

---

*This copy is for your personal, non-commercial use only.*

---

**If you wish to distribute this article to others**, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

**Permission to republish or repurpose articles or portions of articles** can be obtained by following the guidelines [here](#).

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of February 11, 2011):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/312/5770/101.full.html>

**Supporting Online Material** can be found at:

<http://www.sciencemag.org/content/suppl/2006/04/05/312.5770.101.DC1.html>

This article has been **cited by** 105 article(s) on the ISI Web of Science

This article has been **cited by** 27 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/312/5770/101.full.html#related-urls>

This article appears in the following **subject collections**:

Evolution

<http://www.sciencemag.org/cgi/collection/evolution>

22. Y. Nonaka *et al.*, *Eur. J. Biochem.* **229**, 249 (1995).  
 23. H. E. Bulow, R. Bernhardt, *Eur. J. Biochem.* **269**, 3838 (2002).  
 24. M. Weisbart, J. H. Youson, *J. Steroid Biochem.* **8**, 1249 (1977).  
 25. Y. Li, K. Suino, J. Daugherty, H. E. Xu, *Mol. Cell* **19**, 367 (2005).  
 26. J. M. Smith, *Nature* **225**, 563 (1970).  
 27. J. W. Thornton, E. Need, D. Crews, *Science* **301**, 1714 (2003).  
 28. N. Gompel, B. Prud'homme, P. J. Wittkopp, V. Kassner, S. B. Carroll, *Nature* **433**, 481 (2005).  
 29. J. Piatigorsky, *Ann. N.Y. Acad. Sci.* **842**, 7 (1998).  
 30. M. J. Ryan, *Science* **281**, 1999 (1998).  
 31. We thank S. Sower and S. Kavanaugh for agnathan plasma and explant cultures, D. Anderson and B. Kolaczowski for technical expertise, and P. Phillips for manuscript comments. Supported by NSF-IQB-0546906, NIH-F32-GM074398, NSF IGERT DGE-0504627, and a Sloan Research Fellowship to J.W.T.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5770/97/DC1  
 Materials and Methods  
 Figs. S1 and S7  
 Tables S1 to S4  
 References and Notes

2 December 2005; accepted 13 February 2006  
 10.1126/science.1123348

# Phylogeny of the Ants: Diversification in the Age of Angiosperms

Corrie S. Moreau,<sup>1\*</sup> Charles D. Bell,<sup>2</sup> Roger Vila,<sup>1</sup> S. Bruce Archibald,<sup>1</sup> Naomi E. Pierce<sup>1</sup>

We present a large-scale molecular phylogeny of the ants (Hymenoptera: Formicidae), based on 4.5 kilobases of sequence data from six gene regions extracted from 139 of the 288 described extant genera, representing 19 of the 20 subfamilies. All but two subfamilies are recovered as monophyletic. Divergence time estimates calibrated by minimum age constraints from 43 fossils indicate that most of the subfamilies representing extant ants arose much earlier than previously proposed but only began to diversify during the Late Cretaceous to Early Eocene. This period also witnessed the rise of angiosperms and most herbivorous insects.

Ants are a ubiquitous and dominant feature of the terrestrial landscape, playing key roles in symbiotic interactions, soil aeration, and nutrient cycling. They have a rich fossil record (1), yet the evolutionary history of the ~11,800 described modern species remains poorly resolved.

Bolton's (2) recent revision of ants (Hymenoptera: Formicidae) recognized 288 genera in 21 [subsequently reduced to 20 (2, 3)] subfamilies. Several phylogenies have been proposed based primarily on morphological characters, but these reflect disagreement about the positions of major lineages (fig. S2) (4–7). Recent molecular analyses have included only a moderate number of taxa and recovered only weak support for most clades (8, 9), although more comprehensive studies are under way (10).

To evaluate competing phylogenetic hypotheses, we analyzed 4.5 kb of sequence data (Fig. 1) from portions of five nuclear genes and one mitochondrial gene from 139 ant genera and six Aculeata Hymenoptera outgroups ( $n = 149$  specimens) representing 19 of the 20 currently recognized extant subfamilies. The only ant subfamily not included was Aenictogitoninae, a rare group known only from males collected at lights in equatorial Africa. The monophyly of the Formicidae itself was strongly supported in all analyses (Table 1).

Analyses with several methods (11) resulted in a well-resolved phylogeny that divided the family into three groups: the leptanilloid clade, a basal lineage containing 1

subfamily (Leptanillinae) and sister to all other ants; the poneroid clade, containing 5 subfamilies (Agroecomyrmecinae, Amblyoponinae, Paraponerinae, Ponerinae, and Proceratiinae); and the formicoid clade, containing the remaining 13 subfamilies sampled in this study. All three clades were supported by 100% Bayesian posterior probability (bpp) support, but only the formicoid and leptanilloid clades were well supported in the maximum likelihood analyses [ $\geq 94\%$  maximum likelihood bootstrap (ml bs)].

Of the 19 subfamilies investigated here, 14 were recovered as monophyletic with strong support, and none of the three monotypic taxa, each represented by a single extant species (Agroecomyrmecinae, Aneuretinae, and Paraponerinae), nested within another lineage, validating their status as separate subfamilies. However, the three sampled genera of Cerapachyinae were paraphyletic in all analyses. The eight genera of Amblyoponinae grouped together in a clade that lacked support, although the monophyly of Amblyoponinae genera was well supported in an earlier molecular study (2).

The monophyly of the Leptanillinae was strongly supported (100% bpp and ml bs), and its basal position was recovered in all analyses. Ward (6) noted that a basal position of *Leptanilla* within the poneroid group implies that tergo-sternal fusion of abdominal segments III and IV in the worker caste occurred early in ant evolution and was lost secondarily in many lines. Our results indicate that these characters are indeed labile and homoplasious. Although the basal position of Leptanillinae was suggested in other molecular studies (2, 10), previous phylogenetic hypotheses based on morphology had failed to place it in a basal position among extant ants.

Bolton (2) proposed a "poneromorph" clade, including Amblyoponinae, Ectatomminae, Het-

eroponerinae, Paraponerinae, Ponerinae, and Proceratiinae; our results exclude Ectatomminae and Heteroponerinae but add Agroecomyrmecinae. The latter is represented by a single extant species, *Tatuvidris tatusia*, and two fossil genera, and its placement within the poneroid clade is entirely novel. Both Ectatomminae and Heteroponerinae nested within the formicoid clade. Although the poneroid clade received less support in the maximum likelihood and maximum parsimony analyses (Table 1), it was strongly supported in the Bayesian analysis (100% bpp). It seems likely that the five included subfamilies form a monophyletic group or, alternatively, a basal polytomy, but in either case they remain outside both the leptanilloid and the formicoid clades.

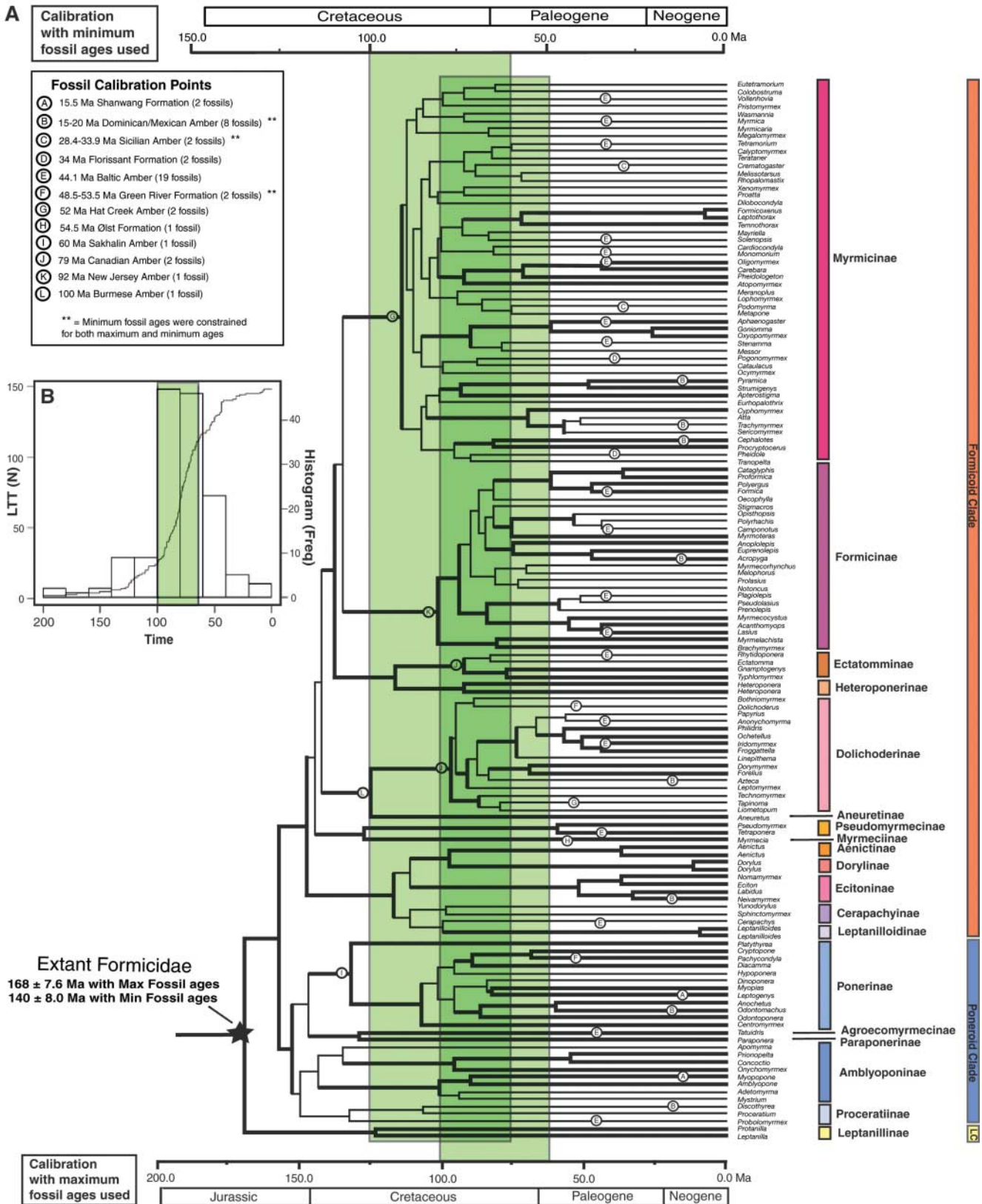
The inclusion of Heteroponerinae within the formicoid clade is also unexpected. As suggested by their name, heteroponerines have historically been placed in the poneromorph clade. Moreover, Ectatomminae, until recently also considered poneromorphs, appear to be closely related to Heteroponerinae. These findings, combined with the lack of stability for the "poneromorphs" observed in morphological analyses (4–7), underscore the extent to which our understanding of ancestral ant morphology and behavior must be revised.

The phylogenetic position of *Aneuretus*, today restricted to Sri Lanka, has been hypothesized to be basal either to the Dolichoderinae or to the Dolichoderinae + Formicinae (12, 13). We recover *Aneuretus* as basal to the Dolichoderinae, with both groups separated from Formicinae, implying that the sting has been reduced independently at least twice in the ants (Dolichoderinae and Formicinae).

The ant fossil record is extensive, with more than 60 extant and 100 extinct genera. The oldest reliably dated fossils are ~100 million years (My) old, from Early Cretaceous French and Burmese ambers (14, 15). These include both *Gerontiformica* and *Burmomyrma* (Aneuretinae), with features typical of modern "crown group" ants, as well as Sphecomyrminae, with features typical of basal "stem group" ants. Although no older sphecomyrminae are known, the presence of stem and crown group ants in these roughly coeval ambers implies an earlier history of Formicidae. The status of the Armaniinae/-idae as stem group ants is controversial (1, 3, 15), but if they are viewed as sister to Formicidae, this also implies an extension of the minimum age of ants to the maximum age of Armaniinae/-idae, which has been estimated to be ~125 My (16).

<sup>1</sup>Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA. <sup>2</sup>School of Computational Science, Florida State University, 150-R Dirac Science Library, Tallahassee, FL 32306-4120, USA.

\*To whom correspondence should be addressed. E-mail: cmoreau@oeb.harvard.edu



**Fig. 1.** Phylogenetic chronogram of ants (A). Proportional lineages-through-time (LTT) plot for ants and distribution of clade ages for crown group ants (B). Bayesian posterior probability clade support of 100% is indicated by thick lines on topology. Fossil calibration points are indicated on topology. Shaded areas (green) delineate the rise of angiosperm dominance depending on whether minimum or maximum ages are used for fossil calibrations

(respective time scales are indicated above and below topology), and the overlap between these two regions corresponds to the greatest period of ant diversification. The LTT plot for ants shows the number of lineages present (as a proportion of terminals) at sequential time points. The shaded area (green) on the LTT plot and histogram likewise correspond to the overlapping region of the origin of angiosperm-dominated forests [(A) and (B)].

**Table 1.** Clade support for phylogenetic analyses and divergence time estimates. Support for clades recovered under Bayesian posterior probabilities, maximum likelihood bootstrap, and maximum parsimony bootstrap (bpp/ml bs/mp bs). Divergence time estimations  $\pm 1.96$  SD of the bootstrapped samples. \*, not applicable because only one taxon represents clade or outgroup; –, support less than 50%; †, minimum age of fossils used as calibration points (otherwise, maximum age of fossils used as calibration points); nm, not monophyletic; PL, penalized likelihood.

Clade	Support for combined analyses	Support for no missing data analyses	Support for Bayesian mixed model analysis	PL divergence time estimates	PL† divergence time estimates
Myrmicinae	100/100/100	100/100/98	100	114.0 $\pm$ 4.5	99.8 $\pm$ 4.2
Formicinae	100/100/100	100/100/100	100	101.4 $\pm$ 3.8	92.0 $\pm$ 0.2
Ectatomminae	100/100/98	100/100/95	100	92.3 $\pm$ 0.6	79.5 $\pm$ 0.9
Heteroponerinae	100/100/100	*/*/*	100	91.8 $\pm$ 2.7	79.0 $\pm$ 3.0
Dolichoderinae	100/100/98	100/100/98	100	96.6 $\pm$ 1.9	85.6 $\pm$ 2.2
Aneuretinae	*/*/*	*/*/*	*	124.6 $\pm$ 4.8	107.7 $\pm$ 5.4
Pseudomyrmecinae	100/100/100	100/100/100	100	59.2 $\pm$ 0.9	50.5 $\pm$ 1.2
Myrmeciinae	*/*/*	*/*/*	*	127.2 $\pm$ 2.2	108.3 $\pm$ 3.0
Aenictinae	100/100/100	*/*/*	100	37.3 $\pm$ 0.8	31.6 $\pm$ 1.1
Dorylinae	100/100/100	100/100/100	100	12.0 $\pm$ 0.4	10.2 $\pm$ 0.7
Ecitoninae	100/100/100	100/100/100	100	52.1 $\pm$ 1.0	44.2 $\pm$ 2.0
Cerapachyinae	76/–/–	*/*/*	–	nm	nm
Leptanilloidinae	100/100/100	*/*/*	100	9.7 $\pm$ 0.4	8.0 $\pm$ 0.3
Ponerinae	100/77/–	100/97/–	100	131.5 $\pm$ 5.9	110.7 $\pm$ 6.3
Agroecomyrmecinae	*/*/*	*/*/*	*	128.7 $\pm$ 4.5	108.2 $\pm$ 5.0
Paraponerinae	*/*/*	*/*/*	*	128.7 $\pm$ 4.5	108.2 $\pm$ 5.0
Amblyoponinae	–/–/–	–/–/–	100	143.1 $\pm$ 5.2	113.3 $\pm$ 4.9
Proceratiinae	89/71/–	*/*/*	99	131.9 $\pm$ 3.9	111.0 $\pm$ 3.5
Leptanillinae	100/100/100	100/100/100	100	123.0 $\pm$ 3.4	102.4 $\pm$ 4.1
Formicoid clade	100/100/93	100/55/93	100	147.0 $\pm$ 8.2	124.7 $\pm$ 6.5
Poneroid clade	100/64/–	100/97/–	95	152.4 $\pm$ 6.2	128.2 $\pm$ 5.9
Leptanilloid clade	100/94/60	100/87/65	100	123.0 $\pm$ 3.4	102.4 $\pm$ 4.1
Formicidae	100/100/100	*/*/*	100	168.8 $\pm$ 7.6	140.6 $\pm$ 8.0

Because the stratigraphic positions of some fossils in our analyses are not resolved within their dated formations, we conducted all analyses with both maximum and minimum ages for those fossils (Table 1). Our divergence time estimates (11) suggest that crown group ants last shared a common ancestor during the Early Cretaceous to Middle Jurassic:  $140 \pm 8.0$  million years ago (Ma) (using minimum ages) to  $168 \pm 7.6$  Ma (using maximum ages) (Fig. 1A). This is considerably older than the  $\sim 125$ -My age estimate based on fossil data. Our findings partially overlap with those of Crozier *et al.* (17), who used about six taxa and mitochondrial sequence data to estimate the age of Formicidae at  $185 \pm 36$  My.

Brady (18) and Ward and Brady (19) used molecular clock evidence to arrive at an age estimate of 130 to 140 My for crown group ants. Their studies were primarily aimed at dating specific lineages and sampled a limited number of fossils to provide minimum age calibration points. Our dates for the origin of the army ant clade ( $\sim 110$  Ma) are similar to those in Brady's study, but the inclusion of wider sampling and additional fossils leads us to an older estimate for the origin of extant ants.

From our analyses (11), we find that much of the diversification of the major ant lineages (Fig.

1A) occurred from the beginning of the Early Paleocene to the Late Cretaceous, 60 to 100 Ma, with ancestors of the major subfamilies present as early as 75 to 125 Ma. The fossil record, however, indicates that ants were relatively rare in the Cretaceous, with their march toward ecological dominance only beginning in the Eocene; they are represented by more than 90 species in  $\sim 45$  genera in Baltic amber, including many extant genera (15, 20). Our data suggest that most of the subfamilies representing extant ants arose much earlier than previously proposed but only began to diversify during the Late Cretaceous to Early Eocene. If ancestors of the major subfamilies were present as early as 75 to 125 Ma, why were they so slow to diversify?

We infer that the rise in angiosperm-dominated forests was harbinger to the diversification of the ants. The window encompassing angiosperm dominance shifts on our chronogram depending on whether we accept the minimum or maximum ages for the ant fossil calibration points (Fig. 1A, shaded green areas). A lineage-through-time (LTT) plot shows a dramatic accumulation of ant lineages at  $\sim 100$  Ma, either toward the end or immediately following the radiation of the angiosperms (Fig. 1B). These analyses indicate that ant diversification closely tracks the rise of angiosperm-dominated forests, between the Early

Paleocene and the Late Cretaceous, 60 to 100 Ma (21–24). The proliferation of angiosperms is thought to have driven the diversification of major herbivorous groups such as beetles (25, 26) and hemipterans (16), and it would appear that ant diversification, too, closely tracks the rise of angiosperm-dominated forests.

At least two explanations could account for these correlated patterns of diversification, although other, as yet unidentified causative factors may have been involved. First, the litter of angiosperm forests is more diverse, providing a wider array of habitats. Modern ant diversity is highest in the soil and ground litter of the world's angiosperm forests, particularly in the tropics (27). Second, the expansion of herbivorous insects provided both a direct food resource for hunting ants and an indirect one in the form of honeydew and larval secretions that “agricultural” ants could harvest. A substantial proportion of arboreal ants in modern Amazonian forests have been found to feed on secretions from Hemiptera and extrafloral nectarines (28, 29). In their dynastic-succession hypothesis of ant evolution, Wilson and Hölldobler (27) similarly stressed the importance of complex habitats provided by angiosperms and the transition from predation to harvesting secretions (16). Presumably this shift in diet also contributed to the evolution of associated social behaviors necessary to exploit and defend these food resources.

A robust hypothesis for the phylogeny of ants permits evolutionary investigation of life history, ecology, and biogeography in generating observed patterns of distribution and diversification of one of the most dominant animal groups. Our phylogenetic and molecular clock analyses of DNA from ants indicate that ants began to diversify much earlier than previously hypothesized and that the rise of the angiosperms may have directly influenced the diversification of this group. Since the mid-Mesozoic, ants have become the insect world's major predators, scavengers, and mutualists. Despite their dominance, we are only beginning to appreciate factors shaping the evolution of this group, highlighting the need for conservation of habitats harboring ant biodiversity, as well as further research on those lineages with poorly understood life histories.

#### References and Notes

- B. Bolton, *Synopsis and Classification of Formicidae* (American Entomological Institute, Gainesville, FL, 2003).
- C. Saux, B. L. Fisher, G. S. Spicer, *Mol. Phylogenet. Evol.* **33**, 457 (2004).
- M. S. Engel, D. A. Grimaldi, *Am. Mus. Novit.* **3485**, 1 (2005).
- B. Hölldobler, E. O. Wilson, *The Ants* (Harvard Press, Cambridge, MA, 1990).
- C. Baroni Urbani, B. Bolton, P. S. Ward, *Syst. Entomol.* **17**, 301 (1992).
- P. S. Ward, *Syst. Entomol.* **19**, 159 (1994).
- D. Grimaldi, D. Agosti, J. M. Carpenter, *Am. Mus. Novit.* **3208**, 1 (1997).
- H. Ohnishi, H. T. Imai, M.-T. Yamamoto, *Genes Genet. Syst.* **78**, 419 (2003).
- C. Astruc, J. F. Julien, C. Errard, A. Lenoir, *Mol. Phylogenet. Evol.* **31**, 880 (2004).
- P. S. Ward, S. G. Brady, B. L. Fisher, T. R. Schultz, *Myrmecologische Nachrichten* **7**, 87 (2005).



11. Materials and methods are available as supporting material on *Science Online*.
12. R. W. Taylor, *Science* **201**, 979 (1978).
13. S. O. Shattuck, *Syst. Entomol.* **17**, 199 (1992).
14. A. Nel, G. Perrault, V. Perrichot, D. Néraudeau, *Geol. Acta* **2**, 23 (2004).
15. G. M. Dlussky, A. P. Rasnitsyn, *Russ. Entomol. J.* **11**, 411 (2003).
16. D. Grimaldi, M. S. Engel, *Evolution of the Insects* (Cambridge Univ. Press, New York, NY, 2005).
17. R. H. Crozier, L. S. Jermin, M. Chiotis, *Naturwissenschaften* **84**, 22 (1997).
18. S. G. Brady, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6575 (2003).
19. P. S. Ward, S. G. Brady, *Invert. Syst.* **17**, 361 (2003).
20. G. M. Dlussky, *Paleontol. J.* **31**, 616 (1997).
21. P. R. Crane, E. M. Friis, K. R. Pedersen, *Nature* **374**, 27 (1995).
22. H. Schneider *et al.*, *Nature* **428**, 553 (2004).
23. C. D. Bell, D. E. Soltis, P. S. Soltis, *Evolution Int. J. Org. Evolution* **59**, 1245 (2005).
24. C. C. Davis, C. O. Webb, K. J. Wurdack, C. A. Jaramillo, M. J. Donoghue, *Am. Nat.* **165**, E36 (2005).
25. B. Farrell, *Science* **281**, 555 (1998).
26. P. Wilf *et al.*, *Science* **289**, 291 (2000).
27. E. O. Wilson, B. Hölldobler, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7411 (2005).
28. J. E. Tobin, in *Nourishment and Evolution in Insect Societies*, J. H. Hunt, C. A. Nalepa, Eds. (Westview Press, Boulder, CO, 1994), pp. 279–307.
29. D. W. Davidson, S. C. Cook, R. R. Snelling, T. H. Chua, *Science* **300**, 969 (2003).
30. We thank the following for the use of specimens: G. D. Alpert, A. N. Andersen, C. J. Burwell, S. P. Cover, L. Davis, M. A. Deyrup, D. Donoso, R. Eastwood, K. Eguchi, X. Espadaler, P. R. Fernández, B. L. Fisher, D. M. General, R. A. Johnson, J. E. Lattke, D. J. Lohman, J. T. Longino, D. B. Merrill, H. G. Robertson, C. Schöning, M. A. Travassos, E. O. Wilson, and K. Yeo. E. O. Wilson, S. P. Cover, G. D. Alpert, and A. J. Berry gave useful suggestions and comments on earlier versions of the manuscript. We thank M. Cornwall, S. P. Cover, S. V. Edwards, B. D. Farrell, K. M. Horton, C. Labandeira, J. E. Moreau, B. A. Perry, J. B. Plotkin, S. Peck Quek, E. O. Wilson, and J. Zhang for assistance during the preparation of this manuscript and J. M. Girard for assistance in the laboratory. For access to computational resources, we thank D. L. Swofford (NSF Information Technology Resources Program grant EF 03-31495, Florida State University). This research was supported by a grant from the Green Fund to C.S.M. and an NSF DEB-0447242 grant to N.E.P.

### Supporting Online Material

[www.sciencemag.org/cgi/content/full/312/5770/101/DC1](http://www.sciencemag.org/cgi/content/full/312/5770/101/DC1)

Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 to S4

References and Notes

Appendices S1 to S2

12 January 2006; accepted 1 March 2006

10.1126/science.1124891

# Platelet-Derived Serotonin Mediates Liver Regeneration

Mickael Lesurtel,<sup>1</sup> Rolf Graf,<sup>1</sup> Boris Aleil,<sup>3</sup> Diego J. Walther,<sup>4</sup> Yinghua Tian,<sup>1</sup> Wolfram Jochum,<sup>2</sup> Christian Gachet,<sup>3</sup> Michael Bader,<sup>5</sup> Pierre-Alain Clavien<sup>1\*</sup>

The liver can regenerate its volume after major tissue loss. In a mouse model of liver regeneration, thrombocytopenia, or impaired platelet activity resulted in the failure to initiate cellular proliferation in the liver. Platelets are major carriers of serotonin in the blood. In thrombocytopenic mice, a serotonin agonist reconstituted liver proliferation. The expression of 5-HT<sub>2A</sub> and 2B subtype serotonin receptors in the liver increased after hepatectomy. Antagonists of 5-HT<sub>2A</sub> and 2B receptors inhibited liver regeneration. Liver regeneration was also blunted in mice lacking tryptophan hydroxylase 1, which is the rate-limiting enzyme for the synthesis of peripheral serotonin. This failure of regeneration was rescued by reloading serotonin-free platelets with a serotonin precursor molecule. These results suggest that platelet-derived serotonin is involved in the initiation of liver regeneration.

**S**erotonin (5-hydroxytryptamine, 5-HT) is not only a neurotransmitter but also a hormone with various extraneuronal functions (1). It is a potent mitogen and modulates the remodeling of tissue (2–5). Platelets (thrombocytes) carry serotonin in the blood and release it at sites of tissue injury as part of their action on hemostasis (6–8). However, platelets are also involved in the inflammatory reaction after tissue injury, which is independent of coagulation (9). In the liver, platelets interact with leukocytes in response to cold ischemia and induce them to adhere to the endothelium of blood vessels, thereby enhancing tissue injury (10, 11). Concurrent activation of liver macrophages called Kupffer cells leads to further endothelial cell damage and hepatocyte apoptosis (12). Depending on the extent of initial

tissue injury, the liver can regenerate in a highly synchronized and organized fashion. Because platelets interact with endothelial cells in the early phase after injury, they might also have an effect on the initiation of liver regeneration.

To establish the role of platelets and their secretory products in liver regeneration, partial hepatectomy was performed in mice in which platelet function was inhibited pharmacologically or platelets were depleted. Initially, thrombocytopenia was induced by injecting busulfan, an alkylating agent that causes massive loss of platelets (13). Furthermore, platelets were functionally targeted by the application of clopidogrel, which selectively and irreversibly antagonizes the P2Y<sub>12</sub> adenosine diphosphate (ADP) receptors on platelets, leading to the inhibition of platelet aggregation (14). After injection of these drugs in mice, a 70% hepatectomy was performed to study regeneration of the liver. Although control animals reacted with an increase in hepatic proliferation [5-bromo-2'-deoxyuridine (BrdU)-, Ki67-, and proliferating cell nuclear antigen (PCNA)-positive] 2 days after hepatectomy, busulfan-injected mice exhibited a reduced response (Fig. 1, A to C, and E). In busulfan-treated mice, the number of platelets was reduced in a dose-

dependent fashion and the leukocyte count was decreased, but erythrocytes were unaffected (Fig. 1D). Thus, these mice exhibited a combined thrombocytopenia and leukopenia. The impairment of hepatocyte proliferation after hepatectomy may be attributed to a lack of each cell type alone or a combination of both.

To investigate the role of platelets more selectively, an antibody to GPIIb $\alpha$  recognizing an epitope on platelets was injected into mice before hepatectomy (15). The number of platelets fell below 10% (Fig. 2A), whereas leukocyte and erythrocyte counts were not affected (Fig. 2, B and C), indicating a specific thrombocytopenia. After 70% hepatectomy, all markers of hepatocellular proliferation were reduced (Fig. 2, D to F) in thrombocytopenic mice.

We also tested whether the inhibition of platelet activity, without affecting the number of platelets, was sufficient to block liver regeneration. Clopidogrel, which inhibits the aggregation response to ADP without affecting platelet stability, reduced hepatocyte proliferation in partially hepatectomized livers, but this effect was less pronounced than in busulfan-treated mice. In mice treated with an enantiomer of clopidogrel, which lacks antiaggregation properties, proliferation was not different from controls (Fig. 1, A to C).

Platelets store and release serotonin. About 95% of all serotonin found in blood is stored in platelets. In vitro, serotonin is a potent mitogen and stimulates hepatocyte mitosis (3, 16). The 5-HT<sub>2A</sub> and 1C receptors appear to mediate mitogenic effects in fibroblasts (17, 18), and the 5-HT<sub>2B</sub> receptor is involved in the development of the heart (19) and the enteric nervous system (20). To test whether serotonin induces hepatocyte proliferation in vivo, thrombocytopenic mice were treated with the serotonin receptor 5-HT<sub>2A/2C</sub> agonist ( $\pm$ )-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI-hydrochloride). The application of this drug had no effect on the extent of thrombocytopenia (Fig. 2G) induced by concurrent treatment with the antibody to GPIIb $\alpha$ . In the presence of the serotonin agonist, proliferation was completely restored (Fig. 2, D to F).

<sup>1</sup>Department of Visceral and Transplantation Surgery and <sup>2</sup>Department of Pathology, University Hospital of Zurich, Switzerland. <sup>3</sup>Institut National de la Santé et de la Recherche Médicale 311, Etablissement Français du Sang-Alsace, Strasbourg, France. <sup>4</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany. <sup>5</sup>Max Delbrück Center for Molecular Medicine, Berlin, Germany.

\*To whom correspondence should be addressed. E-mail: [clavien@chir.unizh.ch](mailto:clavien@chir.unizh.ch)