PNAS RESEARCH ARTICLE | ENVIRONMENTAL SCIENCES

Insect herbivory within modern forests is greater than fossil localities

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Edited by Douglas Futuyma, Stony Brook University, Stony Brook, NY; received February 16, 2022; accepted August 12, 2022

Fossilized leaves provide the longest running record of hyperdiverse plant–insect herbivore associations. Reconstructions of these relationships over deep time indicate strong links between environmental conditions, herbivore diversity, and feeding damage on leaves. However, herbivory has not been compared between the past and the modern era, which is characterized by intense anthropogenic environmental change. Here, we present estimates for damage frequencies and diversities on fossil leaves from the Late Cretaceous (66.8 Ma) through the Pleistocene (2.06 Ma) and compare these estimates with Recent (post-1955) leaves collected via paleobotanical methods from modern ecosystems: Harvard Forest, United States; the Smithsonian Environmental Research Center, United States; and La Selva, Costa Rica. Total damage frequency, measured as the percentage of leaves with any herbivore damage, within modern ecosystems is greater than any fossil locality within this record. This pattern is driven by increased frequencies across nearly all functional feeding groups within the Recent. Diversities of total, specialized, and mining damage types are elevated within the Recent compared with fossil floras. Our results demonstrate that plants in the modern era are experiencing unprecedented levels of insect damage, despite widespread insect declines. Human influence, such as the rate of global climate warming, influencing insect feeding and timing of life cycle processes along with urbanization and the introduction of invasive plant and insect species may drive elevated herbivory. This research suggests that the strength of human influence on plant–insect interactions is not controlled by climate change alone but rather, the way in which humans interact with terrestrial landscape.

plant–insect interactions | paleoecology | herbivory | conservation paleobiology | human influence

Significance

Plants and insects are the most diverse lineages on earth, but their interactions in the face of climate and other global changes are poorly understood. Here, we find that despite insect declines, insect damage to plants is elevated in the modern era compared with other time periods represented in the fossil record. Plants today are experiencing unprecedented levels of insect herbivory, with unknown consequences for plant fitness and evolution.

Plant and insect herbivore interactions have been evolving and responding to abiotic and biotic variables for millennia (1). Today, terrestrial plants represent ∼80% of global biomass, and arthropods dominate animal biomass (2, 3), making them central to understanding terrestrial ecosystems and specifically, to what extent coevolved interactions are maintained or lost in the face of environmental change. Although insect herbivores are hyperdiverse, a growing number of studies demonstrate that, at least in some parts of the world, insects are in decline (4). Understanding insect herbivory is important as it directly relates to biodiversity (5), plant mortality via tissue loss (6), net primary productivity (NPP) (7, 8), and carbon balance (9). Much of the environmental change occurring today is likely to influence plant–insect associations: rapidly increasing global temperatures and atmospheric CO_2 (10); land use change; urbanization; biogeographic range shifts, including the introduction of nonnative species; and biodiversity loss (11–14). As global climate change continues to promote nonanalog environments, understanding how modern insect herbivory compares with the geologic record provides insight into these important relationships. To bridge the knowledge gap, modern plant–insect interactions can be placed against the backdrop of the geologic record to investigate how herbivory has changed through time.

Here, frequencies and diversities of insect herbivory via feeding damage types (DTs) (15) were compared between fossil and Recent (post-1955) datasets. These methods are well established in the paleobotanical record, and studies of DTs on leaf compression fossils have effectively documented insect herbivore diversity and feeding intensity in the geologic past (e.g., ref. 16). Insect herbivore DTs have been linked to living insect herbivore diversity (17), supporting interpretations of changes to insect diversity across epochs, local environments, and global climate perturbations. One benefit of using leaves and DTs to document plant–insect herbivore trophic relationships in modern ecosystems is that it offers an alternative method to quantifying insect herbivore diversity and abundance through insect sampling across landscapes, which is labor intensive (17–19). Data collected using this method can then be compared with the geologic record, which encompasses considerable global climate variability and biotic turnover (e.g., refs. 20–22).

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Author contributions: L.A.-S. and E.D.C. designed research; L.A.-S. performed research; L.A.-S. contributed new reagents/analytic tools; L.A.-S. analyzed data; E.K.M. and E.D.C. interpreted data; and L.A.-S., E.K.M., and E.D.C. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2202852119/-/DCSupplemental.

The geologic record contains a vast assortment of environments and climates, providing a largely untapped opportunity to compare modern plant–insect herbivore relationships with these same relationships across environmental conditions and prior to human influence. The last 66 My are punctuated by climatic and tectonic changes coupled with extinctions and speciation events, greatly influencing ecosystems, with the plant–insect communities responding in various ways (16). The Paleocene and early Eocene (66 to 48 Ma) constitute a "hothouse" interval, including the Paleocene Eocene Thermal Maximum (PETM; 56 Ma), an analog for current anthropogenic climate change (23), and the Early Eocene Climatic Optimum [EECO; 52.6 to 50.3 Ma (24)], which encompasses the highest sustained temperatures of the last 66 Ma. Earth's temperature increased $\sim$5 °C during the PETM relative to the Paleocene and was between 6 °C and 12 °C warmer during the EECO than in the present day (23, 24). These events were followed by cooling and a transition to the "icehouse" Oligocene and Miocene. The early and middle Pliocene were dominated by warm and stable climates; however, increased glaciation during the late Pliocene increased climate variability into the Pleistocene.

Comparative methods for analyzing insect herbivory (i.e., DTs) (15) between fossil and Recent datasets are necessary. DTs are morphologically distinct records of insect feeding, which differ in pattern, shape, and size (ref. 15 and *Materials and Methods* have further information). As many insects can make the same or similar DT due to similarities in mouthparts (25), the DT system does not assign damage to specific insect species. Rather, DTs made by folivorous insects are assigned to seven functional feeding groups: hole feeding, margin feeding, skeletonization, surface feeding, piercing and sucking, mining (Fig. 1 *F* and *G*), and galling (Fig. 1*E*). Each functional feeding group encompasses varying numbers of morphologically distinct DTs, which can also be categorized as generalist, often made by polyphagous insects and present on many plant species, or specialist, occurring on specific plants or related hosts (15). The presence of a distinct rim of thickened tissue distinguishes leaf-chewing damage from damage created after leaf abscission (including during fossilization, collection, and/or processing) or by detritivores.

To investigate how herbivory has changed through time, we compared folivore damage frequency and diversity on Recent leaf packs, mimicking paleobotanical fossil outcrops and sampling methods (Fig. 1 *A* and *B*), with that observed in 64 fossil leaf assemblages (Cretaceous–Pleistocene; 66.8 to 2.06 Ma). Frequency is quantified as the percentage of leaves in an assemblage with damage, and diversity is reported as the number of DTs observed at a site, standardized for sample size. Recent leaf packs were also compared with previously published leaf litter (26, 27) to assess the impact of transport and burial on the results.

Results

Leaves collected from within modern sediment and across various depositional environments in Harvard Forest, the Smithsonian Environmental Research Center (SERC), and La Selva were radiocarbon dated to verify ages. All exhibit excess ^{14}C from atmospheric thermonuclear weapons testing and therefore, are from the midtwentieth century or later (1955 to present). Modern fractions measured range from 1.0094 to 1.0307, with minimum and maximum values occurring within the SERC swamp and small tributary, respectively.

All fossil data were binned to compare herbivory data from the fossil record with data from Recent leaf packs and litter (Fig. 2). Total (difference in mean = 51.88), specialized (difference in mean = 43.18), mining (difference in mean = 5.90), galling (difference in mean = 13.14), hole feeding (difference in mean = 36.51), margin feeding (difference in mean = 39.72), skeletonization (difference in mean = 6.78), and surface feeding damage (difference in mean = 28.78) frequencies were significantly higher, often three times greater or more, in Recent leaf packs than in the binned fossil data (Fig. 2*A*) (all P values $\leq$ 0.01). Although hole feeding can be made by many different insect species, making it incredibly common within fossil and Recent datasets, it is not the sole driver of these results as mining and gall damage frequency are also significantly elevated within the Recent (Fig. 2*A*). Only piercing and sucking damage frequencies did not differ between Recent and fossil assemblages (difference in mean = -0.61; $P = 0.47$); this functional feeding group is likely underestimated in both Recent and fossil data as distinguishable scars are not always visible on leaves. Diversities of total (difference in mean = 11.03), specialized (difference in mean = 6.87), and mining damage (difference in mean = 1.68) were higher within the Recent ($P \leq 0.01$, $P \leq 0.01$, and $P =$ 0.02, respectively) (Fig. 2*B*). Gall diversity was not significantly different between fossil and Recent floras (difference in mean = 0.41; $P = 0.66$) (Fig. 2*B*). Lastly, plant species diversity (rarefied richness and Shannon diversity) and evenness were similar in fossil and modern assemblages (difference in mean = -9.62, -0.32, and -0.03, respectively; $P = 0.13$, 0.29, and 0.73, respectively).

To assess the effect of preservation bias on Recent leaf packs, they were compared with Recent leaf litter as we would expect to see a decrease in preservation between litter (no transport, greater preservation) and pack (transported, less preservation). Leaf-litter (26, 27) and leaf-pack total damage frequencies were nearly identical to one another (difference in mean = -0.94; $P = 0.99$) (Fig. 2*A*), and both were greater than herbivory at fossil sites (difference in mean = 55.20 and 54.25, respectively; all P values $\leq$ 0.01) (Fig. 2*A*).

Because Recent leaf packs display significantly higher herbivory than fossil leaf sites, fossil data were parsed into epochs to investigate whether there is an increase in insect herbivory through time or trends related to climate, mass extinctions, or tectonic events. Averages of total, specialized, and other functional feeding group damage frequencies were binned by time (Cretaceous–Recent), showing similarities between the Cretaceous and the Pleistocene (Fig. 3*A* and *SI Appendix*, Table S1). However, average total, specialized, hole feeding, margin feeding, surface feeding, mining, gall, and skeletonization damage frequencies are all higher in Recent floras than in any other geologic epoch (all P values $\leq$ 0.01) (Fig. 3*A* and *SI Appendix*, Table S1). Average total and specialized damage diversities within Recent assemblages are high relative to the Paleocene to the Miocene ($P \leq 0.01$) (Fig. 3*B* and *SI Appendix*, Table S1) but similar to the Cretaceous, Pliocene, and Pleistocene. Average mine diversity within the Recent is unlike the Paleocene and the Oligocene but similar to all other time intervals (*SI Appendix*, Table S1). There are no significant differences in average gall diversity (Fig. 3*B*), plant diversity (rarefied richness; Shannon diversity) (Figs. 2 and 3*A*), or evenness (Pielou's J) (*SI Appendix*, Table S1) across time bins.

As both modern and fossil studies have shown a relationship between herbivory and mean annual temperature (MAT; degrees Celsius), with elevated insect damage frequency and diversity at higher temperatures (20, 29, 30), we examined this relationship within our dataset (Fig. 4). Fossil MAT values given are paleoclimate, not modern-day values (Dataset S1; *Materials and Methods*). Recent samples fall within the range of MAT encompassed by the fossil assemblages, yet their damage frequencies and diversities fall well above values for fossil floras that lived at similar MAT (Fig. 4). As has been previously demonstrated

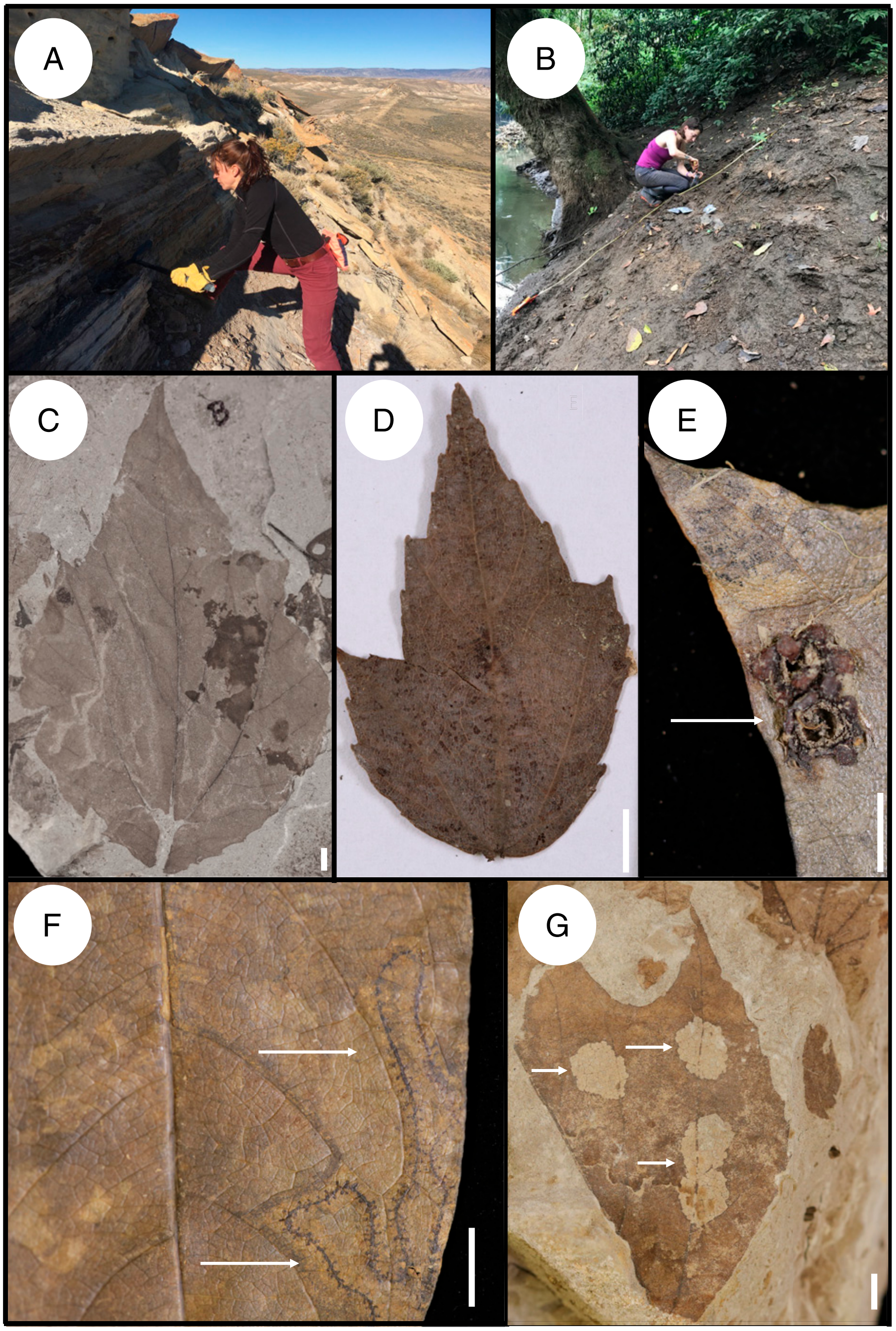

Fig. 1. Leaf collection from fossil (*A*) and modern quarries (*B*). Leaf layers are identified, and overburden (fossil) or sediment (modern) is removed using a paleopick or trowel and sieve. A leaf compression fossil, *Platanites raynoldsii*, of Paleocene age (*C*; University of Wyoming Geologic Museum [UWGM] specimen no. PB968) and Recent *Acer rubrum* (*D*) are examples of excavated leaves. Insect damage (*E*; Gall functional feeding group [FFG], MD1903.2 no. 161; *F*, unknown Mine FFG, LS1901.2 no. 116) is abundant on Recent leaves across all depositional environments and forest types. Fossil insect damage on *P. raynoldsii* (*G*; DT38, Mining FFG, UWGM specimen no. PB922) was previously hypothesized to be made by the insect family [*Incurvariidae*; Lepidoptera (28)]. Arrows are used to clarify insect damage (*E*–*G*). Modern specimens are deposited in the Rocky Mountain Herbarium. (Scale bars: 0.5 cm.)

(e.g., ref. 16), generalized linear models show that total damage frequency, total damage diversity, and specialized damage diversity at fossil localities are positively correlated with MAT ($P = 0.01$, $P \leq 0.01$, and $P \leq 0.01$, respectively) (Fig. 4). There is variability in the relationship between MAT and damage metrics within epochs, but an overall trend is present within fossil assemblages. No relationship exists between MAT and herbivory within the Recent, and furthermore, when Recent values were added to the fossil dataset, there is a weaker correlation or no longer a significant correlation between MAT and herbivory ($P = 0.05$, 0.11, and 0.11, respectively).

Discussion. Damage frequencies within modern ecosystems exceed those in the geologic record by two times on average across functional feeding groups (Fig. 2*A*). Total damage diversity and the diversity of specialized and mining damage are higher in Recent leaf packs than in the fossil record (Fig. 2*B*). While damage diversities and frequencies are both elevated in the Recent compared with any other time period, higher damage frequencies do not appear to be a result of higher damage diversity (Figs. 2 and 3). Damage frequencies and diversities are not directly correlated across epochs, and total damage frequency is much more elevated in the Recent than total damage diversity (Fig. 3). This disconnect

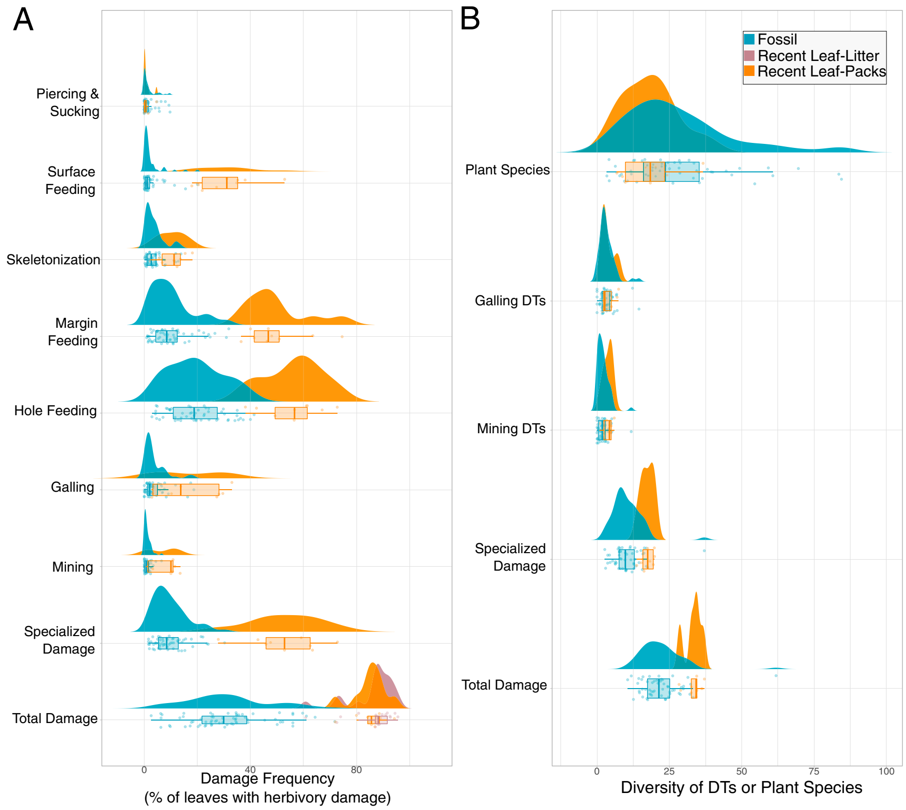

Fig. 2. (*A*) Frequency of herbivory damage and (*B*) diversity of DTs (total, specialized, mining, or galling) or plant species in fossil and Recent assemblages. Total damage frequencies for fossil and Recent assemblages were compared with published leaf-litter studies (*A*) (26, 27). Diversity is reported as the number of DTs or plant species observed and was standardized to 300 leaves to account for variance in sampling size. Rain cloud plots show density distribution (normalized), and box and whisker plots show quantile 1, mean, and quantile 3 along with raw data points. Colors correspond to fossil (blue), leaf-litter (mauve), or leaf-pack (orange) samples.

in total damage frequency and total damage diversity may be caused by higher per-capita insect feeding within the Recent, an increase in feeding within a group(s) of insects, or increased population densities across landscapes. Modern drivers of increased insect herbivory remain unknown but may include changes in atmospheric CO_2, rapidly increasing global temperatures, urbanization and invasive species release from natural enemies, and/or biodiversity loss. Although not directly tested here, we hypothesize that the various aspects of human influence have a strong effect on plant–insect interactions.

When comparing data from fossilized and Recent specimens, preservation bias must be estimated to account for the possibility that fossilization affects the frequencies and/or diversities of leaf DTs that can be observed (i.e., taphonomic bias). To create a modern insect damage dataset parallel to the fossil-derived data, Recent leaf packs were excavated and collected from within the sediment, mimicking a fossil outcrop (*SI Appendix*, *SI Text*). Leaf transport and burial, depositional setting, sediment grain size, and spatiotemporal averaging of the Recent leaf packs are the closest known analog to fossilized leaves. Given millions of years, leaf packs will likely become fossils, and compaction and lithification are unlikely to affect the preservation of the DTs analyzed in this study. Of the 80 DTs present within the Recent leaf packs, 73 of those have been observed on fossils. New DTs are regularly added as new fossil sites are studied, consistent with our discovery of new DTs within the Recent samples, and our new DTs are sufficiently distinct and robust to be preserved on fossils.

Total damage frequency in Recent leaf packs was compared with a second modern dataset, leaf litter, to further investigate the effects of transport, burial, and the excavation process on DT preservation. Leaf litter accounts for "fresh fall" (i.e., leaves from the current season and the canopy directly above); these leaves will not necessarily become fossils as the forest floor undergoes various stages of decomposition (31, 32). Leaf packs undergo transport and burial within anoxic environments and must be excavated. If leaf litter displayed higher herbivory than leaf packs, we would conclude that insect herbivory on leaf packs and thus, fossils is negatively influenced by fossil preservation via transport, burial, and the excavation process. Conversely, if herbivory damage on packs was higher than litter, we could assume that preservation biases inflate herbivory metrics. Our results demonstrate no such preservation bias; total damage frequencies recorded in Recent leaf litter and packs did not differ from one another. However, both Recent litter and pack did differ from the fossil record (Fig. 2*A*), and we conclude that elevated damage frequencies and diversities in the Recent cannot be explained by preservation bias.

Geologic epochs and time bins are temporal groupings that allow us to compare biotic and abiotic character states through time. Epochs are most often delineated by drastic changes in communities [i.e., extinction events (33)], whereas time bins typically consist of finer-scale time intervals that are based within the epoch schema. As epochs and bins represent variable lengths of time and also, differ in sampling effort (sample sizes), comparing broad-scale changes through time must be thoughtfully addressed. In particular, analyzing specimens from both deep time and the Recent presents unique challenges, as the Recent represents a magnitude smaller time interval than that of deep time bins. However, time averaging within both Recent and deep time fossil deposits offsets some of these concerns, as fossilized leaf layers encompass ∼100 to 1,000 y (31), while Recent leaf

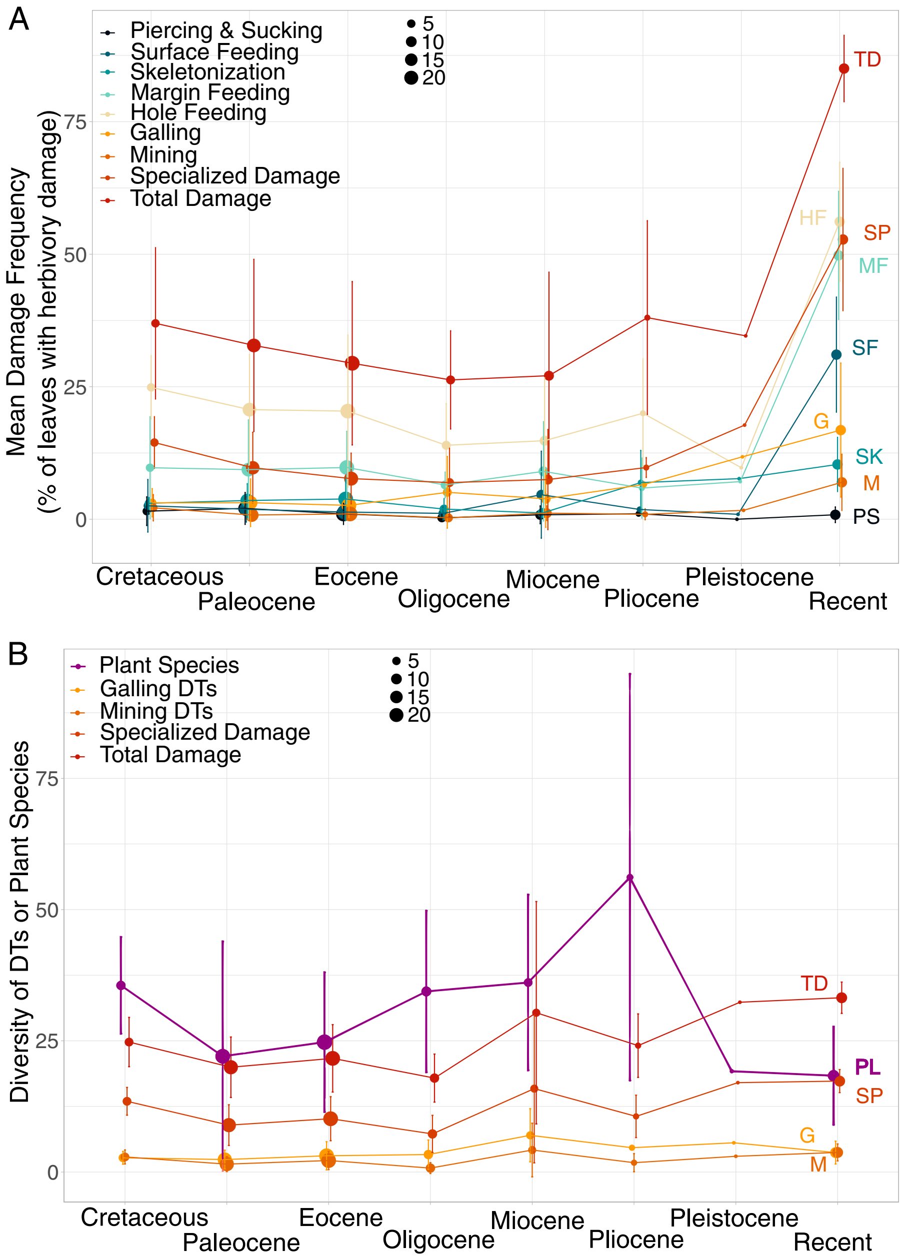

Fig. 3. (*A*) Mean damage frequency (percentage of leaves with herbivory damage) on bulk assemblages through time. All identifiable leaves at a site were scored for herbivory, and damage frequency was calculated for each assemblage. Sites were binned by geologic epoch, and the mean was calculated. Total (TD) and specialized (SP) damage frequencies are plotted, as are frequencies of each functional feeding group: piercing and sucking (PS), surface feeding (SF), skeletonization (SK), margin feeding (MF), hole feeding (HF), galling (G), and mining (M). (*B*) Diversity of DTs (total, specialized, mine, or gall) or plant species (PL) is plotted, with each point representing the mean value for each epoch. Frequency and diversity at individual sites were calculated as in Fig. 2. Colors of points and lines correspond with the legend (upper left corner) and are shared between panels; the size of the points represents the sample size, and SDs are shown as whiskers extending from each point.

packs presented here represent approximately 64 y. Furthermore, they both capture comparable amounts of time during which an insect could feed upon a leaf: the life span of a single leaf and its ecological associations prior to burial. Overall, the trends we present herein are based on data that represent snapshots (leaf life span) from floras that grew throughout the past 66 My through today. While the nature of the fossil record is sporadic through time and space (ex. 1 Pleistocene sample vs. 23 Eocene samples), the broad patterns of plant and insect associations detailed in this study document their evolutionary trajectories coupled with the punctuated record of climate change over the last 66 My. Overall, a long-term perspective is required to understand these ancient organisms and their longstanding ecological associations, as well as pinpoint where future collecting efforts should focus.

Major insect diversification events predate this study. Thus, high insect damage frequency is unlikely to arise from insect or plant diversification. Insect diversification at higher taxonomic levels, resulting in the development of new major mouthparts and functional feeding groups, predates the fossil data presented here and thus, is unlikely to drive herbivory across fossil and Recent time periods in this study. Major family-level insect diversification events occurred during the Early Carboniferous (323.2 to 298.9 Ma), Permian (298.9 to 251.0 Ma), and Paleocene [∼66 to 56 Ma (34, 35)], with insect diversity at the family level peaking within the Early Cretaceous [∼113 to 100 Ma (36)]. This is followed by

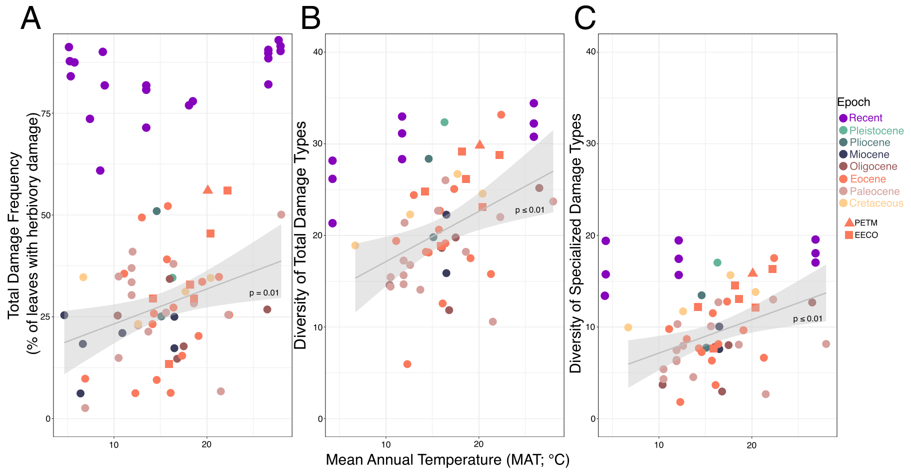

Fig. 4. General linear models of MAT and total damage frequency (*A*), diversity of total (*B*), and specialized (*C*) damage types (calculated as in Fig. 2) show positive correlations between herbivory and MAT when considering fossil sites only. Herbivory at Recent (post-1955) sites exceeds that observed at fossil sites of similar MAT, highlighting the oddity of modern ecosystems. Recent data points (purple) include three leaf-pack samples each from Harvard Forest, SERC, and La Selva as well as published total damage frequency data for 17 leaf-litter samples collected using similar methods (26, 27). The legend is in geologic order (oldest to youngest), with colored points assigned to different epochs and shapes corresponding to climatic events (triangles, PETM; squares, EECO). The gray lines show the linear relationships, with CIs represented by the shaded gray areas. *P* values are given at the bottom right corners of the CIs.

a steady decline through time, sporadically altered by extinction events (37). Genus- and species-level diversification through the Cenozoic remains uncertain (25, 36, 38), but if significant insect herbivore diversification occurred through the Cenozoic, we would expect a steady increase in damage diversity through time. This pattern did not emerge in our data. Instead, we documented oscillations through the Cenozoic and similar total damage diversity between the Cretaceous and Recent (*SI Appendix*, Table S1). This suggests that insect herbivore diversities are similar between the Cretaceous and Recent, providing further evidence of comparable records. Additionally, plant community composition could bias the records as we would expect insect diversity to increase concurrent with plant diversity; however, plant diversity between fossil and Recent leaf packs did not differ (Fig. 2*B*).

While damage diversity did not drastically change through time, instead we observed a sharp increase in damage frequencies in the Recent (Fig. 3), when humans became a dominant force affecting the biosphere (39). Our youngest fossil sample is from Europe (2.06 Ma), which predates the migration of early humans out of Africa ∼1.75 Ma (40). Thus, it is unlikely that early human populations influenced plant–insect interactions during the fossil time period presented here. Additional Pleistocene and Holocene sites are needed to constrain when exactly levels of leaf damage increased and how increases might relate to human activity. Pleistocene leaf fossil sites are rare due to widespread glaciation during the epoch, but leaf packs that predate the industrial revolution are known (e.g., ref. 41). We hypothesize that humans have influenced damage frequencies and diversities within modern forests, with the most human impact occurring after the Industrial Revolution. Consistent with this hypothesis, herbarium specimens from the early 2000s were 23% more likely to have insect damage then specimens collected in the early 1900s, a pattern that has been linked to climate warming (42).

Insects are highly sensitive to climate, such that warmer temperatures generally elevate insect fitness as long as conditions do not exceed insect thermal maxima (43). Thus, rising temperatures can directly lead to larger insect populations in species that can survive climate change. Elevated CO_2 can also enhance insect feeding rates via shifts in nutrient ratios (44, 45). A correlation between MAT and herbivory is observed within the fossil record as a whole, although not necessarily within individual epochs where sample sizes can be small and have uneven geographic and climatic distributions. The variability in the MAT–herbivory relationship is unsurprising, as modern ecologists often see large variability in global datasets that aim to compare MAT with biotic processes, such as NPP (46). What is remarkable is that herbivory metrics are significantly greater in the Recent, particularly when compared against fossil sites with similar MAT (Fig. 4). If climate change alone was the primary driver of increased herbivory in the Recent, hothouse worlds present within the fossil record—with temperatures exceeding those of Recent climate—should have supported comparable or higher levels of damage frequency and diversity than in the Recent. As it is not the case that MAT drives the elevated damage metrics observed in the Recent, we propose that the comparatively rapid warming trends of the Recent era may be responsible for its higher herbivory frequencies, such that rapid warming benefits insects in the arms race against their food source: plants.

Plants represented in the fossil record likely had time to adapt to changes in climate and any associated elevation in insect feeding, even during geologically rapid global warming events, such as the PETM, where warming occurred over 2,000 to 20,000 y (23). The rate of Recent warming, however, is relatively accelerated. In such a scenario, insect herbivores are more likely than their host plants to respond quickly due to their sensitivity to temperature (plastic responses) and their short generation times (evolutionary responses), potentially putting plants at a disadvantage in the evolutionary arms race against insect herbivores. It is also possible that plastic responses result in phenological changes that result in more herbivory in Recent floras. For instance, as the rate of climate change alters leaf phenology, it appears that some herbivorous insects are decreasing the gap between egg hatch and bud burst (47, 48), allowing for more herbivory to occur on developing and vulnerable new leaves. Additionally, a cross-continental study demonstrates that earlier springs—and thus, longer growing seasons that are one result of climate change—are associated with elevated herbivory across a wide range of plant species (49).

Although rapid climate change is one mechanism that may drive elevated herbivory in the Recent, other anthropogenic mechanisms may contribute to this observed pattern. Urbanization and farming increase landscape fragmentation and edge effects, thereby increasing impacts on plant and insect species pools and community composition (50, 51). Insect herbivores are heavily influenced by landscape fragmentation and edges, with bottom-up edge effects benefiting generalist feeding and top-down edge effects benefiting specialist feeding (52). Generalist insect herbivores do not rely on specific food sources, and thus, changes to plant species composition (bottom up) do not negatively impact feeding, unlike host-specific specialist insects (e.g., refs. 53 and 54). Conversely, predators of herbivorous insects (top down) are sensitive to land fragmentation, leaving or avoiding fragmented patches and benefiting specialist insects (55). Movement and migration are impacted by urbanization, which disrupts the gene flow of species, decreasing diversity (56). Additionally, some studies have shown that specialization of insect herbivory decreases with land fragmentation (52). Leaf packs sampled here are all located within research stations, which preserve the natural habitat of the region but are surrounded by roads, housing developments, and farming. Specialist insect herbivory is higher within Recent leaf packs than in the geologic record (Fig. 2), suggesting that top-down effects may be benefiting specialist insect communities within the research station and that a decrease in predation is more important than the fragmentation of the surrounding land. Perhaps urbanization has created insect biodiversity hot spots within research forests by decreasing predation on specialist insect herbivores in addition to generalist feeders. As such, funding and protecting research stations are important for the preservation of insect communities as biodiversity loss is threatening Earth's ecosystems. Finally, humans have introduced new plant and insect species, both intentionally and unintentionally (57). Nonnative ornamental plantings in urban areas surrounding these research stations may harbor nonnative insects that escape urban areas into nearby research stations (57). The introduction of invasive plant species is concurrent with changes in insect communities, mainly in pollinators and insect herbivores (58–60).

Plants and their insect herbivores have a close ecological relationship, which we hypothesize to have been altered by human influences. The increased insect feeding frequencies found here (Figs. 2 and 3) are likely to influence plant fitness (49). Insect damage to leaves negatively affects leaf photosynthesis by removing leaf tissue (direct effect) and decreasing photosynthetic rates in the surrounding leaf tissue (indirect effect) (6). These direct and indirect effects of decreased photosynthesis have been shown to influence fruit and seed production [*Quercus lobata* (61)] in addition to competitive exclusion and increased mortality rates (62). Forests play an important role in the global carbon cycle, pulling carbon from the atmosphere via photosynthesis and when healthy, acting as a carbon sink (e.g., ref. 63). Increased herbivory diminishes plant fitness and raises tree mortality rates (9), altering carbon sink dynamics with the potential to turn forests into carbon sources (64). The effects of insect herbivory on forest carbon source/sink dynamics should not be dismissed as a negligible process in light of anthropogenic atmospheric CO_2 rises.

Summary. Our study demonstrates that plant–insect herbivore interactions recorded on Recent (post-1955) leaf-pack samples are strikingly different from on leaf compression fossils (Cretaceous–Pleistocene; 66.8 to 2.06 Ma). Damage frequencies are drastically higher within the Recent (Fig. 2), a surprising result given current global insect decline and biodiversity loss, which have been attributed to anthropogenic change (4). Recent insect feeding damage metrics and MAT do not directly track as has been demonstrated in the fossil record (Fig. 4), highlighting the anomalous nature of modern ecosystems. Lastly, it is also clear from the current study that damage frequency does not track damage diversity and therefore, is unlikely to be driven by insect diversity at the scale of our study (i.e., 10^6 to 10^4 y for fossil data and 10^2 y for Recent data) within the context of anthropogenic change. Elevated damage frequencies and diversities in the Recent suggest that humans dramatically influence plant and insect herbivore communities (Figs. 2 and 3), with unknown consequences for plants and overall forest communities. This work bridges the persistent gap in the literature between those who study plant–insect interactions over deep time using fossils and those who study such interactions in a modern context with fresh leaf material. Although more research needs to be done to understand the specific mechanistic drivers of Recent increases in herbivory across and within modern forests, human influence via rate of climate change and urbanization is hypothesized to be an important driving factor. In order to assess the full strength of these factors on damage frequencies and diversities, additional research is needed to compare ecosystems that predate the Industrial Revolution (e.g., ref. 41) with this study and future studies.

Materials and Methods

The modern ecosystems and leaf packs sampled within this study are the Harvard Forest, the SERC, and the Organization of Tropical Studies La Selva. Harvard Forest is located near the town of Petersham, Massachusetts, United States and is one of North America's oldest managed forests. This cool, moist temperate forest has low plant diversity and is dominated by eastern hemlock (*Tsuga canadensis*) and red oak (*Quercus rubra*). The SERC is a coastal warm, moist temperate ecosystem in Maryland, United States, bounded on one side by the Chesapeake Bay, making the ecosystem a mixture of fresh and brackish water. Plant species are moderately diverse, with tulip poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), and various oak species dominating the forest. Lastly, the wet tropical ecosystem of La Selva, Costa Rica is highly diverse in both plant and insect species. The forest is located at the confluence of the Sarapiquí and Puerto Viejo Rivers, which flood during the wet season. This ecosystem is very dynamic, with a mixture of old growth swamps and forests in addition to secondary growth forests.

In order to mimic paleobotanical methods within modern forests to the best of our abilities, three sampling locations were chosen within each modern ecosystem to correspond to the dominant depositional environments that preserve leaves within the geologic record. Leaf packs, leaves that have undergone transport and burial within the sediment, were collected from swamps (low transport, autochthonous), small fluvial tributaries (midtransport), and dynamic fluvial channels (high transport, allochthonous), with three lateral samples at each to account for small-scale spatial heterogeneity. Following paleobotanical methods, ∼400 leaves deposited within the sediment were excavated per site, rinsed clean, pressed, and dried (60 °C to 70 °C for 24 to 72 h). Bulk leaf material from each depositional setting in each forest was sent to University of California Irvine's Keck Carbon Cycle Accelerator Mass Spectrometer (KCCAMS) facility for ^{14}C dating. All organic material was rinsed with acid–base–acid (1 N HCl and 1 N NaOH, 75 °C) prior to combustion with radiocarbon concentrations given as fractions of the modern standard (65). Ages were calibrated using Northern Hemisphere Zone 1 or 2 and the fraction of modern ^{14}C and calculated uncertainty (66). *SI Appendix*, *SI Text* has full sampling methods and site descriptions.

All Recent leaves were analyzed for insect damage ($n = 10{,}941$), and all DTs were documented using the damage guide of Labandeira et al. (15) and subsequent revisions. The presence of reaction rims (i.e., thickened tissue around the feeding event[s]) is imperative to the DT system as they allow us to distinguish damage that occurred when the leaf was still attached to the tree vs. after leaf abscission. No reaction rim will occur if the leaf has been shed from the tree, eliminating the possibility of detritivore damage. Additionally, only damage that could have been made by insect herbivores was considered; oviposition and pathogen damage were omitted from our dataset. While folivory damage is made by other arthropods or mollusks, insects are far more diverse and have greater biomass (2), making it highly likely that the dominant group responsible for leaf feeding damage is insects. Damage frequency was calculated as the percentage of leaves at each site that have a given type of damage (e.g., total damage, specialized damage, each functional feeding group). Damage diversity is given as the number of DTs observed at a site (i.e., damage richness) and standardized for sample size using the extension of analytical rarefaction applied by Gunkel and Wappler (67) (standardized to 300 leaves).

Recent data were then compared with the established framework of fossil plant–insect interactions as described above and leaf-litter data from Adams et al. (26) and Smith and Nufio (27). The fossil dataset was previously compiled (16) using fossil plant–insect census data published before 2021. Key words, such as "insect herbivory," "plant–insect interactions," and "fossil," were used within Web of Science and Google Scholar. Furthermore, sites were only included if collections were made in an unbiased manner and if the leaf sample count was at least 300 dicot leaves. Fossil floras ($n = 64$) span $\sim$66.8 My, the Late Cretaceous (66.8 Ma) through the Pleistocene (2.06 Ma). Fossil localities span a wide variety of latitudes, with the majority of sites within the midnorthern latitudes; temperate, subtropical, and tropical forests are represented within the dataset. Reassuringly, Currano et al. (16) found no relationship between herbivory metrics and depositional environments (e.g., fluvial, lacustrine), suggesting that the depositional environment does not influence the results presented here. Lastly, fossil MAT values are reconstructed paleotemperature estimates, not the modern-day values. Leaf-litter data were collected by Adams et al. (26) from five sites in Malaysia (lowland tropical evergreen forest; 2 °N to 3 °N, MAT ca. 26.5 °C); five sites in northern Florida, United States (subtropical; 30 °N, MAT ca. 19.5 °C); and five sites in the northeastern United States [temperate; 40 °N to 42 °N, MAT ca. 7 °C to 9 °C (26)]. The presence and absence of DTs were determined using Labandeira et al. (15) for all 10 modern sites. Forests were dominated by native tree species and had to be composed of approximately 50% broad-leaved angiosperms. Leaves were collected from forest floor leaf litter and analyzed. Published tropical leaf-litter data from La Selva and a second lowland Costa Rican forest, Parque Nacional Corcovado (27), was also used in our comparative analyses; leaf litter was randomly sampled to 30 dicotyledonous leaves at 10-m intervals for 10 sampling locations in 1998, 1999, and 2001 [300 leaves per forest per year, except for 200 leaves from Corcovado in 2001 (27)].

Linear models and Tukey statistical tests (CI = 0.95; Tukey HSD function from the stats package) were used to identify significant differences across time intervals and when comparing fossil, Recent leaf packs, and litter samples with one another. Generalized linear models (CI = 0.95; family = Gaussian; glm from the stats package) were used to quantify relationships between mean annual precipitation (MAT) and all herbivory metrics. Recent leaf packs and litter were combined to compare MAT with total damage frequency. All statistical methods were run in R (version 3.6.1).

Leaf-pack data are available in our GitHub repository (https://github.com/lazevedoschmidt/modern_fossil_herbivory) (68). Fossil leaf data used in Currano et al. (16) are also available in GitHub (https://github.com/anshuman21111/review-paleoherbivory) (69).

Data, Materials, and Software Availability. Data used in this work is publicly accessible and can be found in GitHub (https://github.com/lazevedoschmidt/modern_fossil_herbivory and https://github.com/anshuman21111/review-paleoherbivory) (68, 69). Previously published data were also used for this work (16, 26, 27).

ACKNOWLEDGMENTS. We thank the anonymous reviewers who helped strengthen this manuscript; Orlando Vargas Ramírez for identifying tropical leaves; and Bernal Matarrita Carranza for laboratory coordination at Organization of Tropical Studies in La Selva, Costa Rica. Additionally, we thank the Costa Rican government for permitting the transport of organic leaf material to the University of Wyoming and undergraduate research assistants Sarah Fanning and Rosemary Hopson. This research was funded by NSF Grant EAR-145031 and the University of Wyoming Botany Department. This work was supported by Harvard Forest, the SERC, and the Organization of Tropical Studies La Selva field research stations.

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