



**Breakdown of an Ant-Plant Mutualism Follows the Loss of Large Herbivores from an African Savanna**  
Todd M. Palmer, *et al.*  
*Science* **319**, 192 (2008);  
DOI: 10.1126/science.1151579

***The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of January 10, 2008 ):***

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:  
<http://www.sciencemag.org/cgi/content/full/319/5860/192>

**Supporting Online Material** can be found at:  
<http://www.sciencemag.org/cgi/content/full/319/5860/192/DC1>

This article **cites 29 articles**, 4 of which can be accessed for free:  
<http://www.sciencemag.org/cgi/content/full/319/5860/192#otherarticles>

This article appears in the following **subject collections**:  
Ecology  
<http://www.sciencemag.org/cgi/collection/ecology>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:  
<http://www.sciencemag.org/about/permissions.dtl>

8. D. Sahagian, O. Pinous, A. Olferiev, V. Zakharov, *Am. Assoc. Pet. Geol. Bull.* **80**, 1433 (1996).
9. B. U. Haq, J. Hardenbol, P. R. Vail, *Science* **235**, 1156 (1987).
10. K. G. Miller *et al.*, *Science* **310**, 1293 (2005).
11. S. Voigt, F. Wiese, *J. Geol. Soc.* **157**, 737 (2000).
12. B. T. Huber, R. D. Norris, K. G. MacLeod, *Geology* **30**, 123 (2002).
13. A. Forster, S. Schouten, M. Baas, J. S. Sinninghe Damsté, *Geology* **35**, 919 (2007).
14. A. B. Herman, R. A. Spicer, *Nature* **380**, 330 (1996).
15. A. Bornemann, R. D. Norris, *Mar. Micropaleontol.* **65**, 32 (2007).
16. P. N. Pearson *et al.*, *Nature* **413**, 481 (2001).
17. S. Schouten, E. C. Hopmans, E. Schefuss, J. S. Sinninghe Damsté, *Earth Planet. Sci. Lett.* **204**, 265 (2002).
18. C. Wuchter, S. Schouten, M. J. L. Coolen, J. S. Sinninghe Damsté, *Paleoceanography* **19**, 10.1029/2004PA001041 (2004).
19. J. I. Antonov, S. Levitus, T. P. Boyer, M. E. Conkright, T. O'Brien, *World Ocean Atlas 1998* (National Oceanic and Atmospheric Administration, Silver Spring, MD, 1998), vol. 1.
20. M. A. Arthur, J. H. Natland, in *Deep Drilling Results in the Atlantic Ocean: Continental Margins and Paleoenvironment*, M. Talwani, W. W. Hay, W. B. F. Ryan, Eds. (American Geophysical Union, Washington, DC, 1979), pp. 375–401.
21. J. Hardenbol, J. Thierry, M. B. Farley, P.-C. De Graciansky, P. R. Vail, in *Mesozoic and Cenozoic Sequence Chronostratigraphic Framework of European Basins*, P. C. De Graciansky, J. Hardenbol, T. Jacquin, P. Vail, Eds. (Society for Sedimentary Geology, Tulsa, OK, 1998), vol. 60, pp. 3–13.
22. Absolute ages are adopted from TSCreator ([www.stratigraphy.org](http://www.stratigraphy.org)), based on (21), recalibrated after (4).
23. B. B. Sageman, J. Rich, M. A. Arthur, G. E. Birchfield, W. E. Dean, *J. Sediment. Res.* **67**, 286 (1997).
24. J. M. Hancock, *Proc. Geol. Assoc.* **100**, 565 (1989).
25. A. S. Gale, *Geol. Soc. Spec. Publ.* **103**, 177 (1996).
26. I. Jarvis, A. S. Gale, H. C. Jenkyns, M. A. Pearce, *Geol. Mag.* **143**, 561 (2006).
27. R. G. Fairbanks, R. K. Matthews, *Quat. Res.* **10**, 181 (1978).
28. H. K. Coxall, P. A. Wilson, H. Pälike, C. H. Lear, J. Backman, *Nature* **433**, 53 (2005).
29. P. Huybrechts, D. Steinhage, F. Wilhelms, J. Bamber, *Ann. Glaciol.* **30**, 52 (2000).
30. R. M. DeConto, D. Pollard, *Nature* **421**, 245 (2003).
31. P. Fitzgerald, *Roy. Soc. New Zeal. Bull.* **35**, 453 (2002).
32. U. S. ten Brink, R. I. Hackney, S. Bannister, T. A. Stern, Y. Makovsky, *J. Geophys. Res.* **102**, 27603 (1997).
33. Materials, methods, and data are available as supporting material on Science Online.
34. This research used samples and data provided by the ODP. The ODP was sponsored by NSF and participating countries under the management of Joint Oceanographic Institutions (JOI). We thank P. Worstell for sample preparation and C. Charles (Scripps Institution of Oceanography) and D. Andreasen (University of California Santa Cruz) for their assistance with the mass spectrometers. Financial support was provided by the German Research Foundation (A.B., O.F., and T.W.) and JOI/U.S. Science Support Program (R.D.N.). T.W. acknowledges the Royal Society–Wolfson Research Merit Award.

### Supporting Online Material

[www.sciencemag.org/cgi/content/full/319/5860/189/DC1](http://www.sciencemag.org/cgi/content/full/319/5860/189/DC1)

Materials and Methods

Figs. S1 and S2

Tables S1 to S4

References and Notes

3 August 2007; accepted 16 November 2007

10.1126/science.1148777

# Breakdown of an Ant-Plant Mutualism Follows the Loss of Large Herbivores from an African Savanna

Todd M. Palmer,<sup>1,2,4\*</sup> Maureen L. Stanton,<sup>2,3,4</sup> Truman P. Young,<sup>2,4,5</sup> Jacob R. Goheen,<sup>1,4,6</sup> Robert M. Pringle,<sup>4,7</sup> Richard Karban<sup>8</sup>

Mutualisms are key components of biodiversity and ecosystem function, yet the forces maintaining them are poorly understood. We investigated the effects of removing large mammals on an ant-*Acacia* mutualism in an African savanna. Ten years of large-herbivore exclusion reduced the nectar and housing provided by plants to ants, increasing antagonistic behavior by a mutualistic ant associate and shifting competitive dominance within the plant-ant community from this nectar-dependent mutualist to an antagonistic species that does not depend on plant rewards. Trees occupied by this antagonist suffered increased attack by stem-boring beetles, grew more slowly, and experienced doubled mortality relative to trees occupied by the mutualistic ant. These results show that large mammals maintain cooperation within a widespread symbiosis and suggest complex cascading effects of megafaunal extinction.

Obligate mutualistic relationships among species are ubiquitous and central to ecological function and the maintenance of biodiversity (1–5). The symbiosis between ants and plants, which involves many species throughout the tropics, was the first coevolved mutualism to be thoroughly elucidated by ecol-

ogists (6). Many studies have shown the efficacy of ant mutualists in deterring herbivory (7) and explored the costs and benefits accruing to the interacting partners (8). However, although the importance of large herbivores in the evolution and maintenance of these interactions has been hypothesized (9, 10), it has never been shown.

We investigated the effects of large mammalian herbivores on an ant-*Acacia* mutualism in an African savanna. The whistling-thorn tree, *Acacia drepanolobium*, dominates heavy-clay soils across large expanses of upland East Africa (11). At branch nodes, *A. drepanolobium* produces either slender stipular thorns or hollow swollen thorns that serve as ant housing (“domatia”). The tree also secretes a carbohydrate-rich nectar from extrafloral glands located near the bases of leaves (12). At our study site in Kenya, four species of ants (*Crematogaster mimosae*,

*C. sjostedti*, *C. nigriceps*, and *Tetraponera penzigi*) compete for exclusive possession of host trees and vary strongly in both their defense of the host trees and their use of the tree’s “rewards” [i.e., domatia and nectar (11, 13)]. *Crematogaster mimosae* aggressively defends host trees from herbivores and relies heavily on swollen-thorn domatia, where they raise brood, house workers, and occasionally tend honeydew-producing scale insects (Coccidae) (11). In contrast, *C. sjostedti* is a less-aggressive defender of host plants (13) and, exclusively among the four ant species, does not nest in domatia but rather in stem cavities excavated by the larvae of long-horned beetles (Cerambycidae). Under natural conditions, *C. mimosae* is the most abundant ant symbiont, occupying ~52% of all trees at our sites, whereas *C. sjostedti* occupies ~16% of host plants.

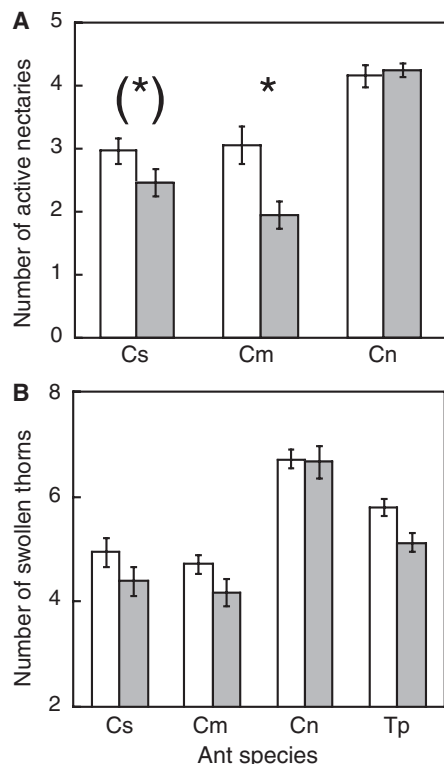
The remaining two ant species, *C. nigriceps* and *T. penzigi*, also occur in relatively low abundance (~15% and ~17% of trees, respectively), and each uses distinctive behaviors that reduce the likelihood of hostile takeover by the competitively superior *C. mimosae* and *C. sjostedti*. *Crematogaster nigriceps* is an effective defender (13, 14) but also prunes axillary buds and kills apical meristems, which reduces lateral canopy spread and thus the likelihood of contact with trees occupied by hostile colonies (15). *Tetraponera penzigi*, an intermediate protector, destroys its host-plants’ nectaries: a “scorched-earth” strategy that reduces the probability of takeover by neighboring nectar-dependent *Crematogaster* colonies (16). All three *Crematogaster* species derive at least some of their energy by foraging off-tree for insects; in contrast, *T. penzigi* seldom leaves its host and appears to subsist by gleaning small food items (e.g., pollen and fungal spores) from the surfaces of its host (17).

In 2005, we sampled *A. drepanolobium* trees (1.8 to 3.0 m in height) in 12 plots (4 ha each)

<sup>1</sup>Department of Zoology, University of Florida, Gainesville, FL 32611, USA. <sup>2</sup>Center for Population Biology, University of California, Davis, CA 95616, USA. <sup>3</sup>Section of Evolution and Ecology, University of California, Davis, CA 95616, USA. <sup>4</sup>Mpala Research Centre, Box 555, Nanyuki, Kenya. <sup>5</sup>Department of Plant Environmental Sciences, University of California, Davis, CA 95616, USA. <sup>6</sup>Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. <sup>7</sup>Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA. <sup>8</sup>Department of Entomology, University of California, Davis, CA 95616, USA.

\*To whom correspondence should be addressed. E-mail: [tmpalmer@zoo.ufl.edu](mailto:tmpalmer@zoo.ufl.edu)

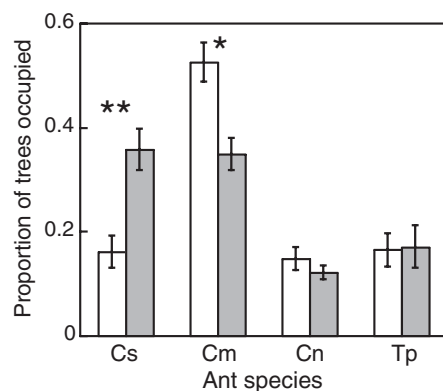
situated within three replicate blocks of the Kenya Long-Term Exclosure Experiment (KLEE) (18). In each block, two plots were accessible to all wildlife, and in the other two (hereafter called “exclosures”), all wild herbivores >15 kg had



**Fig. 1.** Rewards produced in the presence (white bars) and absence (gray bars) of large herbivores by *A. drepanolobium* occupied by different species of *Acacia* ants. Ant species' abbreviations are indicated as: Cs, *C. sjostedti*; Cm, *C. mimosae*; Cn, *C. nigriceps*; Tp, *T. penzigi*. All means shown are averages across six plots  $\pm$  SEM. “Ant species,” herbivore-exclusion treatment (“treatment”), and their interaction all had significant effects on the production of host-tree rewards [multivariate analysis of variance (MANOVA), Wilk's lambda; for ant species:  $F_{6,74} = 80.7$ ,  $P < 0.0001$ ; for treatment:  $F_{2,37} = 7.54$ ,  $P < 0.002$ ; for the interaction:  $F_{6,74} = 2.3$ ,  $P = 0.04$ ]. There was no significant block effect on overall reward production by host plants ( $P > 0.30$ ). (A) ANOVA revealed a significant ant species  $\times$  treatment effect ( $F_{2,28} = 4.3$ ,  $P = 0.01$ ) on mean production of active nectaries by host plants. There was no significant block effect on mean nectary production ( $P = 0.4$ ). Asterisks indicate significant differences in planned contrasts of mean production of active nectaries by species between treatments. (\*),  $P = 0.06$ ; \*,  $P < 0.001$ . Nectary data are not shown for *T. penzigi* because this species actively destroys all host-plant nectaries (16). (B) Both ant species (ANOVA,  $F_{3,38} = 39.5$ ,  $P < 0.0001$ ) and herbivore-exclusion treatment (ANOVA,  $F_{1,38} = 8.0$ ,  $P < 0.01$ ) were significant sources of variation in the mean number of swollen thorns produced by host plants. There were no ant species  $\times$  treatment ( $P > 0.5$ ) or block ( $P > 0.2$ ) effects on mean swollen-thorn production.

been excluded continuously since 1995 (18, 19). This gave us six replicates per treatment, divided among three blocks in a stratified random design (19, 20).

In the absence of browsing by large herbivores, *A. drepanolobium* trees decreased their investment toward supporting symbiotic ants. A single decade of herbivore exclusion resulted in reduced rewards (both extrafloral nectaries and swollen thorns) provided to ants by trees (Fig. 1). Within exclosures, reduction of leaf nectar gland rewards was strongest for trees occupied by *C. sjostedti* and *C. mimosae*, whereas the abundance of “active” nectaries (11) did not decline significantly on trees occupied by *C. nigriceps* (Fig. 1A). From the plant's perspective, the pruning behavior of *C. nigriceps* may simulate browsing by herbivores, inducing host trees to provide more rewards to *C. nigriceps* symbionts even in the absence of browsers (11, 20, 21). Production of domatia by host trees was also significantly reduced by herbivore exclusion (Fig. 1B). Similar to the observed reductions that occurred for nectaries, reductions in swollen-thorn densities for host plants occupied by *C. nigriceps* were negligible relative to trees occupied by the other three ant species. Differences in the density of active nectaries and swollen thorns between exclosure treatments almost certainly do not result from trait-specific variation in tree recruitment or survivorship over the 10-year duration of this experiment; sampled host trees are estimated to be 47 to 70 years old (18), and there were no significant differences in tree density among treatments within this size class of trees [analysis of variance (ANOVA), for treatment:  $F_{1,6} = 0.37$ ,  $P =$



**Fig. 2.** The proportion of host trees occupied by the four *Acacia*-ant species in the presence of large herbivores (white bars) and in plots from which large herbivores had been excluded (gray bars) for 10 years. Means shown are averages across six plots  $\pm$  SEM. MANOVA revealed significant differences in *Acacia*-ant species composition in plots with and without large herbivores (Wilk's lambda,  $F_{4,3} = 17.9$ ,  $P < 0.02$ ). There were no significant block ( $P = 0.13$ ) or treatment  $\times$  block ( $P = 0.65$ ) effects on *Acacia*-ant species composition. Asterisks indicate significant differences in planned contrasts of the relative abundance of each ant species between treatments. \*\*,  $P < 0.003$ ; \*,  $P < 0.01$ .

0.56] (18). Rather, reductions in swollen-thorn and nectar provisioning likely represent phenotypically plastic responses by host plants to the reduction of browsing pressure (18), as with previous studies demonstrating both the relaxation and experimental re-inducibility of stipular thorns in *A. drepanolobium* in the mammal exclosures (21).

The experimental exclusion of large herbivores caused marked shifts in the community of symbiotic ants occupying *A. drepanolobium* (Fig. 2). In the absence of browsing by large herbivores, the proportion of trees occupied by *C. sjostedti* doubled, with this species becoming the most abundant symbiont (18). In contrast, the proportion of trees occupied by *C. mimosae* decreased by more than 30% (Fig. 2). There were no significant differences between treatments in the proportion of host trees occupied by the less-common guild members *C. nigriceps* ( $P = 0.4$ ) and *T. penzigi* ( $P = 0.9$ ) (18). Herbivore exclusion also reduced the average size of individual *C. mimosae* colonies by 47% (planned contrasts,  $P < 0.001$ ), while having no significant effect on average colony size for *C. sjostedti* ( $P = 0.5$ ), *C. nigriceps* ( $P = 0.9$ ), or *T. penzigi* ( $P = 0.5$ ) (18).

In addition to shifting the community structure and competitive relationships among ant symbionts, the exclusion of large herbivores increased the frequency of apparently antagonistic behavior by *C. mimosae*. After reductions in the production of leaf nectar glands within the herbivore-exclusion treatment, *C. mimosae* was twice as likely, on average, to tend sap-sucking homopteran scale insects (scale insects were tended on 19.4 versus 9.7% of branches surveyed for exclosures versus open plots, respectively; logistic regression, for treatment: chi-square = 12.30,  $df = 1$ ,  $P = 0.007$ ) (18).

In addition, aggressive recruitment in response to experimental disturbances of host plants was 50% lower for *C. mimosae* workers in herbivore exclosures versus unfenced plots (fig. S1; planned contrasts,  $P < 0.0001$ ) (18), whereas herbivore exclusion had no significant effects on host-defending behaviors in the three other *Acacia*-ant species (planned contrasts,  $P > 0.4$  for all comparisons) (18). Thus, trees occupied by *C. mimosae* within exclosures are likely to experience both increased costs (due to increased tending of scale insects by their associate ants) and decreased benefits (due to the ants' reduced aggression in response to experimentally induced disturbance).

Changes in *Acacia*-ant community structure and *C. mimosae* colony size appear to be driven by differences among the four ant species in the degree to which they depend on host-plant rewards. The greater reliance of *C. mimosae* and *C. nigriceps* on nectar rewards from host trees, relative to that of *C. sjostedti*, is indicated by their much higher rates of attendance at active nectaries (mean number of workers attending nectaries ( $\pm$  SEM) = 2.02 ( $\pm$  0.37), 3.76 ( $\pm$  0.38), and 0.1 ( $\pm$  0.06) for *C. mimosae*, *C. nigriceps*,

and *C. sjostedti*, respectively; ANOVA,  $F_{2,148} = 61.04$ ,  $P < 0.0001$ ] (18) and by their relatively lower values of  $\delta^{15}\text{N}$  [a stable-isotope signature indicating that *C. sjostedti* derives a greater proportion of its diet from higher trophic levels (22), namely other arthropods (23, 24)]. When large herbivores are excluded, a reduction in the availability of active nectaries on trees appears to reduce average colony size, defense of host plants, and the relative abundance of nectar-dependent *C. mimosae*, all in favor of its parasitic competitor, *C. sjostedti*. In contrast, although *C. nigriceps* also depends heavily on nectaries, branch pruning by this species appears to maintain the production of active nectaries by its host trees (Fig. 1A), even where herbivores are absent (15, 20, 21).

The reduction in ant domatia on host trees that resulted from herbivore exclusion also had a disproportionate negative impact on *C. mimosae*. This species depends entirely on swollen thorns for nest space, whereas *C. sjostedti* houses eggs, larvae, and pupae exclusively within stem cavities excavated by cerambycid beetle larvae. Again, although *C. nigriceps* also raises young exclusively in domatia, pruning by this species may help to maintain domatia production by host trees. Finally, the relative abundance of *T. penzigi* was not influenced by herbivore exclusion. Because this species destroys its host-plant's nectaries (16), very rarely undergoes aggressive colony expansion (25), and is often able to resist invasion by neighboring ant colonies (13), *T.*

*penzigi* is less affected by variation in host-plant rewards than *C. mimosae*.

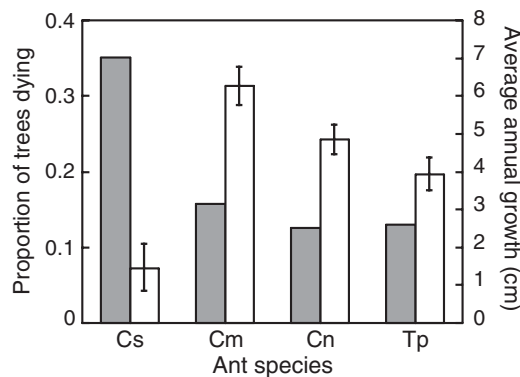
Because *C. sjostedti* derives much of its diet from terrestrial invertebrates, increased arthropod abundance after mammal exclusion might favor that species. However, this is unlikely to explain our results. Extensive sampling within experimental plots (18) demonstrated that neither the mean abundances of arboreal, herbaceous, and terrestrial non-ant arthropods, nor the mean biomass of herbaceous and terrestrial non-ant arthropods, differed significantly between herbivore-exclusion and control plots ( $t$  tests,  $P > 0.1$  for all comparisons) (18). This finding is consistent with a previous study showing no differences in total arthropod abundance across these treatments (26).

Thus, herbivore exclusion effects dramatic changes in the *Acacia*-ant community, but how do these changes feed back to the host plants themselves? Counterintuitively, the loss of large herbivores that browse on *A. drepanolobium* is likely to have strong negative impacts on the growth and survival of these trees, mediated through changes in the abundance of dominant ant symbionts. Eight years of longitudinal data for >1750 marked *A. drepanolobium* individuals (18) show that trees occupied by *C. sjostedti* grow markedly more slowly and suffer twice the mortality as compared with trees occupied by the other three ant species (Fig. 3). The reduced vigor and higher mortality of *C. sjostedti*-occupied trees are likely tied to stem damage

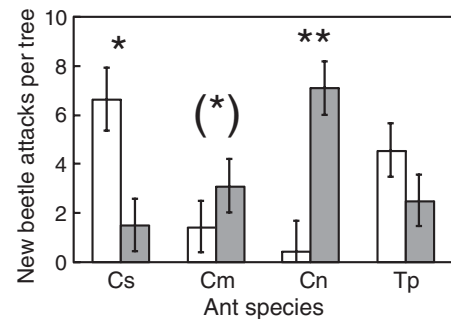
by wood-boring cerambycid beetles that create nesting spaces for *C. sjostedti*. An ant-removal experiment (18) revealed that *C. sjostedti* actively facilitates attack of its host trees by cerambycid beetles. After 18 months, attack by long-horned beetles decreased by 77% on trees from which *C. sjostedti* had been removed, relative to trees where colonies were left intact (Fig. 4). In contrast, *C. nigriceps* and *C. mimosae* appear to actively protect their host trees from attack by these beetles (Fig. 4). The facilitation of these highly destructive (27) tree-boring insects by *C. sjostedti* provides a mechanism for the negative impact of this ant on tree growth and survival.

Our results indicate that the large herbivores typical of African savannas have driven the evolution and maintenance of a widespread *Acacia* mutualism and that their experimentally simulated extinction rapidly tips the scales away from mutualism and toward a suite of antagonistic behaviors by the interacting species. Browsing by large herbivores induces greater production of nectary and domatia rewards by trees, and these rewards in turn influence both the behavior of a specialized, mutualistic ant symbiont and the outcome of competition between this mutualist and a non-obligate host-plant parasite. Where herbivores are present, the carbohydrate subsidy provided by host trees plays a key role in the dominance of the strongly mutualistic *C. mimosae*, which is consistent with the hypothesis that plant exudates fuel dominance of canopy ant species that are specialized users of these abundant resources (28). In the absence of large herbivores, reduction in host-tree rewards to ant associates results in a breakdown in this mutualism, which has strong negative consequences for *Acacia* growth and survival. Ongoing anthropogenic loss of large herbivores throughout Africa (29, 30) may therefore have strong and unanticipated consequences for the broader communities in which these herbivores occur.

**Fig. 3.** Average annual growth (white bars  $\pm$  SEM) and cumulative mortality (gray bars) for host trees occupied by the four *Acacia*-ant species over an 8-year observation period. Average annual growth increments were calculated for trees continuously occupied over an 8-year period by each ant species, with  $n = 158, 192, 162,$  and  $75$  for trees occupied by *C. sjostedti*, *C. mimosae*, *C. nigriceps*, and *T. penzigi*, respectively.



**Fig. 4.** Long-horned beetle (Cerambycidae) attack on ant-removal (gray bars  $\pm$  SEM) and control trees (white bars  $\pm$  SEM) occupied by the four *Acacia*-ant species ( $n = 9$  to  $12$  trees per ant species per treatment). Significance levels for effects of ant removal on new beetle damage sites are shown for one-way ANOVA models conducted separately on each ant species, after the discovery of a highly significant interaction (18) between ant species and the ant-removal treatment. (\*),  $P = 0.07$ ; \*,  $P = 0.02$ ; \*\*,  $P = 0.005$ . The left panel shows cavities excavated by boring beetle larvae within a longitudinal cross section of a main stem of *A. drepanolobium*. An adult cerambycid beetle discovered within this stem is also shown.



**References and Notes**

1. D. A. Wardle *et al.*, *Science* **304**, 1629 (2004).
2. G. P. Jones, M. I. McCormick, M. Srinivasan, J. V. Eagle, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8251 (2004).
3. A. M. Klein *et al.*, *Proc. R. Soc. London Ser. B* **274**, 303 (2007).
4. R. W. F. Hardy, U. D. Havelka, *Science* **188**, 633 (1975).
5. J. L. Sachs, E. L. Simms, *Trends Ecol. Evol.* **21**, 585 (2006).
6. D. H. Janzen, *Univ. Kans. Sci. Bull.* **47**, 315 (1967).
7. M. Heil, D. McKay, *Annu. Rev. Ecol. Syst.* **34**, 425 (2003).
8. M. E. Frederickson, *Oecologia* **143**, 387 (2005).
9. D. H. Janzen, *Ecology* **53**, 885 (1972).
10. W. L. Brown Jr., *Ecology* **41**, 587 (1960).
11. T. P. Young, C. H. Stubblefield, L. A. Isbell, *Oecologia* **109**, 98 (1997).
12. B. Hocking, *Trans. R. Entomol. Soc. London* **122**, 211 (1970).
13. T. M. Palmer, T. P. Young, M. L. Stanton, E. Wenk, *Oecologia* **123**, 425 (2000).
14. L. Stapley, *Oecologia* **115**, 401 (1998).
15. M. L. Stanton, T. M. Palmer, T. P. Young, A. Evans, M. L. Turner, *Nature* **401**, 578 (1999).
16. T. M. Palmer, T. P. Young, M. L. Stanton, *Oecologia* **133**, 372 (2002).
17. W. M. Wheeler, I. W. Bailey, *Trans. Am. Philos. Soc.* **22**, 235 (1920).

18. Materials and methods are available as supporting material on *Science* Online.
19. T. P. Young, B. D. Okello, D. Kinyua, T. M. Palmer, *Afr. J. Range Forage Sci.* **14**, 94 (1998).
20. M. Huntzinger, R. Karban, T. P. Young, T. M. Palmer, *Ecology* **85**, 609 (2004).
21. T. P. Young, M. L. Stanton, C. E. Christian, *Oikos* **101**, 171 (2003).
22. T. M. Palmer, *Ecology* **84**, 2843 (2003).
23. B. J. Peterson, B. Fry, *Annu. Rev. Ecol. Syst.* **18**, 293 (1987).
24. D. M. Post, *Ecology* **83**, 703 (2002).
25. M. L. Stanton, T. M. Palmer, T. P. Young, *Ecol. Monogr.* **72**, 347 (2002).
26. R. M. Pringle, T. P. Young, D. I. Rubenstein, D. J. McCauley, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 193 (2007).
27. E. G. Brockerhoff, A. M. Liebhold, H. Jactel, *Can. J. For. Res.* **36**, 263 (2006).
28. D. W. Davidson, *Biol. J. Linn. Soc.* **61**, 153 (1997).
29. J. T. Du Toit, D. H. M. Cumming, *Biodiversity Conserv.* **8**, 1643 (1999).
30. W. K. Ottichilo, J. De Leeuw, A. K. Skidmore, H. H. T. Prins, M. Y. Said, *Afr. J. Ecol.* **38**, 202 (2000).
31. We thank J. Lemboi and the excellent staff at Mpala Research Centre and Mpala Ranch for logistical support, students from The Kenya Wildlands Program for field assistance, and the Gordon Lab (Stanford University), Koch Lab (University of California), Silliman Lab (University of Florida), K. Rudolph, and four anonymous reviewers for helpful comments on the manuscript. This work was funded by NSF grants DEB-0089706 and DEB-0444741 to T.M.P., M.L.S., and T.P.Y. The KLEE plots were built and maintained with grants from the James Smithson Fund of the Smithsonian Institution (to A. Smith), the National Geographic Society (4691-91), NSF (BSR-97-07477 and BSR-03-16402), and the African Elephant Program of the U.S. Fish and Wildlife Service (98210-0G563) (to T.P.Y.). This research was carried out under the auspices of the Ministry of Education, Science, and Technology of the Republic of Kenya (Permit number MOEST 13/001/34 17). This paper is dedicated to the memory of Otis Trout Palmer, a true mutualist.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/319/5860/192/DC1  
Materials and Methods

Fig. S1  
References

10 October 2007; accepted 28 November 2007  
10.1126/science.1151579

# Endothelial Progenitor Cells Control the Angiogenic Switch in Mouse Lung Metastasis

Dingcheng Gao, Daniel J. Nolan, Albert S. Mellick, Kathryn Bambino, Kevin McDonnell, Vivek Mittal\*

Angiogenesis-mediated progression of micrometastasis to lethal macrometastasis is the major cause of death in cancer patients. Here, using mouse models of pulmonary metastasis, we identify bone marrow (BM)-derived endothelial progenitor cells (EPCs) as critical regulators of this angiogenic switch. We show that tumors induce expression of the transcription factor *Id1* in the EPCs and that suppression of *Id1* after metastatic colonization blocked EPC mobilization, caused angiogenesis inhibition, impaired pulmonary macrometastases, and increased survival of tumor-bearing animals. These findings establish the role of EPCs in metastatic progression in preclinical models and suggest that selective targeting of EPCs may merit investigation as a therapy for cancer patients with lung metastases.

Disseminated malignant primary tumor cells colonize target secondary organs, through bone marrow (BM)-derived premetastatic niches (1, 2), to form dormant micrometastases (3). In some cases, these micrometastases activate the angiogenic switch and progress to macrometastases (4, 5). The cellular and molecular mechanisms regulating the angiogenic switch and the dynamics of vessel assembly during the progression of micrometastases to macrometastases remain poorly understood, which limits the utility of antiangiogenic approaches to controlling metastasis. In this study, we have investigated whether BM-derived endothelial progenitor cells (EPCs) contribute to angiogenesis-mediated progression of micrometastases into deadly macrometastases.

To facilitate tracking of both metastatic tumor cells and BM-derived cells *in vivo*, we implanted Lewis lung carcinoma cells stably expressing red fluorescent protein (LLC-RFP) into syngeneic mice reconstituted with BM cells expressing green fluorescent protein (GFP<sup>+</sup> BM) (fig. S1A) (6). After primary tumor resection (fig. S1B),

numerous RFP<sup>+</sup> pulmonary micrometastases (<1 mm in diameter) were detected by stereomicroscopic imaging at day 14 after tumor inoculation (12 on average per animal) (fig. S1C). The total number of metastases increased with time (average 22 and 35 per animal at day 21 and day 28, respectively) (Fig. 1A), with a concomitant increase in macrometastases ( $\geq 1$  mm in diameter, 47% at day 28) (Fig. 1A), which indicated a time window of micrometastasis to macrometastasis progression. We next determined whether this window of metastasis progression was associated with the angiogenic switch. Immunohistochemical staining showed that the micrometastatic foci (day 14) were largely avascular, as determined by a lack of CD31<sup>+</sup> vessels (Fig. 1B, top). In contrast, macrometastatic foci (days 21 to 28) were infiltrated with many CD31<sup>+</sup> neovessels of various sizes (Fig. 1B, bottom), which suggested that these lesions had undergone an angiogenic switch during their expansion in size. As expected, many BM-derived GFP<sup>+</sup> cells were recruited to both micro- and macrometastases (fig. S1C and Fig. 1B). Although a majority of these cells represented hematopoietic lineages, as previously described in primary tumors (7) (fig. S2A), we focused on BM-derived endothelial cells that directly contribute to neovascularization (8). Microscopic

analysis of macrometastases showed that a subset of neovessels had incorporated BM-derived endothelial cells [GFP<sup>+</sup>CD31<sup>+</sup> (Fig. 1C)]. Luminal incorporation was confirmed by optical sectioning microscopy, which showed that the GFP and CD31 signals were localized to the same individual cell in all three dimensions [supporting online material (SOM) text, Note 1, and (fig. S2B)]. Functional incorporation of BM-derived endothelial cells was quantified by systemic perfusion of fluorescently labeled isolectin GS-IB4, which specifically binds to the luminal surface of endothelial cells in vessels with active blood circulation (8, 9). Macrometastases were dissected from the lungs, and fluorescence activated cell sorting (FACS) analysis showed that the lumenally incorporated BM-derived endothelial cells (GFP<sup>+</sup>Lectin<sup>+</sup>CD31<sup>+</sup>CD11b<sup>-</sup>) represent on average  $12.7 \pm 2.9\%$  of total endothelial cells (Lectin<sup>+</sup>CD31<sup>+</sup>CD11b<sup>-</sup>) (Fig. 1, D and E).

To confirm that these events also occur in a model of spontaneous metastasis, we transplanted syngeneic GFP<sup>+</sup> BM into MMTV-PyMT transgenic mice, a model of breast cancer. Pulmonary micrometastases were detected in the mice at 12 weeks of age, and these lesions progressed into numerous macrometastases by week 16 (Fig. 2A). Notably, GFP<sup>+</sup> BM-derived cells colocalized with the metastatic lesions (Fig. 2B). As observed in the LLC model, the micrometastases were avascular and lacked CD31<sup>+</sup> vessels (Fig. 2C), whereas macrometastases were infiltrated by CD31<sup>+</sup> neovessels (Fig. 2D), which indicated that these lesions had undergone an angiogenic switch at this defined window. Histology revealed vessel-incorporated GFP<sup>+</sup> CD31<sup>+</sup> BM-derived endothelial cells (Fig. 2E). Further quantification showed that  $11.7 \pm 3.7\%$  of vessels in the metastases contained incorporated GFP<sup>+</sup> BM-derived endothelial cells (Fig. 2F).

We have previously shown that the BM-derived endothelial cells are derived from progenitor cells defined by cell surface expression of vascular endothelial (VE)-cadherin, vascular endothelial growth factor receptor 2 (VEGFR2), dim CD31, and Prominin I and lack various hematopoietic markers (8). Analysis of micrometastases showed infiltration of BM-derived GFP<sup>+</sup> VE-cadherin<sup>+</sup> EPCs in the peripheral region of the lesions (Fig. 3A). FACS analysis of the lungs bearing micro-

Cancer Genome Research Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

\*To whom correspondence should be addressed. E-mail: mittal@cshl.edu