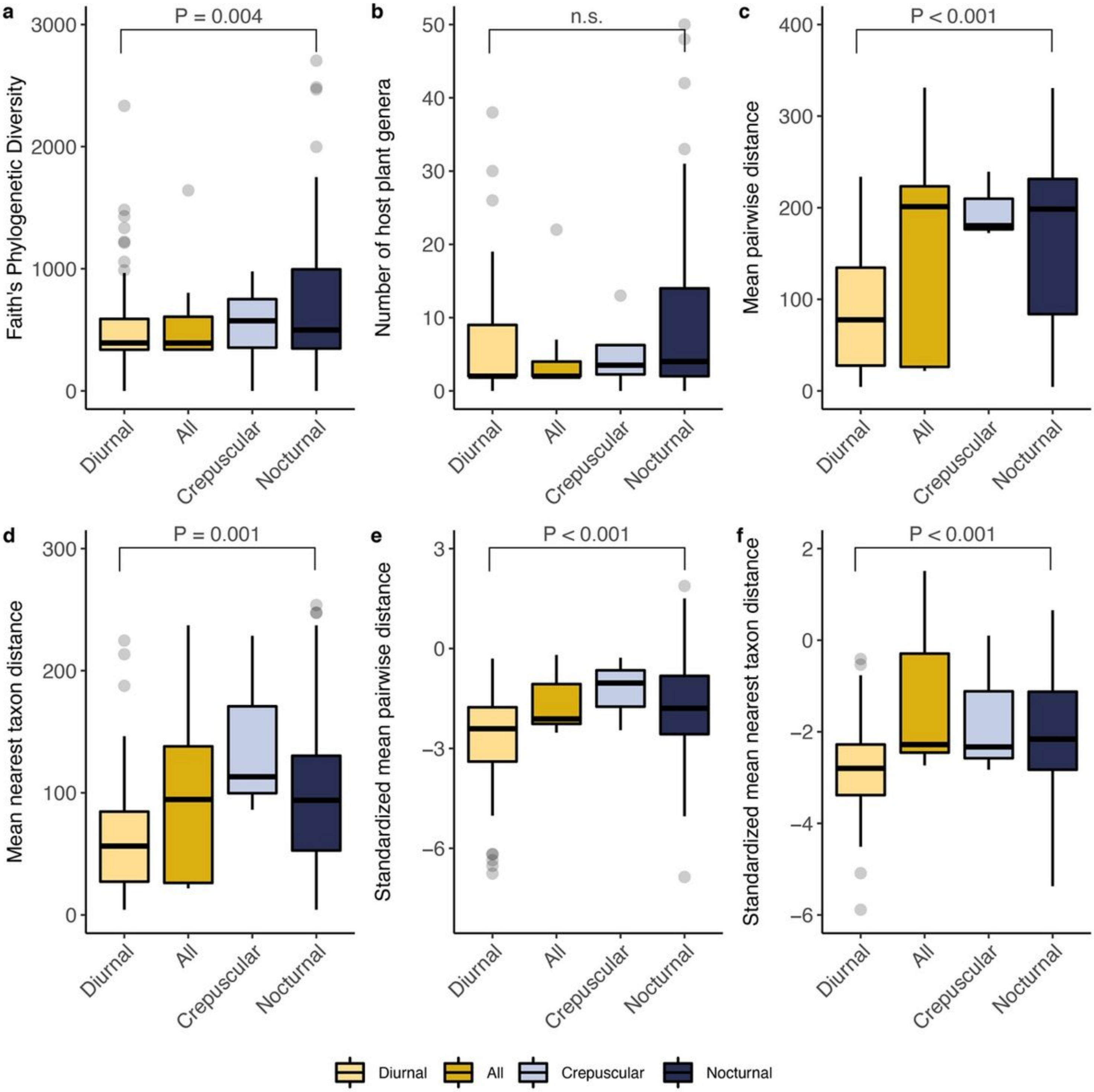

Figure 2

Diel activity and host specificity of Lepidoptera

Associations between host specificity and adult diel activity preference. (a) Faith’s phylogenetic diversity of host plants of Lepidoptera species. (b) Host plant genera per number of Lepidoptera species. (c) Mean pairwise distance of host plants of Lepidoptera species. (d) Mean nearest taxon distance of host plants of Lepidoptera species. (e) Standardized mean pairwise distance of host plants of Lepidoptera species. (f) Standardized mean nearest taxon distance of host plants of Lepidoptera species. Box plots are generated from 136 species of Lepidoptera, where records of host plant genera were available. P-values are generated from comparisons between diurnal and nocturnal species using the Wilcoxon rank sum test.

Figure 3

Ancestral character state reconstruction of Lepidoptera

Ancestral character state reconstruction of Lepidoptera. Pie charts represent host specificity at the tips and the nodes. Dark green indicates species that feed on a single plant family. Light green indicates species that feed on more than one plant family (posterior probability). Adult diel activity preference is illustrated by the color of the branch (posterior density map): Yellow indicates that adults are active in the light. Black indicates that adults are active in the dark. Shaded light green areas indicate the few clades that are ancestrally generalist. Shaded grey areas indicate early diverging lineages.

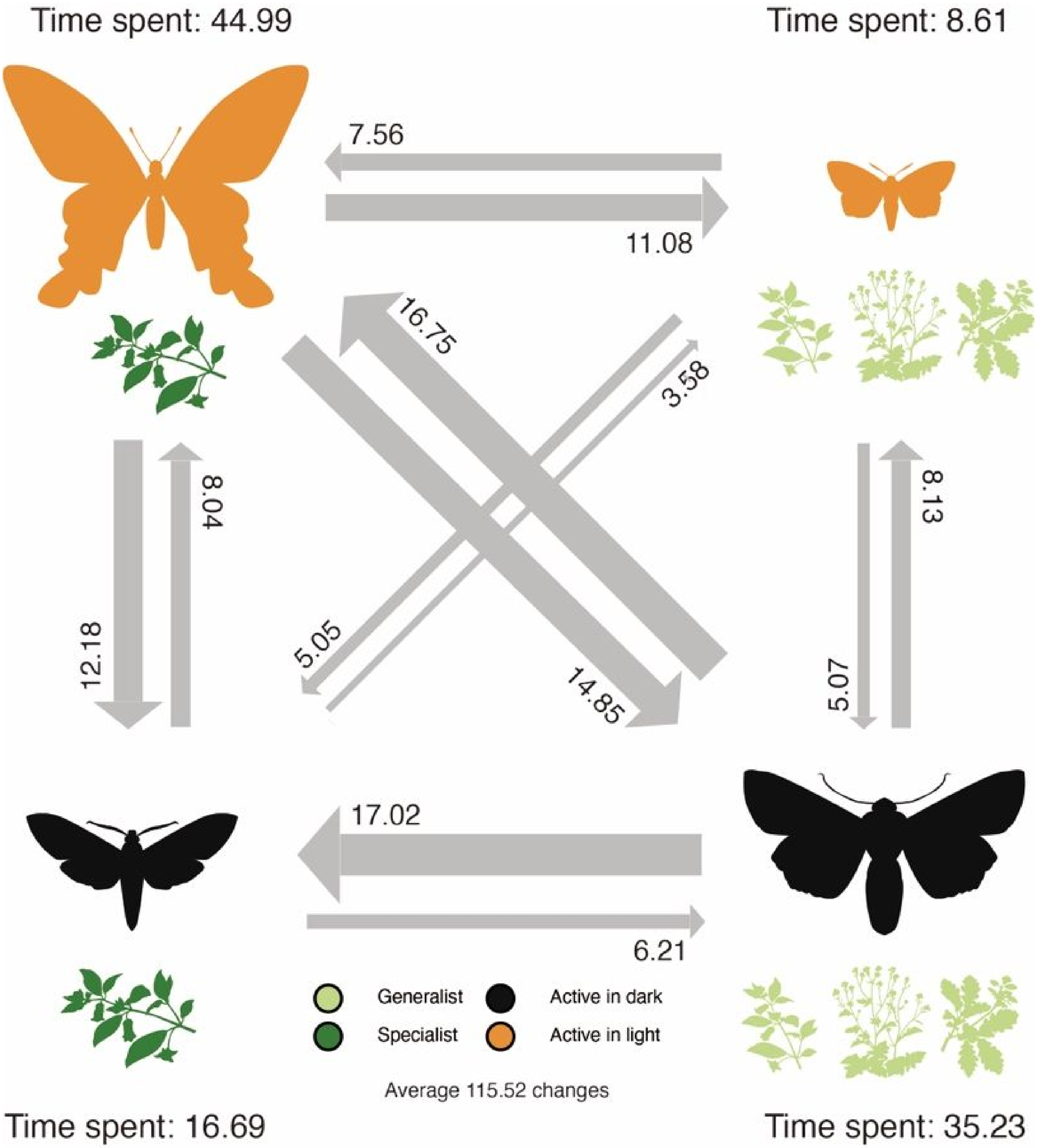

Figure 4

Evolutionary pathway analysis

Evolutionary pathway analysis of adult diel activity preference and host specificity. Illustrated are the four states of diel activity and host specificity (from top-left, clockwise): light-active specialists, light-active generalists, dark-active specialists, dark-active generalists. Numbers next to arrows indicate the frequency of respective transitions. The icons’ size and lines’ width are proportional to the time spent and the transition number.

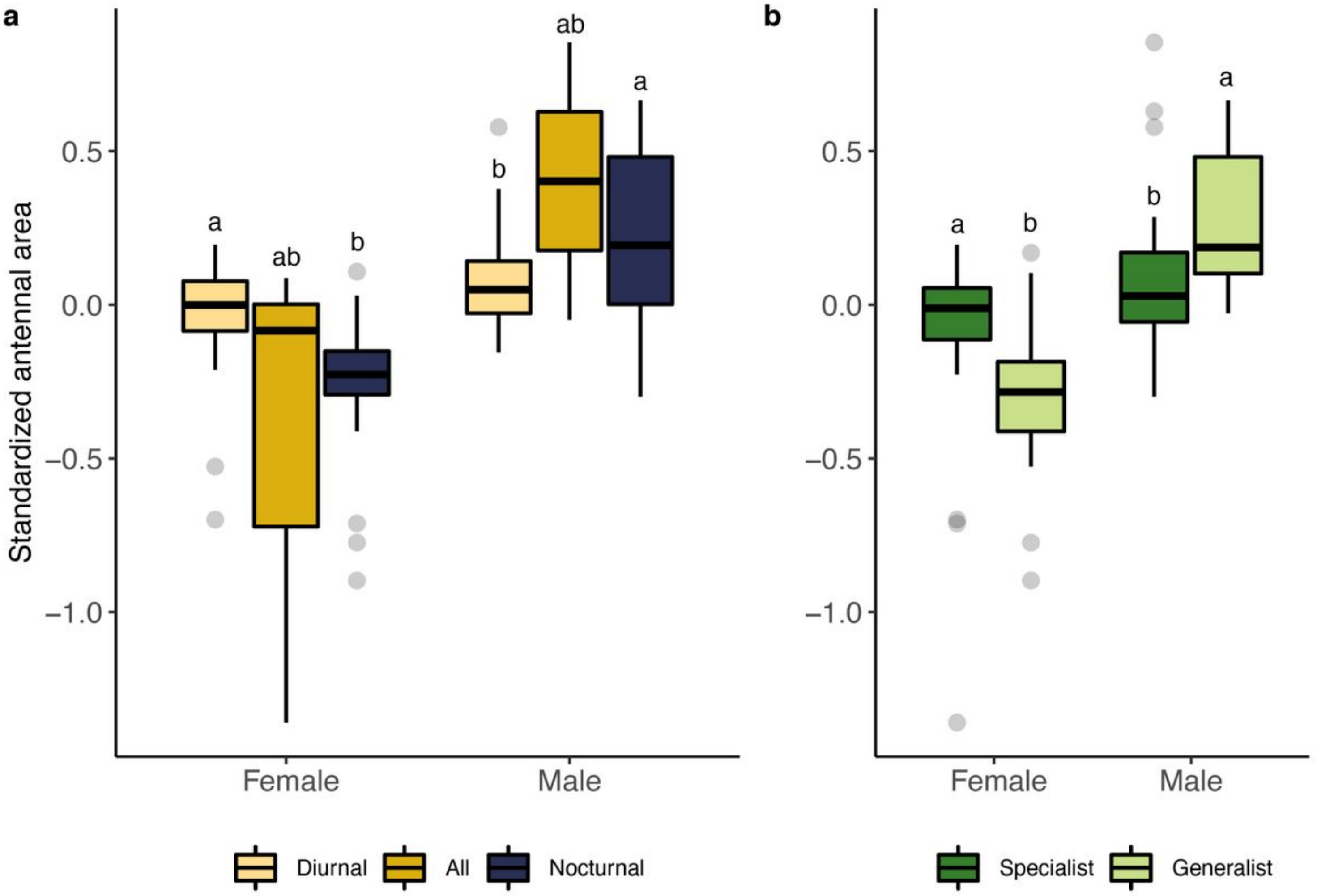

Figure 5

Relative antennal area of Lepidoptera with different diel activity preference and host specificities

Boxplots of the antennal area as a function of body size among Lepidoptera species. (a) Differences in the antennal area relative to body size between species with distinct diel activity preference. (b) Differences of the relative antennal area between species with different host specificities. Different letters indicate statistical differences using a Wilcoxon sum rank test between categories within each sex. Relative antennal areas are calculated by considering body size and phylogenic association. Crepuscular species were removed due to a lack of specimens. All: species that are active during the day and at night.

Supplementary Files

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