

Scars on fossil leaves: An exploration of ecological patterns in plant–insect herbivore associations during the Age of Angiosperms

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ABSTRACT

Ancient plant–insect herbivore associations can be studied directly through observation of feeding damage scars on well-preserved leaf adpression fossils. Early work on insect herbivory was largely qualitative and descriptive. The establishment of the insect damage census protocol by Wilf and Labandeira in 1999 modernized the study of ancient insect herbivory, formalized the functional feeding group–damage type (DT) system, and allowed rigorous quantitative analyses. In this review, we first discuss how to recognize insect herbivore damage and conduct insect damage censuses, as well as the importance of collecting these data.

We then present a compilation of all published insect damage census data from angiosperm-dominated ecosystems that included at least 300 fossil dicot leaves. These 66 datasets range from Late Cretaceous to Pleistocene in age and include the majority of continents. Damage richness, damage frequency, relative abundance distributions of functional feeding groups, and ecological network metrics were computed for each site. We investigated spatio-temporal variations and tested potential drivers using various statistical analyses. Reassuringly, herbivory metrics do not appear to be influenced by publication date or depositional setting. There is no linear correlation between herbivory and geologic age, and the few significant differences that occur among time bins document the importance of the K-Pg extinction event on insect herbivory. When sites are partitioned into latitudinal bins, the mid southern hemisphere (60°S to 23°27'S) stands out as having frequent and diverse damage. High latitudes tend to have low damage richness and frequency, although not all differences are significant. Mean annual temperature influences herbivory more than any potential abiotic or biotic driver explored in our analyses and has significant positive relationships with total, specialized, and galling DT richness at 300 leaves, as well as specialized damage frequency. We close our paper by discussing best practices and promising avenues for future insect herbivory research.

1. Introduction

The evolutionary history of plants and their insect adversaries is a story told by the scars on fossil leaves. Insect damage on a single leaf captures one moment in time when an insect fed upon a plant, but it also reflects the millions of years of evolution leading up to that moment. The effects of insect herbivory on terrestrial ecosystems through time have been immeasurable over the past 400 million years (Labandeira, 2006,

2007), and the diversity of ecological, biochemical, behavioral, physiological, genetic, and evolutionary underpinnings of plant–insect associations create an almost boundless prospective dataset (Strauss and Zangerl, 2002). Only recently have scientists begun to understand major themes of the diversity and intensity of plant and insect associations in the fossil record, including temporal and spatial variations in ancient food-web structure (e.g., Wilf, 2008; Prevec et al., 2009; Dunne et al., 2014; Feng et al., 2017; Shaw et al., 2021), the evolution of insect

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feeding guilds and specific insect clades (e.g., Labandeira, 2006; Sarzetti et al., 2008; Sohn et al., 2019a; Maccracken, 2020), host plant specialization (e.g., Wilf et al., 2000; Winkler et al., 2010; Jud and Sohn, 2016), and the impact of climate change (e.g., Wilf and Labandeira, 1999; Winkler et al., 2009; Currano et al., 2010; Wappler et al., 2012) and mass extinction events (e.g., Labandeira et al., 2002; Wilf et al., 2006; Wappler et al., 2009; Donovan et al., 2016) on plant–insect associations.

In general, the study of deep time insect herbivory has become more sophisticated, detailed, and rigorous through time. Among the first instances of fossil insect herbivory recognized and reported in the scientific literature were descriptions of galls on fossil leaves from the Cretaceous (Lesquereux, 1892), Eocene (Cockerell, 1910), Oligocene (Scudder, 1886; Brues, 1910), and Pleistocene (Berry, 1909), and now dubious leaf mines on Permian ferns (Potonié, 1893). Systematic descriptions of insect damage on fossil leaves began in the mid-1970s through the 1980s (e.g., Opler, 1973, 1982; Hickey and Hodges, 1975; Crane and Jarzembowski, 1980; Liebhold et al., 1982; Scott and Taylor, 1983; Scott and Paterson, 1984; Scott et al., 1985; Rozefelds and Sobbe, 1987). A renaissance of fossil insect herbivory studies occurred in the 1990s, when paleobiologists began methodically analyzing and quantifying insect damage across periods of time and for entire floras (Beck and Labandeira, 1998; Labandeira et al., 1994; Labandeira, 1998a, 1998b; Wilf and Labandeira, 1999). Insect damage research became more widespread, systematic, and formalized with the functional feeding group–damage type system of herbivory analysis, first introduced in a seminal paper by Wilf and Labandeira (1999) and later codified in the *Guide to Insect (and Other) Damage Types on Compressed Fossil Plants* (Labandeira et al., 2007). During the 2000s and 2010s, fossil insect herbivory studies expanded into previously unstudied geographic regions and time periods, and many utilized new statistical analyses and sampling regimes which more closely align with techniques used by neontologists. Importantly, studies of modern ecosystems were conducted to ground-truth observations from the fossil record (Adams et al., 2009; Carvalho et al., 2014; Sohn et al., 2019b).

In this contribution, we first review how to recognize insect herbivory on fossil leaves and to analyze the composition, frequency, diversity, and intensity of ancient herbivory. We then discuss the taxonomic and phylogenetic significance of identifying insect culprits, noting the difficulty in making these assignments. Finally, we conduct a meta-analysis of fossil insect herbivory in angiosperm-dominated ecosystems from the Late Cretaceous through the Pleistocene. We focus specifically on herbivory on non-monocotyledonous angiosperms (i.e., “dicots”), both because more data exist and because extant herbivores overwhelmingly target dicot leaves (Grubb et al., 2008). We examine spatial and temporal variations in herbivory on bulk floras and test potential drivers of these patterns, using a combination of linear models, bipartite network methods, co-occurrence patterns, and various other statistical analyses.

2. Identification, application, and quantification of insect herbivory

Evidence for the damage that herbivorous insects inflict—the punctures, skeletonization, galls and leaf mines in fossil leaves—constitute one of the richest ecological sources of evidence available on species interactions of any kind from the distant past. Herbivore-induced insect damage can be distinguished from physical damage, such as tearing, detritivory, or taphonomic processes, using the following criteria. First is the presence of reaction tissue, in which cells are enlarged or multiplied along the site of damage (Brues, 1924; MacKerron, 1976; Vincent et al., 1990; Johnson and Lyon, 1991). Second is the targeting of a specific host-plant taxon or a particular plant organ that can be attributed to insect-specific patterns of damage, such as linear rows of punctures on or along primary veins or small cusps occurring on the cut edge of a plant tissue (Keen, 1952; Kazakova, 1985; Gangwere, 2017). Third is the repetition of a damage pattern based on

shape, size, and position of the damage on the plant (Bodnaryk, 1992; Heron, 2003).

The *Guide to Insect (and other) Damage Types on Compressed Fossils* (Labandeira et al., 2007) provides a system for classifying damage by feeding guild, or functional feeding group (FFG), and damage type (DT). The guide additionally includes DTs for oviposition and fungal/pathogen damage. Functional feeding groups that occur on leaves include hole feeding, margin feeding, surface feeding, skeletonization, piercing and sucking, galling, and leaf mining (Fig. 1). Discrete, diagnosable DTs are documented within each FFG and assigned a DT number. For example, DT2 is within the hole feeding FFG and is defined as a circular perforation 1–5 mm in diameter. Damage types are defined by the distinctive shape, size, extent, and location of herbivore damage on the affected leaf or other plant organ, and each is rated for host specificity based on comparison with extant insects and their damage. Generalized DTs are most often made by polyphagous insects, capable of eating many plant species. Specialized DTs are made by oligophagous or monophagous insects that eat only one or a few closely related plant species (Labandeira et al., 2007). Although some insects can make multiple DTs and some DTs can be made by many taxonomically unrelated insects, neontological work by Carvalho et al. (2014) demonstrated a strong correlation between the number of DTs observed on a plant host and the number of insect herbivores captured on that host. As such, leaf damage has been used to investigate changes in ancient insect diversity for intervals and geographic regions where few insect body fossils are known.

Insect herbivory can be best assessed in ancient ecosystems using unbiased damage censuses. First, a bench quarry is created, exposing a productive leaf-bearing layer. That layer is then excavated in as large blocks as possible, and the blocks are split to expose fossil leaves. Every identifiable leaf(let) is carefully examined using a hand lens and/or a stereoscope, and the DTs occurring on that specimen are recorded. Many studies only include leaves (or leaflets) that are at least 50% complete, and we advocate for this approach. Whenever possible, three or more quarries should be excavated from the same stratigraphic level to account for spatial heterogeneity in both plant composition and herbivory (Burnham et al., 1992; Currano, 2009). The total number of specimens collected from a site is often determined by the extent of exposed fossiliferous strata, the density of identifiable specimens within those strata, and the amount of time that can be spent at a site. While best data collection practices are known to differ between geologic eras and plant clades (e.g., Schachet et al., 2020), large sample sizes are often favorable. In our previous work, our goal has been to census at least 1000 specimens per site; on average, about one third of leaf specimens have herbivory on them, and so this large sample size increases the chances of finding rarer damage types, such as mines and galls.

Unbiased damage censuses produce a data matrix of fossil leaf specimens, their botanical identifications (either a formal Linnaean assignment or an informally named morphospecies), and the presence/absence of every DT. From this matrix, herbivory can be quantified for the bulk flora, as well as for individual species/morphotypes of host plants and individual specimens. Commonly used herbivory metrics include damage frequency, composition, and diversity. Damage frequency is simply the proportion of leaves with damage; total damage frequency (all DTs) and specialized damage frequency (only specialized DTs) are often reported. Damage composition refers to the relative abundances of the different DTs or FFGs, and ordination analyses like nonmetric multidimensional scaling (NMDS) provide an effective means of visualizing compositional differences among sites or plant hosts. Damage diversity is quantified as DT richness, or the number of damage types observed, standardized for sample size. Many papers report total, specialized, mine, and gall diversity. An additional herbivory metric that is not as frequently employed is the herbivory index, or the percent of leaf surface area herbivorized by insects. This index is important for quantifying changes in insect consumption of plant tissue and is frequently measured by ecologists studying modern insect herbivory. These data are time-consuming to collect, as each specimen must be

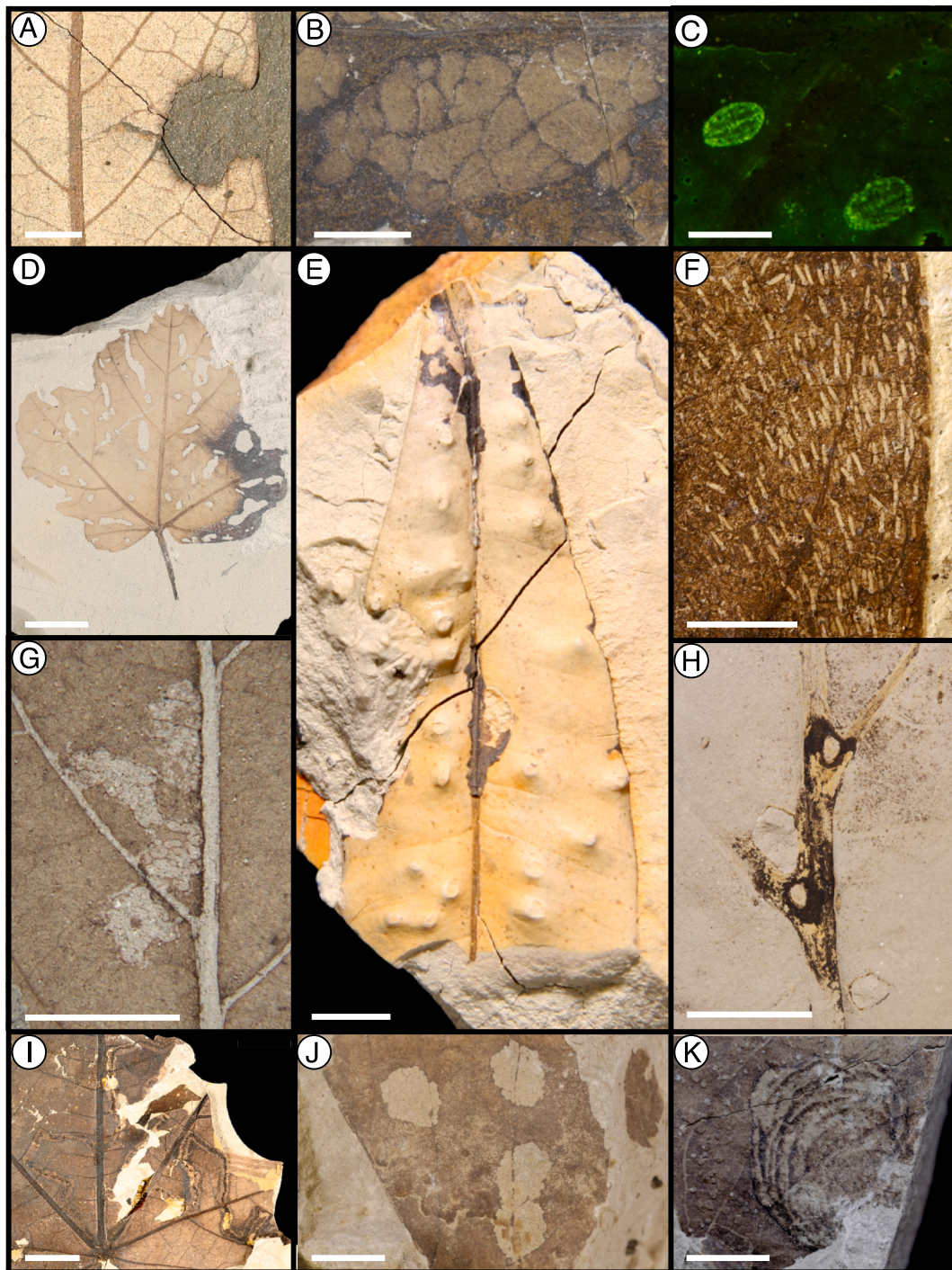


Fig. 1. Examples of insect damaged leaves during the Age of Angiosperms. A) Leaf-cutter bee (*Megachilidae*) margin feeding (DT81) from the Eocene Messel Formation (Wedmann et al., 2009). B) Surface feeding (DT33) on a eudicot from the Late Cretaceous of Utah, USA (Maccracken, 2020). Scale bar = 0.5 cm. C) Scale insects imaged using epifluorescent microscopy on a fossil legume leaflet from the early Miocene of Ethiopia. D) Hole feeding and margin feeding on a fossil angiosperm of unknown affiliation from the Late Cretaceous of Utah, USA (DT1, DT2, DT3, DT7, DT12, DT15) (Maccracken, 2020). E) Cynipid galls on Sapoteae sp. from the late Oligocene Chilga Basin, Ethiopia. F) Oviposition (ichnospecies *Paleoovoidus rectus*) on *Laurophyllum pseudoprinceps* from the Late Oligocene Rott fossilagerstätte (Petrulevičius et al., 2011). G) Skeletonization (DT61) adjacent to the primary and secondary venation on “*Myrica*” *premira* from the latest Cretaceous of Argentina (Donovan et al., 2018). H) Acarodomatia (DT339) from the Late Cretaceous of Utah, USA (Maccracken et al., 2019). I) Coleopterid leaf mine (DT164) on “*Dombeya*” *novi-mundi* from the Eocene of Wyoming, USA. J) Circular mined areas that appear skeletonized (DT38) on *Platanites raynoldsii* from Wyoming, USA (Schmidt et al., 2019). K) Ichnofossil of *Leucopteroptera spirala*, a lyonetiid moth leaf-mine (DT178) from the Late Cretaceous Kaiparowits Formation, USA (Maccracken et al., 2021). Scale bars are 1 cm in A,D, E, I, J and 0.5 cm in C, F, G, H, K.

photographed and then leaf area and area damaged must be manually traced and measured using image analysis software (e.g., ImageJ). A video demonstration of conducting an unbiased damage census accompanies this paper.

3. Leaf damage informs about insects present

The earliest unequivocal arthropod herbivory consists of sporangial and stem consumption from the earliest Devonian Period (ca. 410 Ma) of Europe (Kevan et al., 1975; Labandeira, 2007; Labandeira et al., 2014). Very limited herbivory has been documented for the Mississippian, 359–323 Ma (Iannuzzi and Labandeira, 2008). By the Pennsylvanian, 323–299 Ma, arthropods herbivorized roots, leaves, seeds, and wood, and all major herbivorous arthropod feeding guilds had evolved (Labandeira, 1998a, 2006; Scott and Taylor, 1983) except for leaf mining, which first unequivocally appears in the earliest Triassic of Russia (Krassilov and Karasev, 2008) and the Late Triassic of South Africa (Labandeira et al., 2018). By the Early Cretaceous, the damage types created by insect herbivores appear essentially modern (Labandeira, 1998c). Hole feeding, margin feeding, skeletonization, surface feeding, piercing-and-sucking phloem and xylem sap feeding, leaf mining, galling, wood boring, and seed predation are extensive and well known before the advent of angiosperms (see Labandeira et al., 2007 and recent addenda for a record of Paleozoic and Mesozoic damage types; Labandeira, 2021). However, the Mesozoic Era in general and Cretaceous Period in particular is the poorest-sampled interval for quantitative angiosperm–insect associational studies (Pinheiro et al., 2016), and we expect that many Cenozoic damage types may have first occurrences in the Mesozoic.

Assigning damage to DTs, rather than simply FFGs, is well worth the time investment. First, DT richness provides a proxy for insect herbivore diversity (see Section 2 above). Second, tracking distinct DTs in the fossil record significantly improves our understanding of evolution and ecology, even when the insect culprits cannot be identified. For example, Donovan et al. (2020) surveyed DTs on Cretaceous through Eocene fossil *Agathis* leaves from Patagonia and compared the suite of DTs observed with that on extant *Agathis*, using herbarium specimens collected in Malaysia and Australia. By discovering a similar suite of DTs on fossil and modern specimens, they demonstrated persistence of ecological guilds over tens of millions of years and across great distances. Further, the DTs observed on herbarium specimens document a new diversity of insect herbivory, and potentially insect herbivores, associated with extant *Agathis*. Third, certain DTs are distinctive enough to be assigned extant insect lineages, although convergence of herbivore mouthparts and feeding behaviors often makes genus- or species- level identification of the insect culprit rare. Below, we provide two examples where fossil leaf damage significantly improved our understanding of insect evolution, phylogeny, and host plant preferences.

The Nepticulidae (pygmy moths) are an early-diverging, species-rich lepidopteran family. Adult nepticulid body fossils are rare, with just 11 confirmed specimens in the review of Doorenweerd et al. (2015), the oldest of which are between 43 and 45.2 Ma. Including leaf mines assignable to Nepticulidae (see Doorenweerd et al., 2015 for descriptive characters) increases the number of fossil occurrences of the family by more than five times and pushes the earliest appearance back 60 million years, to 102 Ma (Labandeira et al., 1994; Sohn et al., 2012). Fossil nepticulid leaf mines occur on a wide variety of angiosperm families (Doorenweerd et al., 2015). Some of these lineages are not known to host nepticulid larvae today (e.g., Laurales and Austrobaileyales), suggesting diverse extinct nepticulids. Other cases, like the fossil nepticulid mine discovered on Miocene *Fagus* leaves from South Korea (Sohn et al., 2019a) record long-term associations between nepticulids and host plants.

Other Lepidopteran leaf mines have also provided important calibration points for phylogenetic analyses. The recent description of *Leucopterosa spiratae* (Fig. 1K), a fossil lepidopteran leaf mine from the

Late Cretaceous of western North America, is the earliest (75.6 ± 0.18 Ma) and only record of a fossil Lyonetiid leaf-mining moth (Maccracken et al., 2021). This discovery is among the oldest known fossils assignable to the Yponomeutoidea–Gracillarioidea (Y–G) clade. The mine morphology is associated with subfamily Cemiostominae, and it may be a close relative to members of extant *Leucoptera*. *Leucopterosa spiratae* provides a much-needed calibration point for the Y–G clade, in which further phylogenetic work is required to determine the monophyly, placement, and evolutionary timing of the family Lyonetiidae within the Y–G clade.

4. Compilation and exploration of published studies on angiosperm-dominated fossil ecosystems

4.1. Data and associated metadata collection

We compiled fossil insect damage census data published prior to 2021 using Web of Science and Google Scholar and keywords “insect herbivory,” “plant–insect interactions,” and “fossil.” We included in our compilation all sites that were collected in an unbiased manner (i.e., all identifiable leaves scored for herbivory) and had at least 300 dicot leaves. We chose this value to maximize the number of sites that could be included in the meta-analysis and thus spatial and temporal coverage, but we reiterate that new work should target 1000 specimens per census. For each site, we recorded basic site information (age, geographic location, depositional setting, publication date) and paleoclimate reconstructions (mean annual temperature, or MAT, and mean annual precipitation, or MAP). We searched the literature for the most recently published geologic ages and paleoclimate reconstructions for each site. When only a geologic stage was given, we used the midpoint date of the stage range. In some cases, dates were estimated from locality placement on stratigraphic columns. We assigned each site to the latitude bins High-S (90°S to 60°S), Mid-S (60°S to 23°27′S), Low (23°27′S to 23°27′N), Mid-N (23°27′N to 60°N), and High-N (60°N to 90°N). We also grouped sites more finely by region: Antarctica, New Zealand, Patagonia, Tropical South America, Africa, Eurasia, North America, Alaska, Iceland, and Spitsbergen. Paleoclimatic reconstructions such as MAT and MAP were calculated using various methods across many research groups. In an effort to reconcile different methods, we chose leaf margin analysis (MAT) and leaf area analysis (MAP) results when possible; however other methods (e.g., CLAMP, nearest living relative) were included when necessary. Differing methods are assumed to not alter results in a meaningful way.

4.2. Quantitative analyses

4.2.1. Exploring variations in herbivory metrics in the dataset

Due to heterogeneity in reported methods for quantifying insect herbivory among publications, we chose to do all quantitative analyses for this study using the raw census datasets. We obtained these datasets either from the publication or by contacting the authors. We first eliminated all non-dicots and unidentifiable specimens and then standardized the formatting of each site's data to be able to batch run analyses in R. Dicot diversity was reported using Shannon's Index and rarefied richness at 300 leaves, and dicot evenness was quantified as Pielou's J. We analyzed insect damage frequency, diversity, and composition on each bulk flora. We considered total damage (all insect feeding DTs), specialized damage (insect feeding DTs typically made by monophagous or oligophagous insects), and damage within functional feeding groups. We also calculated the percent of damage type occurrences that were specialized. Damage diversity was standardized for sample size using the extension of analytical rarefaction applied by Gunkel and Wappler (2015), and we computed total, specialized, mine, and gall diversity at 300 dicot leaves. Only 11 sites include herbivory index data (% of surface area removed for the whole flora), and so we did not utilize this metric. Differences in damage typing accuracy and

specimen preservation likely occur between datasets, but we assume those differences also do not alter the results in a meaningful way.

We began our exploration of the dataset by testing for differences in each herbivory metric across geologic time (binned by epoch), latitude bin (High-S, Mid-S, Low, Mid-N, High-N), and depositional setting (lacustrine, fluvial, coastal, mixed). Outliers were identified using the Rosner statistical test ($\alpha = 0.05$; `rosnerTest` from the `EnvStats` package; Millard, 2013) and removed from analyses. The Tukey statistical test (confidence interval = 0.95; `TukeyHSD` from the `stats` package) was used to identify significant differences in mean values. Boxplots were created using R version 3.6.1 and a combination of base R, `tidyverse` (Wickham et al., 2019), and `ggplot` (Wickham et al., 2016). Last, we used nonmetric multidimensional scaling (NMDS) to visualize differences in FFG distributions among sites, and ANOSIM to test for differences among time bins, latitude bins, and depositional settings. NMDS was conducted using function `metaMDS` (distances = Bray-Curtis; `vegan` package; Oksanen et al., 2013) on a data matrix of the proportion of leaves at each site with each FFG that had been arcsin square root transformed.

We then constructed generalized linear models to evaluate the

relationships between various DT metrics and associated site metadata. The DT metrics were specialized DTs, galling DTs, mining DTs, and all DTs measured at 300 leaf specimens, while site metadata included publication date, MAT, MAP, and plant diversity statistics. These factors were analyzed at different levels of complexity: (1) simple linear regression model, (2) multiple linear regression model, and (3) mixed effects model with varying slope and/or intercept (with latitude and depositional environment as random effects). Linear models were based on the gaussian family, or generalized normal, of distributions. A Benjamini-Hochberg correction was used on the simple linear regression analyses to limit the false discovery rate, or the rate of Type I errors that occur when making multiple comparisons.

4.2.2. Bipartite network construction and analyses

In order to analyze the patterns in and among interactions, we constructed weighted bipartite networks using plant-insect associations for each flora (Fig. 2; for detailed methodology see Swain et al., 2021). In each bipartite network, nodes were either plant taxa (at the most highly resolved rank possible) or herbivory DTs (Labandeira et al., 2007). In these networks, an edge can only be present between a DT and a plant

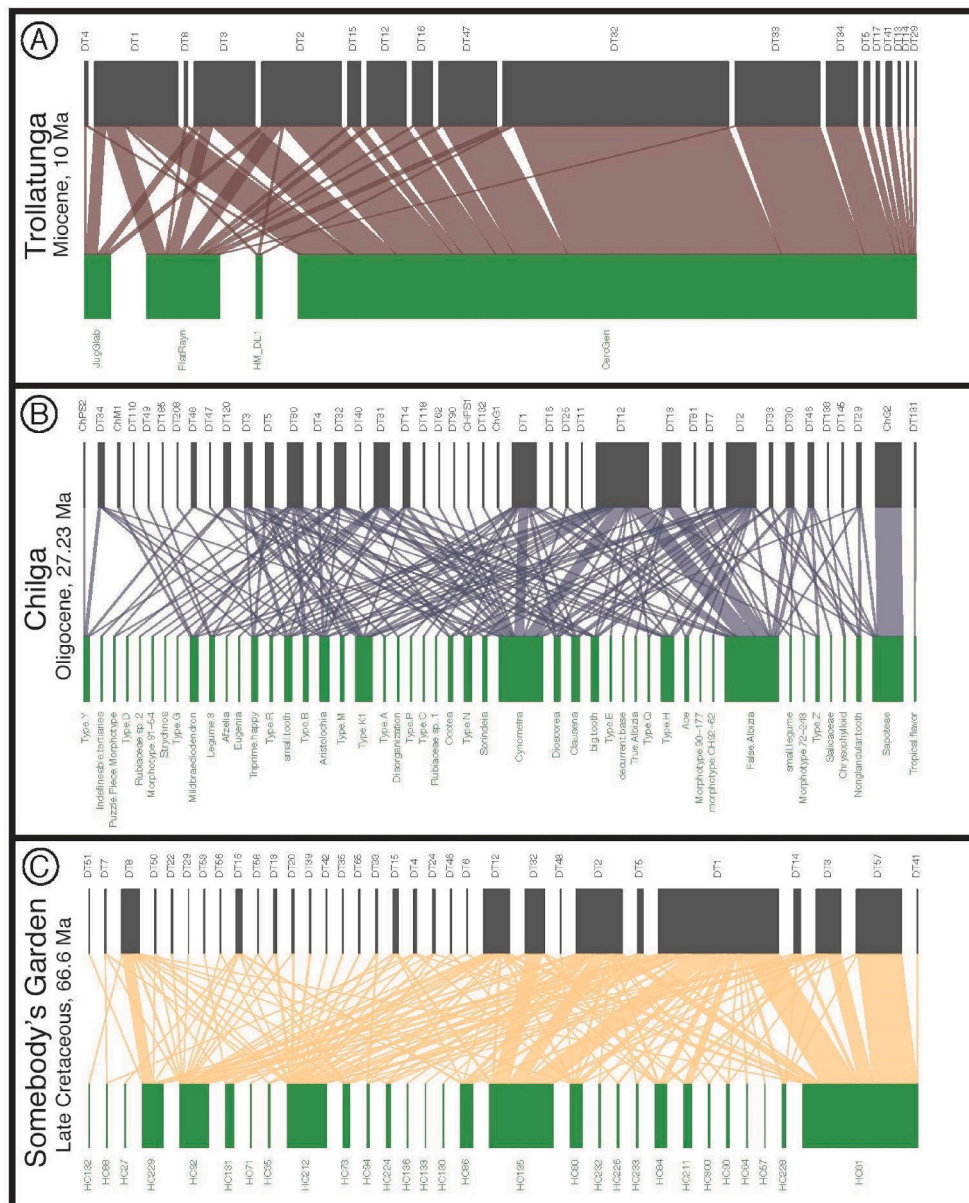


Fig. 2. Bipartite network representations of the fossil plant-DT associations in A) Trollatunga (Miocene of Iceland), B) Chilga (Oligocene of Ethiopia), and C) Somebody's Garden (Late Cretaceous of USA). Note that A has high nestedness, where plant species are associated with DTs in a such a fashion that some are subsets of others (i.e., a high nestedness occurs when there is a sequence of plant species where each species is a subset of the previous species in the sequence) and moderately high connectance (number of associations realized out of all possible associations), but a low number of species. An interesting aspect of B is that it shows a unique 'directed' association or specialization between the "Tropical flavor" leaf morphospecies and DT131 (see right end); it has low connectance, low nestedness, but a high C score (how overlapping or unique are the associations; higher values denote high uniqueness), high interaction evenness (how balanced the associations are with respect to the Shannon diversity; higher values denote more evenness) and low niche overlap. C has intermediate C score, high interaction evenness, low niche overlap, low connectance, and low nestedness.

taxon, and represents the presence of a particular DT on the plant taxon. The width (or weight) of an edge represents the number of occurrences of a given DT in a plant taxon. The width of each node denotes their respective number of occurrences. Each network is normalized according to its total number of DT occurrences and leaf specimens for each node-class. For each site, we constructed 500 bootstrapped networks using 300 leaves (without replacement, see Gunkel and Wappler, 2015) to standardize the comparisons.

Although these network representations provide an effective visualization tool for understanding inter-relationships among plant species and DTs, the complexities of these interactions can quickly make the patterns indiscernible to the naked eye. Therefore, in order to investigate and compare the properties of these networks quantitatively, we use several well-documented ecological (network) metrics from the literature and in many cases, these statistics can be intuitively/qualitatively understood from network visualizations (see Tables 1, S1; Fig. 2; Swain et al., 2021). For each of the 500 networks at each site, we calculated these metrics at three scales: at the whole network level, each node-class level (either all plants or all DTs), or at node level (see Dormann et al., 2009). These metrics measure different properties of the ecological networks such as centrality, specialization, co-occurrence, nestedness, and robustness (see Swain et al., 2021 for a detailed overview; see Tables 1, S1).

Network-level statistics were compared across time and latitude bins, as described above for herbivory metrics. We constructed linear models of important network properties with environmental covariates such as mean annual temperature and precipitation (and performed multiple correlation correction on the same using the Benjamini-Hochberg method). We also performed a PCA analysis of node-level statistics to discern which factors explain the greatest variance among properties of plants and DTs.

4.2.3. Co-occurrence analyses

The abundance values for the DTs at a leaf specimen level for each site were used to calculate strength of co-occurrence using a metric-free, distribution-free, and randomization-free model based on a

hypergeometric approach of probabilistic occurrences (see Veech, 2013; Griffith et al., 2016). In this model, we compared the observed co-occurrence to the expected co-occurrence (where the latter is the product of the occurrence probability of two species and sampling site count). Employing a hypergeometric distribution, this method determines whether the probability of observed co-occurrence count is significantly greater than expected (positive association), significantly less than expected (negative association), or not significantly different and approximately equal to expected (random association; for details of implementation and review/comparison with previous co-occurrence tools, see Griffith et al., 2016). By adding the presence or absence of the positive/negative co-occurrence of DTs at a site level, we collated the data in a single matrix to see consensus co-occurrence patterns in the data.

5. Meta-analysis results

Our dataset consists of 77,763 leaves from 66 sites (Fig. 3). Two thirds of the sites are in the northern hemisphere mid-latitudes, with 31 sites in North America and 13 sites in Eurasia. The tropics ($n = 3$) and high southern latitudes ($n = 2$) are severely underrepresented in the dataset. Most research has focused on the Paleocene ($n = 19$) and Eocene ($n = 23$), with only two Pliocene and one Pleistocene sites. Herbivory metrics for each site are available in the Supplementary Materials and visualized in Fig. S1. Total damage frequency ranged tremendously from 2.6% of leaves damaged at the Paleocene Seymour Island site in Antarctica to 71.8% at the Miocene Hindon Maar site in New Zealand, with a mean of 30.4% across all sites. Specialized damage frequency ranged from 0.89% (Eocene Chickaloon site, Alaska) to 30.3% (Hindon Maar), with mean value 8.8%. Damage diversity, standardized to 300 leaves, was lowest at Chickaloon (5.9 DTs; 1.8 specialized DTs) and highest at Hindon Maar (61.9 total DTs; 37.1 specialized DTs); the mean across the 62 sites for which diversity data was collected was 21.3 total DTs and 9.9 specialized DTs.

Hole feeding is the most abundant FFG (mean frequency of 19.1%), followed by margin feeding (9.0%). Leaf mining is the least abundant FFG in our dataset, with a mean site frequency of 1.0% and eight sites without a single leaf mine. Our exploration of DT co-occurrence patterns at a leaf resolution and at site level (collated across sites), revealed pairs of DTs that co-occur at different levels of commonality (Fig. 4). Hole feeding DTs 1, 2, 3, and 5 and margin feeding DT12 form a definitive cluster with positive co-occurrences in more than 20 sites. Other positively co-occurring pairs are rare and exhibit a site-specificity, albeit a second cluster consisting of DTs (such as hole feeding DTs 4, 7, and 8 and margin feeding DTs 14–16) positively co-occurs with this former cluster in a large number of sites, but not as strongly among themselves. Negative co-occurrences are rare.

We calculated the network metrics of DTs and host plants at a node level (see Table 1 for detailed description) and performed a PCA on them separately for DTs and plants (Fig. S2). The axes that differentiate and explain the most variance show two aspects of specialization of associations. The primary axis shows the number of associations (plants/DTs) associated with the other trophic level and its related metrics (such as species partner diversity, effective partners, species strength, and degree). The secondary axis was represented by interaction asymmetry (Blüthgen's d) and closeness metrics. Incidentally, this representation of PCA axes is true for each of the sites in isolation and also in previous studies incorporating these metrics in fossil plant–DT associations (Swain et al., 2021).

We investigated how the field of fossil plant–insect associations has changed over the past several decades by constructing simple generalized linear models of the gaussian family of distributions and tested for correlations between publication date and both herbivory metrics and geologic age of the sites. We found that, in general, the richness of damage types, including specialized, galling, and mining damage types, were not affected by the date of publication or geologic age of the

Table 1
Brief description of the major network metrics used in this work. For a more detailed list, please refer to Supplementary Table S1.

Metric	Type	Definition
C score	Co-occurrence	Mean (normalized) number of checkerboard combinations across all nodes in a given node-class (either DTs or plants). It measures the randomness of the distribution of associations of plants/DTs. (see Stone and Roberts, 1990)
Connectance	–	The fraction of all possible links that are realized in a network (Dunne et al. 2002)
Interaction evenness	Specialization	Measures how balanced the distribution of interactions is across species, based on Shannon's diversity (see Tylianakis et al., 2007)
Niche overlap	Co-occurrence	Mean similarity in interaction pattern between species of the same node-class, and is calculated using Horn's index (see Dormann et al., 2009)
Nestedness	Nestedness	Measures 'nestedness' based on the overlap of interactions. A perfectly nested assemblage can be arranged in a sequence of DTs which are associated with plants such that the associations of one DT in the sequence is a subset of its preceding DT. (see Rodríguez-Gironés and Santamaría, 2006)
H2'	Specialization	Measures the degree of specialization or partitioning among two node classes (DTs and plants) for the full network. It is calculated in comparison to no specialization in a network (complete overlap of associations) (see Blüthgen et al., 2006)

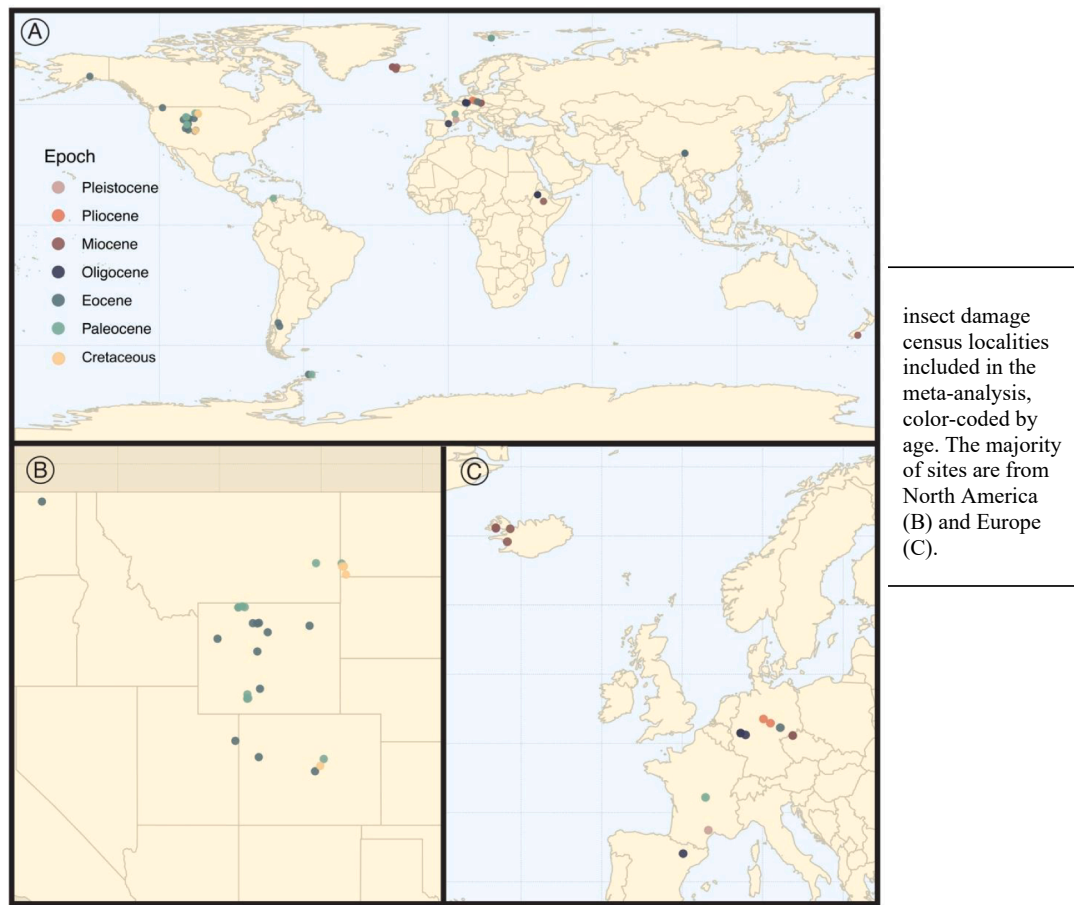


Fig. 3. Map of insect damage census localities included in the meta-analysis, color-coded by age. The majority of sites are from North America (B) and Europe (C).

localities (Tables S2–S3, Fig. 5A); however, there was a positive relationship between the publication date and the geologic ages of the localities ($t = -4.569$, $p < 0.001$).

5.1. Temporal variations in herbivory

Boxplots, Rosner and Tukey statistical tests, and ANOSIM show that only a few damage metrics differ significantly through time (Fig. S3). Correlation analyses revealed no significant relationships between damage metrics and the geologic ages of the localities (Tables S2–S3). There are significant differences in specialized damage frequency and percent of DT occurrences that are specialized across epochs (Fig. 6A,B). Cretaceous specialized damage frequency is higher (mean frequency of ~15%, Fig. 6A) than all other time bins except the Pleistocene, which is only represented by a single sample (the Bernasso site in France). Focusing on the Cretaceous, Paleocene, and Eocene, specialized damage frequency decreases between the Cretaceous and Paleocene, and there is high variability in Paleocene insect feeding specialization. Eocene insect specialization is similar to the Paleocene with slightly lower mean values. Percent of DT occurrences that are specialized shows a similar pattern; the Cretaceous is high, although not statistically distinct from other time bins (Fig. 6B).

Network analyses output for connectance, C score, and niche overlap for plants (lower trophic level or LL) show significant differences across time bins (Fig. 6 C–E) and echo the findings above of a dynamic transition from the Cretaceous to the Eocene. The Cretaceous and Eocene have lower connectance than the Paleocene, signifying more network specialization (Fig. 6C); this relationship is strongly supported when we remove all other time intervals (Tukey test $p = 0.0020$). The C score

measures the randomness of species distribution across an ecosystem (Stone and Roberts, 1990); simply put, a low C score indicates high randomness and a lack of species influencing each other across the ecosystem. The average Paleocene Plant C score is lower than during the Cretaceous and Eocene, although this is not a statistically strong signal (Fig. 6D). Niche overlap is a measure of how species use resources or other environmental resources; overlap occurs when two or more species utilize the same resources or environment (Pocheville, 2015). Here, we found no statistical overlap between the Paleocene and Eocene plant species (Fig. 6E). Interestingly, Cretaceous values of plant niche overlap are statistically similar to both the Paleocene and Eocene, although the mean value is lower than the Eocene. As none of the network metrics is strongly correlated with environmental parameters (MAT or MAP; after statistical corrections; see Table S3), we believe that these differences of network properties among different time bins reflect ecological manifestations rather than simple environmental variations.

5.2. Spatial variations in herbivory

We examined herbivory variations across latitudes, independent of time, using boxplots, Rosner and Tukey tests, and NMDS. Generally speaking, the mid-S stands out as having high total and specialized damage frequency and diversity (Fig. 7 A–D; Rosner test results indicate that Hindon Maar is an outlier, and it is not included in these analyses). High-latitude sites tend to have lower damage frequencies and diversities than those at mid- to low-latitudes, although not all differences are significant. Simple boxplots show significant differences at various latitudes in percent of leaves with surface, margin, piercing and sucking, and hole feeding damage (Fig. 8 A–D). Low latitudes have abundant

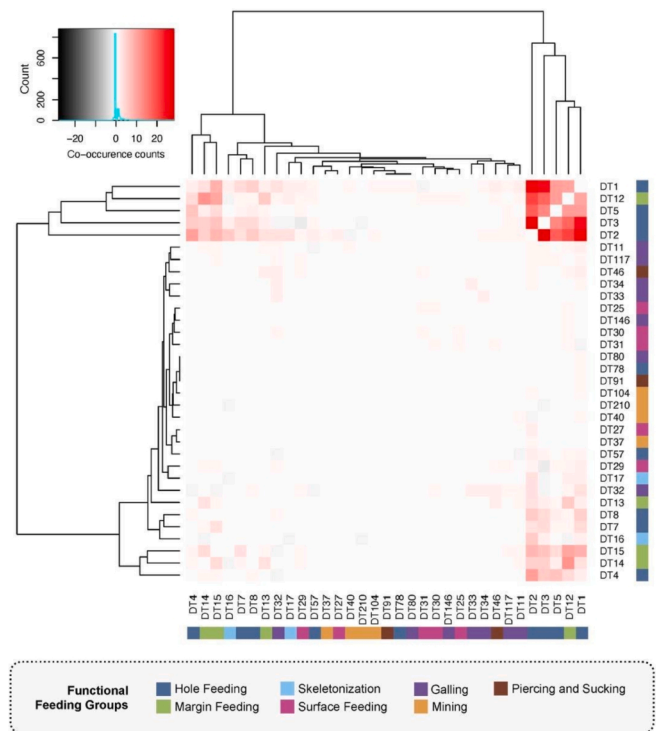
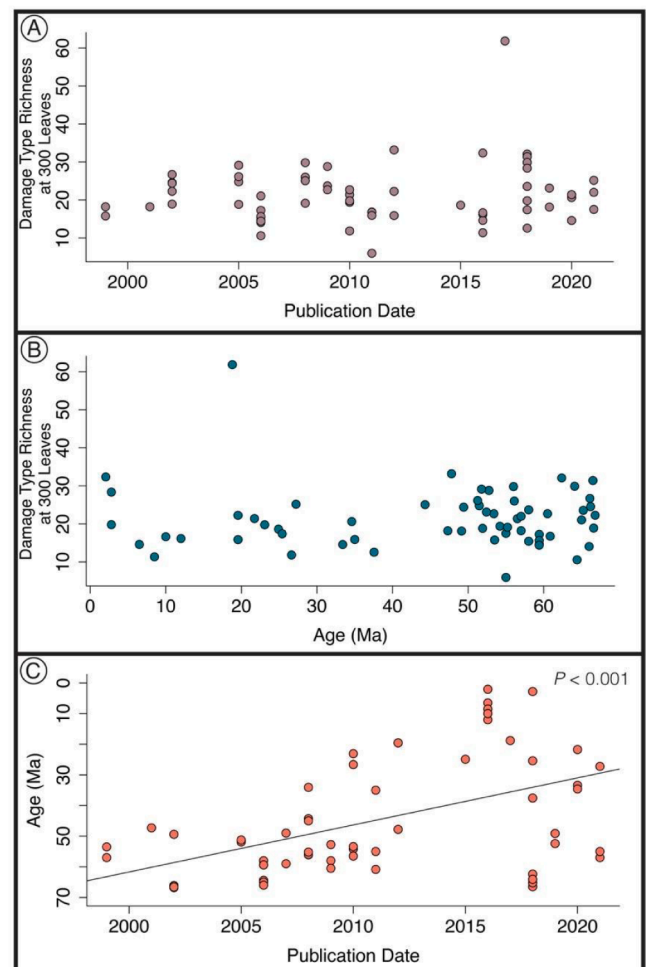


Fig. 4. A heat map of co-occurrences of damage types (DTs) at a single leaf resolution, calculated at a site level, and then collated over all the sites in this study. The color represents the number of sites where the co-occurrence was present either significantly more (red; positive co-occurrence) or less than expected (gray; negative co-occurrence). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

surface feeding and piercing and sucking marks. The mid-S has high margin and hole feeding frequency, although there are similarities with the low latitudes in margin feeding and the low latitudes and mid-N in hole feeding. Unlike the mid-S, the mid-N has similar FFG composition to all other latitudes. The latitudinal extremes of high-S and high-N have the lowest percentages of all FFGs. Our ability to compare the low-latitudes with other regions is limited due to small sample size ($n = 3$).

The relative abundance distribution of FFGs can be compared among sites using NMDS and ANOSIM analyses (Fig. 8E). While there is considerable overlap among latitude bins (ANOSIM $R = 0.18$, $p = 0.02$), there is some consistency in where a particular latitudinal bin plots. Axis 1 appears to separate sites with high frequencies of all FFGs (negative axis 1 scores) from those with low values of all FFGs (positive axis 1 scores). Most mid-S sites have negative axis 1 scores, mid-N sites range from about -0.5 to 0.25 , and high-latitude sites all have positive axis 1 scores. The North American sites with the lowest axis 1 scores, which plot closest to the highly damaged mid-S sites, are from the Paleocene–Eocene Thermal Maximum, the Early Eocene Climatic Optimum, and the diverse early Eocene Republic flora. Axis 2 appears to separate sites based on the relative representation of different FFGs; sites with high axis 2 scores have abundant galling, surface feeding, and piercing and sucking.

Network metrics of ecological significance show a higher resolution of interactions within our data (Fig. 7 E–G). Interaction evenness is a measure of uniformity of interaction strengths (based on Shannon diversity), with higher values being more uniform (Tylianakis et al., 2007). The mid-S has higher interaction evenness (mean of ~ 0.7) than other latitudes, and the difference between the mid-S and mid-N is statistically significant. Given the absence of any correlation between interaction evenness and environmental measures such as MAT and MAP, this might denote regional effects (as all mid-S sites are from



5.1. Temporal variations in herbivory

Fig. 5. Major trends, or lack thereof, within the field of fossil plant–insect associations. A) The richness of damage types (DTs), standardized to 300 leaves, at each locality by date of peer-reviewed publication. B) DTs richness standardized to 300 leaves per locality spanning the past 70 million years. C) The positive relationship between the date of publication and geologic age of each locality.

Patagonia). H2, an inverse measure of specialization (Blüthgen et al., 2006), is lowest in the mid-S (i.e., signifying higher specialization). Nestedness is higher among the high-N latitudes and is dissimilar to the other latitudinal groups (Rodríguez-Gironés and Santamaría, 2006). Both of these measures (nestedness and H2) were statistically correlated with MAT, but we did not have enough data to hold the significance after multiple correlation correction, signifying a possible, yet unproven, relationship between environmental variables (such as temperature), which depend upon latitudes and their effect on the association structure of plant–insect assemblages.

5.3. Driving factors

Simple generalized linear models of the gaussian family of distributions were used to further explore relationships among insect damage metrics and climatic variables, latitude, and plant diversity (Tables S2, S3). There was a positive, statistically significant relationship between the total richness of DTs at 300 leaves and MAT ($^{\circ}\text{C}$) ($t = 3.985$, $p = 0.003$) (Fig. 9A). We also found a statistically significant, positive slope

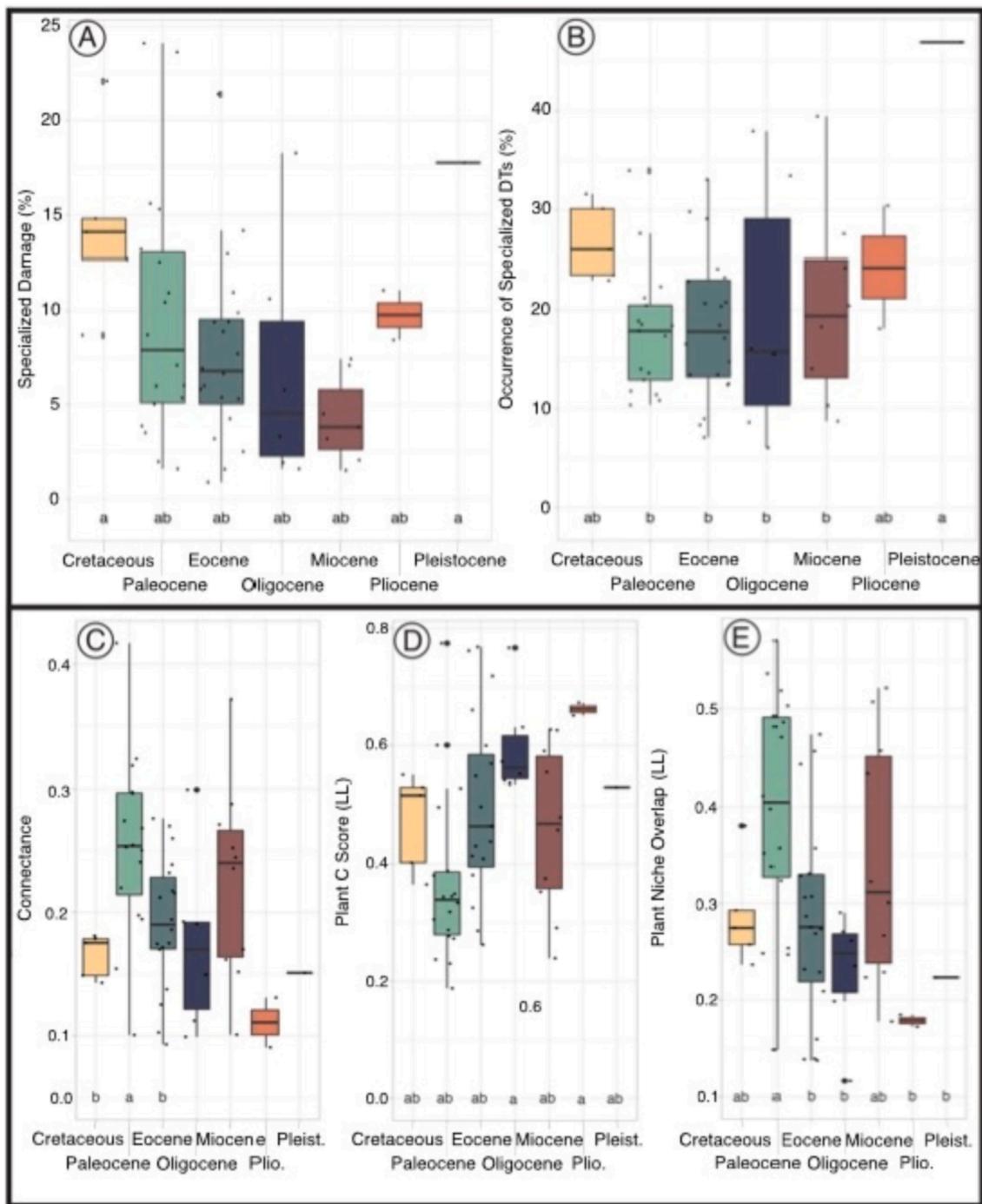


Fig. 6. Box and whisker plots of significant changes in insect herbivory and network properties through time: A) specialized damage frequency expressed as the percent of leaves at a site with specialized damage, B) percent of damage occurrences that are specialized, C) connectance, D) plant (LL) C score, and E) plant (LL) niche overlap. Points represent individual samples with whiskers representing the lower and upper limits of the data. Boxes show the lower (Q1) and upper (Q3) quantiles with the median represented by the bar across the box. Tukey statistical tests assessing similarities across epochs (lowercase letters) are shown at the bottom of each box plot. Connectedness (panel C) was significantly different only when comparing Cretaceous-Eocene. LL stands for lower trophic level (i.e. host plants).

for specialized DTs ($t = 3.768$, $p = 0.0031$) and galling DTs ($t = 3.014$, $p = 0.0287$) in relation to the MAT (Fig. 9B,C). The relationships between DT diversity, including total, specialized, mining, and galling DTs, and MAP (mm/year) were not statistically significant, as well as those for plant diversity measured at 300 specimens, Pielou's evenness, and Shannon's diversity index (Table S2). Similar to the DT richness analyses, the frequency of specialized DTs was positively correlated with MAT ($t = 3.768$, $p < 0.01$) (Table S3). In contrast to DT richness analyses, there was not a significant relationship between MAT and the

frequency of total, mining and galling DTs, or between Shannon's diversity index and the percentage of leaves with total DTs, gall DTs, or mine DTs.

To account for additional parameters that may have affected the relationships between herbivory metrics and abiotic or biotic factors, we utilized mixed effect models that included the random effects of latitude or depositional environment. When the random effect of the depositional environment (either fluvial, lacustrine, or mixed; coastal had an insufficient sample size) was included in a mixed effects model, we

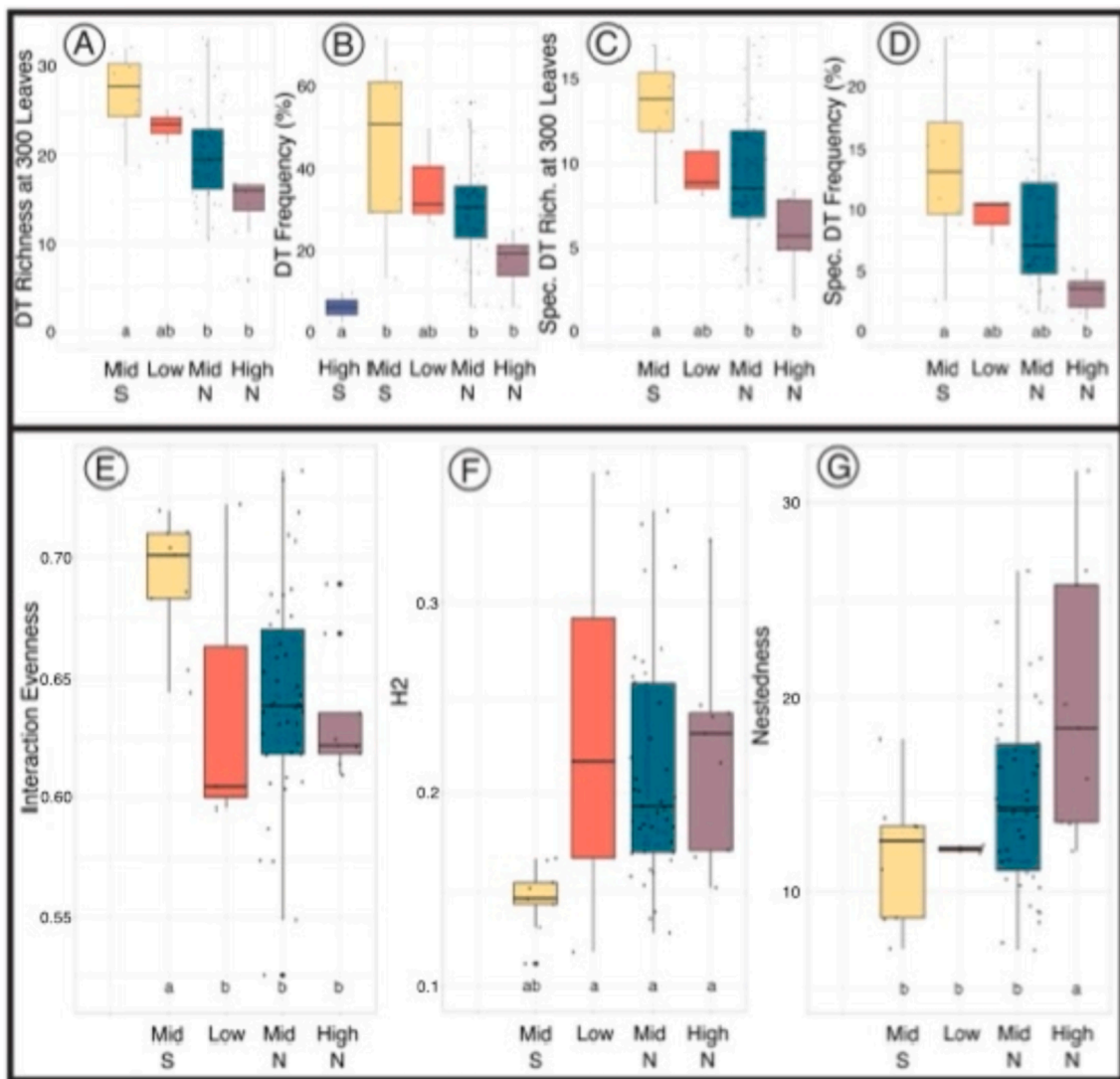


Fig. 7. Box and whisker plots of insect herbivory metrics and network properties across latitudinal bins: A) number of DTs standardized to 300 leaves, B) damage frequency expressed as the percent of leaves with any damage, C) number of specialized DTs standardized to 300 leaves, D) specialized damage frequency, E) interaction evenness, F) H2, G) nestedness. Points represent individual samples with whiskers representing the lower and upper limits of the data. Boxes show the lower (Q1) and upper (Q3) quantiles with the median represented by the bar across the box. Tukey statistical tests assessing similarities across latitudes (lowercase letters) are shown at the bottom of each box plot.

found that depositional environment explained very little of the variation (Table S5; ANOSIM analyses also found no relationship between depositional environment and FFG relative abundance distributions). This result is both surprising and reassuring, as different depositional settings capture different source areas and therefore also floral diversity (e.g., Ellis and Johnson, 2013; Spicer, 1981). The greatest amount of variance was instead explained by MAT or Shannon's diversity index, respectively, and those relationships remained statistically significant (Table S5). When latitude (high S sites were not included due to insufficient sample sizes) was included as a random effect, the results indicated that latitude was important in some instances (Tables 2, S5). The analysis of DTs and MAT with latitude as a random effect indicated that a significant amount of variance was detected for the mid-S ($t = 2.026$, $p = 0.048$). When analyzing specialized or galling DTs and MAT, latitude did not account for a significant amount of variance. However, there was a significant amount of variance detected at low-latitude localities for mining DTs and MAT ($t = -2.222$, $p = 0.031$). Similarly, a significant amount of variation was found for the mid-S in our mixed effects model of DTs and Shannon's diversity index ($t = 4.350$, $p < 0.001$), and specialized DTs and Shannon's diversity index ($t = 3.853$, $p < 0.001$).

6. Discussion

6.1. Insect herbivory tracks environmental conditions more strongly than time

Analyses for if and how the field of fossil plant–insect associations has changed over the past few decades yielded interesting and reassuring results. First, there was not a significant relationship between DT richness and publication date (Fig. 5A). The number of DTs has necessarily and steadily increased since the inception of the damage type schema, which now includes over 400 DTs (Labandeira et al., 2007 and subsequent addenda). We had hypothesized that as the field of study advanced, DTs might become increasingly finely partitioned, leading to an increase in DT richness in more recent publications. To our relief, this is not found to be the case; the increase in DTs over the past several decades appears to be the result of many new DTs discovered in the fossil record, as opposed to DTs being splintered into ever more constrained descriptions. Second, there was a significant, positive relationship between publication date and site geologic age (Fig. 5C). The first papers utilizing the DT system considered Cretaceous through Eocene floras (e.

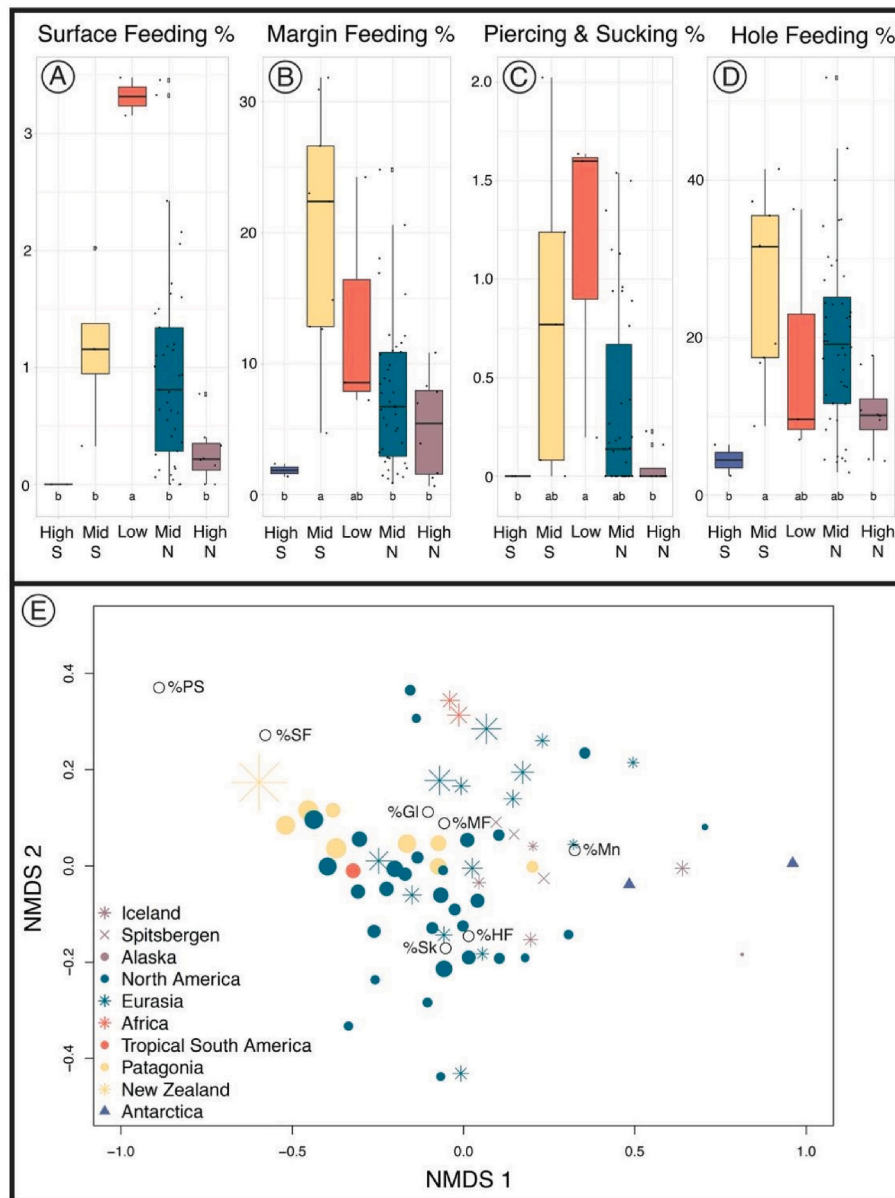


Fig. 8. Variations in functional feeding groups (FFGs) across latitudes. Box and whisker plots, made as in Fig. 7, show significant differences in the percent of leaves at a site with surface feeding (A), margin feeding (B), piercing and sucking (C), and hole feeding (D). Tukey statistical test results are shown in lowercase letters at the bottom of each box plot. A nonmetric multidimensional scaling (NMDS, panel E) ordination visualizes differences in the relative abundances of functional feeding groups among sites. Stress value for the NMDS is 0.16. Point shape and color indicate geographic region, and point size scales with DT richness. FFGs are plotted on the same ordination axes as open circles and abbreviated as galling (Gl), hole feeding (HF), margin feeding (MF), mining (Mn), piercing and sucking (PS), skeletonization (S), and surface feeding (SF).

g., Wilf and Labandeira, 1999; Labandeira et al., 2002). As the field of fossil plant–insect associations has increased in the number of practitioners and the geographic extent of studies, so too has there been a shift towards younger floras and a general expansion of time intervals investigated (ex. Wappler, 2010; Gunkel and Wappler, 2015; Adroit et al., 2016, 2018; Wappler and Grímsson, 2016; Moreno-Domínguez, 2018), although we note that the Late Neogene remains understudied. We expect the representation of younger floras to continue to increase due to their greater availability (increased proportion of older rocks being destroyed by geologic processes), as well as the ease of taxonomic identification of younger floras (Raup, 1979).

DT1 (circular holes less than 1 mm in diameter), DT2 (2–5 mm circular holes), DT3 (2–5 mm polylobate holes), DT5 (>5 mm polylobate holes) and DT12 (non-specific margin feeding) are the damage types that most commonly co-occur on the same leaf. Other hole and margin feeding DTs (4, 7, 8, 14, and 15) also positively co-occur with these, although not as often. All of these DTs require chewing mouthparts, and the same insect could have been responsible for all occurrences of these DTs on a leaf. It is important to remember, though, that richness of leaf-chewing insects is correlated to DT richness (Carvalho et al., 2014).

Negative co-occurrences are rare, and we cannot point to any relationships that might indicate avoidance of damaged leaves by insects. Some of the weak negative co-occurrences may be due to researcher choices. For example, skeletonization DT16 and DT17 differ only in the strength of the reaction rim; in our experience, researchers tend to choose either DT16 or DT17 for all occurrences of skeletonization on a leaf and attribute differences in the reaction rims to preservation.

Herbivory has varied considerably over the past 70 million years, but the overall pattern does not show an increase from the Cretaceous to the Quaternary (Figs. 5B, 6). Rather, insect damage analyses record ecosystem responses to abiotic changes, such as the K–Pg bolide impact and climate change. Specialized feeding damage is high in the Cretaceous relative to the Paleocene, and variability among Paleocene sites is high. These results concur with regional analyses that documented high extinction of specialist herbivores across the K–Pg boundary (Labandeira et al., 2002; Donovan et al., 2016, 2018) and prolonged recovery times (Wilf et al., 2006). Network analyses provide additional insight on the K–Pg mass extinction event. Connectance and niche overlap for plants are high in the Paleocene relative to both the Cretaceous and the Eocene, and the C score for plants is low in the Paleocene

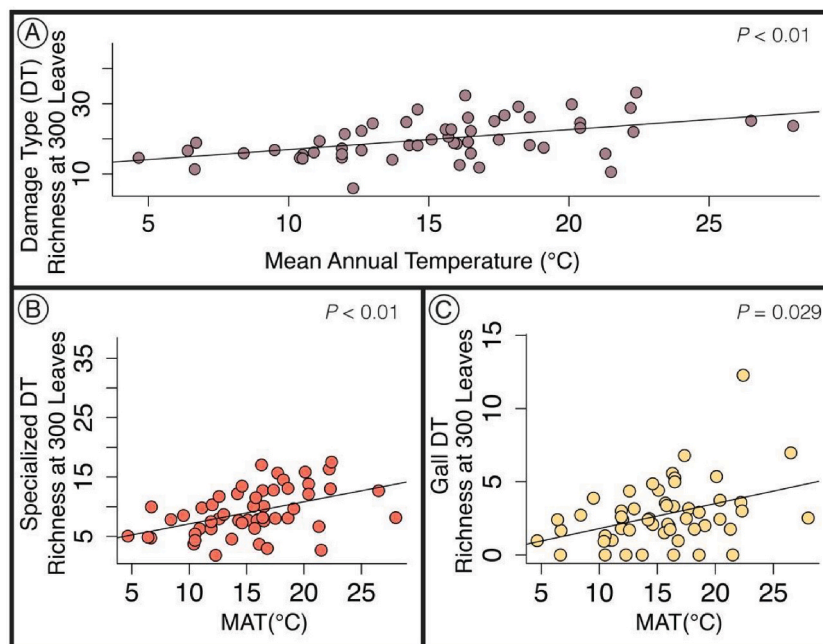


Fig. 9. Statistically significant relationships between: A) the richness of DTs per locality and mean annual temperature (MAT); B) the richness of specialized DTs per locality and MAT; and C) the richness of galling DTs per locality and MAT. All richness values are standardized to 300 leaves.

Table 2

Mixed effects models with significant results.

Independent variable	Dependent variable	Mixed effects regressor	Coefficients	Regression coefficient	Standard error	T-value	p-value
DT Richness	MAT	Latitude	Intercept	10.267	2.499	4.108	0.0002 ***
			MAT	0.4493	0.198	2.265	0.0280 *
			Mid-North Latitudes	3.031	2.370	1.279	0.207
			Low Latitudes	1.9250	5.363	0.359	0.721
			Mid-South Latitudes	6.939	3.424	2.026	0.0482 *
Mining DT Richness	MAT	Latitude	Intercept	0.152	0.703	0.216	0.8301
			MAT	0.174	0.056	3.121	0.0030 **
			Mid-North Latitudes	-1.080	0.667	-1.620	0.1117
			Low Latitudes	-3.353	1.509	-2.222	0.0309 *
			Mid-South Latitudes	0.085	0.963	0.088	0.9301
DT Richness	Shannon	Latitude	Intercept	12.383	3.237	3.825	0.0003 ***
			Shannon	1.509	1.033	1.460	0.1498
			Mid-North Latitudes	4.803	2.651	1.812	0.0754
			Low Latitudes	6.996	4.529	1.545	0.1280
			Mid-South Latitudes	14.483	3.330	4.350	5.83e-05 ***
Specialized DT Richness	Shannon	Latitude	Intercept	4.770	2.295	2.078	0.0423 *
			Shannon	0.824	0.733	1.124	0.2657
			Mid-North Latitudes	2.855	1.880	1.519	0.1343
			Low Latitudes	2.924	3.210	0.911	0.3663
			Mid-South Latitudes	9.096	2.361	3.853	0.0003 ***

relative to the Cretaceous and Eocene (Fig. 6C–E). Connectance measures the proportion of interactions between DTs and host plants realized out of all the possible interactions in a given assemblage; both the niche overlap and C score measures the overlap of interactions among the DTs and host plants in different contexts (Swain et al., 2021). These results relate to the loss of specialists in the aftermath of the extinction and the advent of generalists (and therefore, increase in connectance and niche overlap and a decrease in C score) early on in the Paleocene, but is later followed by an increased in specialists in Eocene as the environments ‘stabilize’.

Spatially, the mid-S latitudes stand out as a hotspot for insect damage diversity, particularly when compared with the mid-N and high latitudes. Network analyses show that interaction evenness is high and H2 low compared with the mid-N and high latitudes, symbolizing high degree of specialization in associations (lower H2 values denote high specialization and high interaction evenness denote a balanced

distribution of interaction as per Shannon's diversity index) at these mid-S sites. The mid-S sites included in this analysis all come from the Cretaceous through Eocene of Patagonia, which has been previously proposed as a hotspot for biodiversity and a refugia during the K–Pg mass extinction (Wilf et al., 2005; Donovan et al., 2016, 2018; Stiles et al., 2020), and therefore providing a chance for high degree of association specialization. There is a need for more tropical sites of this age to determine what the low latitudes were like during this interval. Were they even more diverse than Patagonia? Alternatively, was tropical plant and insect herbivore diversity depressed relative to Patagonia due to either high temperatures or proximity to the Chicxulub impact site (Wing et al., 2009; Carvalho et al., 2021)?

Our spatial analyses also demonstrate the need for additional high-latitude sites to understand how polar forest ecosystems functioned. While the relatively low frequency and diversity of insect damage at high latitude (Figs. 7, 8) may partially be due to relatively low

temperatures, plants and insects also would have experienced seasonal darkness, which may have further depressed herbivory. The high northern latitudes also show high H2 and nestedness compared with lower latitudes, indicating a low degree of association specialization, but high level of (nested) overlap of associations. High nestedness is sometimes indicative of highly generalized associations in some DTs (with respect to plants affected) and high specialization in some, existing on a continuum although in a nested structure (i.e., highly general DTs interact with more plants, and less general DTs are associated only with a subset of those plants). This can result from either an adaptation to reduce competition or temporally induced high levels of generalized feeding (either due to sudden emergence or migrations) above the spatiotemporally persistent specialized insects. Although speculative, these patterns invoke interesting questions such as: were polar ecosystems dominated by holometabolous insects capable of dormancy, or might there have been significant migrations of insects? Did wood-boring insect diversity and abundance increase relative to folivorous insects due to lower availability of leaf tissue (Royer et al., 2003) during the dark winters?

We found that temperature played a significant role in the richness and frequency of insect damage from the Late Cretaceous to the Quaternary. These results have implications for both future studies on fossil plant–insect associations and the future of terrestrial ecosystems facing anthropogenic global climate change. Modern insect herbivores are influenced by, and sensitive to, changes in temperature (Jamieson et al., 2012). Also coupled to global temperature are levels of atmospheric carbon dioxide, which impact the quality and quantity of plant material consumed by insect herbivores due to changes in plant nutritional content (Knepp et al., 2005).

Regional changes in deep time insect herbivory have been detected in response to fluctuating temperatures. For instance, Currano et al. (2010) measured insect damage richness against floral diversity during the late Paleocene, Paleocene–Eocene Thermal Maximum, and early Eocene in the Bighorn Basin of Wyoming, USA. They found that insect damage richness was influenced by MAT ($R^2 = 0.89$; $p < 0.01$) more so than floral diversity ($R^2 = 0.38$; $p = 0.08$). Wappler and Grímsson (2016) found that in Icelandic Neogene deposits, plant diversity and DT richness were positively related ($r = 0.76$; $p = 0.0001$), and that the plant diversity changed through time in response to long-term global cooling. In particular, they found that the structural complexity of diverse herbaceous assemblages increased insect herbivory, even when the patterns of global cooling led the authors to predict otherwise. At a global scale, Pinheiro et al. (2016) postulated that herbivory was associated with changes in temperature, atmospheric CO₂, and O₂ availability, not simply an increase of herbivory from the Paleozoic to the Cenozoic. These results mirror our finding of a positive relationship between MAT and DT, specialized DT, and galling DT richness, as well as MAT and the frequency of specialized DTs (Fig. 9, Tables 2, S2, S3).

The signal between MAT and insect herbivory across the 66 localities analyzed herein, which span over 64 million years, demonstrates that large-scale patterns in insect herbivory can be detected in the fossil record of angiosperms. Our results indicate that broadly speaking, MAT is key to predicting DT richness (a proxy for insect herbivore richness), whereas other biotic and abiotic factors affect DTs to varying degrees. As mentioned above, the mid-S latitudes were a hotspot for biodiversity and potentially a refugia for both plants and herbivorous insects during the K–Pg mass extinction (Wilf et al., 2005; Donovan et al., 2016, 2018). The relationship between MAT and richness of mining DTs, however, peaks in the low latitudes. Although neontological studies have not measured the global patterns of leaf miner diversity by latitude, regional studies have found either no relationship or a negative relationship between latitude and leaf miner diversity (ex. Sinclair and Hughes, 2008; Adams et al., 2010; Bairstow et al., 2010; Hiura and Nakamura, 2013; Kozlov et al., 2013). Our results may indicate that in deep time, low latitudes were an evolutionary museum and/or cradle of leaf miner biodiversity (McKenna and Farrell, 2006).

6.2. Priorities for future research

The immediate future of research on deep time plant–insect associations will hopefully be shaped by an even more diverse pool of scientists, studying new time periods, geographic areas, and implementing novel and visionary analyses. We particularly encourage research at low and high latitudes, as discussed above. We predict that forthcoming studies will continue to expand and identify the evolutionary origins of ecological occurrences and structures, such as domatia (O'Dowd et al., 1991; Nelsen et al., 2018; Maccracken et al., 2019), extra-floral nectaries, florivory, insectivorous plants, pollination traps, and importantly, plant secondary metabolites (ex. McCoy et al., 2021). Furthermore, machine learning and paleobotanical databases will increase our ability to taxonomically identify and unite floras through time and space to enhance our understanding of deep time plant–insect associations. Plant morphology is complex and highly variable, which coupled with our limited ability to identify the taxonomy of many fossil plants based on isolated macrofossils, presents a substantial and time-consuming problem. Advances in machine learning, such as rapid classification by computer programs of 1) fossil leaf morphology to detect phylogenetic affinity (ex. Wilf et al., 2016) or 2) insect damage followed by measurement of surface area damage (Meineke et al., 2020), will allow entire floras to be analyzed more efficiently and accurately. We predict that the dissemination of this type of information will also become increasingly accessible to practitioners. The Integrative Paleobotany Portal, or PBot (<https://paleobot.github.io/PBot/>), will provide both a private workbench for describing plant fossils and a publicly accessible database of fossil plant descriptions. PBot will allow paleobotanists to compare and synonymize formally named species and informal leaf morphospecies across time, space, and research groups, increasing our ability to examine spatio-temporal variations in herbivory on individual plant lineages.

Our meta-analysis results, together with previous studies (e.g., Wilf and Labandeira, 1999; Currano et al., 2010; Wappler and Grímsson, 2016; Pinheiro et al., 2016), document a significant effect of mean annual temperature on insect herbivory in natural ecosystems over long time scales. The inability to replicate rates of anthropogenic warming in deep time, though, makes it challenging to infer future rates of herbivory and biodiversity loss. Herbarium specimens can act as a bridge between paleontological (10⁴–10⁶ yrs) and ecological time scales (<10² yrs) and have shown promise in quantifying impacts of anthropogenic climate change on plant–insect interactions (Meineke et al., 2018). Further, these specimens hold key information for understanding how changes in herbivory relate to nutrient content, phenology, along with many other traits that cannot be measured in the fossil record (Meineke et al., 2018). We hope to see an increasing number of studies utilizing herbarium collections and integrating data from seasons, to decades, to thousands and millions of years.

Development and application of novel quantitative methods for exploring the structure of plant–DT associations in the fossil record can pave the way for an understanding beyond singular central tendency (mean/median) or occurrence metrics and help us understand assemblages as a system. The use of bipartite networks in this meta-analysis showcases important insights that can be gained by using such methods, which are just getting applied to the field. Further explorations can be performed on the co-occurrence patterns among the DTs at a leaf level for particular sites, which can inform us more about the nature of insects that cause herbivory (site-specific). Moreover, most quantifications of leaf damage only focus on the mean damages incurred on any species or site, but do not take into account the variation among leaves or species. Network analyses solve the problem of the latter, but a variational analysis of how the distribution of various DTs change across leaves, species, and sites can help in understanding the variance in leaf traits, nutrition and possibly in some cases, insect outbreaks. Insect outbreaks are of particular interest as some studies have shown an increase in outbreaks with increasing temperatures (Nelson et al., 2013).

This problem is complex as it has been linked to a shortening of insect life cycles (Bale et al., 2002) and drought stress of plants (Gely et al., 2020).

6.3. Best practices for studying fossil insect herbivory

Sampling regimes should be carefully considered and explicitly stated for all paleobotanical endeavors, especially those for plant–insect associational studies. If we can create reproducible and comparable datasets, future meta-analyses will become increasingly successful and informative. First and foremost, it is crucial to collect data in a systematic and unbiased way. Census collections, or those in which data is collected on every identifiable fossil, and in an ideal world those fossils are all specimens are reposit in an institution, provide the most unbiased evidence for fossil plant–insect associations. Identification of insect damage on fossil leaves is relatively easy (see Supplementary Materials for a short video on insect damage identification) and requires only a small expenditure of time during the census, but drastically increases the ecological data associated with the fossil flora.

The authors of this paper, and indeed many practitioners of fossil insect damage, are happy to help paleobotanists incorporating plant–insect associational data into their studies, either with damage type identification or best practices. These best practices include 1) if possible, increasing sample sizes to at least 1000 specimens and statistically correcting for differences in sample size; 2) use the Labandeira et al. (2007) *Guide to insect (and other) damage types on compressed plant fossils (version 3.01)* and any later versions to describe insect damage on fossil leaves and contribute to the guide with new damage types; 3) publish raw data as a .csv file that includes specimen number, plant ID, and DT occurrences; 4) publish the code used for statistical analyses on Github or other platform; and 5) try to publish in open access journals, and if not feasible, use online platforms to make research available and/or be responsive to online requests for publications.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.palaeo.2021.110636>.

Supplementary materials, code and data availability

Our video demonstration of conducting an unbiased damage census, “So, you want to damage type a flora,” is available on YouTube at <https://youtu.be/zFkL52ND0kc>

All other supplementary materials are available at github.com/anshuman21111/review-paleoherbivory. This includes:

1. Table of herbivory data and bibliographic information for all sites used in the meta-analysis
2. All code and accessory data files required to replicate our analyses
3. Bipartite network diagrams for all sites
4. Supplementary Figures
 - a. Fig. S1. Herbivory metrics plotted vs. geologic age.
 - b. Fig. S2. PCA for the node-level metrics of DTs and host plants across assemblages.
 - c. Fig. S3. Insect herbivory compared among epochs.
5. Supplementary Tables
 - a. Table S1. Extended definitions and descriptions of network properties.
 - b. Table S2. Full generalized linear model results examining relationships between damage type richness and possible drivers.
 - c. Table S3. Full generalized linear model results examining relationships between damage frequency and possible drivers.
 - d. Table S4. Full generalized linear model results examining relationships between network metrics and environmental variables (MAT and MAP).

e. Table S5. Full mixed effects model results.

f. Table S6. Acknowledging the indigenous peoples at insect damage census localities

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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