Richness of plant–insect associations in Eocene Patagonia: A legacy for South American biodiversity

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South America has some of the most diverse floras and insect faunas that are known, but its Cenozoic fossil record of insects and insect herbivory is sparse. We quantified insect feeding on 3,599 leaves from the speciose Laguna del Hunco flora (Chubut, Argentina), which dates to the early Eocene climatic optimum (52 million years ago) and compared the results with three well preserved, rich, and identically analyzed early- and middle-Eocene floras from the following sites in North America: Republic, WA; Green River, UT; and Sourdough, WY. We found significantly more damage diversity at Laguna del Hunco than in the North American floras, whether measured on bulk collections or on individual plant species, for both damage morphotypes and feeding groups. An ancient history of rich, specialized plant–insect associations on diverse plant lineages in warm climates may be a major factor contributing to the current biodiversity of South America.

paleobotany | Argentina | herbivory | Laguna del Hunco | paleoecology

South America is well known for its highly diverse** floras (1–3) and associated insect faunas (4–7). Observations in South America and elsewhere show strong positive linkage between plant and insect-herbivore diversity (8–14). Proposed mechanisms include dependency of insect diversity on plant diversity (8, 9, 13), coevolution of plants and insects (15–21), herbivore selection against host density (22–25), and herbivore intensification of abiotic factors that select for habitat specialization (26, 27). Paleontological data can be used to test whether plant and herbivore diversity were correlated in the past (28–30), although data bearing on the primary mechanisms are not typically available from fossils. However, throughout South America, insect body fossils have a limited Cenozoic record (31), and insect damage on fossil plants has rarely been reported (32, 33). Here, we seek to determine whether a diverse fossil flora from South America has an elevated level of plant–insect associations. We quantify insect-feeding damage on one of the most speciose known assemblages of fossil plants, from early-Eocene Patagonia and, for comparison, on three well preserved, including several phytophagous taxa (43).

Although not previously reported, the LH flora has well preserved insect damage on a broad spectrum of host plants, and some individual leaves bear an extremely diverse array of herbivory (Fig. 1). Individual feeding associations, under separate investigation, show varied phylogenetic and biogeographic affinities for the insect herbivores. These data reveal part of the mostly undocumented heritage of the insect herbivore fauna of South America, and we briefly note three examples. The first example is a distinctive frass-filled blotch mine in an araucarian or podocarpaceous conifer leaf, very similar to damage inflicted today by the basal ditrysian moth genus Paracoleurostoa (Graecillariidae) and Chrysorthenches (Platellidae) on the same respective hosts in New Zealand (44, 45). This damage is similar to feeding by basal chrysomeloid clades on these plant hosts in Australia and South America (19, 46, 47). Collectively, this and biogeographic evidence suggest a Gondwanan interaction that originated in the Mesozoic Era. The second example is an angulate-serpentine mine on an unidentified dicot produced by a nepticulid moth, almost identical to extant Stigmella (Nepticulidae) mines on Urticaceae in Micronesia and Polynesia (48) and Rubiaceae in southern Africa (49). These mine types also are known from Eurasian Fagaceae, Rosaceae, and Rhamnaceae from a separate Stigmella lineage (50). Third, an occurrence of cecidomyiid galls on a probable Sterculiaceae may indicate tropical affinities.

During macrofloral sampling, all specimens with insect damage were collected to generate an unbiased data set for direct comparison with North American floras that were collected and analyzed by using identical methods. Determination of herbivory, as contrasted to postmortem detritivory or physically induced damage, was based on four explicit criteria, detailed in ref. 51. These criteria were (i) detection of callus or other reaction tissue along or adjacent to a leaf area with removed tissue; (ii) presence of veinal stringers, necrotic tissue flaps and cuspathe chewed margins consistent with known patterns of insect feeding; (iii) stereotyped patterns of leaf damage that are nearly identical or identical to known modern feeding; and (iv) distinctive host specificity patterns that would be highly unusual if the causative agent were not biological. Most leaf specimens typically preserved the same insect damage on both part and counterpart, indicating that the capture of damage types from single specimens was robust. All voucher specimens from LH are

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Abbreviation: LH, Laguna del Hunco.
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**In this article, “richness” and “diversity” have the same, traditional meaning: the number of species or other biological entities.
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created by multiple biological species, or a single biological species may have produced multiple damage types (53). However, damage-type data record trophic interactions at varying levels of host specialization and thus most directly indicate the diversity of ecological associations, rather than the actual diversity of herbivores (28, 30). Many relatively rare damage types are typically made by host-specialized insects (54) and can be diagnostic of particular herbivore clades when fossilized (55–57); their diversity may have a relatively direct proportional relationship to herbivore diversity. Nonfeeding damage, such as oviposition scars and fungal infections, was excluded from analysis.

The three North American samples are as follows: 1,019 dicot leaf specimens from the early Eocene, lacustrine Republic flora, deposited at the Museo Paleontológico Egidio Feruglio, as described in ref. 36.

We scored 3,599 identified dicot leaves for presence or absence of 52 discrete damage types, belonging to four functional feeding groups: external feeding, mining, galling, and piercing-and-sucking. The damage types are expanded from our previous work on Cretaceous and Paleogene floras from the Western Interior USA (28–30, 51, 52). Some damage types may have been created by multiple biological species, or a single biological species may have produced multiple damage types (53). However, damage-type data record trophic interactions at varying levels of host specialization and thus most directly indicate the diversity of ecological associations, rather than the actual diversity of herbivores (28, 30). Many relatively rare damage types are typically made by host-specialized insects (54) and can be diagnostic of particular herbivore clades when fossilized (55–57); their diversity may have a relatively direct proportional relationship to herbivore diversity. Nonfeeding damage, such as oviposition scars and fungal infections, was excluded from analysis.

The three North American samples are as follows: 1,019 dicot leaf specimens from the early Eocene, lacustrine Republic flora,
Klondike Mountain Formation, northeastern Washington (58, 59); 894 specimens from the middle Eocene, lacustrine Green River flora, Green River Formation, northeastern Utah (29, 60); and 792 specimens from the early Eocene, fluvial Sourdough flora, Wasatch Formation, southwestern Wyoming (28, 29, 61), which constitute a total of 2,705 North American identified dicot floras have been reported in refs. 28 and 29. The Green River Adjusted for sample size, Republic has the most speciose (52). Insect-damage data for the Green River and Sourdough floras have been reported in refs. 28 and 29. The Green River assemblage represents a seasonally dry climate, which had characteristic effects on herbivory discussed in ref. 29, and the Sourdough sample is from or very near the beginning of the early Eocene climatic optimum (61, 62). The Sourdough flora, by comparison with the late Paleocene in the same area of Wyoming, records a diversification of plants and herbivory with warming temperatures (28).

We analyzed the insect damage data for bulk collections from single quarries (Fig. 2, including all four major quarries at LH) and for individual host species (Fig. 3). To maximize sample size and decrease noise in the species-level analyses, each species was lumped over all 25 LH quarries. The data were tabulated and processed separately for total damage types and functional feeding groups (Figs. 2 and 3). Analysis of damage types treats all kinds of damage equally, including the damage that is often made by unspecialized insects (such as hole and margin feeding), but it is sensitive to the full morphological spectrum of insect damage. Analysis of feeding groups, because only four categories are scored, is more sensitive to relatively rare damage typically inflicted by host-specialized insects in the mining, galling, and piercing-and-sucking categories (54). We analyzed the data both including and excluding undamaged leaves, and we found that confining analyses to damaged leaves decreases noise and is a major analytical improvement, although sample size is reduced as a consequence. From each data matrix of herbivorized specimens by damage types, we generated resampling curves for insect damage richness at individual quarries, by using all host species combined (see legend to Fig. 2 for details). For individual
host species with ≥10 total specimens with insect damage, we show the resampled mean numbers of damage types and feeding groups for 10 herbivorized specimens (see legend to Fig. 3 for details). These host species means were also combined into floral grand means (Fig. 4).

**Results and Discussion**

The resampled number of damage types at each of the four major LH quarries significantly exceeds all three North American samples (Fig. 2a). The number of functional feeding groups is greater than all North American samples for three of the four LH quarries (Fig. 2b); piercing-and-sucking damage was not found at LH-6 or Green River). These LH samples saturate quickly on the resampling curves with respect to feeding groups (Fig. 2b): LH-2 reaches 3.95 of the 4 possible feeding groups (41 leaf specimens, whereas Sourdough reaches this value at 261 and Republic at 481 leaf specimens. Notably, the quarry with the greatest plant diversity, LH-2 (36), also has the maximum richness of both damage types and feeding groups.

Elevated diversity of both damage types and feeding groups is also clear on individual hosts (Fig. 3), where LH species occupy most of the highest values. The two measures are not well correlated among hosts (Spearman rank-order correlation coefficient, 0.46), showing variance in herbivore accommodation within vs. among herbivore guilds (damage type vs. feeding group richness). Only two species, both from LH, rank in the top five in both categories: Lauraceae sp. and “Cassia” argentinensis, a legume (Fig. 3). Among the heavily herbivorized species at LH are both typically tropical (Lauraceae, Fabaceae, Malvaceae, Myrtaceae, and Sapindaceae) and temperate or tropical montane (two species of Lomatia) lineages. The conspicuous exception to the LH hosts ranking highest in damage diversity is Populus wilmatteae from the Green River flora, which has the maximum feeding group diversity (Fig. 3); elevated herbivory on P. wilmatteae may result from its adaptations to moister, riparian habitats within the seasonally dry Green River climate, as described in ref. 29.

Floral grand means of the host data in Fig. 3 show the 16 LH species to have more damage types and feeding groups than either the single or pooled North American samples (Fig. 4). The difference is highly significant for the pooled sample and significant for four of six single North American samples, despite the low numbers of species available in the single samples (Fig. 4). Among the North American floras, Green River and especially Sourdough are low in damage-type diversity but high in feeding-group diversity, whereas Republic has a relatively high number of damage types; these results from individual host species are mostly similar to their encompassing bulk floras (Fig. 2).

The LH flora has the maximum scores for both damage types and feeding groups, whether scored on bulk floras from several distinct stratigraphic levels or on individual host species (Figs. 2–4). The bulk floral results (Fig. 2), especially for feeding groups, are consistent with the pronounced host-specificity of extant herbivorous insects (20, 48, 54) and the possible accumulation of herbivore diversity from distinct component communities (63, 64) of herbivore species on many different plant hosts. Separately, herbivory data from individual hosts (Figs. 3 and 4) indicate greater richness within component communities at LH than at the North American sites.

Our results reinforce modern observations that plant and insect-herbivore diversity are positively linked (8–10, 13, 14) and demonstrate an elevated richness of plant–insect associations within the Patagonian Eocene ecosystem. This diversity is an ancient legacy for the dominant ecological pattern seen in extant South American tropical and subtropical forests; highly diverse, specialized insect herbivores on speciose floras. To test the generality of our results temporally and spatially, comparable data are needed from other South American sites with well-preserved paleofloras, especially at low paleolatitudes.

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