

The impact of herbivore–plant coevolution on plant community structure

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Coevolutionary theory proposes that the diversity of chemical structures found in plants is, in large part, the result of selection by herbivores. Because herbivores often feed on chemically similar plants, they should impose selective pressures on plants to diverge chemically or bias community assembly toward chemical divergence. Using a coevolved interaction between a group of chrysomelid beetles and their host plants, I tested whether coexisting plants of the Mexican tropical dry forest tend to be chemically more dissimilar than random. Results show that some of the communities are chemically overdispersed and that overdispersion is related to the tightness of the interaction between plants and herbivores and the spatial scale at which communities are measured. As coevolutionary specialization increases and spatial scale decreases, communities tend to be more chemically dissimilar. At fairly local scales and where herbivores have tight, one-to-one interactions with plants, communities have a strong pattern of chemical disparity.

herbivore specialization | insect–herbivore interaction | plant chemical diversity

Researchers have long been interested in the question of whether coevolution, the reciprocal evolutionary influences of interacting groups of organisms, can shape patterns of divergence among related species within communities. Studies of interspecific competition have often looked at community-wide patterns of divergence among related species (1, 2). Likewise, a number of studies of pollinators and plants have focused on community-wide patterns of floral traits (3, 4). Yet, relatively less work has been focused on the role of plant–herbivore coevolution on community patterns of plant divergence. One interesting example is the work of Gilbert (5), who proposed that coevolution of *Heliconius* butterflies and *Passiflora* vines resulted in the diversification of leaf shapes of cooccurring *Passiflora* species.

Coevolution has been proposed as a major factor promoting the diversity of chemical compounds in plants (6, 7). The continuous selective effects of herbivore attack and plant defense are thought to be largely responsible for the incremental elaboration, proliferation, and intricacy of plant secondary compounds and insect detoxification mechanisms (8–11). Yet, the study of plant–herbivore coevolution and its impact on plant chemistry has focused primarily on interactions that involve a small number of species or populations. Such studies provide evidence that plants often produce distinctive chemicals that protect them against herbivory (11, 12). However, this approach has shed little light on the question of whether coevolution has created patterns of chemistry and interactions powerful enough to structure plant chemical diversity (6, 13).

Because related phytophagous insects often feed on plants that share common chemical compounds to which they are adapted (14–16), it is meaningful to ask whether herbivory might structure community chemical profiles, either biasing community assembly or imposing selective pressure for divergence of defensive compounds. This issue can be addressed by looking for community-level patterns caused by herbivory and coevolution (17, 18). If host shifts tend to occur between plants with similar chemistry, herbivory could limit the coexistence of plants that share common chemical compounds. If chemically similar plants cooccur, selection would

favor divergence. Likewise, if a plant in a regional species pool is chemically similar to an already present species, the probability of successful invasion would be lower. Such processes of divergence and assembly should lead to chemically overdispersed communities, that is, communities with chemical defenses that are more dissimilar than expected by chance.

The effect of herbivory on diversification of chemistry should be stronger for narrowly coevolved systems involving fewer interacting species. In such associations, species tend to develop specific adaptations to the features of their counterparts (19). Thus, a chemically similar plant should be more vulnerable to a specialized herbivore able to handle many of its compounds. More diffuse associations, involving groups of herbivores interacting with a group of plants, may have more generalized adaptations because selection pressures on chemistry may conflict with multiple herbivores (20, 21). In such systems, chemical divergence is less likely to have an effect on herbivore host invasion, and community-level overdispersion is less likely to occur.

Resource competition is an alternative factor that is widely thought to influence the attributes of species and community assembly. If closely related species require the same limiting resources (i.e., water, nutrients, and pollinators), competitive exclusion may limit their coexistence (1). Several studies have shown that sympatric species are more phylogenetically distant than expected, presumably because related species have similar competitive niches (1, 22).

Here, I use the tropical genus *Bursera* to test whether coevolution with specialized herbivores could have resulted in community-level chemical overdispersion. I also test for community-level phylogenetic overdispersion which could suggest that resource competition is a structuring factor.

*Bursera*s are typically low- to medium-size trees (Fig. 1). The genus includes ≈ 100 species distributed from the southern United States to Peru (23). It reaches its maximum diversity and abundance in the tropical dry forests of Mexico where, with ≈ 85 endemic species, it is one of the major elements of the flora (24, 25). The predominance of *Bursera* is particularly striking along the deep canyons of the Balsas River basin, which is one of the major extensions of the dry forest. On the floors of these canyons, this genus often becomes the absolute dominant woody taxon, surpassing legumes and other groups in diversity and abundance and validating the name “cuajotales” given to many of these forests, from the Aztec name “cuajote” (leprous tree) given to *Bursera* species (25–27). In the eastern region of the Balsas depression, for example, in an area of $\approx 50,000$ km² that includes Zopilote Canyon, ≈ 45 species of *Bursera* occur and 9–15 *Bursera* species commonly

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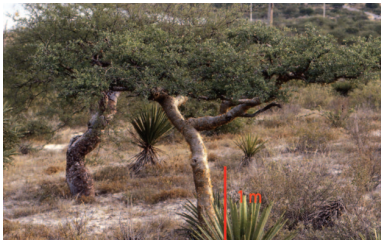


Fig. 1. *Bursera aptera*. The genus *Bursera* reaches its maximum diversity and abundance in the tropical dry forests of Mexico where, with ≈ 85 endemic species, it is one of the major elements of the flora.

coexist in single localities. There are high levels of endemism in the genus, and 65% of the species have a geographic distribution of $< 50,000$ km². Because the genus is old, highly adapted to the ecological and climatic conditions of these forests, and of great physiognomic importance, its evolution and diversification has been linked to the history of the Mexican dry forests (24).

Bursera produces an array of terpenes, mostly mono- and sesquiterpenes, and alkanes (28–30). These compounds are toxic or repellent to insect herbivores and, in *Bursera*, decrease the survival and growth of their specialized herbivores, the chrysomelid genus *Blepharida* (31, 32). The impact of *Blepharida* on *Bursera* often depends on the defensive status of the plants, and poorly defended individuals with relatively low concentration of terpenes can be completely defoliated by these beetles (33).

Blepharida includes ≈ 45 species that feed on *Bursera* [Fig. 2 and supporting information (SI) Table 2]. *Blepharida* species have been observed to be the most frequent and abundant herbivores of *Bursera* in visits to multiple field sites in Mexico over the past 15 years. Most *Blepharida* are narrowly specialized with one *Bursera* host (which I will refer to as “monophagous”) or two to four hosts (“oligophagous”) (Fig. 3) (32). A few, relatively more generalized (“polyphagous”) beetles feed on many *Bursera* hosts (32). Time-calibrated phylogenies of these insects and plants suggest that they have interacted for at least 112 million years (8) and that plant defensive chemical traits and the insects’ counterdefensive feeding strategies have evolved in response to concurrent reciprocal selective pressures (32, 34).

These herbivores show a preference for colonizing chemically similar plants that are not necessarily phylogenetically close (14). This preference for chemically similar plants should impose pressures on plants to develop divergent chemistries. Therefore, this is a good system by which to examine whether chemical overdispersion is an aspect of plant community structure. Because related *Bursera* species are often chemically dissimilar, hypotheses about chemical traits can be tested without the concern that chemistry is completely correlated with plant phylogeny (14).



Fig. 2. *Blepharida pallida*. The *Blepharida* genus includes ≈ 45 species, which all feed only on *Bursera*.

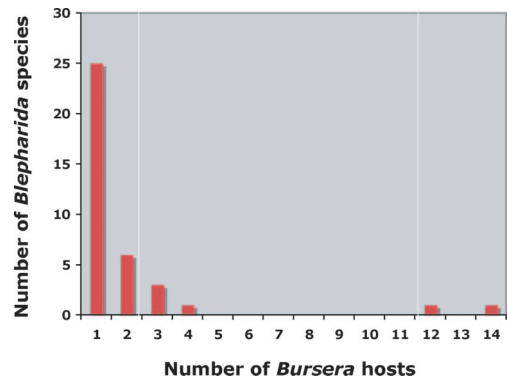


Fig. 3. Number of hosts attacked by *Blepharida* species. Most *Blepharida* species are highly specialized and are known to feed on only one (monophagous) or up to four hosts (oligophagous). But two of them are more generalized, feeding on 12 or 14 hosts (here called “polyphagous”).

Results and Discussion

Testing for Community-Level Chemical Overdispersion. To investigate whether *Bursera* communities are chemically overdispersed, I analyzed the chemistry of 57 species by using gas chromatography–mass spectrometry (see *Methods*). Analyses were directed at volatile terpenes and alkanes that are the most abundant secondary compounds in *Bursera* and have known impact on *Blepharida* (28–30). A matrix of chemical dissimilarity among species was constructed based on the presence and relative concentration of 74 chemical compounds found in these plants.

I quantified the average chemical distance among species in 18 *Bursera* communities in four areas of the tropical dry forests located in the Balsas basin, and three communities in one area in the Papaloapan river basin (Fig. 4). These five areas were selected because they contain the highest diversity of *Bursera* and *Blepharida*, and because the *Blepharida* species found there tend to be monophagous or oligophagous (35).

Six other *Bursera* communities from two coastal areas in the Pacific were also included in the analysis (Fig. 4). These two areas were chosen because they have high numbers of *Bursera* species and many of them are attacked by the polyphagous *Blepharida*, *B. alternata*, and *B. pallida*, which are abundant there (35). All areas were $\approx 10,650$ km² (1° latitude and longitude), except areas in the Pacific that were smaller because part of the targeted 1° latitude and longitude was occupied by the Pacific Ocean.

Bursera tends to grow in deep canyons. Species occupy different

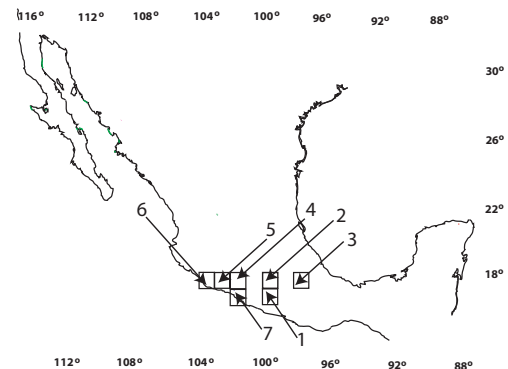


Fig. 4. Geographic localization of selected areas of study. Areas 1–5 contain the highest diversity of *Bursera* and monophagous and oligophagous *Blepharida*. In areas 6 and 7, polyphagous *Blepharida* are more frequent. 1, Chilpancingo; 2, Cañón del Zopilote; 3, Tehuacán; 4, Infiernillo; 5, Aguililla; 6, Zihuatanejo; 7, Aquila.

altitudinal ranges with the highest diversity usually occurring at the canyon bottoms (35). Within areas, I used communities with increasingly large altitudinal bands (and hence geographic area) with the smaller ones nested within the larger. For all areas, the smallest altitudinal band is located at the bottom of the canyons and increasing bands add species and habitats by moving up in altitude, still within the 1° target area. Finally, for each area, I expanded the geographic extent to 3° of latitude and longitude using the full altitudinal range.

To determine which species were present in an area and at what elevations, species lists for all of the communities were constructed by using information from the major Mexican herbaria [Herbario Nacional de Mexico (MEXU), Herbario de la Escuela Nacional de Ciencias Biológicas, and Herbario del Centro de Ecología, region del Bajío], from the on-line biodiversity information of the Mexican Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (www.conabio.gob.mx), and from repeated visits over 15 years to these areas.

I also estimated the degree of specialization in the interaction between *Bursera* and *Blepharida* species in the communities studied. For this, I calculated the percent of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida*.

The percent of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida* tended to be high in all communities located in the Balsas and Papaloapan river basins [Table 1; Chilpancingo (area 1), Cañón del Zopilote (area 2), Tehuacán (area 3), Infiernillo (area 4), and Aguililla (area 5)]. In most of these communities, this percentage was >58, and in most communities of small geographical scale (situated below 1,000 m of altitude), the percentage was >78. The highest level of specialization was at the community located at the bottom of the canyon in Chilpancingo, in which only one *Bursera* species hosts a polyphagous beetle species and all other burseras are attacked by a single monophagous or oligophagous *Blepharida* species each. Considerably lower levels of specialization were found in areas 6 and 7 (Zihuatanejo and Aquila) (Table 1), where the percentage of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida* ranged from 22 to 53. These results are congruent with previous observations that polyphagous species tend to attack *Bursera* species distributed along the Pacific coast (35).

I tested for chemical overdispersion with randomization tests. I examined whether the particular configurations of species found in these real communities are more chemically diverse than communities randomly chosen from the same overall set of species. The average chemical distance for a given community was compared with the distribution of average chemical distances of many random communities constructed by choosing the same number of species randomly from the full list of species for all communities. Results show that in those areas where coevolutionary specialization with *Blepharida* is high (as measured by the percent of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida*), communities were significantly overdispersed (Table 1). High specialization and overdispersion occur at smaller scales in all of the areas of the Balsas and Papaloapan basins: Chilpancingo, Cañón del Zopilote, Tehuacán, Infiernillo, and Aguililla (Table 1 and Fig. 5). This pattern is consistent with the idea that beetles impose stronger selective pressure for chemical divergence of the locally present plant species. However, increasing scale in this study also results in increasing habitat diversity by including higher-elevation habitats. So, the decline in overdispersion with scale could be due to weaker species interactions or a greater importance of overdispersion at canyon bottoms than at higher elevations. In both areas on the Pacific coast (Zihuatanejo and Aquila) where coevolutionary specialization is low, average chemical distances did not differ much from the corresponding random communities at any scale (Table 1 and Fig. 5).

The key role of herbivore specialization for chemical distance can be appreciated by plotting the deviation of chemical distance from

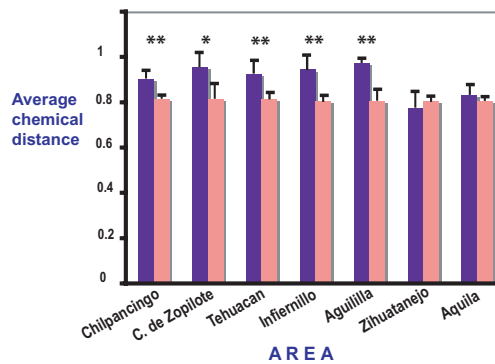


Fig. 5. Average chemical distance for *Bursera* communities that develop at the lowest altitudes in each of the seven areas studied (purple bars) compared with the average chemical distance of 10,000 random *Bursera* communities of equal size (pink bars). Black lines above bars indicate confidence intervals. *, $P < 0.1$; **, $P < 0.05$.

that expected for random communities (calculated as Z scores) (36) against the percentage of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida* species (Fig. 6). As specialization increases, the Z scores for chemical distance increase. This happens equally for large areas and small areas (slope = 0.0234, $P < 0.0001$) although small areas have higher Z scores at any given percent monophagy/oligophagy (0.749 SD higher, $P < 0.0003$). Thus, whereas the degree of specialization also affects chemical distance at larger spatial scales, the lower average chemical distance at larger scales explains why fewer large areas had significant overdispersion.

Testing for Phylogenetic Overdispersion. A matrix of phylogenetic distance was also constructed for 58 *Bursera* species, and average phylogenetic distance was calculated for the same communities that were tested for chemical overdispersion. Randomization tests were also performed to test for phylogenetic overdispersion.

Higher average phylogenetic distances than those expected by chance were rare in the 27 communities studied. It never occurred at the smaller scales where resource competition might be expected to be strongest (22) (Table 1). Probability values even increased at smaller scales in some cases, as if closely related species were more likely to cooccur as communities became smaller. If we assume, as others have, that a close phylogenetic relationship reflects competitive similarity, resource competition does not seem to structure

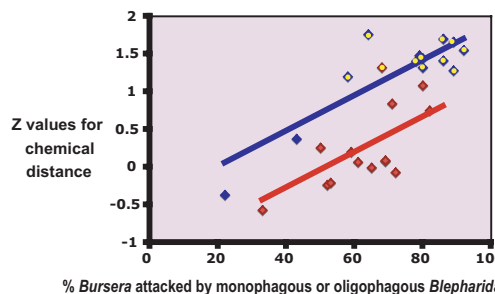


Fig. 6. Standardized average chemical distances for 27 *Bursera* communities in the seven geographic areas studied vs. percentage of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida*. Blue squares indicate communities that develop at altitudes up to 1,000 m. Red squares indicate larger communities that develop at altitudes up to 2,200 m. Yellow dots indicate communities in which chemical defenses are statistically more dissimilar than random. Regression lines for small and large communities were fit simultaneously adjusting for correlated error structure (assuming local, compound symmetric error covariance; see *Methods*).

Table 1. Results of randomization tests on phylogenetic and chemical overdispersion of *Bursera* communities

Area	Localization	Elevation, m	Phylogenetic distance				Chemical distance				% species attacked by monophagous or oligophagous <i>Bllepharida</i>		
			Observed, mean	Random, mean	Z score	P	No. of species sampled	Observed, mean	Random, mean	Z score		P	No. of species sampled
Chilpancingo	lat 17–18°, long 99–100°	400–650	40.530	38.795	0.473	0.338	12	0.909	0.815	1.548	0.031**	13	92
		400–1,000	38.614	38.705	–0.034	0.548	18	0.879	0.815	1.404	0.058*	19	78
		400–2,200	37.020	38.735	–0.993	0.847	29	0.814	0.815	–0.009	0.500	28	65
Cañón del Zopilote	lat 16–19°, long 98–101°	400–2,200	36.674	38.726	–1.428	0.913	34	0.817	0.815	0.066	0.435	36	61
		400–500	40.800	38.700	0.288	0.355	5	0.960	0.184	1.280	0.060*	5	89
		400–750	40.056	38.796	0.276	0.424	9	0.904	0.815	1.321	0.064*	11	80
Tehuacán	lat 17–20°, long 98–101°	400–1,000	39.181	38.714	0.151	0.469	15	0.896	0.815	1.480	0.044**	15	79
		400–2,200	38.537	38.785	–0.110	0.577	22	0.812	0.815	–0.073	0.562	23	72
		400–2,200	39.389	38.717	0.636	0.275	42	0.817	0.815	0.076	0.489	39	69
Infiernillo	lat 18–19°, long 97–98°	550–800	37.000	38.788	–0.393	0.696	9	0.935	0.815	1.413	0.036**	9	86
		550–2,200	36.894	38.687	–0.487	0.726	12	0.884	0.815	1.08	0.125	12	80
		550–2,200	39.330	38.719	0.282	0.415	24	0.849	0.815	0.749	0.236	19	82
Aguililla	lat 17–20°, long 100–103°	200–500	43.267	38.588	0.734	0.234	6	0.957	0.816	1.709	0.007**	8	86
		200–1,000	44.278	38.690	1.222	0.084*	9	0.927	0.816	1.776	0.011**	12	64
		200–2,200	40.627	38.710	0.719	0.223	18	0.876	0.815	1.328	0.075*	19	71
Zihuatanejo	lat 17–20°, long 101–104°	200–2,200	37.403	38.723	–0.757	0.784	29	0.843	0.815	0.835	0.209	27	68
		150–300	43.867	38.888	0.781	0.221	6	0.981	0.813	1.445	0.028**	5	80
		150–600	42.607	38.636	0.788	0.204	8	0.954	0.816	1.659	0.015**	8	89
Aquila	lat 18–19°, long 103–104°	150–1,000	43.033	38.685	1.350	0.073*	14	0.888	0.814	1.204	0.094*	13	58
		150–2,000	43.331	38.755	1.879	0.017**	20	0.818	0.814	0.087	0.506	17	69
		150–2,000	39.786	38.714	0.685	0.254	32	0.821	0.815	0.200	0.444	30	59
Zihuatanejo	lat 17–18°, long 101–102°	0–1,000	38.611	38.682	–0.014	0.547	9	0.786	0.815	–0.369	0.699	9	22
		0–2,000	39.439	38.767	0.186	0.461	12	0.778	0.813	–0.570	0.745	12	33
		0–2,000	42.053	38.739	1.851	0.018**	28	0.806	0.814	–0.213	0.612	26	53
Aquila	lat 18–19°, long 103–104°	0–1,000	40.717	38.682	0.693	0.245	16	0.837	0.815	0.373	0.415	15	43
		0–2,000	41.987	38.704	0.466	0.054*	22	0.826	0.815	0.256	0.433	19	50
		0–2,000	39.468	38.711	0.431	0.349	29	0.806	0.814	–0.237	0.620	26	52

For each community examined, the observed average chemical or phylogenetic distance between species was compared to a distribution of average chemical or phylogenetic distances of an equal number of randomly selected species (the null model). *P* values are reported for one-tailed tests; i.e., the observed chemical distance was greater than all but the proportion *P* of 1,000 null communities. *, *P* < 0.1; **, *P* < 0.05.

these communities. Alternatively, competitive niches among sympatric species may not be phylogenetically conservative.

Results show that there is a nonrandom pattern of chemical overdispersion in *Bursera* communities that seems to be tied to the tightness of the interaction with their herbivores. As coevolutionary specialization with *Blepharida* increases, communities tend to be more chemically dissimilar. A possible alternative explanation for this pattern of overdispersion of chemistry, phylogenetic overdispersion driven by plant competition, was tested and can be rejected as an explanation for the observed patterns. Terpenes may have alternative functions besides antiherbivore defense, and it is possible that chemical overdispersion could be due to other causes. Terpenes may have other physiological functions, such as protection from high temperatures (37). They may also have other ecological roles, such as attraction to pollinators (38). It is unlikely, however, that these physiological or ecological alternative functions are behind *Bursera*'s community patterns of chemical differentiation. If the main function of *Bursera*'s chemistry was physiological, then we would expect the community to exhibit the opposite pattern: plants should converge in chemistry to adapt to similar conditions, and communities should have a lower average distance than random. Likewise, there is little floral diversity in the genus, and pollination is accomplished by generalized insects. Thus, there is no *a priori* reason to expect a correlation between leaf chemistry and pollination. Hence, whereas it is not possible to completely exclude a role for other factors, degree of herbivore specialization seems to be the strongest candidate for explaining chemical overdispersion. This explanation is reinforced by the strong relationship of herbivore specialization and degree of overdispersion.

This investigation provides evidence that *Blepharida* can affect *Bursera*'s community structure. Whereas other studies have also shown that the structure of vegetation may be modified by herbivores (39), the impact of plant–herbivore coevolution on plant chemical profiles at the community level has rarely been examined. My results suggest that coevolution, by arranging the chemical features of plants, may act in concert with herbivory to influence community structure. Previous research indicates that throughout their evolution, closely related species of *Blepharida* have invaded more chemically similar *Bursera* plants (14). The present study shows that *Blepharida*'s selective pressures may have favored the evolution or assembly of chemically divergent *Bursera* communities.

That chemical overdispersion exists in areas inhabited by highly specialized, monophagous *Blepharida* species, but not in areas mostly inhabited by polyphagous species, suggests a possible positive feedback loop in the evolution of herbivore specialization. Monophagous species appear to favor the assembly or evolution of *Bursera* communities with chemically different species. But then, chemical disparity in the plant community would make the adoption of new hosts more difficult, thus reinforcing *Blepharida*'s specialization. This positive feedback could also create a link between specialization and the strength of herbivory. High herbivore pressure may favor the assembly or evolution of dissimilar defensive chemistry among members of the plant community, thus perpetuating the cycle of specialization. Low herbivore pressure, such as when coevolution is diffuse and damage is inflicted by less virulent polyphagous herbivores (40, 41), may permit the assembly or evolution of more chemically similar coexisting plant species, perpetuating the cycle of generalization. Plants that are completely (or almost completely) defoliated by monophagous or oligophagous *Blepharida* species are frequently observed in the field. Yet, no instances of this kind of extreme damage have been observed with the polyphagous *B. pallida* or *B. alternata* (33). This observation suggests that highly specialized *Blepharida* tend to inflict greater damage to their hosts. However, more data are needed to ascertain whether herbivore pressure and plant community chemical structure feed back into *Blepharida*'s feeding specialization.

Coevolutionary theory predicts that specialized herbivores select for chemical divergence (7, 10). Generalized herbivores are less

likely to do so because they are adapted to a range of plant chemistries. Thus, the average chemical distance among plants in communities should depend on the proportion of specialized herbivores. Results here demonstrate that plant chemical overdispersion occurs in local communities in cases in which herbivores are more specialized and, thus, that plant–herbivore coevolution may be a significant factor promoting chemical diversity at a community level.

Methods

Collection of Plant Tissues. Samples of leaves from 57 *Bursera* species were collected from live plants in natural populations in Mexico and immediately extracted in dichloromethane. Collection of species was concentrated in mainland Mexico and represents $\approx 82\%$ of the species present in the area. Endemic species from the Baja California and Yucatan peninsulas were not sampled because *Blepharida* beetles from the mainland are not likely to encounter them. Because plant chemistry may vary within and among populations, I sampled several individuals and populations for each species. Samples consisted of three to five individuals in each of one to two populations for species of restricted geographic distributions and up to five populations for species of more widespread distributions. For a few of the species, sampling consisted of only two individuals because the species are rare and difficult to find in a limited amount of time. Sampling was restricted to mature, full-grown individuals, concentrating on new, fully developed leaves during time of active growth of plants (June, July, and August).

Chemical Analysis. All extracts were analyzed by gas chromatography–mass spectrometry performed on a Hewlett–Packard 5890 gas chromatograph linked to a Hewlett–Packard 5970B mass selective detector (Hewlett–Packard, Palo Alto, CA) at 70 eV ($1 \text{ eV} = 1.602 \times 10^{-19} \text{ J}$), m/z 40–600 full scan with a DB-5 column (J & W Scientific, Folsom, CA; 15 m long, 0.32-mm inside diameter, and 0.25- μm film). Helium was the carrier gas at a linear velocity of 20 cm/sec. The splitless injector temperature was 200°C, the flame ionization detector was held at 240°C, and the oven temperature was 40°C for 4 min and then increased 8°C/min to 230°C and held for 5 min. Detected volatile compounds in each species were identified by matching the obtained spectra with standard mass spectral libraries (NBS 75.K). Many of the major compounds were also identified by comparing obtained spectra with spectra and retention times of authentic standards.

Chemical Variation Within Species. To examine the degree of chemical variation within species and populations, I calculated the average Pearson correlation of the relative abundances of chemical compounds between pairs of individuals in the same species by using the statistical package JMP (42). For most of the species, there was little variation among individuals in the same population and among populations, confirming previous results that showed high chemical cohesiveness within species (SI Table 3). When correlation coefficients among individuals of same or different populations were high (usually true), individuals in a species were put in one single population. When individuals from different populations had average correlation coefficients of <0.4 , the populations were not combined. This happened with some species that have relatively wide geographic ranges, such as *B. grandifolia*, *B. discolor*, *B. fagaroides*, and *B. glabrifolia*.

Chemical Distance Between Species. I calculated averages of abundance of all constituents for each species. For the species that had populations that were chemically different, I calculated averages for each population. A matrix of chemical distance between all species and populations considered was constructed on the basis of the relative abundance of 74 chemical compounds by calculating Pearson correlations between species pairs. Each correlation was then subtracted from 1 to convert it to a distance. For species with chemically different populations, average distances for a community included only the populations in that area.

Scaling of Chemical Overdispersion in *Bursera* Communities. Testing for coevolution-mediated chemical overdispersion is complicated by the ability of herbivores to fly and potentially colonize plants far from where their presence has been reported. When communities are too small, herbivores are likely to affect larger communities than the actual community tested. Measuring communities that are too large, on the other hand, increases the possibility that rare or isolated plant species can be missed and unaffected by herbivores. A solution to this problem was to measure the same community at increasing scales. The smallest communities measured were at canyon bottoms. Canyon bottoms are the prime habitat for the genus *Bursera*, overall (35); they are where diversity and overall abundance of the genus are highest. Both *Bursera* and *Blepharida* are tropical and highly adapted to the dry and warm conditions that are prevalent at the base of the basins. They do not stand up well to freezing weather, which although infrequent, does occur at the tops of the basins (25). Habitats radiating outward (and upward) from canyon bottoms, though more favorable for some adapted species, are less favorable for *Bursera*s, overall, so that diversity and overall abundance decline. Thus, within the 1° target area, I chose canyon bottoms as central targets and increased the scale by working outward and upward.

Randomizations. To determine whether community chemical profiles are overdispersed, I determined the probability that the average chemical distance of a community would be as large or larger than that expected by chance alone. For each real community, the observed average chemical distance between species was compared with the distribution of average chemical distances of an equal number of randomly selected species (the null model). The pool from which species were randomly selected was all of the species and populations included in the matrix of chemical distances. These species are all of the species and populations that grow in the regions studied. Thus, I tested whether the particular configurations of species found in communities are more chemically diverse than communities randomly chosen from the same overall set of species. The null distributions of average chemical distance were generated with 10,000 randomizations for each test. *P* values are for one-tailed tests, i.e., the observed chemical distance was equal to or greater than all but the proportion *P* of the 10,000 null communities. Randomizations were done with a program written in R (43).

Effect of Specialization on Overdispersion. I performed a regression analysis to determine the relationship between chemical overdispersion and insect–host specialization. Most *Bursera* species are attacked by only one species of *Blepharida* (SI Table 2). I measured

the degree of specialization in a particular community as the percentage of *Bursera* species that are attacked by monophagous or oligophagous *Blepharida*. For each community, chemical overdispersion was measured as the difference between average chemical distance from the average null expectation in terms of SDs (calculated as *Z* scores) (36).

Because the communities at different scales from the same location are not independent, I could not rely on the standard regression assumption of independent errors. Using the latitude and longitude coordinate system, I constructed a spatial model with SAS Proc MIXED to explicitly model correlated error structure (44). The initial models permitted spatial correlation among all communities and sites. They indicated significant correlation among errors ($P < 0.047$), but the error correlations dissipated at $<1^\circ$ latitude or longitude (i.e., parameter estimates for the “range” of correlated errors were less than the distances between the seven study areas). This justified remodeling the correlated error structure as block-diagonal (assuming correlated errors within, but not between, the seven principle areas of study). Akaike’s information criterion and Schwartz’s Bayesian criterion (44) were used to select an appropriate block diagonal error structure (compound symmetric). Regression coefficients and associated probabilities reported here have all been adjusted for correlated errors by using the above assumptions (local, compound symmetric error covariance).

Testing for Resource Competition Within Communities. To measure phylogenetic overdispersion, I used a previously published molecular phylogeny of *Bursera* (23, 34), reconstructed by using sequences from the internal transcribed spacer region and the external transcribed spacer. Parsimony and likelihood methods as well as Bayesian techniques were implemented for its reconstruction. Phylogenetic distances between 60 species were calculated as their patristic distances by using PAUP* 4.0b10 (45).

A matrix of phylogenetic distance was constructed for 58 *Bursera* species, and the average phylogenetic distance was calculated for the same communities that were tested for chemical overdispersion. Randomization procedures to test for phylogenetic overdispersion were the same as those to test for chemical overdispersion. The observed average phylogenetic distance between species was compared with the null model by using a one-tailed test.

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- Losos JB, Leal M, Glor RE, de Queiroz K, Hertz PE, Schettino RL, Lata AC, Jackman TR, Larson A (2003) *Nature* 424:542–545.
- Weihner E, Keddy PA (1999) *The Search for Assembly Rules in Ecological Communities* (Cambridge Univ Press, Cambridge, UK).
- Aizen MA, Vazquez DP (2006) *Ecography* 29:357–366.
- Armbruster WS, Edwards ME, Debevec EM (1994) *Ecology* 75:315–329.
- Gilbert LE (1980) in *Coevolution of Animals and Plants*, eds Gilbert LE, Raven PH (Univ of Texas Press, Austin).
- Berenbaum M, Zangerl AR (1995) in *Phytochemical Diversity and Redundancy in Ecological Interactions*, eds Romeo JT, Saunders JA, Barbosa P (Plenum, New York), pp 1–24.
- Ehrlich PR, Raven PH (1964) *Evolution (Lawrence, Kans)* 18:586–608.
- Becerra JX (2003) *Proc Natl Acad Sci USA* 100:12804–12807.
- Benderoth M, Textor S, Windsor A, Mitchell-Olds T, Gershenzon J, Kroymann J (2006) *Proc Natl Acad Sci USA* 103:9118–9123.
- Berenbaum M, Feeny P (1981) *Science* 212:927–929.
- Berenbaum MY, Zangerl AR (2006) *Ecology* 87:3070–3081.
- Lokvam J, Brenes-Arguedas T, Lee JS, Coley PD, Kursar TA (2006) *Am J Bot* 98:1109–1115.
- Price PW (1997) *Insect Ecology* (Wiley, New York).
- Becerra JX (1997) *Science* 276:253–256.
- Berenbaum M (1983) *Evolution (Lawrence, Kans)* 37:163–179.
- Feeny P (1992) in *Herbivores: Their Interactions with Secondary Metabolites*, eds Rosenthal GA, Berenbaum MR (Academic, San Diego), pp 1–46.
- Agrawal AA, Lau JA, Hamback PA (2006) *Q Rev Biol* 81:349–376.
- Strauss SY, Sahli H, Conner JK (2005) *New Phytologist* 165:81–90.
- Van Zandt PA, Agrawal AA (2004) *Oikos* 104:401–409.
- Leimu R, Koricheva J (2006) *Am Nat* 168:E15–E37.
- Strauss SY, Irwin RE (2004) *Annu Rev Ecol Syst* 35:435–466.
- Cavender-Bares J, Ackerly DD, Baum DA, Bazzaz FA (2004) *Am Nat* 163:823–843.
- Becerra JX, Venable DL (1999) *Am J Bot* 86:1047–1057.
- Becerra JX (2005) *Proc Natl Acad Sci USA* 102:10919–10923.
- Rzedowski J (1978) *Vegetación de México* (Limusa, México D.F., México).
- Trejo I (1998) PhD thesis (Universidad Nacional Autónoma de México, México D.F., México).
- Trejo I, Dirzo R (2002) *Biodiversity Conserv* 11:2063–2084.
- Becerra JX, Venable DL, Evans PH, Bowers WS (2001) *Am Zool* 41:865–876.
- Evans PH, Becerra JX (2006) *Flavour Fragrance J* 21:616–618.
- Evans PH, Becerra JX, Venable DL, Bowers WS (2000) *J Chem Ecol* 26:745–754.
- Becerra JX (1994) *Ecology* 75:1991–1996.
- Becerra JX (2004) *Mol Phylogenet Evol* 30:107–117.
- Becerra JX (1993) PhD thesis (Univ of Arizona, Tucson).
- Becerra JX (2003) *Mol Phylogenet Evol* 26:300–309.
- Becerra JX, Venable DL (1999) *Proc Natl Acad Sci USA* 96:12626–12631.
- Sokal RR, Rohlf FL (1995) *Biometry* (Feeman, New York).
- McGarvey DJ, Croteau R (1995) *Plant Cell* 7:1015–1026.
- Dudareva N, Negre F, Nagegowanda DA, Orlova I (2006) *Crit Rev Plant Sci* 25:417–440.
- Fine PVA, Mesones I, Coley PD (2004) *Science* 305:663–665.
- Coley PD, Barone JA (1996) *Annu Rev Ecol Syst* 27:305–335.
- Hamilton WD, Brown SP (2001) *Proc R Soc London Ser B* 268:1489–1493.
- SAS Institute (2005) JMP (SAS Institute, Cary, NC).
- R Development Core Team (2004) R: A Language and Environment for Statistical Computing (Univ of California, Berkeley), Version 2.0.1.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) *SAS System for Mixed Models* (SAS Institute, Cary, NC).
- Swofford DL (2000) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods) (Sinauer, Sunderland, MA).