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Origin of extant domesticated sunflowers in eastern North America

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Eastern North America is one of at least six regions of the world where agriculture is thought to have arisen wholly independently^{1–5}. The primary evidence for this hypothesis derives from morphological changes in the archaeobotanical record of three important crops—squash, goosefoot and sunflower—as well as an extinct minor cultigen, sumpweed^{1,3}. However, the geographical origins of two of the three primary domesticates—squash and goosefoot—are now debated^{6,7}, and until recently sunflower (*Helianthus annuus* L.) has been considered the only undisputed eastern North American domesticate. The discovery of 4,000-year-old domesticated sunflower remains from San Andrés, Tabasco^{8,9}, implies an earlier and possibly independent origin of domestication in Mexico and has stimulated a re-examination of the geographical origin of domesticated sunflower. Here we describe the genetic relationships and pattern of genetic drift between extant domesticated strains and wild populations collected from throughout the USA and Mexico. We show that extant domesticates arose in eastern North America, with a substantial genetic bottleneck¹⁰ occurring during domestication.

There are two hypotheses regarding the origin of agriculture in eastern North America. One hypothesis holds that agriculture arose independently in this region with the domestication of four to seven indigenous species^{1–3,8,11}. The alternative states that most major cultigens originated in Mesoamerica and were dispersed northwards to the eastern woodlands of North America, triggering the domestication of minor indigenous crops^{1–3,8,11}.

Before the discovery of domesticated sunflower remains in Mexico, the sunflower provided the most convincing evidence for an independent origin of agriculture in eastern North America. Although the progenitor of domesticated sunflower, *H. annuus*, is now distributed across North America from southern Canada to northern Mexico¹², wild populations from the east-central USA are most similar morphologically to the domesticates, and domesticated sunflower remains are found at several archaeological sites in

this region. Thus, early authors placed the origin of domestication in eastern North America^{1,3,11–16} and identified the east-central wild form as a probable progenitor^{13–16}. Molecular genetic studies completed before the Tabasco, Mexico, discovery were inconclusive regarding both the number of origins and geographical source of the domesticated sunflower, but in these studies wild populations were insufficiently sampled^{10,17–19} and the molecular markers employed were insufficiently variable to resolve genetic relationships^{17–19}.

To determine the geographical origin(s) of sunflower domestication and to account for the genetic composition of extant domesticates, we have used model-based methods to evaluate genetic relationships and reconstruct the pattern of genetic drift among 21 populations of wild *H. annuus* and eight Native American landraces from the USA and Mexico, as well as two modern cultivars (USDA and Mammoth) (Fig. 1, Supplementary Table 1). The results described are based upon data from 18 microsatellite loci distributed across the sunflower genome (Supplementary Table 2).

To identify ancestral source populations for extant domesticates we used the ‘admixture model’ included in the software program STRUCTURE^{20,21} to infer population structure in wild *H. annuus* and assign domesticates to inferred populations. In this bayesian approach, multilocus genotypic data are used to define a set of populations with distinct allele frequencies, hereafter referred to as clusters, and assign individuals probabilistically to these defined clusters with or without prior knowledge of sampling location. Also, the admixture model assumes that loci are unlinked and can freely recombine.

Without specifying prior information concerning sampling location, and allowing for admixed individuals, we estimated the number of genetic clusters of wild *H. annuus* to which we would assign the domesticates (Methods, Supplementary Methods). Combining the results from these tests with geography, we modelled the assignment on two scales: regional and local. Using our estimate of population structure on a regional scale, we defined all Mexican populations plus Arizona as one potential source cluster and central US populations as a second potential source cluster (Supplementary Fig. 1). Although alleles are widespread across both regions and there are no significant differences in heterozygosity ($P > 0.42$, two-sided) or allelic richness ($P > 0.18$, two-sided) between the two regions and each domesticated individual was allowed to have originated from more than one source, all domesticates were assigned to the US cluster in all ten runs of the algorithm. The proportion of each domesticated individual’s genome having ancestry in the USA was ≥ 0.985 for all individuals, and for each domesticated strain, the average estimated ancestry in the US cluster was ≥ 0.997 (Fig. 2).

Again using the results of our analysis of population structure in wild *H. annuus*, we further modelled the assignment of the domesticates by subdividing the regional groups into four local area source clusters corresponding to west Mexico, east-central Mexico, the US Great Plains and the east-central USA (Supplementary Fig. 2). In all ten runs of the algorithm, again allowing for admixed origins, all domesticates were assigned to the east-central USA. The proportion of each individual’s genome having ancestry from this area was ≥ 0.896 , and for each domesticated strain, average estimated ancestry in the east-central USA was ≥ 0.994 (Fig. 2). Thus, the results of both our ancestry analyses indicate that the ten strains of domesticated *H. annuus* are genetically most similar to wild populations from the central USA, particularly the easternmost populations in our sample.

When considered as a group, the genetic diversity in domesticated sunflower is significantly less than the genetic diversity in wild *H. annuus* (Supplementary Table 3). We hypothesized that the domesticates’ low genetic diversity is a consequence of strong genetic drift from central US populations, a scenario compatible with bottlenecks that would have occurred owing to strong selection during domestication¹⁰. To investigate the historical processes

underlying this pattern of reduced diversity, we compared the rate of drift away from a common ancestor for each wild population and domesticated strain, using the 'F model' as implemented with the program STRUCTURE²¹. The model assumes that each population in the comparison has undergone independent drift away from the allele frequencies found in their common ancestor. A bayesian approach is used to make inferences about ancestral allele frequencies as well as the rates of drift away from the ancestral allelic state in each population, hereafter referred to as *F* values. In wild-domestication comparisons, wild populations whose allele frequencies most closely resemble those found in the common ancestor of wild and domesticated *H. annuus* should exhibit little drift away from the ancestral state, that is, they should have low *F* values. Furthermore, if domestication was associated with a genetic bottleneck, drift in the domesticated strains should be significantly higher than drift in the wild populations.

For each pairwise comparison of wild population and domesticated strain, the estimated *F* values were consistent across three runs of the model and a clear geographically correlated pattern of drift was detected in the wild populations (Supplementary Table 4). The 90% credibility regions around the estimated *F* values for four wild populations—Kansas, South Dakota, Oklahoma² and Iowa—

include zero drift away from the ancestral allele frequencies across all comparisons. These results are consistent with the genetic composition of these modern populations being nearly identical to that of the wild progenitors of domesticated sunflowers.

The drift values estimated for the domesticates are much higher than those found in the wild populations and consistent with strong genetic drift during domestication (Supplementary Table 4). The lowest mean value for any domesticate (Havasupai) is 0.2864, which is 220 times the lowest mean drift value for any wild population (0.0014) (Fig. 3). The confidence limits for these values are relatively narrow, implying that the genetic composition of the domesticates has changed at least 50-fold faster than the wild populations since they diverged. Furthermore, the variation in extant genetic diversity among domesticated lineages suggests that multiple founder events and bottlenecks¹⁰ subsequent to the initial domestication event are responsible (Supplementary Table 3). Again, this scenario is borne out in our genetic drift analysis: although drift in all domesticates is very high, it ranges from around 0.3 for Arikara, Havasupai, Maíz de Tejas, Mammoth and Seneca to around 0.5 for Hopi, Mandan and USDA.

The *F* model results are consistent with the ancestry analysis presented above, supporting a central US origin for domesticated

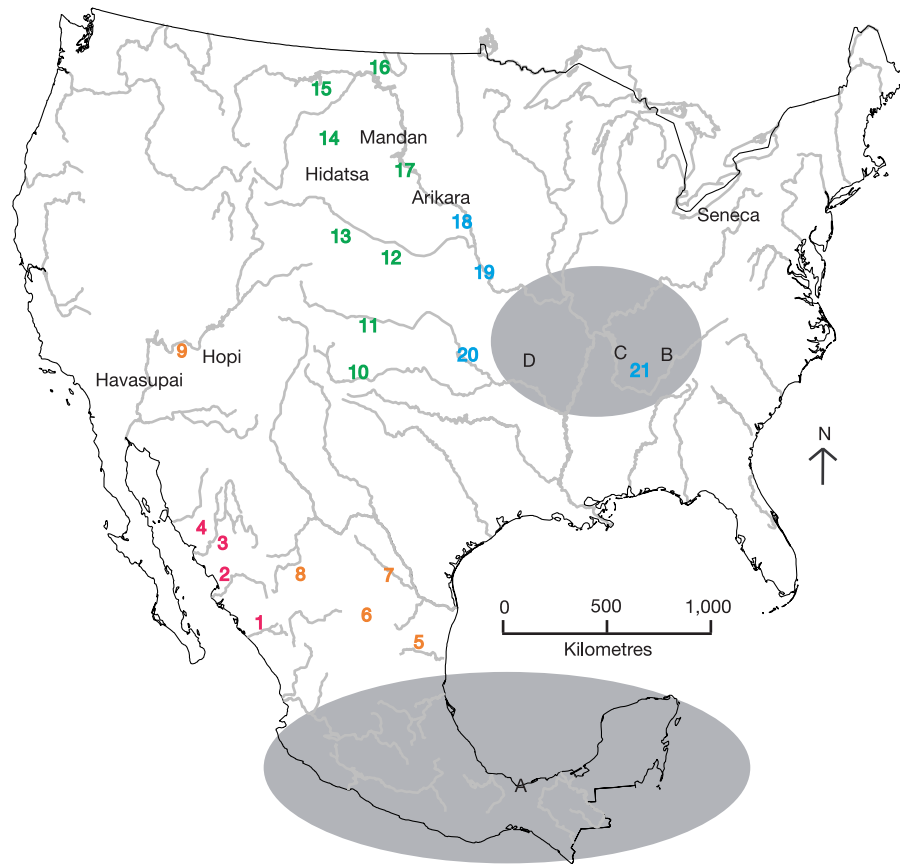


Figure 1 Map of sampling locations, archaeological sites and Native American groups. Shaded areas indicate centres of domestication with eastern North America¹ to the north and Mesoamerica (as defined in ref. 26) to the south. Numbers indicate sampling locations of wild populations where 1 is Sinaloa, 2 is Sonora5, 3 is Sonora4, 4 is Sonora6, 5 is Tamaulipas, 6 is Zacatecas, 7 is Nuevo León, 8 is Chihuahua, 9 is Arizona, 10 is Texas, 11 is Oklahoma², 12 is Kansas, 13 is Colorado, 14 is Montana¹, 15 is Montana², 16 is North Dakota, 17 is South Dakota, 18 is Iowa, 19 is Missouri, 20 is Oklahoma¹ and 21 is Tennessee (colours correspond to local area model ancestry analysis). Letters indicate archaeological sites with the oldest remains of domesticated sunflower, where A

is San Andrés, Tabasco, Mexico ($4,130 \pm 40$ radiocarbon years before present (BP)), B is Higgs, Tennessee, USA ($2,850 \pm 85$ radiocarbon years BP), C is Hayes, Tennessee, USA ($4,265 \pm 60$ radiocarbon years BP) and D is Marble Bluff, Arkansas, USA ($2,843 \pm 44$ radiocarbon years BP); and names indicate the historical locations of Native American groups. We note that although they were collected in Mexico, the identities of indigenous groups associated with Maíz de Tejas and Maíz negro are unknown. USDA and Mammoth are modern cultivars derived from Russian stock. Therefore, these strains do not appear on the map.

sunflowers (Fig. 3). The nine lowest mean F values are all for central US populations. Seven out of the eight Mexican populations including Arizona have mean F values many times higher than those found in this group of nine central US populations, and their 90% credibility regions do not overlap with this group in any of the domesticate–wild comparisons (Supplementary Table 4). Only the northeasternmost Mexican population (Nuevo León) has an intermediate mean F value; an analysis of ancestry using the admixture model estimated 70% Mexican and 30% US ancestry for this population, indicating that its intermediate mean F value is caused by genetic exchange with wild central US populations. Three of the central US populations—North Dakota, Oklahoma1 and Tennessee—have diverged from the population ancestral to domesticated sunflowers, as indicated by intermediate or high mean F values relative to other wild populations in the central USA. These populations nevertheless cluster with other US populations in our analysis of population structure in wild *H. annuus* and on the neighbour-joining tree, which is consistent with a localized drift event in these locations.

Given the substantial genetic bottleneck common to all domesticated sunflowers and the evidence for genetic fluidity among individual wild populations, it may be that genetic evidence alone

will be of limited value in further narrowing down the site or region of origin of modern domesticated sunflowers, so that a multi-disciplinary approach may be required. Archaeological evidence of sunflower domestication has been reported at multiple sites in the region extending from the Appalachian west wall to the prairie margin in the modern-day states of Tennessee, Kentucky, Iowa, Missouri, Arkansas and Ohio^{11,15,22,23}. Historical records suggest that wild *H. annuus* was introduced by humans to this area from the adjacent central plains and subsequently brought under cultivation¹⁴. Geographically distant indigenous groups have developed extant Native American landraces for the seed as a source of food and a source of oil or for the achene coat as a source of dye¹³.

We have shown that genetic relationships and historical drift between wild populations and extant domesticates support the hypothesis that extant domesticated sunflowers arose from wild populations in the central USA via strong genetic drift as would be expected from a selective bottleneck during domestication in eastern North America. Thus, our findings are consistent with earlier archaeological and morphological conclusions and agree with current research from other continents indicating that the transition to agriculture involves complex regional models rather than simple diffusion^{2–4}. The possibility of an earlier and independent domes-

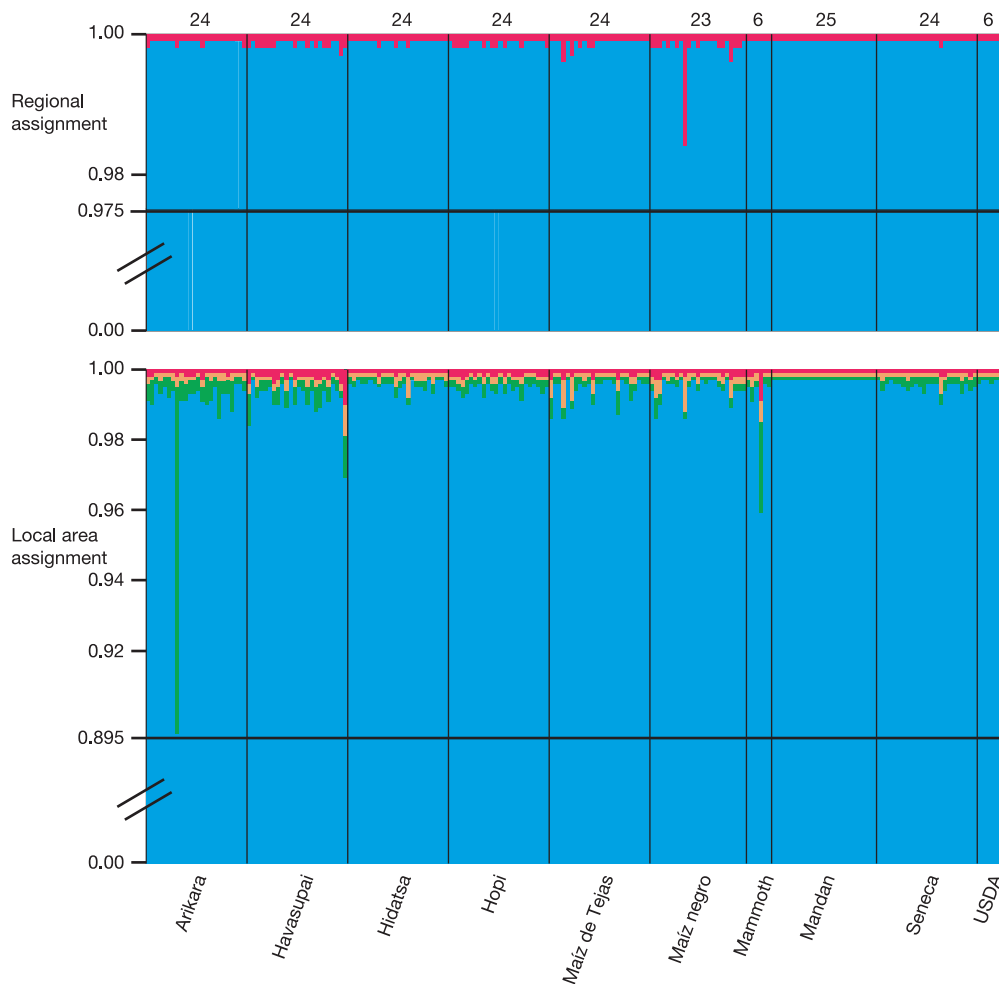


Figure 2 Results of domesticated *H. annuus* ancestry analysis. Each domesticated individual's genome is represented by a thin vertical line partitioned into coloured segments in proportion to the estimated ancestry in each source cluster. Strains are separated by black lines, with names below and sample sizes above. Results are shown

for one run each of: regional model (upper frame) with Mexico plus Arizona (red) and central USA (blue), local area model (lower frame) with west Mexico (red), east-central Mexico (orange), US Great Plains (green), east-central USA (blue). Each run of the algorithm for each model yielded nearly identical results.

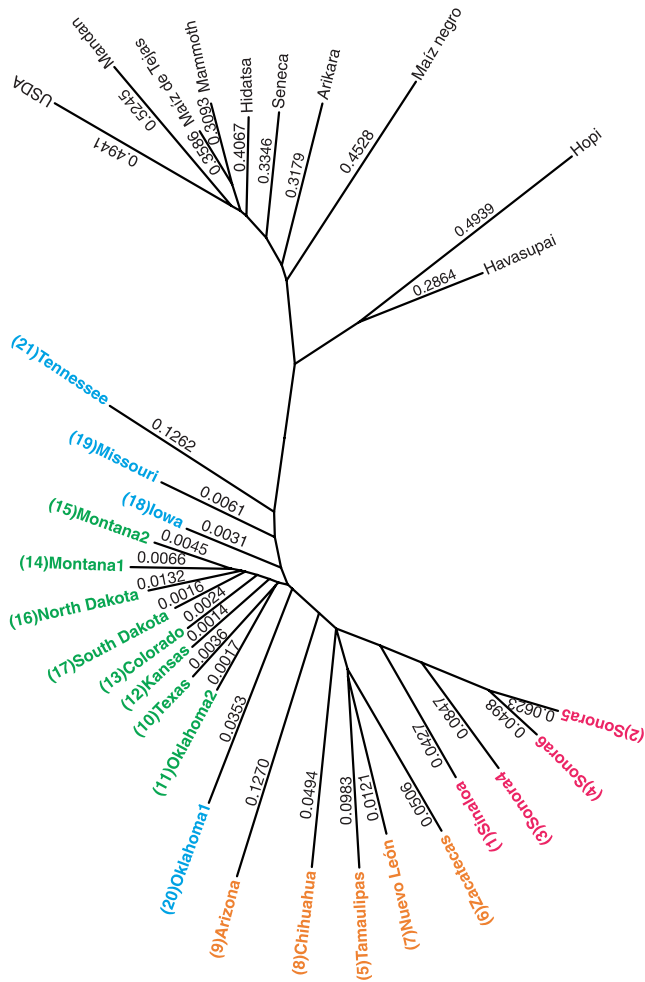


Figure 3 Comparisons of genetic drift in wild populations (colours correspond to local area model source clusters) and domesticated strains (in black). The lines represent a neighbour-joining tree summarizing the genetic distances, D_A , between groups. Mean F values for each domesticated strain averaged across all wild comparisons and mean F values for each wild population averaged across all domesticate comparisons appear along lines. Numbers in brackets correspond to those on Fig. 1 for sampling locations.

tication event in Mexico, as suggested by the discovery in Tabasco, is not ruled out. However, our results indicate that a Mexican domesticate did not contribute to extant domesticated germplasm. To determine the significance of the Mexican palaeoethnobotanical sunflower findings, further archaeological work elucidating the origin and fate of the domesticated sunflower in Mexico is strongly recommended. □

Methods

Sampling and data collection

For DNA extraction, leaf tissue was sampled in the field from twenty-one wild populations of *H. annuus* and from greenhouse plants grown from seed, representing eight Native American landraces (Arikara, Havasupai, Hidatsa, Hopi, Maiz de Tejas, Maiz negro, Mandan and Seneca) and two modern cultivars (USDA, Mammoth) (Fig. 1 and Supplementary Table 1). Microsatellite loci were amplified by polymerase chain reaction (PCR) with fluorescence-labelled primers and surveyed in all sampled wild populations and domesticates (Supplementary Table 2). Of these 21 loci, data for three were excluded from further analysis owing to an apparent excess of homozygosity, presumably due to null alleles. PCR amplifications were carried out according to standard protocol and electrophoresis was carried out on an ABI Prism 3700 DNA analyser (PE Biosystems). Peak data were analysed using Genescan 3.5 and scoring of markers was performed with Genotyper 3.6NT (PE Biosystems programs).

Genetic diversity estimation

Gene diversity (average heterozygosity) in each wild population and each domesticated strain was estimated using DISPAN²⁴. Diversity measures were calculated and comparison tests were performed using FSTAT version 2.9.3 (ref. 25; Supplementary Table 3). P values were estimated using a permutation method.

Inferring population structure in wild populations of *H. annuus*

Using the admixture model^{20,21} (STRUCTURE version 2) we estimated the number of genetic clusters, K , to which we would assign the domesticated individuals without specifying prior information concerning sampling location. Using the full data set, three to ten parallel Markov chains were run for all models of K with a burn-in of 50,000 iterations and a run length of 10^6 iterations following the burn-in. For each run, the ln likelihood of each model was calculated. The full data set was analysed for all models from $K = 1$ through to 12 and also for $K = 21$ clusters (21 is the total number of sampled populations). For all $K = 21$ runs, individuals were assigned to no more than 12 clusters. Therefore, in additional analyses we tested only models of $K = 1$ through to 12. For the full data set, a single splitting solution was found for $K = 2$ in which the sampled populations were clustered into two geographical regions: all Mexican populations plus Arizona versus central US populations (Supplementary Fig. 1). For further analysis the data set was subdivided into the Mexico plus Arizona subsample and the central US subsample (Supplementary Methods). The former was tested for $K = 1$ through to 9 and the latter was tested for $K = 1$ through to 12 running the algorithm three to five times for each K . At $K \geq 2$, the central USA was split into two clusters corresponding to the upper Great Plains and the east-central USA (Supplementary Fig. 2). For the Mexico plus Arizona region, $K = 2$ yielded two solutions that divided the sampled populations into a west coastal area and an east-central area (Supplementary Fig. 2).

Ancestry analysis of the domesticated *H.annuus* individuals

Using STRUCTURE version 2, ten parallel Markov chains were run for each model with a burn-in of 30,000 iterations and a run-length of 10^5 iterations following the burn-in. All models allowed for admixed individuals (Supplementary Methods). For each run, wild individuals were specified as belonging to pre-determined source clusters, but no prior information was specified as to the origin of domesticated individuals. We estimated the ancestry in each source cluster averaged across all individuals per domesticated strain and the proportion of each domesticated individual's genome having ancestry in each cluster.

Genetic drift analysis

For all pairs of wild populations and domesticated strains, we used the F model²¹ (STRUCTURE version 2) to estimate the amount of drift that has occurred in each lineage since their divergence. We specified in advance the origin of each individual as either a domesticate or wild with a prior probability of misassignment to these categories of 0.001. We set the mean and variance of the prior on F to 0.1, which puts approximately equal weight on low and high values. Three parallel Markov chains were run for each comparison with a burn-in of 50,000 iterations and a run length of 10^6 iterations following the burn-in. Regions of 90% credibility were calculated from the distribution of F values estimated during the final run (Supplementary Table 4). Mean drift values for each wild population were calculated across all runs and all domesticates. Mean drift values for each domesticated strain were calculated across all runs and all wild populations.

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Evolutionary change from induced to constitutive expression of an indirect plant resistance

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Induced plant resistance traits are expressed in response to attack and occur throughout the plant kingdom^{1,2}. Despite their general occurrence, the evolution of such resistances has rarely been investigated³. Here we report that extrafloral nectar, a usually inducible trait, is constitutively secreted by Central American *Acacia* species that are obligately inhabited by ants. Extrafloral nectar is secreted as an indirect resistance⁴, attracting ants that defend plants against herbivores⁵. Leaf damage induces extrafloral nectar secretion in several plant species^{6–8}; among these are various *Acacia* species and other Fabaceae investigated here. In contrast, *Acacia* species obligately inhabited by symbiotic ants⁹

nourish these ants by secreting extrafloral nectar constitutively at high rates that are not affected by leaf damage. The phylogeny of the genus *Acacia* and closely related genera indicate that the inducibility of extrafloral nectar is the plesiomorphic or ‘original’ state, whereas the constitutive extrafloral nectar flow is derived within *Acacia*. A constitutive resistance trait has evolved from an inducible one, obviously in response to particular functional demands.

Induced resistances to herbivores have been described for more than 100 plant species² and can greatly benefit plants^{7,10–13}. They are generally regulated by the octadecanoid pathway, in which the plant hormone jasmonic acid forms a central signal^{2,14,15}. This pathway is taxonomically widely distributed and thus has to be regarded as evolutionarily highly conserved. Therefore, the question arises as to whether the expression regime of induced resistance traits can be evolutionarily adapted to particular functional demands.

We used extrafloral nectar (EFN) secretion by Central American *Acacia* (Fabaceae subfamily Mimosoideae) species to investigate whether a resistance trait can be differently expressed in closely related species. EFN is secreted by all species through glands on the leaf stalks, but it serves two different functions. The obligate myrmecophytes among these species permanently house ant colonies in their hollow thorns⁹. These symbiotic ants defend their host against herbivores and competing vegetation. They are nourished by plant-derived cellular protein-rich food bodies¹⁶ and by EFN, and both the ants and the plants seem to be highly adapted to this mutualism^{9,17}. Other, non-myrmecophytic species secrete EFN that is consumed by non-specialized ants from the vicinity. Agrawal & Rutter¹⁸ stated that ants obligately inhabiting myrmecophytes, in general recruit actively to parts of their host that currently require defence. In contrast, attraction of unspecialized ants to non-myrmecophytes was predicted to be controlled by the plants, for example, by short-term increases in the provisioning of food rewards. EFN secreted by *Acacia* functions as a regular food source for specialized mutualists of myrmecophytes and as a ‘bait’ attracting ants facultatively to non-myrmecophytes, thus allowing a test of these predictions.

We studied five myrmecophytic and four non-myrmecophytic *Acacia* species (all of the subgenus *Acacia*) as well as three species of related genera of the Mimosoideae. Study sites were located at the Pacific coast and in the Isthmus of Tehuantepec (state of Oaxaca, Mexico). Only non-myrmecophytes that grew at the same sites as myrmecophytes were selected, so that putative site effects on EFN secretion patterns could not cause differences among species (see Methods for a description of how EFN flow was induced and quantified). EFN secretion by all non-myrmecophytes (*Acacia cochliacantha*, *A. farnesiana*, *A. macracantha*, *A. pennatula*, *Leucaena leucocephala*, *Piptadenia flava* and *Prosopis juliflora*) increased after mechanically damaging leaves or after jasmonic acid application, and was five to more than ten times higher on treated than on control twigs (Fig. 1). In contrast, EFN secretion did not respond to leaf damage in any of the myrmecophytes (*Acacia chiapensis*, *A. collinsii*, *A. cornigera*, *A. globulifera* and *A. hindsii*, Fig. 1); all species secreted EFN constitutively at high rates. Therefore, the same trait was inducible in some, but constitutive in other species of the same genus, and there was an obvious relation to its different functions that matches the predictions of Agrawal & Rutter¹⁸. The induction of EFN secretion in non-myrmecophytes can guide non-specialized ants to plant parts that are currently under attack, whereas ants inhabiting myrmecophytes are provided with a permanent EFN flow at rates which in most cases are higher than those in induced non-myrmecophytes (Fig. 1).

EFN secreted by only one myrmecophyte (*A. collinsii*) responded to exogenous jasmonic acid application (Fig. 1), thus raising the question of whether the octadecanoid pathway is active in the species investigated here. Leaves of four myrmecophytes and four non-myrmecophytes were damaged mechanically in the field to