

Fossil insect folivory tracks paleotemperature for six million years

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Abstract. Paleocological studies enhance our understanding of biotic response to climate change because they consider timescales not accessible through laboratory or ecological studies. From 60 to 51 million years ago (Ma), global temperatures gradually warmed to the greatest sustained highs of the last 65 million years. Superimposed on this gradual warming is a transient spike of high temperature and pCO₂ (partial pressure of carbon dioxide in the atmosphere; the Paleocene-Eocene Thermal Maximum 55.8 Ma) and a subsequent short-term cooling event (~54 Ma). The highly resolved continental fossil record of the Bighorn Basin, Wyoming, USA, spans this interval and is therefore uniquely suited to examine the long-term effects of temperature change on the two dominant groups in terrestrial ecosystems, plants and insect herbivores. We sampled insect damage on fossil angiosperm leaves at nine well-dated localities that range in age from 52.7 to 59 Ma. A total of 9071 leaves belonging to 107 species were examined for the presence or absence of 71 insect-feeding damage types. Damage richness, frequency, and composition were analyzed on the bulk floras and individual host species. Overall, there was a strong positive correlation between changes in damage richness and changes in estimated temperature, a weak positive relationship for damage frequency and temperature, and no significant correlation for floral diversity. Thus, insect damage richness appears to be more sensitive to past climate change than to plant diversity, although plant diversity in our samples only ranges from 6 to 25 dicot species. The close tracking of the richness of herbivore damage, a presumed proxy for actual insect herbivore richness, to both warming and cooling over a finely divided, extended time interval has profound importance for interpreting the evolution of insects and plant–insect associations in the context of deep time. Our results also indicate that increased insect herbivory is likely to be a net long-term effect of anthropogenic warming.

Key words: Bighorn Basin, Wyoming, USA; climate change; global warming; herbivory; paleobotany; Paleocene-Eocene Thermal Maximum; paleoecology; plant–insect interactions.

INTRODUCTION

Paleocological studies provide essential deep-time context for biotic response to climate change. The long-term response of plants and insect herbivores, the two dominant groups in terrestrial ecosystems (Price 2002), can be interpreted by analyzing insect herbivore damage on well-preserved fossil angiosperm fossil leaves (Fig. 1; Wilf and Labandeira 1999, Wilf et al. 2001, Currano et al. 2008, Smith 2008, Wilf 2008). Because paleocological studies have different limitations from ecological or laboratory studies, they are an important component in the understanding of the effects of warming on life. Paleocological studies are limited by the nature of the fossil record. Fossiliferous rocks of the appropriate age are only exposed in certain places, and the maximum possible temporal resolution is 10⁴–10⁵ years. Conversely, the complexity of plant–insect food webs and the

possibility of long response times and feedback mechanisms make it difficult to generalize from experiments or short-term ecological studies to the response of natural ecosystems over long timescales. Furthermore, experimental constraints often limit studies to either temperature or carbon dioxide and do not address their combined effects, as in natural systems. Paleocological studies do not have these limitations because they consider the net effects of climate change on whole ecosystems over long (10⁴–10⁷ yr) timescales.

Neocological and laboratory analyses do, however, provide a relevant framework for interpreting paleontological results. Today, overall insect herbivory, plant richness, and herbivorous insect diversity are greatest in the tropics (Moran and Southwood 1982, Stork 1987, Coley and Aide 1991, Price 1991, Coley and Barone 1996), implying broad correlations between temperature and plant diversity, insect diversity, herbivory, or all three. Temperature changes affect both the life histories of insects and the boundaries of their geographic distributions. Recent shifts in critical life history events have been demonstrated for insects in the United Kingdom (Harrington et al. 2001) and in western North

Manuscript received 18 November 2009; accepted 14 January 2010; final version received 15 February 2010.
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America (Hansen et al. 2001). These include changes in the timing of developmental stages and diapause and an increase in the number of generations per year. Nonmigratory European butterfly species have shifted their ranges northward, at times extending beyond the range of their original, primary host plants (Hill et al. 1999, Parmesan et al. 1999). Temperature change has also been suggested as a cause of intensified outbreaks of mountain pine beetle, gypsy moth, spruce beetle, and spruce budworm in North America during the last decade (Logan et al. 2003). Although there is no evidence of carbon dioxide directly affecting insects, its indirect effects are felt via changes in plant chemistry. In general, plants grown under elevated CO₂ tend to accumulate more carbon and have a higher carbon:nitrogen ratio, making them nutritionally poorer (Bazzaz 1990, Lincoln et al. 1993, Whittaker 2001). Insect consumption rates show an average compensatory increase (Watt et al. 1995), but the decrease in food quality can negatively impact insect-herbivore abundance and growth rate (Stiling and Cornelissen 2007). These results are from controlled laboratory experiments of relatively short duration and can be highly species specific (Zvereva and Kozlov 2006); it is not known whether the observed trends persist over longer time periods or how plants compensate.

The fossil record of the late Paleocene and early Eocene in the Bighorn Basin, Wyoming, USA, is ideal for studying the long-term effects of warming. The Bighorn Basin contains the most complete rock record available of terrestrial environments during this time interval (Wing 1998, Gingerich 2001, Clyde et al. 2007). Biostratigraphy, chemostratigraphy, and magnetostratigraphy are well constrained (Rose 1981, Bown et al. 1994, Gingerich 2001, Wing et al. 2005, Secord et al. 2006, Clyde et al. 2007), facilitating correlations between Bighorn Basin fossil localities and to the marine climate record (Koch et al. 1992). Furthermore, fossil plants are preserved in very similar rock types, indicating that edaphic conditions remained relatively consistent throughout the study interval. Paleobotanical and geochemical paleotemperature proxies record four major temperature changes between 59 and 52 million years ago (Ma): a gradual warming during the last three million years of the Paleocene; an abrupt temperature increase at the Paleocene-Eocene Boundary (the Paleocene-Eocene Thermal Maximum [PETM], beginning 55.8 Ma); an early Eocene cool period; and a renewed warming to the Early Eocene Climatic Optimum (EECO) 51–53 Ma (Wing et al. 2000, Zachos et al. 2001, 2008). These sequential trends are documented globally, in deep-sea isotope records (Zachos et al. 2001, 2008), and locally in the Bighorn Basin (Bao et al. 1999, Wing et al. 2000). Unfortunately, a high-resolution record of carbon dioxide concentrations throughout the Paleocene and Eocene does not yet exist, making it impossible to quantitatively correlate the partial pressure of carbon dioxide in the atmosphere (pCO₂) and

herbivory or to decouple the effects of temperature and pCO₂. Carbon isotopic evidence and mass balance equations, however, indicate that pCO₂ increased by a multiple of 3–4 during the PETM (Zachos et al. 2003). The insect herbivore damage studied here records terrestrial ecosystem response to both temperature and pCO₂, which are coupled in natural systems over long timescales (Siegenthaler et al. 2005, Royer et al. 2007a).

The PETM is an abrupt and transient warming event, whereas the EECO is a sustained interval of maximum Cenozoic warmth. The PETM is one of the best deep-time analogs for modern, anthropogenic global warming because of its rate and magnitude of temperature and pCO₂ change (Kennett and Stott 1991, Koch et al. 1992, Zachos et al. 2005). It is marked by a negative carbon isotope excursion in both marine and terrestrial reservoirs, consistent with the release of a large amount of ¹³C-depleted carbon to the atmosphere and ocean (Dickens et al. 1995, Zachos et al. 2005, Higgins and Schrag 2006, Pagani et al. 2006). Global mean surface temperatures rose at least 5°C over ~10 thousand years (kyr) and returned to background levels after ~100 kyr (Röhl et al. 2000, Zachos et al. 2003). Previous work documents transient floral change in the Bighorn Basin (Wing et al. 2005) and a spike in insect herbivory (Currano et al. 2008) during the PETM.

Here, we present the most finely resolved and data-rich record of insect herbivory from a past interval of significant climate change, the late Paleocene and earliest Eocene (59–52.6 Ma) in the Bighorn Basin. Using the fossil record to study one geographic location over several million years, with relatively consistent edaphic conditions, eliminates the effect of latitude and permits direct correlation between herbivory and climate. Previous studies in the western United States have shown an increase in insect damage richness with warming across the Paleocene-Eocene boundary and during the terminal Cretaceous (Wilf and Labandeira 1999, Wilf et al. 2001, Labandeira et al. 2002b, Currano et al. 2008, Wilf 2008) and a decline in leaf area removed by herbivores between two sites before and after the Eocene-Oligocene cooling (Smith 2008). For the first time in a single study, we compare the effects of cooling, gradual warming, and abrupt warming for a large number of sites that are geologically closely spaced in time and within a single basin. We consider the effects of floral diversity on insect herbivore damage and test whether climate is the most likely primary driver of trends in herbivory.

METHODS

Insect damage data

We collected and analyzed 9071 fossil angiosperm leaves (or leaflets in the case of compound leaves) from nine well-dated stratigraphic levels (Smith et al. 2004, Wing et al. 2005, Secord et al. 2006, Clyde et al. 2007) whose ages span the late Paleocene warming, PETM, early Eocene cooling, and the beginning of the EECO

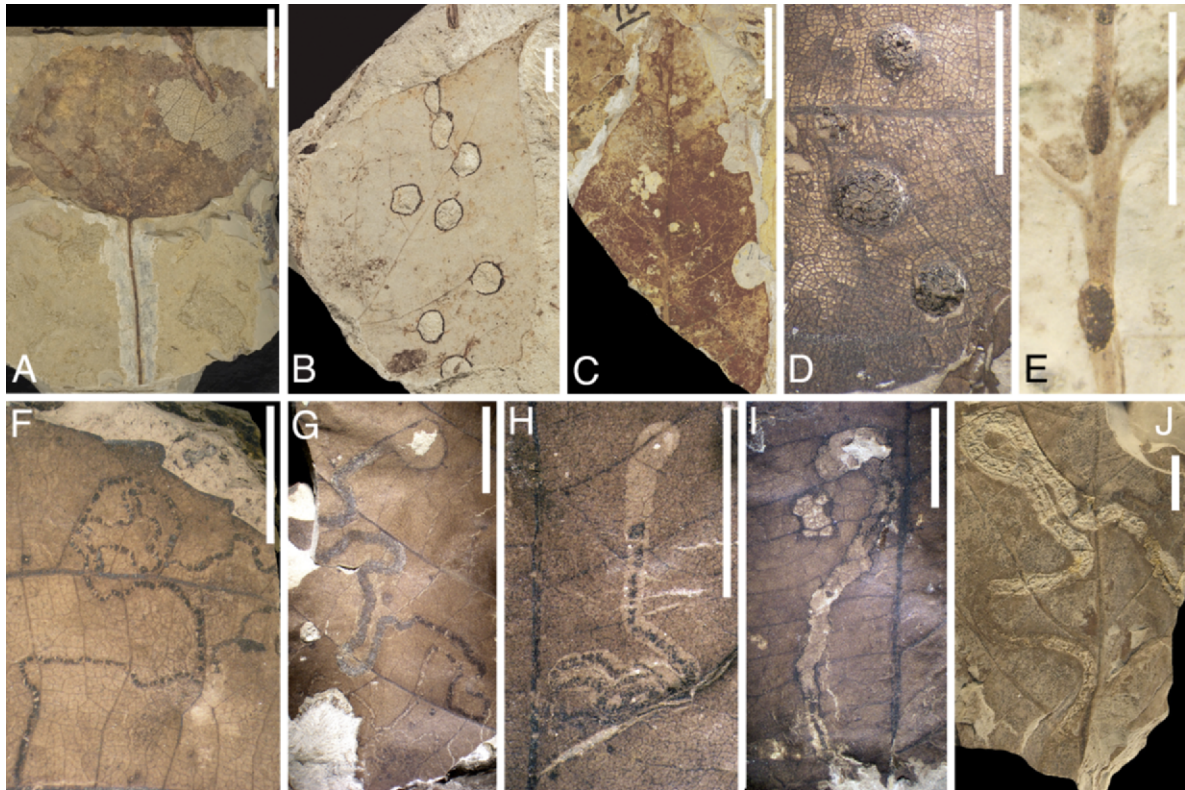


FIG. 1. Insect herbivory on fossil angiosperm leaves, rotated for best display of the damage. (A) Skeletonization (damage type [DT] 16) on *Cercidiphyllum genetrix* (site P4, 55.9 Ma, National Museum of Natural History accession number USNM 539636). (B) Large hole feeding (DT 4) on Dicot sp. WW006 (site E1, Paleocene-Eocene Thermal Maximum, 55.8 Ma, USNM 530968). (C) Margin feeding (DT 81) on Fabaceae sp. FU750 (site P4, USNM 539929). (D) Thick, woody galls (DT153) on “*Platycarya*” *castaneopsis* (site E5, Early Eocene Climatic Optimum, 52.6 Ma, USNM 539923). (E) Galls on a primary vein (DT 33) of *Cercidiphyllum genetrix* (site P4, USNM 539930). (F) Threadlike, serpentine mine packed with frass (DT 33) on *Alnus* sp. (site E5, USNM 539931). (G) Frass-laden serpentine mine with an enlarged terminal chamber (DT 40) on *Alnus* sp. (site E5, USNM 539932). (H) Small serpentine mine with a round terminal chamber and frass confined to the central third of the mine (DT 151) on *Luehea newberryana* (site E5, USNM 539913). (I) Roughly linear serpentine mine with a terminal chamber and intermittent frass (DT 90) on *Populus wyomingiana* (site E5, USNM 539933). (J) Coleoptera mine with a thick outer rim and distinct frass trail (DT 164) on “*Dombeya*” *novi-mundi* (site E3, 54.2 Ma, USNM 539926). Scale bars are 2 cm in panel (A), 1.5 cm in panel (C), and 0.5 cm in panels (B) and (D–J). See Table 1 for an explanation of abbreviations. Photo credits: panels (A, C, E, F, G, I), E. Currano; panel (B), A. Morey; panels (D, H, J), A. Rulis.

(Fig. 2, Table 1; Appendix A). Only “dicots” (i.e., non-monocot angiosperms) were examined in this study because, unlike monocots and ferns, their leaves are often preserved at >50% completeness and are therefore easy to consider as individual units and normalize for sampling effort. Additionally, insect herbivory on monocots is much less abundant than on dicots (Wilf et al. 2000, Grubb et al. 2008), and much of the literature on insect damage in Rocky Mountain basins has focused on dicot leaves (Wilf and Labandeira 1999, Wilf et al. 2001, 2006, Labandeira et al. 2002b, Currano et al. 2008, Smith 2008).

The four Paleocene localities are from the northern Bighorn Basin and are in the Fort Union Formation (Fig. 2, Table 1; Appendix A); for simplicity, we will refer to them as P1 through P4, respectively, from oldest to youngest. The five Eocene sites (E1–E5) are from the central and southern Bighorn Basin and are in the

Willwood Formation. Site E1 occurs in the middle of the PETM (S. L. Wing, E. C. Lovelock, and E. D. Currano, *unpublished data*). Insect herbivory at P1, P2, P4, E1, E2, and E5 is further discussed elsewhere (Wilf et al. 2006, Currano et al. 2008, Currano 2009). An index of mean annual temperature (MAT index) for each stratigraphic level was either obtained from the literature or calculated here. Both previously published and new MAT index values were calculated using leaf margin analysis according to the equation of Wing and Greenwood (1993: Fig. 1; Table 1, Appendix A). Leaf margin analysis is a widely used method that uses empirical correlations of MAT and the percentage of species at a site that have untoothed leaves to estimate paleotemperatures from the corresponding percentages in fossil floras (Bailey and Sinnott 1915, Wolfe 1979). However, leaf margin analysis assumes that environment, not phylogeny, is the primary factor influencing

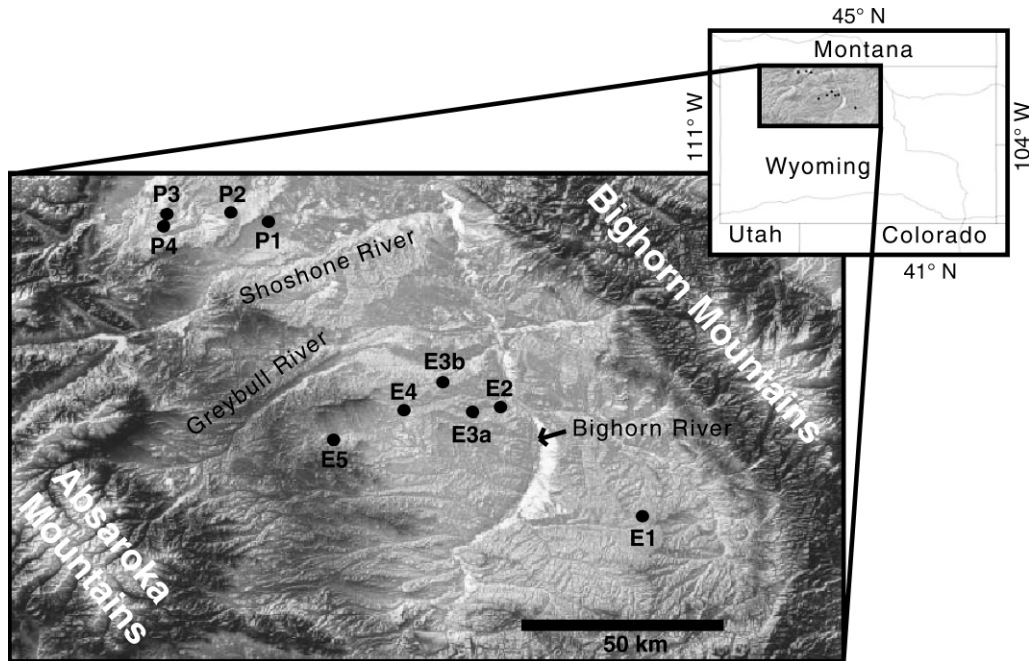


FIG. 2. Map of the fossil localities in Bighorn Basin, Wyoming, USA (see Table 1). Sites are labeled P (Paleocene) or E (Eocene) and numbered chronologically. Detailed geographic information for each site is included in Appendix A. The dot labeled E3a represents National Museum of Natural History (USNM) locality 37654, and E3b represents USNM localities 42407 through 42410.

the presence or absence of leaf teeth and thus that the response of this trait to environmental change should be constant through time. Because a variety of observations cast doubt on this assumption (Bailey and Sinnott 1916, Jordan 1997; S. A. Little, S. W. Kembel, P. Wilf, and D. L. Royer, *unpublished manuscript*), we stress that our

leaf margin analysis results should not be regarded as precise numerical estimates of paleotemperature (and accordingly are plotted without error bars). Rather, the MAT index corresponds to the estimated temperature (in degrees Celsius) if the observed leaf margin percentage were derived from a modern sample within

TABLE 1. Fossil leaf sites from the late Paleocene and early Eocene of the Bighorn Basin, Wyoming, USA.

Site number	Site name	USNM locality number	Epoch and mammal zone	Age (Ma)	MAT index	Lithology	No. leaves in census
E5 ^A	Fifteenmile Creek	42400–42406	Eocene, Wasatchian 7	52.7	22.2 ^B	laterally extensive carbonaceous shale	1821
E4	PN	37560	Eocene, Wasatchian 5	53.4	15.8 ^B	mud/silt lens	693
E3	Cool Period	37654, 42407–42410	Eocene, Wasatchian 3–4	54.2	11.1	USNM 37654 is in mud/silt lens; the rest are in a laterally extensive carb shale	491
E2 ^C	South Fork of Elk Creek	42395–42399	Eocene, Wasatchian 1–2	55.2	16.4 ^B	laterally extensive carbonaceous shale	1008
E1 ^C	Hubble Bubble	42384	PETM (Eocene), Wasatchian 0	55.8	20.1 ^D	mud/silt lens	995
P4 ^C	Daiye Spa	41643	Paleocene, Clarkforkian 3	55.9	16.4	mud/silt lens	843
P3	Dead Platypus	42411	Paleocene, Clarkforkian 2	56.4	12	mud/silt lens	1016
P2 ^E	Lur'd Leaves	42042	Paleocene, Tiffanian 5b	57.5	10.5 ^C	mud/silt lens	1364
P1 ^E	Skeleton Coast	42041	Paleocene, Tiffanian 4a	58.9	10.5 ^C	mud/silt lens	840

Notes: Land mammal zones are widely used in North American biostratigraphy and are especially well resolved in the study area (Woodburne 2004, Secord et al. 2006). Sources are (according to superscripted capital letters): (A) Currano 2009; (B) Wing et al. 2000; (C) Currano et al. 2008; (D) S. L. Wing, E. C. Lovelock, and E. D. Currano, *unpublished data*; (E) Wilf et al. 2006. Other sites and paleoclimate estimates are published for the first time here and are fully described in the Supplement. Abbreviations: Ma, million years ago; MAT, mean annual temperature; PETM, Paleocene–Eocene Thermal Maximum; USNM, National Museum of Natural History.

the extant calibration region (in this case, East Asia; Wolfe 1979, Wing and Greenwood 1993). The MAT index values show relative temperature changes that are well corroborated by local geochemical paleotemperature proxies (Bao et al. 1999, Wing et al. 2000) and faunal turnover events in the Bighorn Basin (Gingerich 1989, Clyde and Gingerich 1998, Secord et al. 2006, Chew 2009), as well as the global deep-sea record (Zachos et al. 2001).

Plant fossils studied here are preserved as compressions and impressions in two similar fluvial depositional environments (Table 1). Sites E2, E5, and most of the E3 localities (National Museum of Natural History [USNM] locations 42407–42410) are in laterally extensive carbonaceous shale deposits (Wing 1984, Davies-Vollum and Wing 1998). The most complete and best-preserved leaf fossils are found in silty claystone subunits, which represent distal overbank deposits that formed during intervals of high sediment discharge. Thus, these fossil assemblages represent locally derived back swamp vegetation accumulating over a season to a few years (Davies-Vollum and Wing 1998). The remaining localities are in lenticular mudstone and siltstone units. These deposits extend less than 300 m laterally and contain multiple fining-upward sequences; many have floating aquatic plants. These sediments accumulated when ponds formed in abandoned channel beds, and changes in lithology and plant composition occurred as the water shallowed (Wing 1984).

The leaves in this study were divided into plant morphotypes using leaf architectural characters (Ellis et al. 2009). Full morphotype descriptions are found in Appendix B. Some of these morphotypes have been formally described and named, though many of the names are historic and require revision. If a listed genus is in quotes, we suspect that although the name is nomenclaturally valid, the generic assignment is incorrect. Morphotypes that have not been formally named were given Fort Union (FU) or Willwood (WW) formation morphotype numbers, and family-level designations were made whenever possible.

Insect damage censuses were conducted at each of the nine stratigraphic levels. For the laterally extensive fossil deposits at E2, E3, and E5, we censused fossil leaves from multiple smaller quarries within the same level (Appendix A: Table A1). The term “locality” here indicates a single quarry, whereas “site” lumps all quarries from the same stratigraphic level. At each locality, the fossil-bearing layer was identified and the overburden removed to make a bench quarry (see Plate 1). Every identifiable dicot leaf (or leaflet for compound leaves) with at least half of the blade intact was scored for the presence or absence of the 71 insect feeding-damage types (DTs) that were found in this study (Wilf and Labandeira 1999, Labandeira et al. 2002a, 2007). Insect herbivory is distinguished from detritivory by: the presence of plant response tissue; the targeting of particular tissues, organs, and plant host species;

elevated stereotypy in the pattern, position, or type of damage; or specific micromorphological features, such as pupal chambers, particular types of mandible bite marks, or exit slits in mines (Labandeira 1998). A representative suite of voucher specimens is deposited in the Department of Paleobiology, National Museum of Natural History, under the collection numbers found in Table 1. The complete data set is available in the Supplement.

Damage types can be subdivided into generalized DTs, made by insects that typically eat many taxonomically unrelated host plants, and specialized DTs, made by insects that typically eat only one or a few closely related host plants (Wilf and Labandeira 1999, Labandeira et al. 2002b, 2007). Specialized damage is recognized by similarity to extant specialized feeders, by morphologically stereotyped damage patterns, and by restricted occurrences confined to particular host plant species or tissue types in either fossil or extant host taxa (Labandeira et al. 2002b, 2007). Here, we consider DTs to be specialized if they have a host specialization value of 2 or 3 on the 1–3 scale in the Wilf and Labandeira (1999) system, as expanded by Labandeira et al. (2007). The DTs present in this study are also divided into seven functional feeding groups and subgroups: external feeding (with the subgroups hole feeding, margin feeding, skeletonization, and surface feeding), galling, leaf mining, and piercing and sucking, as described elsewhere (Labandeira et al. 2002a, 2007). These functional feeding categories refer to the distinctive ways in which insects access foliar tissues, and each category has a spectrum of damage made by phytophagous arthropods with distinctive suites of mouthpart types.

Leaf mass per area

Throughout the study interval, a great deal of floral turnover occurs; no single plant species, or even family, can be traced from 59 to 52 Ma (P1 to E5; Appendix B). Consequently, we needed to establish whether there are significant structural differences among the leaves at these sites that may be driving trends in insect damage. One especially important leaf trait that can be estimated in fossils is leaf mass per area (M_A). Species with high M_A generally have thicker, tougher leaves that are less palatable to insect herbivores (Coley and Barone 1996, Royer et al. 2007b). Plants with low M_A generally have short leaf life spans and high nutrient concentrations, making them more palatable to insect herbivores. Mechanistically, leaves with a low M_A have proportionally more photosynthetic mesophyll to maximize their photosynthetic rate (Wright et al. 2004). Mesophyll is both rich in nitrogen and biomechanically relatively weak, which increases vulnerability to herbivory and physical deterioration and results in a shorter leaf life span.

Fossil M_A can be estimated from petiole width and leaf area using an extensive modern calibration set that demonstrates a robust scaling relationship between

petiole width squared and leaf mass, normalized for leaf area (Royer et al. 2007b). The theoretical explanation for this relationship is that a wider petiole has a greater cross-sectional area that scales to support a heavier leaf. Every fossil leaf that clearly showed the attachment of the petiole to the leaf (or petiolule to the leaflet) blade and had a reconstructable leaf area was analyzed for M_A using the protocol of Royer et al. (2007b). For correlation analyses, only plant species with at least two measurable fossils and 20 leaves in a given census were used, resulting in a data set of 177 leaves belonging to 39 species–site pairs.

Quantitative analyses

We consider damage diversity (also referred to as “richness,” i.e., the number of damage types present at a site or on a host species), frequency, and composition on both the bulk flora and individual plant species within a flora. All three damage metrics indirectly reflect the number of insect species present, the nutrient content of a plant’s leaves, the abundance of structural and chemical defenses in the leaves, the adaptations of insects to plant defenses, and environmental conditions. A study of spatial variation in insect herbivory among the localities making up sites E2 and E5 revealed that differences in diversity and composition between sites were significantly greater than variations within a site (Currano 2009), and intra-site variation was primarily due to differences in floral composition.

Damage diversity is presented here as the number of DTs present in a sample, normalized for the number of leaves sampled, as in previous studies (e.g., Wilf and Labandeira 1999). Because damage diversity is highly influenced by sample size, we standardized our data using resampling routines to select a random subset of 450 leaves without replacement and calculate the mean resampled damage diversity for the subsample. This process was repeated 5000 times, and the results were averaged to obtain the standardized damage diversity for each flora. The standard deviation for the resamples was calculated to provide error bars. The same procedure was used to standardize damage diversity to 20 leaves on each of the 54 species–site pairs with at least 20 leaf specimens. Damage diversity is not perfectly analogous to insect diversity because a single insect species may make multiple types of damage, and some generalized DTs may be made by many taxonomically unrelated insect species or by diverse but similar species within a genus (Johnson and Lyon 1991, Basset and Höft 1994); however, preliminary data show a good correspondence of DT richness to actual insect richness (M. Ramirez-Carvalho et al., *unpublished data*). Damage diversity on a per leaf basis, as done here and in previous studies, is a core measure of how many ecological feeding types are present on a certain amount of foliage and, because fossil leaves are the units excavated, from a particular amount of sampling effort. This metric is frequency dependent to some extent because a variable

proportion of leaves have damage (Wilf et al. 2005). However, if undamaged leaves are excluded from diversity analyses to compensate for the frequency dependence, the critical relationship between observed insect damage and the amount of foliage resource available would be lost.

Damage frequency is the percentage of leaves that are damaged. Because a leaf either has damage or does not, error bars for damage frequency are calculated based on a binomial sampling distribution. Damage frequency depends heavily on the abundance and density of insect populations and therefore is more variable within a stratigraphic level than diversity or composition, which appear to track plant hosts both today (Novotny et al. 2007) and in the past (Currano 2009).

Damage composition here describes the relative abundance distributions of the DTs or functional feeding subgroups across time and host species. It is quantitatively analyzed here by using cluster analysis and nonmetric multidimensional scaling (NMDS) on a matrix of the proportion of leaves in a given sample that are damaged by insects of each functional feeding group/subgroup. Data were analyzed at the level of functional feeding group/subgroup, rather than DT, to increase the signal:noise ratio. The data were first arcsine square-root transformed to improve normality in proportional data by extending the ends of the scale (Sokal and Rohlf 1995). A dissimilarity matrix between all pairs of samples was then computed using the Bray-Curtis distance metric (Bray and Curtis 1957). The “metaMDS” function in R version 2.4.1 (R Development Core Team, Vienna, Austria) was used to ordinate the samples using NMDS, and the “agnes” function in R was used to perform agglomerative hierarchical clustering with Ward’s clustering method.

These damage metrics were correlated with temperature and floral diversity. Additionally, because leaf mass per area can be estimated for fossil leaves (Royer et al. 2007b), damage on individual species can be correlated with leaf mass per area. Because every variable in the data set comprises a time series, there is likely a serial correlation, or nonrandom association of each value with the values immediately preceding and succeeding it in time. In order to test whether two time series are correlated with one another, rather than each one simply being correlated to time, first differences analyses are often used to remove serial correlation. Because our fossil sites are not equally spaced in time, we could not do a true first differences analysis. Instead, we simply calculated the differences in each variable between chronologically adjacent sites, a useful first-order correction for serial correlation we refer to below as “modified first differences analysis.”

RESULTS

Plant diversity and composition

Leaf abundance data collected as part of the insect damage censuses are used here to describe trends in

TABLE 2. Floral diversity and evenness.

Site code	Age (Ma)	<i>S</i>	Diversity at 450 leaves	Diversity error	Pielou's <i>J</i>	PIE
E5	52.7	24	17.6	4.9	0.40	0.77
E4	53.4	14	12.1	3.7	0.30	0.55
E3	54.2	18	17.7	4.2	0.52	0.85
E2	55.2	6	4.8	2.4	0.21	0.31
E1	55.8	25	20.0	5.0	0.39	0.72
P4	55.9	16	14.7	4.0	0.50	0.82
P3	56.4	19	14.3	4.4	0.48	0.84
P2	57.5	15	12.5	3.9	0.39	0.65
P1	58.9	7	6.5	2.6	0.39	0.55

Notes: *S* is the total number of plant species observed in the sample. Floral diversity was rarefied to 450 leaves using analytical rarefaction, and the error on the rarefaction is $\text{SQRT}(S)$. Pielou's *J* (Pielou 1969) and PIE (probability of interspecific encounter; Hurlbert 1971) are two evenness metrics.

floral diversity and composition. Because plants and herbivorous insects have coevolved (e.g., Strong et al. 1984), these patterns are important in our overall understanding of insect herbivory. Many trends from this report compare as expected to previous studies of floral diversity and composition in the Paleocene and Eocene of the Bighorn Basin (Wing et al. 1995, 2000, 2005, Davies-Vollum and Wing 1998, Wing 1998, Wing and Harrington 2001), showing that the sites we investigated are representative of these much larger paleobotanical studies, and, importantly, precisely constraining floral change to the leaf deposits bearing the insect damage quantified here. Floral diversity (also referred to as "richness") and evenness values are shown in Table 2. The highest values occur at the two warmest sites, E1 from the PETM and E5 from the EECO. Richness and evenness at site E3 are probably inflated relative to the other samples because that level includes localities that are 13.7 km apart, rather than being from a single quarry or bed (Appendix A). Sites P2, P3, P4, and E4 have intermediate species richness, and sites P1 and E2 have extremely low floral richness.

Although the two warmest sites also have the highest floral diversity, the data show only a weak, positive correlation between floral richness (rarefied to 450 leaves; Table 2) and MAT index when all sites are included ($R^2 = 0.15$). There is also no correlation between change in temperature and change in floral richness at chronologically adjacent sites ($R^2 = 0.002$). If site E3 is removed from the analysis because it incorporates a greater geographic area than the other sites, the R^2 value increases to 0.32 ($P = 0.14$) and the modified first differences R^2 value to 0.33 ($P = 0.17$). In contrast, previous studies that analyzed stratigraphic range data, rather than abundance data, and included many additional Bighorn Basin collections have shown a positive relationship between floral diversity and temperature (Wing et al. 1995, 2000, Wing 1998, Wing and Harrington 2001). Because the Bighorn Basin stratigraphic range data incorporate a broader geographic area and many more plant fossil collections and time slices than we analyzed, it provides a more complete view of regional vegetation.

Variations in floral composition among sites can be summarized using NMDS (Fig. 3; see also Appendix B for pictures and descriptions of the plant morphotypes). The Paleocene and Eocene floras are well separated on the plot. Sites P1 (58.9 Ma) and P2 (57.5 Ma) contain species that are abundant in Paleocene floras throughout the Rocky Mountain basins (Brown 1962), particularly *Browniea serrata* (Nyssaceae), *Cercidiphyllum genatrix* (Cercidiphyllaceae), *Davidia antiqua* (Nyssaceae), and *Platanus raynoldsi* (Platanaceae). Site P1 is dominated by *C. genatrix* and P2 by *Persites argutus* (Lauraceae). Site P3 (56.4 Ma) has many of the same species as P1 and P2, as well as other common Paleocene plants including *Averrhoites affinis* (?Sapindales), a betulaceous morphotype (Betulaceae sp. FU741), and *Macginitiea gracilis* (Platanaceae). *Ginkgo adiantoides* leaves are also common at P3, although as gymnosperms they are not included in the analysis. The latest Paleocene flora, P4, is a mix of the typical Paleocene species and new species. *Cercidiphyllum genatrix*, *M. gracilis*, and *P. raynoldsi* are still abundant, whereas *B. serrata*, *D. antiqua*, and

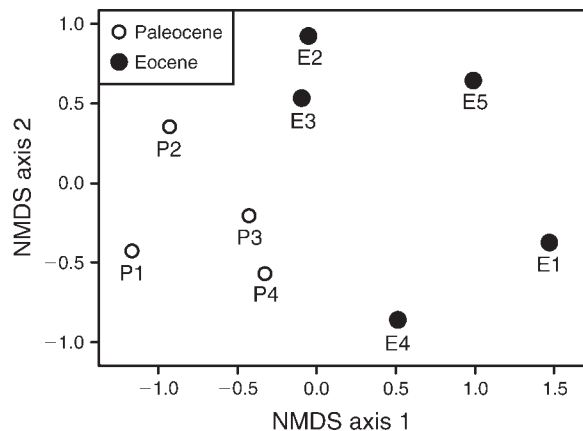


FIG. 3. Nonmetric multidimensional scaling (NMDS) ordination of the Bighorn Basin floras based on the relative abundances of dicot leaf species. NMDs axes 1 and 2 define the two-dimensional ordination space that most accurately represents the differences in floral composition among sites. Ages and other site data are given in Table 1.

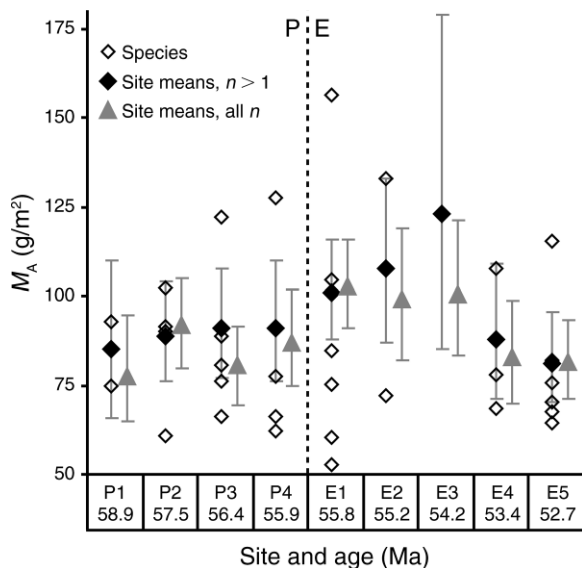


FIG. 4. Estimated leaf mass per area (M_A) at each site, using the method of Royer et al. (2007b). The mean M_A values for all species–site pairs with at least 20 censused leaves and two measurable fossils are plotted (Species). Site means were calculated two ways: as the mean value among all plant species with at least two measurable fossils ($n > 1$) and as the mean value among all plant species that had one or more measurable fossils (all n). Error bars represent the $\pm 95\%$ prediction interval, which depends both on the strength of the regression and the number of leaves measured for each site and is asymmetrical based on log relationships. The dashed line marks the Paleocene–Eocene boundary. The horizontal axis is not to scale. See Table 1 for explanations of site abbreviations.

Zizyphoides flabella (Trochodendraceae) form a much smaller component of the flora. Several of the rarer species at P3, including Dicot sp. FU745 and Dicot sp. FU749, are found in much greater abundance at P4.

The PETM flora from site E1 is both the most diverse and the most distinct flora in this study, depicted graphically by its placement in the NMDS plot. Legumes are an important component of this flora (S. L. Wing, E. C. Lovelock, and E. D. Currano, unpublished data), and conifers are absent as macrofossils (Smith et al. 2007). Many of the species found at E1 have not been identified elsewhere, and the only abundant species found at other sites in this study are Fabaceae sp. WW007 and *Populus wyomingiana* (Salicaceae).

Site E2 (55.2 Ma) is strongly dominated by *A. affinis*, and the only other common dicot species is *Alnus* sp. (Betulaceae). The E2 site probably represents a swamp with a wet substrate that was at least seasonally flooded (Wing 1984). Similarly, E3 localities USNM 42408–42410 are dominated by *A. affinis*, although “*Dombeya*” *novi-mundi* (Malvaceae) is locally abundant. Interestingly, one E3 locality contains *P. wyomingiana*, which was previously thought to be absent in the Bighorn Basin until the warming to the EECO (Wing 1998). Site E3 locality USNM 37654 is dominated by

“*Ampelopsis*” *acerifolia* (?Cercidiphyllaceae, ?Vitaceae) and Dicot sp. WW037. The NMDS 1 scores for Sites E2 and E3 are more similar to the late Paleocene sites than they are to the PETM or EECO, echoing the results of Wing et al.’s (2005) larger study in the Bighorn Basin.

The most abundant plant species at E4 (53.4 Ma) are *M. gracilis*, Fabaceae sp. WW007, Fabaceae sp. WW040, and *P. wyomingiana*. Because most of the abundant species also occur at either sites P3 and P4 or E1, site E4 ordinated between these sites on the NMDS plot. Many of the plant taxa at site E5 (52.65 Ma), particularly “*Platycarya*” *castaneopsis* (Juglandaceae) and the fern *Lygodium kaulfussi*, are characteristic of the warm early Eocene in the region (Wing 1998). The three most abundant species are *Alnus* sp., “*Platycarya*” *castaneopsis*, and “*Dombeya*” *novi-mundi*, and other common species include *P. wyomingiana*, Dicot sp. WW052, and a Lauraceae (sp. WW061).

Leaf mass per area results

Fig. 4 shows leaf mass per area for all species–site pairs having at least 20 leaves in a given census and two fossils that could be measured for petiole width and leaf area (species; Appendix C lists M_A for each species). Among-species site mean M_A was estimated using only these species ($n > 1$) and also using all leaves for which petiole width and leaf area could be measured (all n). Plant species that are found at multiple sites generally have very similar M_A among sites, although there is a large but nonsignificant difference for *C. genatrix* between P1 (93 [+55.8/–34.9 g/m²]) and P4 (66.3 [+25.9/–18.6 g/m²]) (mean and 95% prediction interval, which is asymmetrical based on log relationships). Nearly all plant species have low estimated M_A that would be consistent with high palatability. The only species with reconstructed M_A greater than 100 g/m² are legumes (Fabaceae sp. FU740, WW001, WW002), “*Platycarya*” *castaneopsis*, and *A. affinis*. There are no significant among-site differences in M_A (an ANOVA of M_A by sites yielded an F value of 0.45 and $P = 0.86$, $df = 8, 30$).

Damage diversity

Fig. 5 shows the number of damage types on the bulk floras resampled to 450 leaves and on individual species resampled to 20 leaves. On the bulk floras, total, overall specialized, and mine damage diversity curves mirror the MAT index curve. Site E5 (EECO) has the highest damage richness, followed closely by the PETM E1 locality. Sites E4 and P4 are the next highest in both temperature index and total and specialized damage diversity, followed by E2 and P3. Sites P1, P2, and E3, in rank-order from low to high, have the lowest total and specialized damage diversity and the coldest temperature index. The mine diversity pattern parallels the total and overall specialized pattern, except that E4 is more similar to the earlier Paleocene sites than it is to the later Paleocene or other early Eocene sites.

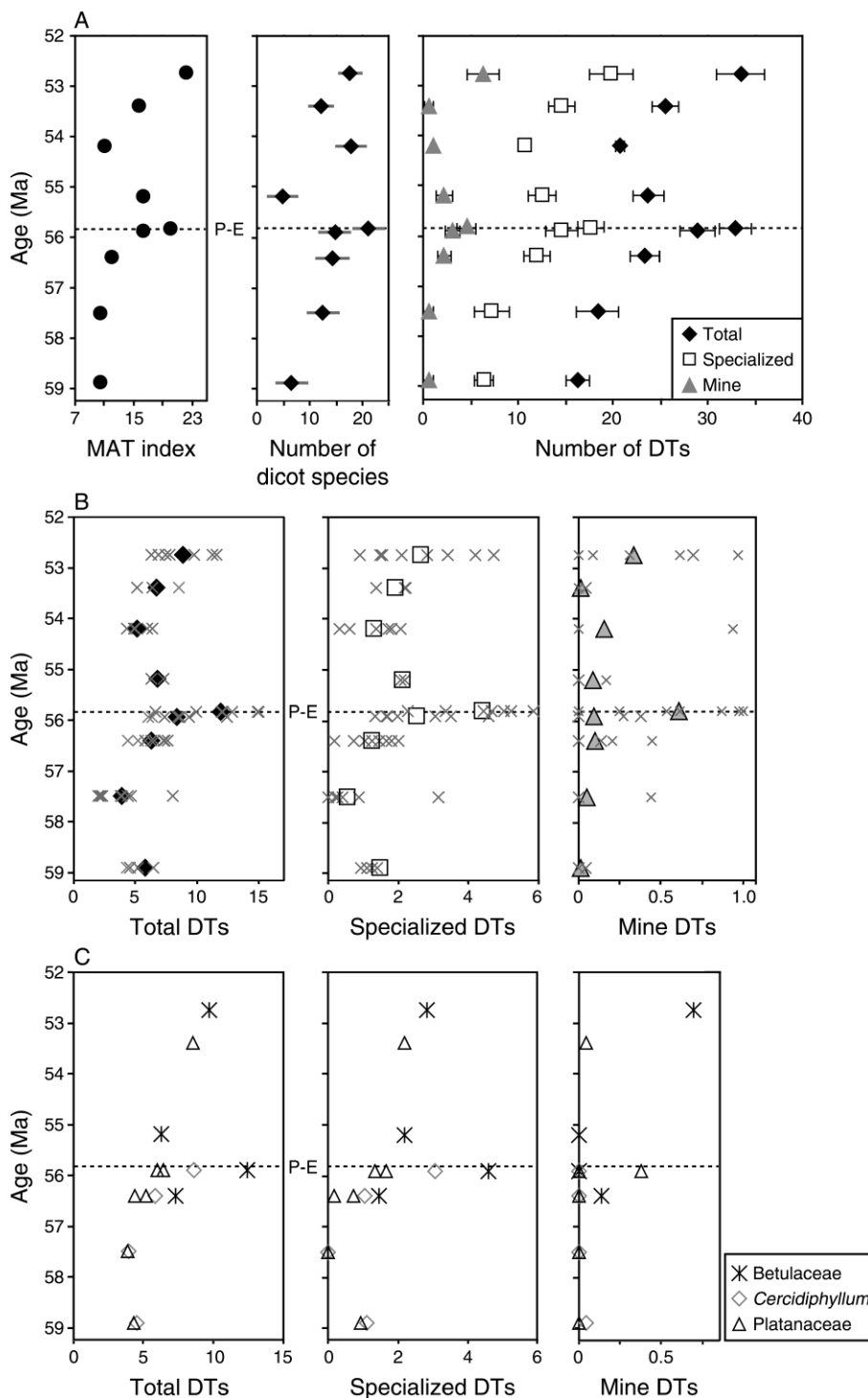


FIG. 5. Damage diversity through time (Ma, millions of years ago). (A) Mean annual temperature (MAT) index (Table 1), floral richness (dicots), and total, specialized, and mine damage type (DT) diversity for the bulk floras. Floral richness and DTs are standardized to 450 leaves. Error bars represent one SD above and below the mean of the resamples and are too small to be visible for some data points. (B) Total, specialized, and mine damage type diversity for individual plant hosts (see Supplement for data). Each “x” symbol represents a plant host with at least 20 leaves in the flora; the circles, squares, and triangles are the means of the X’s at the site. Error bars were omitted to reduce clutter. (C) A taxonomic subset of the data from panel (B), including only *Cercidiphyllum genatrix* and plant species in the Betulaceae and Platanaceae families. Error bars were omitted to reduce clutter. The dashed lines mark the Paleocene–Eocene boundary.

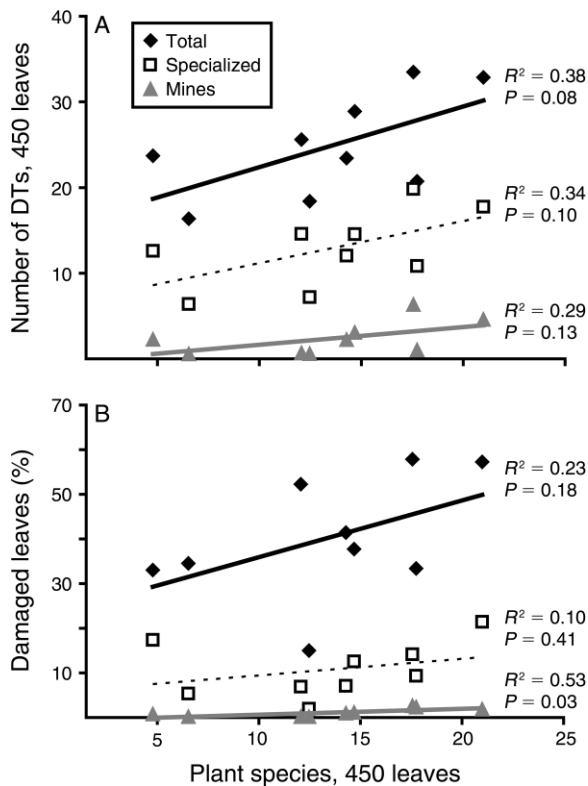


FIG. 6. (A) Insect damage richness and (B) frequency vs. dicot leaf diversity for the bulk floras (damage types [DTs]: total, specialized, and mines). Regression lines are from a linear model, and R^2 values are shown on the plots. Error bars were omitted to reduce clutter. For both panels, dicot leaf diversity is rarified to 450 leaves; in panel (A) damage diversity is standardized to 450 leaves.

Sampling-standardized damage diversity is plotted against dicot leaf diversity (rarefied to 450 leaves) in Fig. 6A and mean annual temperature in Fig. 7A. Floral diversity is positively correlated with insect damage diversity, but the R^2 values are generally low and no P value is less than 0.05. The modified first differences analysis gives lower R^2 and higher P values. However, mean annual temperature has a significant positive correlation with total, overall specialized, and mine damage diversity; all three damage metrics have R^2 values greater than 0.75. Fig. 7B shows change in MAT index vs. change in damage frequency between chronologically adjacent sites (modified first differences analysis). The relationships between MAT index and all three damage diversity metrics remain significant and positive.

In general, damage richness patterns on individual species match those of the bulk flora (Fig. 5). All of the species at P1 and P2 have low total and overall specialized damage diversity, and only *Browniea serrata* at P2 has a moderately high diversity of mines. Total, specialized, and mine damage diversity on individual species gradually increase through the late Paleocene

and reach a peak during the PETM (site E1). Following the PETM, total and specialized damage diversities decrease through E3. Mine diversity at E2 is low, but sharply increases at E3, due to the appearance of a wide, sinusoidal mine packed with frass (DT164; see Appendix D for a full description) on “*Dombeya*” *novi-mundi*. Richness then progressively increases to high values at EECO site E5. Total, specialized, and mine damage diversity are lower in the EECO than in the PETM, although the difference is not significant.

Damage frequency

The percentage of leaves with any damage, specialized damage, and mining damage are shown in Fig. 8, and the percentage of leaves at each site with each functional feeding category is shown in Fig. 9. Table 3 shows the percentage of leaves at each site that has a given number of DTs, which also involves richness. When considering total damage, the bulk floras split into two groups. Sites E1, E4, and E5 have high damage frequencies that exceed 50%. The remaining sites have between 30% and 42% damage, except for P2, where only 15% of leaves are damaged. The mean damage frequency among plant species at site E1 is 70%, followed by 61% at E5 and 51% at E4. Mean damage frequency at the other sites falls between 22% and 42%. Plant species within a site show a range of damage frequencies, probably reflecting their differing investments in anti-herbivore defenses. The range in damage frequency is greatest at E1 and E5. At all nine sites, most of the damaged leaves have only one DT. The PETM flora has a long tail of richly damaged leaves, and one leaf (Dicot sp. WW005) has 10 DTs. No other flora has more than six DTs on a single leaf.

The percentage of leaves with overall specialized damage is significantly higher at the PETM site than at any other site. Based on Fig. 9, this high specialized damage frequency is due to elevated levels in all relevant categories: surface feeding, galling (Fig. 1D, E), leaf mining (Fig. 1F–J), and piercing and sucking. Site E2 has the next highest specialized damage frequency, attributable to the abundance of piercing and sucking DT46. Sites P4 and E5 have intermediate specialized damage frequencies, and the remaining sites are relatively low, particularly P2. Plant species within a site show a greater range of specialized damage frequencies than total damage frequencies. This is particularly apparent at E5, which has plant species with both the highest specialized damage frequency (*Allophylus flexifolia*, Sapindaceae) and lower damage frequencies comparable to the earlier Paleocene sites (*Populus wyomingiana*, “*Platycarya*” *castaneopsis*, “*Dombeya*” *novi-mundi*, and Dicot sp. WW052). The PETM locality (E1) has the greatest number of species with abundant specialized damage. When the individual species values at a single site are averaged to obtain a site mean, E1 is the highest, E5, E2, and P4 (ordered from high to low) have intermediate values, and the remaining

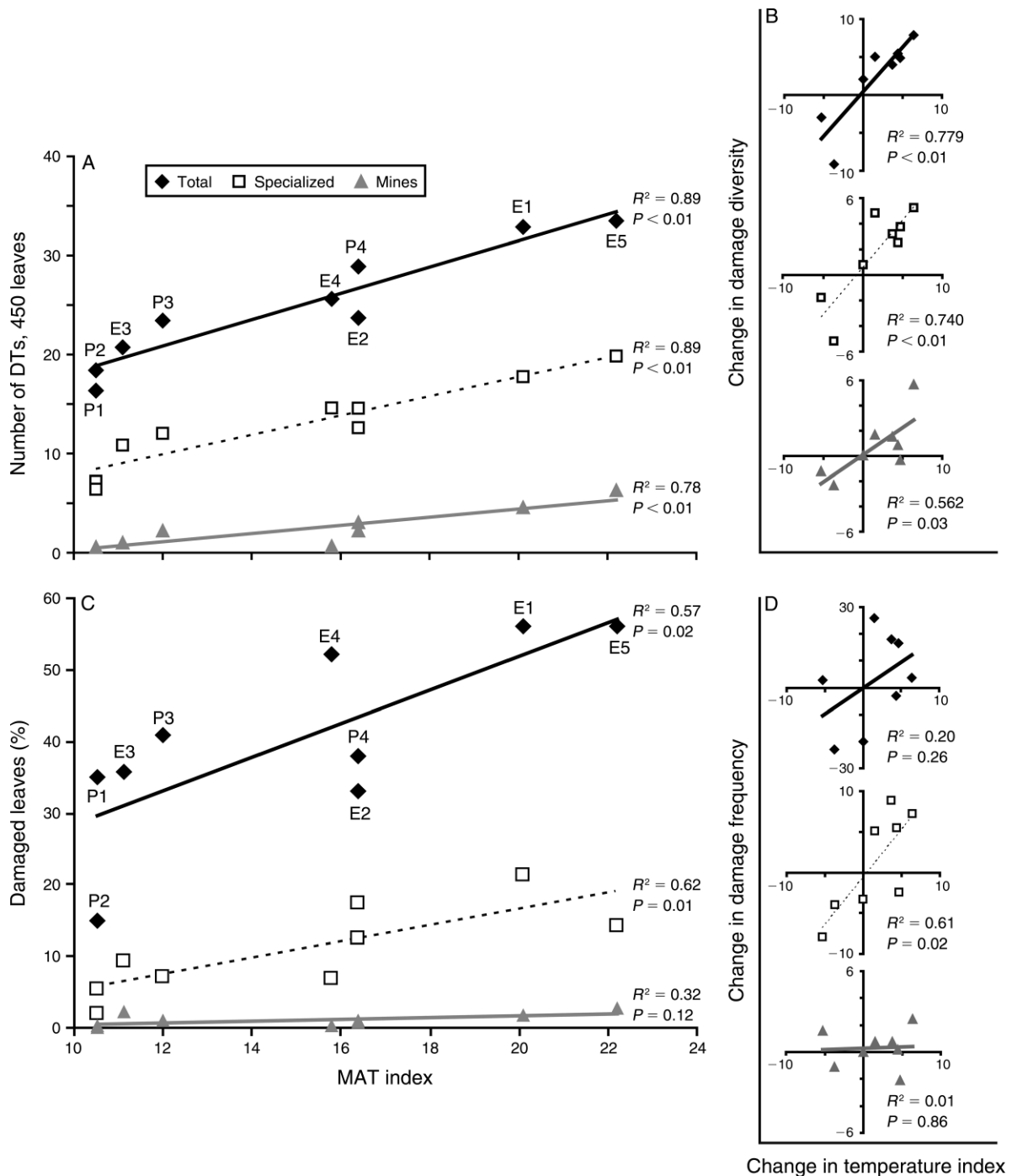


FIG. 7. Insect damage (total, specialized, and mines) vs. the mean annual temperature (MAT) index. Regression lines are from a linear model. (A) Damage diversity, resampled to 450 leaves vs. MAT index. (B) Modified first differences analysis, showing the change in the number of damage types between chronologically adjacent sites vs. change in MAT index. (C) Damage frequency vs. MAT index. (D) Modified first differences analysis, showing the change in percentage of damaged leaves vs. change in the MAT index.

sites are comparatively low. Thus, bulk floras and individual species show the same pattern.

Leaf mining is most abundant at E3 and E5, where 2.6% and 2.2% of the leaves are mined, respectively (Fig. 8). At E5, mines occur on six species: *Allophylus*

flexifolia, *Alnus* sp. (Fig. 1F, G), *Populus wyomingiana* (Fig. 1I), “*Dombeya*” *novi-mundi*, *Luehea newberryana* (Malvaceae; Fig. 1H), and “*Platycarya*” *castaneopsis*. Most of these mines are morphologically similar to those made by lepidopterans (Hering 1951, Steuer 1995), and

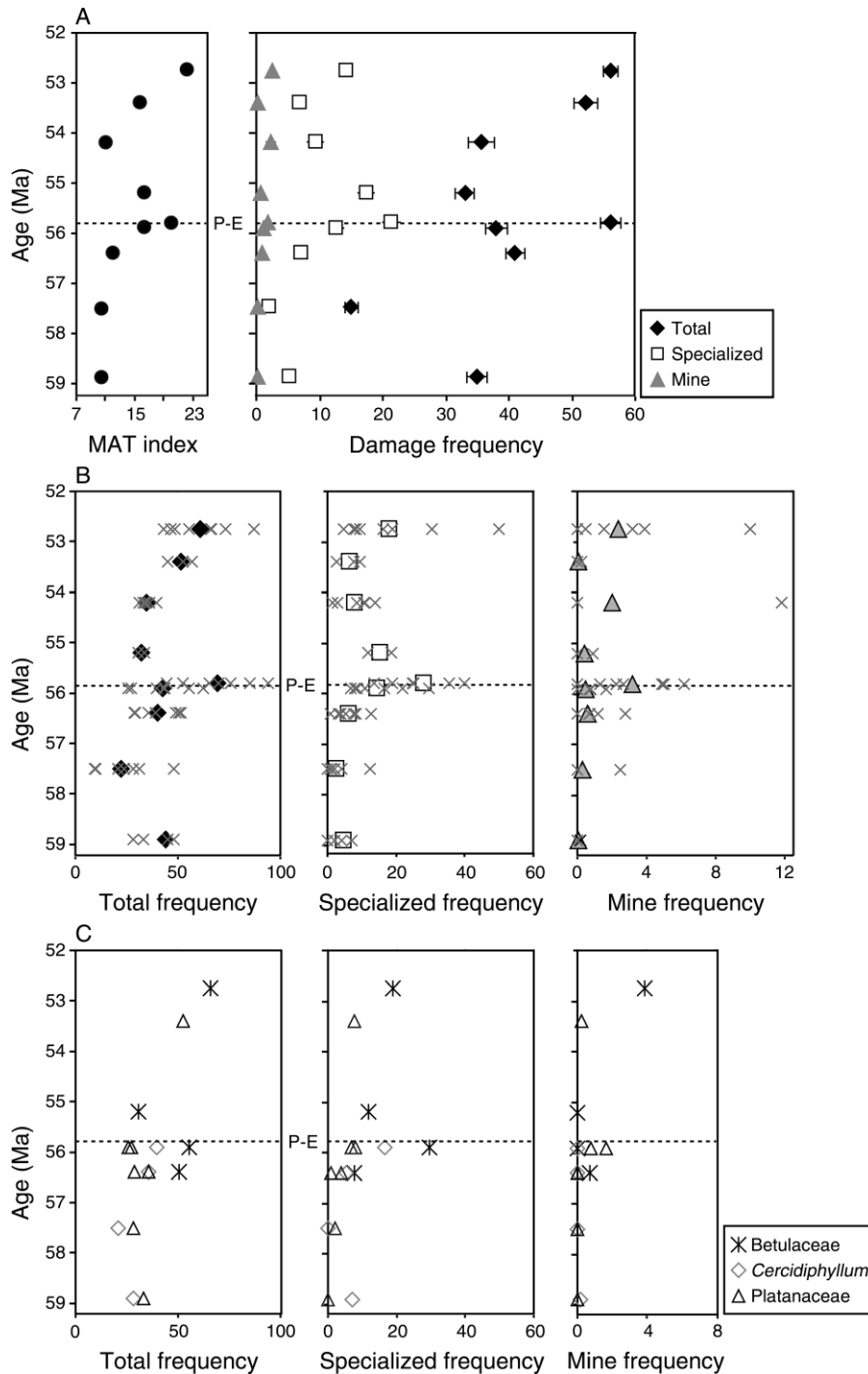


FIG. 8. Damage frequency. (A) Mean annual temperature (MAT) index (Table 1) and total, specialized, and mine damage frequency for the bulk floras. Error bars represent ± 1 SD, based on a binomial sampling distribution, and are too small to be visible for some data points. (B) Total, specialized, and mine frequency for individual plant hosts, as in Fig. 5. (C) Total, specialized, and mine damage diversity for specific host plants, as in Fig. 5. In panels (B) and (C), error bars were omitted to reduce clutter. The dashed lines mark the Paleocene–Eocene boundary.

both *Alnus* sp. and “*Platycarya*” *castaneopsis* have mines characteristic of the lepidopteran family Incurvariidae (fairy moths; DT38; Hering 1951). Mining frequency during the early Eocene cool period (site E3) is high

because of the abundance of a single mine type, DT164, on “*Dombeya*” *novi-mundi* (USNM location 42409; Fig. 1J); this may represent an outbreak over a limited spatial area, perhaps a single tree. Leaf mining

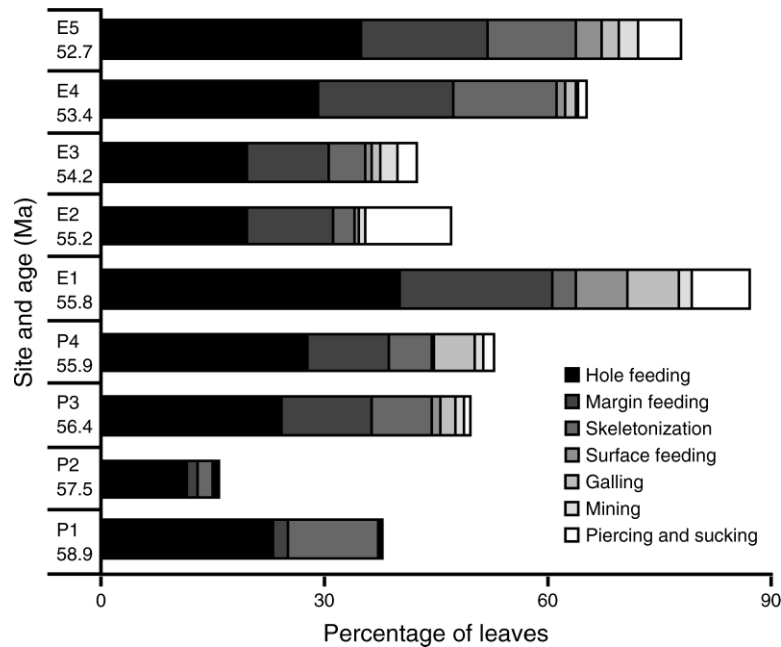


Fig. 9. The percentage of leaves in each bulk flora with damage from each functional feeding group and subgroup. Note that site P1 has no damage attributable to piercing and sucking. See Table 1 for explanations of the site abbreviations.

frequency is third highest in the PETM, where three common plant species have >4.5% of leaves mined and another has >1% leaf mining damage. Blotch mines and lepidopteran serpentine mines are found in approximately equal abundances at E3. Sites P3, P4, and E2 all have an intermediate abundance of mines. At E2, there are three mine types on *Averrhoites affinis*, whereas the other two sites each have mines on three common plant species (*Macginitiea gracilis*, *Platanus raynoldsi*, and Fabaceae sp. FU750 at P4; *A. affinis*, *Zizyphoides flabella*, and Betulaceae sp. FU741 at P3). Sites P1, P2, and E4 all have extremely low leaf-mining frequencies, and all are found on a single plant host: *Cercidiphyllum genatrix* at P1, *Browniea serrata* at P2, and *M. gracilis* at E4.

Linear regression was used to determine the significance of correlations between dicot diversity and damage frequency (Fig. 6B) and also temperature and damage frequency (Fig. 7C, D). There are positive

relationships between plant diversity and damage frequency, but only floral diversity vs. mine frequency has both a high R^2 value and a P value less than 0.05. This relationship remains significant when serial auto-correlation is removed using a modified first differences analysis ($R^2 = 0.55$ and $P = 0.04$). There is a significant positive correlation between MAT index and total and specialized damage frequency. However, only the relationship with specialized damage frequency remains significant when a modified first differences analysis is performed.

Damage composition

An NMDS ordination was performed on a matrix of the percentage of leaves at each site with each of the seven functional feeding categories (Fig. 10), based on the data shown in Fig. 9. Scores for the functional feeding categories are plotted on the same axes as the sites. The oldest sites, P1 and P2, plot far to the right on

TABLE 3. Percentage of leaves at each site with a given number of damage types (DTs).

No. DTs	P1	P2	P3	P4	E1	E2	E3	E4	E5
1	24.4	10.6	28.7	19.5	27.9	16.6	24.8	34.1	35.1
2	8.3	3.3	10.1	8.7	14.0	10.2	6.5	13.1	16.0
3	1.3	0.7	2.4	7.0	8.0	4.9	1.8	3.9	4.8
4	0.5	0.3	0.2	1.8	3.6	1.1		1.0	1.1
5		0.1		0.7	1.8	0.3	0.2	0.1	0.8
6				0.1	0.4				0.2
7					1.1				
8					0.3				
9									
10					0.1				

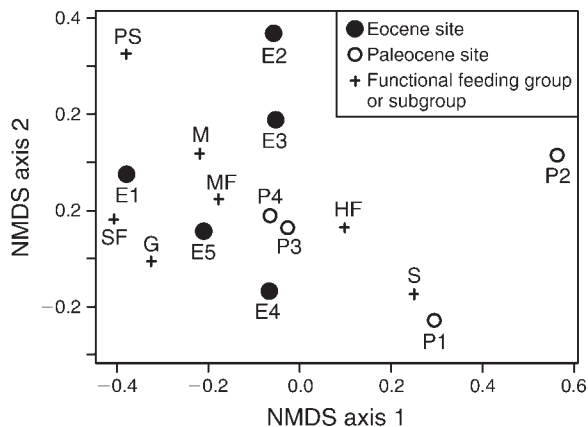


FIG. 10. Nonmetric multidimensional scaling (NMDS) of insect damage on the bulk floras (sites). NMDS axes 1 and 2 define the two-dimensional ordination space that most accurately represents the differences in insect damage composition among sites. The nine sites were ordinated in NMDS based on the percentage of leaves with each functional feeding group or subgroup (as plotted in Fig. 9). The seven functional feeding categories were also ordinated based on their relative abundances at the sites, and they are plotted on the same axes. Abbreviations are: HF, hole feeding; MF, margin feeding; S, skeletonization; SF, surface feeding; G, galling; M, leaf mining; and PS, piercing and sucking.

NMDS axis 1 due to their scarcity of damage in general and specialized damage in particular. The PETM site (E1) plots to the far left on axis 1 because it has abundant specialized feeding damage, especially specialized surface-feeding DTs. The PETM has the highest frequency of four of the seven functional feeding categories. Only E2 has more piercing and sucking, and E3 and E5 have more leaf mining. Insect damage composition during the PETM is most similar to that from E5, the other warm site. The most important differences in insect damage between the two are that E5 has more skeletonization and less surface feeding and galling. Sites P3, P4, E2, E3, and E4 all have similar axis 1 scores, but they vary along axis 2. The abundance of piercing and sucking at E2 pulls it toward the top of axis 2, whereas the abundance of skeletonization at E4 gives it a lower axis 2 score. The remaining sites have intermediate relative abundances of all the functional feeding categories and therefore plot at the center of the NMDS ordination.

Cluster analysis was used to examine changes in damage composition and distribution on individual plant species through time (Fig. 11). All species–site pairs with at least 20 leaves were used in the analysis. We have arranged the clusters so that specialized damage increases from the bottom to the top of the figure. The abundances of the more specialized functional feeding categories, which are strongly affected by temperature (Figs. 6 and 7), drive the clustering. The first break in species–site pairs separates all but one of the early late Paleocene species (cluster 1: P1 and P2) from nearly everything else (cluster 2). As shown previously in the

frequency and diversity data, species from the earlier and cooler late Paleocene have less damage overall and very little specialized damage. However, *Brownia serrata* from P2 has occurrences of galling, leaf mining, and piercing and sucking and is therefore placed in cluster 2. The remaining species–site pairs that fall in cluster 1 are from the latest Paleocene or cool, early Eocene and have abundant hole feeding, margin feeding, and skeletonization, but little other insect damage.

Cluster 2 divides further into two major clusters, which are labeled 3 and 4 (Fig. 11). Cluster 4 contains all the PETM taxa, half of the EECO taxa, and two species from the latest Paleocene. With a few exceptions, these species bear all seven functional feeding categories and highly abundant specialized damage. It is peculiar that *Populus wyomingiana* from site E5 is not in this cluster because it has all seven functional feeding categories in high abundance, but that may simply be because cluster analyses force divisions on a gradient of data. The three other E5 species not in cluster 4 are Dicot sp. WW052, “*Platycarya*” *castaneopsis*, and “*Dombeya*” *novi-mundi*, which have comparatively high M_A and are inferred to have tougher, better-defended leaves than the other E5 species. In summary, the plant hosts in cluster 4 are either PETM species or the most heavily damaged species from other warm time intervals.

The majority of plant hosts in cluster 3 come from intermediate temperature sites P3, P4, E3, and E4. The number of functional feeding categories present and their relative abundances fall between clusters 1 and 4. A split occurs in cluster 3 between species–site pairs that have little skeletonization and no surface feeding (cluster 3A) and those with comparatively abundant skeletonization and surface feeding (cluster 3B). The lower-order clusters either continue to show the pattern of increasing prevalence of specialized damage or are not highly resolved.

DISCUSSION

Leaf mass per area and insect herbivory

At the site mean level, there is no correlation between M_A and damage frequency or diversity (Table 4, Appendix C). Most plant species in this study have leaves with low M_A that should be palatable to herbivores. There are several potential explanations why there is not a significant decrease in damage at higher M_A , as demonstrated in Royer et al. (2007b). First, there are very few species with high M_A , and so this data set may not capture enough of the M_A spectrum to observe a decrease in damage at high M_A . Second, several of the species with high M_A make up >50% of their respective floras by abundance (E2 and E3, *A. affinis*). Therefore, these species should be more apparent to insect herbivores and may, in fact, have been the only abundant dicot species for insects to consume (Feeny 1976). Third, three species with high estimated M_A are legumes (Fabaceae spp. FU750, WW001, WW040). Legumes have pulvinate petioles,

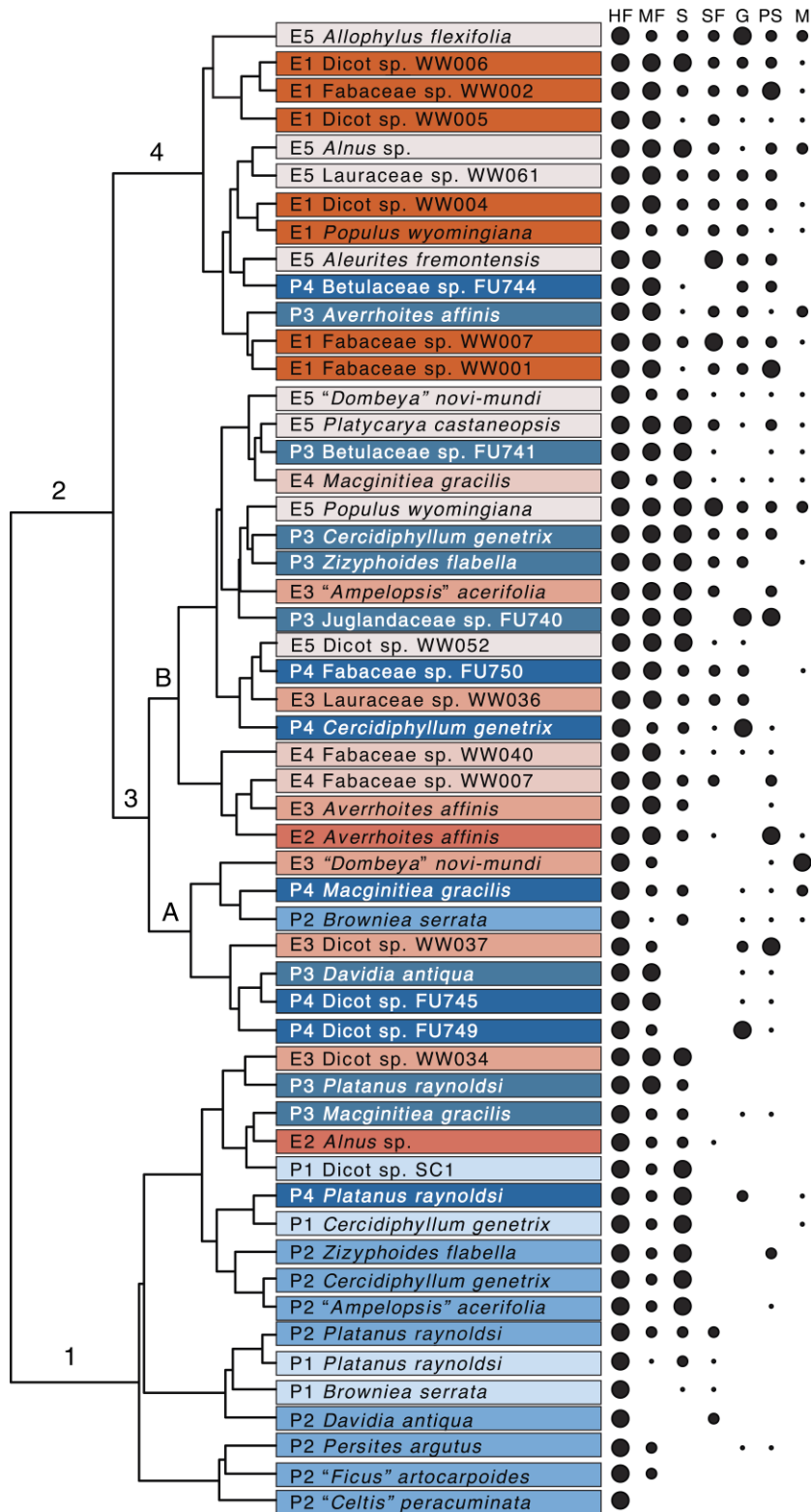


FIG. 11. Cluster analysis of insect damage on species-site pairs, based on the relative abundances of the seven functional feeding groups and subgroups. Each plant host with at least 20 leaf specimens was included in the analysis. Leaf morphotypes that have not been formally named were assigned Fort Union (FU) or Willwood (WW) formation morphotype numbers. The black circles are scaled according to the relative abundances of each functional feeding category on each plant host. The numbered clusters 1, 2, 3A and 3B, and 4 are fully explained in *Results: Damage composition*. The general pattern is that galling, piercing and sucking, and mining damage types are more abundant on host plants from warm Eocene sites. Feeding category abbreviations are: HF, hole feeding; MF, margin feeding; S, skeletonization; SF, surface feeding; G, galling; PS, piercing and sucking; and M, leaf mining. See Fig. 2 for site locations.

TABLE 4. Correlations between leaf mass per area (M_A) and insect damage.

Insect damage measure	R^2	F	P	df
Damage frequency	0.06	1.99	0.17	1, 31
Specialized damage frequency	0.05	1.51	0.23	1, 31
Mine frequency	0.07	2.50	0.12	1, 31
Total damage diversity	0.12	4.15	0.05	1, 31
Specialized damage diversity	0.13	4.52	0.04	1, 31
Mine diversity	0.08	2.60	0.12	1, 31

Notes: Results from the linear model function in R (version 2.4.1; R Development Core Team, Vienna, Austria) with species M_A as the independent variable and damage metrics as the dependent variable. All plant species at each site with at least two petiole width and leaf area measurements were used in the analysis. Total, specialized, and mine diversity were standardized at 20 leaves.

which may artificially inflate the estimated M_A , even though measurements are taken below the swollen portion (Royer et al. 2007b). Additionally, because legumes can fix nitrogen, they may be more palatable to herbivores (Quispel 1954). Finally, two species with relatively high M_A (Fabaceae sp. WW001 from E1 and "*Platycarya*" *castaneopsis* from E5) come from extremely warm time periods, and a correlation between temperature and insect damage may mask a trend between M_A and damage.

Floral diversity and insect damage

Most insect phytophagy is highly host-specific (Bernays and Chapman 1994, Termonia et al. 2001, Farrell and Sequeira 2004). Therefore, one might expect more types of damage, particularly mines and specialized damage, on a more diverse bulk flora (Price 1991, 2002), in accord with the general observation that insect richness correlates with plant species richness (e.g., Siemann et al. 1996, Wright and Samways 1998, Knops et al. 1999, Hawkins and Porter 2003). However, for these data, only a weak positive correlation exists between dicot richness and herbivory (Fig. 6). The only significant correlation is between floral diversity and mine frequency.

There are many reasons why a significant, positive correlation between insect damage and floral diversity is not observed. First, there may not have originally been a correlation between plant diversity and insect herbivory for these floras. Floras that have low plant diversity are generally dominated by a single plant species (e.g., *Averrhoites affinis* at E2). If a single plant dominates the landscape, it is quite apparent to insects. Furthermore, if the plant is dominant over an extended time interval, more insects should evolve or immigrate to eat it. Alternatively, there may have been a correlation between floral diversity and herbivory, but it is hidden by sample size or overshadowed by other variables. Floral richness varies from ~5 to 20 species per 450 leaves (Table 2). Nine floras may be too few to see a significant correlation, and the floras are spread over an interval of roughly six million years. If plants and insects

have different rates of evolutionary change, this may hide a relationship between floral and insect damage diversity. Additionally, each site contains plant species that are represented by fewer than five leaves each; it is extremely unlikely that all the insect damage on the given host will be observed on so few leaves. Also, these floras are composed of different plant species. Although M_A is not significantly different among sites, the proportional representation of chemical defenses may vary. Finally, the strong influence of temperature on insect damage may mask the influence of floral diversity. On the plots of damage diversity vs. floral diversity, the points that fall above the regression lines (P4, E1, E2, E4, E5) are warm sites, whereas the points that fall well below the regression lines (P1, P2, E3) are significantly cooler.

To further examine the effect of floral diversity and composition on insect damage, we considered the relationship between an individual plant species' relative abundance in the flora and its damage frequency and diversity (Fig. 12). As explained in Feeny's (1976) apparency hypothesis, abundant plants are more conspicuous and have a higher likelihood of being found by insect herbivores, a finding borne out in Root's (1973) classic experiment on pure stands of collards. Additionally, it is most beneficial for insect species to adapt to eat abundant plants. Alternatively, one might expect plant species that are abundant at a given site to have relatively low insect damage compared with the less common species. Insect herbivory negatively affects individual plants because of the loss of photosynthetic tissue, nutrients, and stored energy. An abundant plant species may have become dominant because it was better defended than neighboring species. Fig. 12 shows that all of the extremely high damage frequencies and sampling-standardized diversities occur on hosts with a relative abundance <10%, perhaps suggesting that herbivores are responsible for their low abundances. At E5, insect herbivore damage generally decreases as relative abundance in the flora increases. *Alnus* sp., the most abundant plant at E5, may have more damage than expected because it fixes nitrogen and has relatively low M_A , enhancing palatability. *Alnus* was also found to have high damage richness and frequency in the early Eocene of southern Wyoming (Wilf and Labandeira 1999).

Temperature and insect damage

The relationship between mean annual temperature and insect herbivory is evident in Figs. 5 and 8, where damage frequency and diversity are plotted next to MAT index for the Bighorn Basin. Damage increases as temperature increases through the late Paleocene, peaks in the PETM, decreases during the early Eocene cooling, and then increases again during the warming to the EECO. Thus, unlike previous studies (Wilf and Labandeira 1999, Wilf et al. 2001, Currano et al. 2008, Smith 2008), this study captures the response of insect

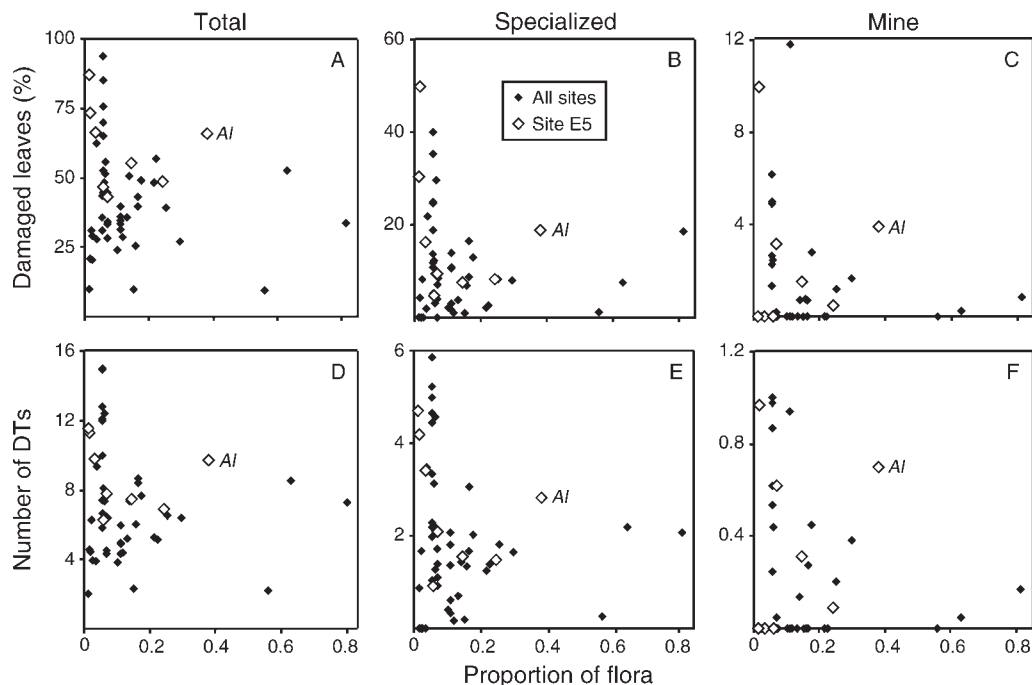


FIG. 12. Insect damage vs. host plant relative abundance. All species–site pairs with at least 20 leaves are plotted. The site E5 species are shown as open symbols to provide an example of the pattern within a single flora. *Al* stands for *Alnus* sp. at site E5. (A) Total damage frequency, (B) specialized damage frequency, (C) mine frequency, (D) total damage diversity, (E) specialized damage diversity, (F) mine diversity. Damage diversity has been resampled to 20 leaves per species.

herbivores to gradual warming, abrupt warming, and cooling within a single basin and depositional setting. Damage diversity is more strongly correlated with MAT than damage frequency (Fig. 7).

Temperature has such a strong effect on insect herbivory because it affects both insect geographic ranges and life history. As climate warms in the temperate zone, speciose insect communities from lower latitudes can migrate northwards. Alternatively, warming could enable insects to migrate across high-latitude land bridges connecting North America, Europe, and Asia. Based on evidence from the Pliocene–Pleistocene fossil record and modern ecological studies, geographic range shifts can occur very rapidly (Coope 1995, Hill et al. 1999, Parmesan et al. 1999). The hypothesis of specialized, thermophilic herbivores migrating to the Bighorn Basin is further supported by the composition analyses (Figs. 9 and 10), which show an increase in the frequency and diversity of specialized herbivore damage on all plant species during the warmer intervals. The increase in herbivory on all host plants produces an increase in damage diversity and frequency on the bulk floras. We note that an increase in bulk-flora damage diversity, as seen here, was not observed in Wilf and Labandeira's initial work (1999) from southern Wyoming, due to the high abundance in their study of an early Eocene Apocynaceae species with very low herbivory.

Temperature-induced changes in insect life history probably have the most effect on damage frequency. As temperature increases, one might predict a greater number of insects per unit area and unit time (Hansen et al. 2001, Harrington et al. 2001, Logan et al. 2003), as well as higher metabolic rates, leading to an increased frequency of insect attack. The positive correlation between damage frequency and temperature may be weakened by the indirect effects of pCO₂ on insect herbivores. Although warming is predicted to increase insect population density, the decreased food quality at high pCO₂ should moderate this increase. However, decreased nutritional value of the leaves also leads to compensatory feeding and an increase in attack frequency, and it is unknown whether the experimentally observed change in food quality is sustained over long time periods. The PETM locality is the only one that we are certain had extremely elevated carbon dioxide levels (Zachos et al. 2003), and it has both high total damage frequency and frequency of leaves with multiple damage types. This suggests that the net effect of warming and elevated pCO₂ is an increase in damage frequency as well as diversity.

CONCLUSIONS

The unique and detailed paleobotanical record of the Bighorn Basin reveals that interactions among plants and insect herbivores are strongly influenced by climate change. Both geochemical and paleobotanical proxies



PLATE 1. Jeff Creamer excavates leaf fossils at 53.4-million-year-old site E4 in the Bighorn Basin, Wyoming, USA. The non-fossiliferous overburden has been removed, exposing the rock layer that contains the best-preserved fossils. Photo credit: E. D. Currano.

show that gradual warming, abrupt warming, and cooling all occurred between 59 and 52.6 Ma, making this an ideal interval to study the net effects of climate change on terrestrial ecosystems. Patterns of insect herbivory through time are visible on the individual host plants within a flora, and these scale up to produce very similar patterns on the bulk flora. Surprisingly, there is not a significant correlation between plant richness and insect herbivory. Instead, total, specialized, and mine damage diversity are strongly correlated with temperature. Insect herbivore damage from warm intervals (the PETM and EECO) suggests the northward migration of highly diverse and specialized insect populations from lower latitudes. Damage frequency is only weakly correlated with temperature, perhaps because other factors such as $p\text{CO}_2$ confound the relationship. In accord with neocological studies, we conclude that temperature affects both insect life history and geographic range, but we show that the net response is visible over long timescales. Based on our results, we predict that present-day anthropogenic warming will alter insect herbivore populations and distributions and cause a cumulative increase in herbivore damage at middle latitudes.

ACKNOWLEDGMENTS

We thank Scott Wing for his assistance on all aspects of this project, Liz Lovelock for morphotyping the PETM flora, and

Amy Morey for photographing the morphotype exemplars. This research was performed on Bureau of Land Management (BLM) land, and we are grateful to Mike Bies and the Worland BLM office for all their assistance. Many students and colleagues assisted us in the field and the laboratory, and we particularly thank B. Cariglino, J. Creamer, K. Galligan, A. Humphries, S. Little, S. Lyles, F. Marsh, E. Perkins, K. Rega, A. Rulis, and K. Werth. The manuscript benefited from thoughtful discussions with J. Bonelli, G. Hunt, M. Patzkowsky, D. Royer, and J. Sessa and comments from two anonymous reviewers. This research was funded by an NSF Graduate Research Fellowship to E. D. Currano, NSF EAR grants 0236489 to P. Wilf and 0120727 to Scott Wing, Petroleum Research Fund grant 40546-AC8 to P. Wilf, the Roland Brown Fund to Scott Wing, and student research grants to E. D. Currano from the Evolving Earth Foundation, the Geological Society of America, the Paleontological Society, and Pennsylvania State University. This is contribution 172 of the Evolution of Terrestrial Ecosystems Consortium at the National Museum of Natural History.

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APPENDIX A

Additional locality and paleoclimate data (*Ecological Archives* M080-019-A1).

APPENDIX B

Woody dicot species and morphotypes in the Bighorn Basin floras (*Ecological Archives* M080-019-A2).

APPENDIX C

Leaf mass per area estimates (*Ecological Archives* M080-019-A3).

APPENDIX D

New insect feeding damage types from the Bighorn Basin floras (*Ecological Archives* M080-019-A4).

SUPPLEMENT

Complete insect damage data set from the nine Paleocene–Eocene Bighorn Basin sites described in this paper (*Ecological Archives* M080-019-S1).