

quently, produces pale yellow seeds (Fig. 4A, left). Transgenic Tas expression restored anthocyanin synthesis (Fig. 4A, right) because of a strong decrease in CHS siRNA levels (Fig. 4B). Tas-expressing plants also exhibited developmental anomalies, including leaf elongation and serration (Fig. 4C), reminiscent of those elicited in *Arabidopsis* by viral suppressors interfering with miRNA functions (3, 4). As in mammalian cells, Tas enhanced miRNA accumulation (Fig. 4D), independently of their nature or mode of action, suggesting that it suppresses a fundamental step shared between the miRNA and siRNA pathways that is conserved from plants to mammals.

These results indicate that RNA silencing limits the replication of a mammalian virus, PFV-1, and that a cellular miRNA contributes substantially to this response. As a counterdefense, PFV-1 produces Tas, a broadly effective silencing suppressor. Because all our experiments were conducted with Tas-expressing viruses, because of the essential role of the protein for replication (15), the strong effect of Tas on siRNA accumulation observed in *Arabidopsis* could account for our failure to detect siRNAs in mammalian cells (fig. S1). Therefore, we do not yet rule out their implication in the antiviral response reported here.

Our findings with miR-32 and PFV-1 were in fact anticipated in plants by Llave, who pointed out several near-perfect homologies between *Arabidopsis* small RNAs and viral genomes (16). The chances of a match between cellular miRNAs and foreign (i.e., viral) RNAs increase proportionally with the size of sampled sequences. The extent to which cellular miRNAs will be selected to target pathogen genomes upon their initial interaction with viruses may vary. Endogenous viruses might effectively coevolve with miRNAs for defensive or developmental purposes (17, 18), such that viral control might eventually constitute the sole function of some cellular miRNAs. Exogenous viruses with high mutation rates could, on the other hand, rapidly escape this miRNA interference through modification of the small RNA complementary regions (19).

Our results support the emerging notion that miRNAs might be broadly implicated in viral infection of mammalian cells, with either positive or negative effects on replication (5, 20). They also indicate that virtually any miRNA has fortuitous antiviral potential, independently of its cellular function. Moreover, because the repertoire of expressed miRNAs likely varies from one cell type to another (11), this phenomenon could well explain some of the differences in viral permissivity observed between specific tissues.

Note added in proof: Recent findings indicate that a single 8-oligonucleotide seed (small RNA positions 1 to 8 from the 5' end)

is sufficient to confer strong regulation by animals miRNAs. Thus, fortuitous targeting of foreign RNAs by cellular miRNAs could be widespread (21, 22).

References and Notes

1. A. J. Hamilton, D. C. Baulcombe, *Science* **286**, 950 (1999).
2. H. Li, W. X. Li, S. W. Ding, *Science* **296**, 1319 (2002).
3. K. D. Kasschau *et al.*, *Dev. Cell* **4**, 205 (2003).
4. P. Dunoyer, C. H. Lecellier, E. A. Parizotto, C. Himber, O. Voinnet, *Plant Cell* **16**, 1235 (2004).
5. S. Pfeffer *et al.*, *Science* **304**, 734 (2004).
6. W. X. Li *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1350 (2004).
7. V. Schramke, R. Allshire, *Curr. Opin. Genet. Dev.* **14**, 174 (2004).
8. M. Heinkel *et al.*, *EMBO J.* **19**, 3436 (2000).
9. K. Ye, L. Malinina, D. J. Patel, *Nature* **426**, 874 (2003).
10. J. M. Vargason, G. Szittyá, J. Burgyan, T. M. Tanaka Hall, *Cell* **115**, 799 (2003).
11. D. P. Bartel, *Cell* **116**, 281 (2004).
12. M. Kiriakidou *et al.*, *Genes Dev.* **18**, 1165 (2004).
13. M. Lagos-Quintana, R. Rauhut, W. Lendeckel, T. Tuschl, *Science* **294**, 853 (2001).

14. J. S. Jepsen, M. D. Sorensen, J. Wengel, *Oligonucleotides* **14**, 130 (2004).
15. G. Baunach, B. Maurer, H. Hahn, M. Kranz, A. Rethwilm, *J. Virol.* **67**, 5411 (1993).
16. C. Llave, *Mol. Plant Pathol.* **5**, 361 (2004).
17. H. Seitz *et al.*, *Nat. Genet.* **34**, 261 (2003).
18. S. Mi *et al.*, *Nature* **403**, 785 (2000).
19. A. T. Das *et al.*, *J. Virol.* **78**, 2601 (2004).
20. S. Lu, B. R. Cullen, *J. Virol.* **78**, 12868 (2004).
21. J. Brennecke *et al.*, *PLoS Biol.* **15**, e85 (2005).
22. B. Lewis, C. Burge, D. Bartel, *Cell* **120**, 15 (2005).
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Materials and Methods

Figs. S1 to S4

References and Notes

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Postsecretory Hydrolysis of Nectar Sucrose and Specialization in Ant/Plant Mutualism

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Obligate *Acacia* ant plants house mutualistic ants as a defense mechanism and provide them with extrafloral nectar (EFN). Ant/plant mutualisms are widespread, but little is known about the biochemical basis of their species specificity. Despite its importance in these and other plant/animal interactions, little attention has been paid to the control of the chemical composition of nectar. We found high invertase (sucrose-cleaving) activity in *Acacia* EFN, which thus contained no sucrose. Sucrose, a disaccharide common in other EFNs, usually attracts nonsymbiotic ants. The EFN of the ant acacias was therefore unattractive to such ants. The *Pseudomyrmex* ants that are specialized to live on *Acacia* had almost no invertase activity in their digestive tracts and preferred sucrose-free EFN. Our results demonstrate postsecretory regulation of the carbohydrate composition of nectar.

Many plants produce nectar in their flowers (floral nectar) and on vegetative parts [extrafloral nectar (EFN)] to mediate their interactions with animals. The chemical composition of nectar strongly affects the identity and behavior of the attracted insects and thus the outcome of the interaction (1–3). Particularly important chemical factors include amino acid content (4–6) and the ratio and amount of the main sugars: glucose, fructose, and sucrose (3). However, previous studies have focused on nectar as a “standing crop,” leav-

ing open the question of how its chemical composition is controlled.

Floral nectar is produced to attract pollinators, whereas EFN acts to defend plants indirectly [see (7) for a description of EFN in more than 80 plant families]. Most interactions among animals and both floral and extrafloral nectars are thus believed to be mutualistic. Highly specialized mutualisms are surprisingly rare in nature, because they are associated with specific coevolutionary problems (8). In mutualisms in general, one partner provides a service for the other and receives some kind of reward (9). In defensive ant/plant mutualisms, the presence of ants serves as an indirect defense mechanism and, in return, they receive food rewards and/or nesting space (10).

Ant/plant mutualisms differ widely in their specificity and thus are particularly suitable for

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studying mechanisms that determine species-specific interactions (11). Whereas foraging ants are attracted by plant-derived food rewards in facultative interactions, obligate ant plants (myrmecophytes) house and nourish specialized ant colonies, which defend their hosts against herbivores, pathogens, and competing vegetation [reviewed in (10)]. Although both partners appear to be vitally dependent on such interactions, the mutualistic association even in the case of obligate myrmecophytes is transmitted horizontally: Both partners reproduce independently, and the mutualism has to be established anew in each subsequent generation (12). Such mutualisms are easily exploited by parasites or “cheaters” (13), and several parasites exploiting the plant-derived food resources have been described in ant/plant mutualisms (14–16). Filters excluding other species have therefore often been predicted (10). However, the existence and/or relevance of such filters for highly specific mutualisms such as ant/plant interactions have rarely been investigated (17, 18). EFN appears to be an easily accessible target for exploitation by nonsymbiotic or even parasitic species, which, if they competed with the resident ants, would reduce the overall efficacy of the mutualistic interaction (19). We therefore assessed whether the EFNs secreted by related plant species having varying degrees of association with ants differ in their chemical compositions and whether those compositions are affected by any postsecretory mechanism.

We compared the EFN of four Central American *Acacia* myrmecophytes to the EFN of related yet nonmyrmecophytic species. Myrmecophytic *Acacia* species secrete EFN constitutively to nourish specialized mutualists, whereas the other species secrete EFN only in response to herbivore attack; this EFN attracts nonsymbiotic ants and thereby functions as an induced defense mechanism (20). Experiments were conducted at two different sites in Mexico. In our “cafeteria”-style experiments, we offered nectars of myrmecophytic and nonmyrmecophytic species, together with solutions of glucose, fructose, sucrose, and a mixture of these three sugars, to nonsymbiotic ants (species foraging in the vegetation and not regularly associated with *Acacia* myrmecophytes). Equal amounts of solutions at a standardized concentration were offered on the natural vegetation. All foraging ants arriving at the experimental site thus could freely choose among them. Ants feeding on the nectar droplets were counted repeatedly over at least 2 hours (21).

The species secreting the EFN was a significant source of variance in the ants’ attendance [$P < 0.01$ according to one-way analysis of variance (ANOVA) for all four feeding experiments (Fig. 1)]. Nonsymbiotic ants (at least 11 species in total) significantly

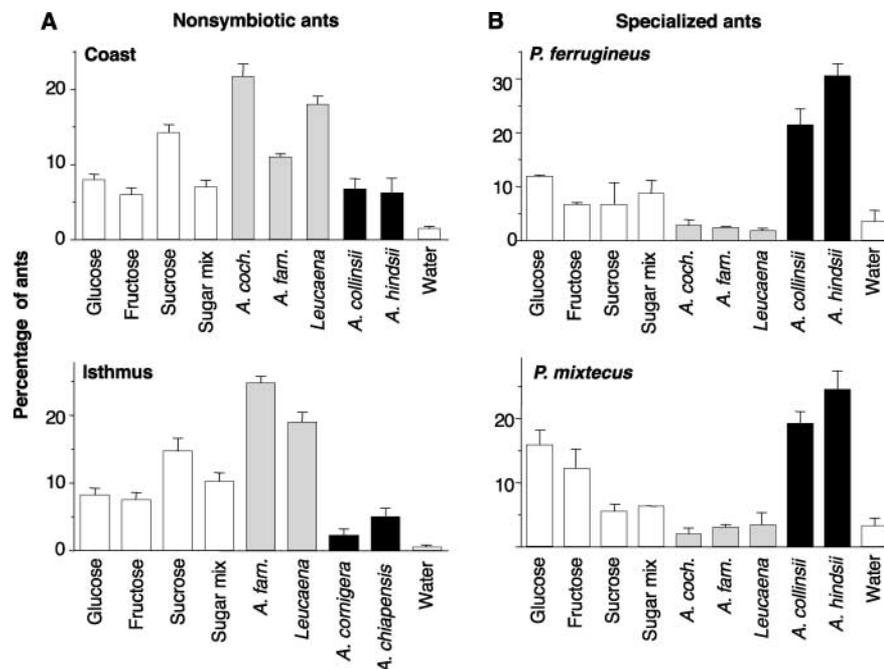


Fig. 1. Ant attendance to sugar solutions and EFNs secreted by certain *Acacia* species. Cafeteria-style experiments were conducted at two sites to test for attendance by the locally occurring nonsymbiotic ant species (A) (21). The same liquids were offered to two species (*P. ferrugineus* and *P. mixtecus*) specifically inhabiting *Acacia* myrmecophytes (B). Ant attendance is expressed as a percentage of all feeding ants that were attracted to one particular solution; bars represent means + SE. Nonmyrmecophytic species (*A. coch.*, *Acacia cochliacantha*; *A. farn.*, *Acacia farnesiana*; and *Leucaena*, *Leucaena leucocephala*) are shown in gray; myrmecophytes are shown in black. The type of sugar solution was a significant source of variance in the number of ants attracted to the different solutions ($P < 0.01$ in all four cases, one-way ANOVA on absolute total ant numbers, $n = 4$ days each for nonsymbiotic ants and $n = 5$ colonies each for specialized ants). Calculation of a priori contrasts revealed a significant ($P < 0.001$) difference between the EFN of myrmecophytes and the EFN of nonmyrmecophytes (see table S1 for all contrasts tested). See supporting online material for localization of the two study sites, coast and Isthmus.

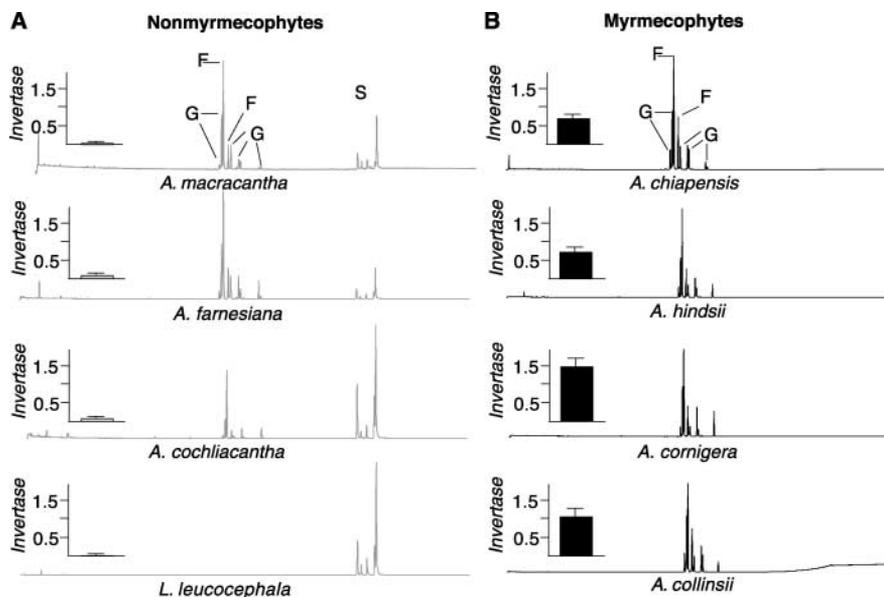


Fig. 2. Sugar profiles and invertase activity in EFNs. Chromatograms give relative abundances of sugars in the EFN of nonmyrmecophytic [(A), gray] and myrmecophytic [(B), black] species. Each sugar resulted in several peaks, which were identified by mass spectrometry (G, glucose; F, fructose; S, sucrose). Inserts at left: Invertase activity in the nectars is expressed as μg of glucose released per μl of EFN per minute, and bars represent means + SE. Species was a significant source of variance in invertase activity ($P < 0.01$ according to univariate ANOVA, five to six samples per species), and post hoc tests [least significant difference (LSD)] revealed that invertase activity in the EFNs of all myrmecophytes was significantly ($P < 0.05$) greater than in EFNs of all nonmyrmecophytes.

preferred the EFN secreted by nonmyrmecophytes to that of myrmecophytes (Fig. 1A). In contrast, two species of specialized *Acacia* ants (*Pseudomyrmex ferrugineus* and *P. mixtecus*) preferred the EFN of myrmecophytes to that of nonmyrmecophytes when they were subjected to a comparable experimental procedure (Fig. 1B).

The EFN secreted by myrmecophytes thus provides a valuable food source for the

resident ants but is not attractive to non-symbiotic EFN-feeding ants. Floral nectar or pollen can contain ant repellants in order to avoid ant/pollinator conflicts (22, 23). Why is the EFN secreted by myrmecophytic *Acacia* species so unattractive to potential competitors of the resident ants? In the choice experiments, solutions of sucrose were much more attractive to nonsymbiotic ants than was the EFN of myrmecophytes (Fig. 1A). Su-

crose, a common component of EFN, is generally very attractive to ants (6, 24–27). Although amino acids can increase the attractiveness of EFN (5, 6), sucrose thus was a likely candidate to cause the strong differences noted in our study. Gas chromatography–mass spectrometry analyses (21) revealed that the EFN of the nonmyrmecophytes always contained sucrose, as well as varying amounts of glucose and fructose (Fig. 2A). In contrast, the EFN of *Acacia* myrmecophytes contained only glucose and fructose (Fig. 2B). Adding sucrose to myrmecophyte EFN significantly increased its attractiveness to generalists but made it less attractive to specialized ants (Fig. 3). The lack of sucrose is thus one important factor (possibly among others) making EFN secreted by Central American *Acacia* myrmecophytes so unattractive to nonsymbiotic ants.

Is the absence of sucrose a consequence of a pre-secretion process, or do any postsecretory regulation mechanisms affect the carbohydrate composition of nectar? To study the underlying mechanism, we quantified invertase activity (encoded as EC 3.2.1.26 by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, catalyzing the hydrolysis of sucrose into glucose and fructose) in the EFN of the study species (21). Invertase activity was detected in all samples of the myrmecophytic species we investigated but in only some samples of nonmyrmecophytic plants. On average, invertase activity ranged from 0.01 to 0.09 μg of glucose released $\mu\text{l}^{-1} \text{min}^{-1}$ in the EFN of nonmyrmecophytes and 0.73 to 1.52 μg of glucose $\mu\text{l}^{-1} \text{min}^{-1}$ in the EFN of myrmecophytes (inserts, Fig. 2). Although it does not exclude a selective secretion, this result shows that the EFN of the myrmecophytes is kept free of sucrose by postsecretory hydrolytic activity.

Because they are highly attractive to many different ant species (6, 24–26), sucrose and other di- and trisaccharides appear to be a particularly important food component for ants. Why is the EFN with sucrose, then, less accepted by *Pseudomyrmex* ants that inhabit the *Acacia* myrmecophytes? Disaccharides and larger oligosaccharides must be cleaved into their monomers before they can be transported through membranes. Consequently, invertase activity has repeatedly been detected in ants (25, 28–30). Invertase activity in extracts of the digestive system of the two specialized *Acacia* inhabitants (21) was significantly lower than in seven other species that live at the same site (Fig. 4). One of the latter species, *P. gracilis*, belongs to the same genus as the two *Acacia* mutualists but has only facultative interactions with *Acacia* myrmecophytes and can live independent from these plants. This species showed significantly greater invertase activity than did the specialized ants (Fig. 4).

Close reciprocal adaptations among *Acacia* myrmecophytes and the inhabiting ants *P.*

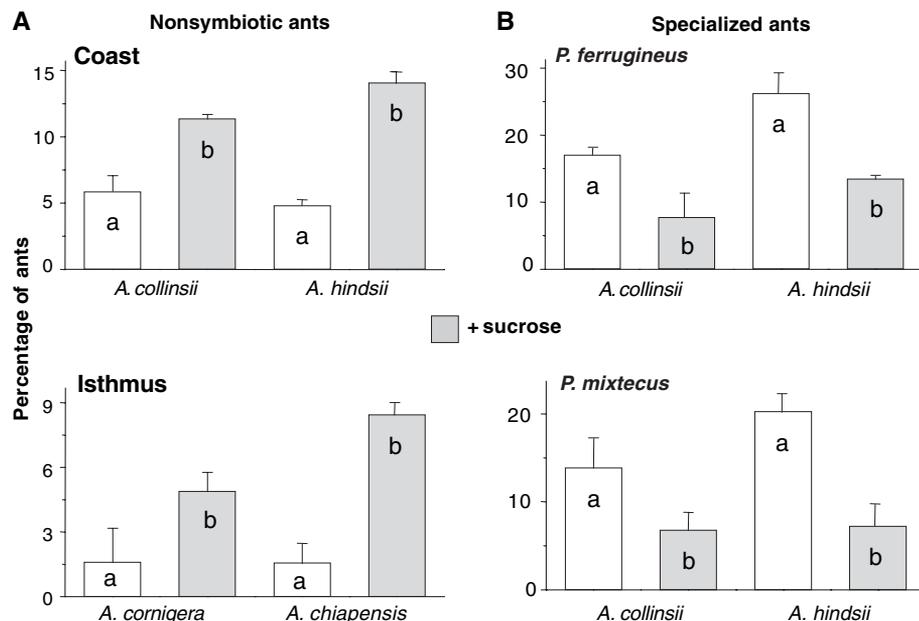
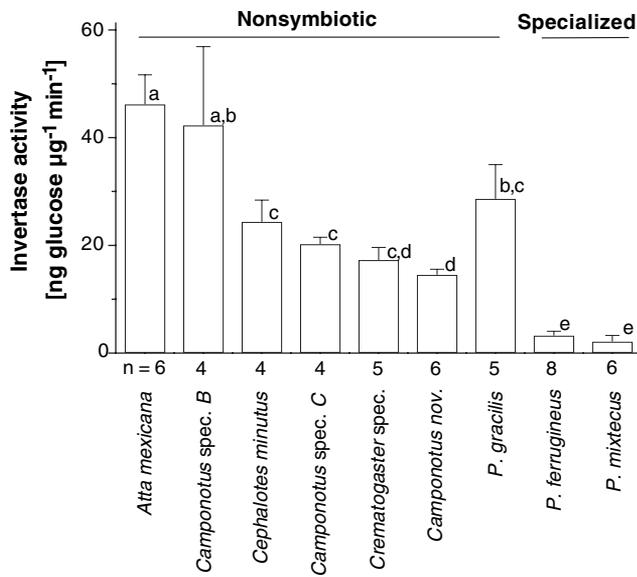


Fig. 3. Responses of ants to EFN nectar of two *Acacia* myrmecophytes (*A. cornigera* and *A. hindsii*) supplemented with sucrose. Responses of nonsymbiotic (A) and specialized (B) ants to nectars without (white bars) and with (gray bars) added sucrose [equal volumes of natural nectar and sucrose solution mixed, concentration 2% (weight/volume)] are given as percentages of ants attracted to these two solutions in cafeteria-style experiments. Nonsymbiotic ants were investigated at two sites (coast and isthmus), and the specialized *Acacia* inhabitants (*P. ferrugineus* and *P. mixtecus*) were investigated on *Acacia collinsii* shrubs. Bars represent means + SE. The numbers of ants attracted to nectar with and without added sucrose differed significantly [indicated by different letters; $P < 0.05$ according to paired *t* test, $n = 7$ feeding sites (A) or colonies (B) in all cases].

Fig. 4. Invertase activity in the digestive tracts of several ant species. Ants feeding on EFN and/or plant sap were collected from several colonies (sample numbers appear below bars representing means + SE) to compare their invertase activity to the activity in *Pseudomyrmex ferrugineus* and *P. mixtecus*, two species obligately inhabiting *Acacia* myrmecophytes. Species was a significant source of variance ($P < 0.001$, univariate ANOVA), and species labeled with different letters are significantly different ($P < 0.05$, LSD post hoc analysis). *Camponotus nov.*, *Camponotus novogradensis*.



ferrugineus and *P. mixtecus* became apparent in this study. Invertase activity, which was very low in the mutualistic ants' digestive tracts, was significantly increased in the EFN that is secreted by these ants' host plants. Because myrmecophytism represents the derived trait among Central American *Acacia* species (20), the enzymatic activity involved in postsecretory regulation of nectar carbohydrate composition must have been greatly intensified during the evolution of these species' life history. This adaptation allows plants to present a valuable food source to their resident mutualists that will seldom be exploited by unspecialized competitors.

Few enzymes are known from any type of nectar, and those that have been characterized function in the antimicrobial defense of floral nectars (31–33). The invertase activity in the EFN of *Acacia* myrmecophytes, in contrast, affects the composition of the main nectar components: carbohydrates. To date, the source of this activity is not known, and even a microbial origin cannot be excluded. However, we detected free proteins in the EFN of myrmecophytes that are absent from the EFN of nonmyrmecophytes. The removal of these proteins from the EFN removes invertase activity as well, and data from preliminary matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry characterization of those proteins are consistent with a putative plant origin (34). Similar patterns in invertase activity have been observed in plants growing in the field (where the EFN is immediately collected by ants) and in a greenhouse in Germany (where the EFN remains on nectaries for days). These observations support the idea that the invertase activity we observed results from an enzyme that is secreted by the plant.

The so-called filters that allow for specificity in horizontally transmitted mutualisms appear to be complex signals comprising elaborate blends of chemical compounds, and they often include cascades of consecutively activated genes. We found that even the presence (or absence) of a seemingly simple and common compound such as sucrose can be used as a filter to stabilize a specific symbiotic mutualism. The absence of sucrose from the EFN of myrmecophytes is an illustration of what had been called “adaptive specialization”: an evolved trait that excludes less desirable partners in a multispecies association (11). Such simple compounds may have easily been overlooked in previous studies, if only because of their ubiquity, and should be considered in future studies.

References and Notes

- H. G. Baker, I. Baker, in *Biochemical Aspects of Evolutionary Biology*, M. Nitecki, Ed. (Univ. of Chicago Press, Chicago, 1982), pp. 131–171.
- B. Bentley, T. S. Elias, *The Biology of Nectaries* (Columbia Univ. Press, New York, 1983).
- V. V. Roshchina, V. D. Roshchina, *The Excretory Function of Higher Plants* (Springer-Verlag, Berlin, 1993).
- H. G. Baker, I. Baker, *Nature* **241**, 543 (1973).
- J. Lanza, *Ecol. Entomol.* **16**, 203 (1991).
- N. Blüthgen, K. Fiedler, *J. Anim. Ecol.* **73**, 155 (2004).
- T. S. Elias, in *The Biology of Nectaries*, B. Bentley, T. S. Elias, Eds. (Columbia Univ. Press, New York, 1983), pp. 174–203.
- J. D. Hoeksema, E. M. Bruna, *Oecologia* **125**, 321 (2000).
- J. L. Bronstein, *Q. Rev. Biol.* **69**, 31 (1994).
- M. Heil, D. McKey, *Annu. Rev. Ecol. Evol. Syst.* **34**, 425 (2003).
- M. L. Stanton, *Am. Nat.* **162**, S10 (2003).
- J. L. Bronstein, *Biotropica* **30**, 150 (1998).
- D. M. Wilkinson, T. N. Sherratt, *Oikos* **92**, 377 (2001).
- D. H. Janzen, *Science* **188**, 936 (1975).
- D. K. Letourneau, *Science* **248**, 215 (1990).
- N. E. Raine, N. Gammans, I. J. Macfadyen, G. K. Scrivner, G. N. Stone, *Ecol. Entomol.* **29**, 345 (2004).
- J. L. Bronstein, *Ecol. Lett.* **4**, 277 (2001).
- C. Brouat, N. Garcia, C. Andary, D. McKey, *Proc. R. Soc. London Ser. B* **268**, 2131 (2001).
- M. Heil, A. Hilpert, R. Krüger, K. E. Linsenmair, *J. Trop. Ecol.* **20**, 201 (2004).
- M. Heil *et al.*, *Nature* **430**, 205 (2004).
- Materials and methods are available as supporting material on Science Online.
- N. E. Raine, P. Willmer, G. N. Stone, *Ecology* **83**, 3086 (2002).
- J. Ghazoul, *Ecol. Lett.* **4**, 295 (2001).
- M. L. Cornelius, J. K. Grace, J. R. Yates, *Anz. Schaedlingskd. Pflanzenschutz Umweltschutz* **69**, 41 (1996).
- J. L. Boevé, F. L. Wackers, *Oecologia* **136**, 508 (2003).
- J. O. Stapel, A. M. Cortesero, C. M. DeMoraes, J. H. Tumlinson, W. J. Lewis, *Environ. Entomol.* **26**, 617 (1997).
- R. K. Vandermeer, C. S. Lofgren, J. A. Seawright, *Fla. Entomol.* **78**, 144 (1995).
- G. L. Ayre, *Can. Entomol.* **99**, 408 (1967).
- B. L. Ricks, S. B. Vinson, *Entomol. Exp. Appl.* **15**, 329 (1972).
- M. Heil, R. Büchler, W. Boland, *J. Chem. Ecol.* **30**, RC177 (2004).
- W. J. Peumans, K. Smeets, K. van Nerum, F. van Leuven, E. J. M. van Damme, *Planta* **201**, 298 (1997).
- R. W. Thornburg *et al.*, *Plant Syst. Evol.* **238**, 211 (2004).
- C. Carter, R. W. Thornburg, *Trends Plant Sci.* **9**, 320 (2004).
- A. Svatos, M. Heil, unpublished data.
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Materials and Methods
Table S1
References

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Retinoic Acid Controls the Bilateral Symmetry of Somite Formation in the Mouse Embryo

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A striking characteristic of vertebrate embryos is their bilaterally symmetric body plan, which is particularly obvious at the level of the somites and their derivatives such as the vertebral column. Segmentation of the presomitic mesoderm must therefore be tightly coordinated along the left and right embryonic sides. We show that mutant mice defective for retinoic acid synthesis exhibit delayed somite formation on the right side. Asymmetric somite formation correlates with a left-right desynchronization of the segmentation clock oscillations. These data implicate retinoic acid as an endogenous signal that maintains the bilateral synchrony of mesoderm segmentation, and therefore controls bilateral symmetry, in vertebrate embryos.

The body plan of vertebrate embryos is overtly symmetric; only later in development do internal organs move into asymmetric positions. Among the most obviously symmetric embryonic struc-

tures are the left and right somitic columns, in which paired epithelial structures arise by segmentation of the paraxial mesoderm. Somite development relies on a “clock and wavefront” mechanism (1), in which a molecular oscillator that depends on Notch and Wnt pathways (the “segmentation clock”) generates cyclic waves of gene expression along the presomitic mesoderm (PSM) (2, 3). In addition, a caudal-to-rostral *Fgf8* (fibroblast growth factor 8) mRNA gradient acts as a moving wavefront (the “determination front”), triggering somite differentiation and setting the intersomitic boundaries (4). Retinoic acid (RA) plays multiple roles during patterning of the vertebrate anteroposterior axis. Altered RA

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