The Plant Cell Wall–Decomposing Machinery Underlies the Functional Diversity of Forest Fungi

Daniel C. Eastwood, *et al.*

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binding site (fig. S8A and fig. S9), Get1 must displace helix $\alpha2^{Get2}$, which is connected to helix $\alpha1^{Get2}$ by the flexible glycine linker. NMR analyses revealed that Get1 binding indeed causes some Get2 interactions with Get3 to disappear. Specifically, interaction with Get2 was observed in the region of L4 to A49, and upon addition of Get1, residues G24 to A49 no longer interacted with Get3 (fig. S10). This shows that helix $\alpha2^{Get2}$ is no longer bound to Get3 in the ternary complex.

On the basis of different crystal structures of Get3, we previously proposed a model for how the Get3 ATPase regulates TA protein insertion (*19*). With structures of different Get3-receptor complexes as well as functional data in hand, distinct docking states can be integrated into this model (Fig. 4). Assisted by Get4/5/Sgt2, TA proteins bind to Get3-ATP-Mg^{2+} (step 1). After ATP hydrolysis, the reaction products stay trapped, and the energy gained from hydrolysis is stored in a strained conformation (*19*). The N terminus of Get2 tethers the Get3/TA protein complex to the ER membrane (step 2). Binding of Get1 displaces $\alpha2^{Get2}$, and the Get3/TA protein complex is now docked to the receptor complex at the membrane (step 3). When the TA protein is released, Get3 relaxes to the closed state, and inorganic phosphate dissociates (step 4). According to the crystal structures, Get1 can stay bound to Get3 during the transition from the closed to the open state. What actually triggers opening of Get3? We favor the idea that the energy from ATP hydrolysis drives Get3 to the open state, and ADP-Mg^{2+} leaves by way of the observed tunnels. In this state, Get1 interferes with nucleotide binding and prevents closure of the dimer. Finally, binding of ATP facilitates dissociation of Get3 (step 5), which sets the stage for the next targeting cycle. As Get1-CD is rigidly linked to the TMDs, structural changes observed in the Get3/Get1 complexes can be extrapolated to the complete membrane receptor (as indicated in Fig. 4 and fig. S11). The opening of Get3 during TA protein insertion may create a force that is directly transferred to the TMDs of the receptor, which could contribute to TA protein insertion. Related structural transitions have been reported for ATP-binding cassette (ABC) transporter proteins (*27*, *28*). In Get1, the coiled-coil domain with the tip helix may have a function similar to the coupling helix in ABC transporters and may directly communicate nucleotide-dependent changes in Get3 to the transmembrane segments as anticipated in the model above. It is now important to dissect the precise mechanism of TA protein insertion and to see whether a general concept can be derived that is shared by different membrane transport systems.

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Supporting Online Material

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Materials and Methods
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The Plant Cell Wall–Decomposing Machinery Underlies the Functional Diversity of Forest Fungi

Daniel C. Eastwood,[1]*† Dimitrios Floudas,[2]* Manfred Binder,[2]* Andrzej Majcherczyk,[3]* Patrick Schneider,[4]* Andrea Aerts,[5] Fred O. Asiegbu,[6] Scott E. Baker,[7] Kerrie Barry,[5] Mika Bendiksby,[8] Melanie Blumentritt,[9] Pedro M. Coutinho,[10] Dan Cullen,[11] Ronald P. de Vries,[12] Allen Gathman,[13] Barry Goodell,[9,14] Bernard Henrissat,[10] Katarina Ihrmark,[15] Hävard Kauserud,[16] Annegret Kohler,[17] Kurt LaButti,[5] Alla Lapidus,[5] José L. Lavin,[18] Yong-Hwan Lee,[19] Erika Lindquist,[5] Walt Lilly,[13] Susan Lucas,[5] Emmanuelle Morin,[17] Claude Murat,[17] José A. Oguiza,[18] Jongsun Park,[19] Antonio G. Pisabarro,[18] Robert Riley,[5] Anna Rosling,[15] Asaf Salamov,[5] Olaf Schmidt,[20] Jeremy Schmutz,[5] Inger Skrede,[16] Jan Stenlid,[15] Ad Wiebenga,[12] Xinfeng Xie,[9] Ursula Kües,[3]* David S. Hibbett,[2]* Dirk Hoffmeister,[4]* Nils Högberg,[15]* Francis Martin,[17]* Igor V. Grigoriev,[5]* Sarah C. Watkinson[21]*

Brown rot decay removes cellulose and hemicellulose from wood—residual lignin contributing up to 30% of forest soil carbon—and is derived from an ancestral white rot saprotrophy in which both lignin and cellulose are decomposed. Comparative and functional genomics of the "dry rot" fungus *Serpula lacrymans*, derived from forest ancestors, demonstrated that the evolution of both ectomycorrhizal biotrophy and brown rot saprotrophy were accompanied by reductions and losses in specific protein families, suggesting adaptation to an intercellular interaction with plant tissue. Transcriptome and proteome analysis also identified differences in wood decomposition in *S. lacrymans* relative to the brown rot *Postia placenta*. Furthermore, fungal nutritional mode diversification suggests that the boreal forest biome originated via genetic coevolution of above- and below-ground biota.

Many Agaricomycete fungi have been sequenced to date (*1*), permitting comparative and functional genomic analyses of nutritional niche adaptation in the underground fungal networks that sustain boreal, temperate, and some subtropical forests (*2*). Through the sequencing of the brown rot wood decay fungus *Serpula lacrymans*, we conducted genome comparisons with sequenced fungi, including species representing each of a range of functional niches: brown rot and white rot wood decay, parasitism, and mutualistic ectomycorrhizal symbiosis.

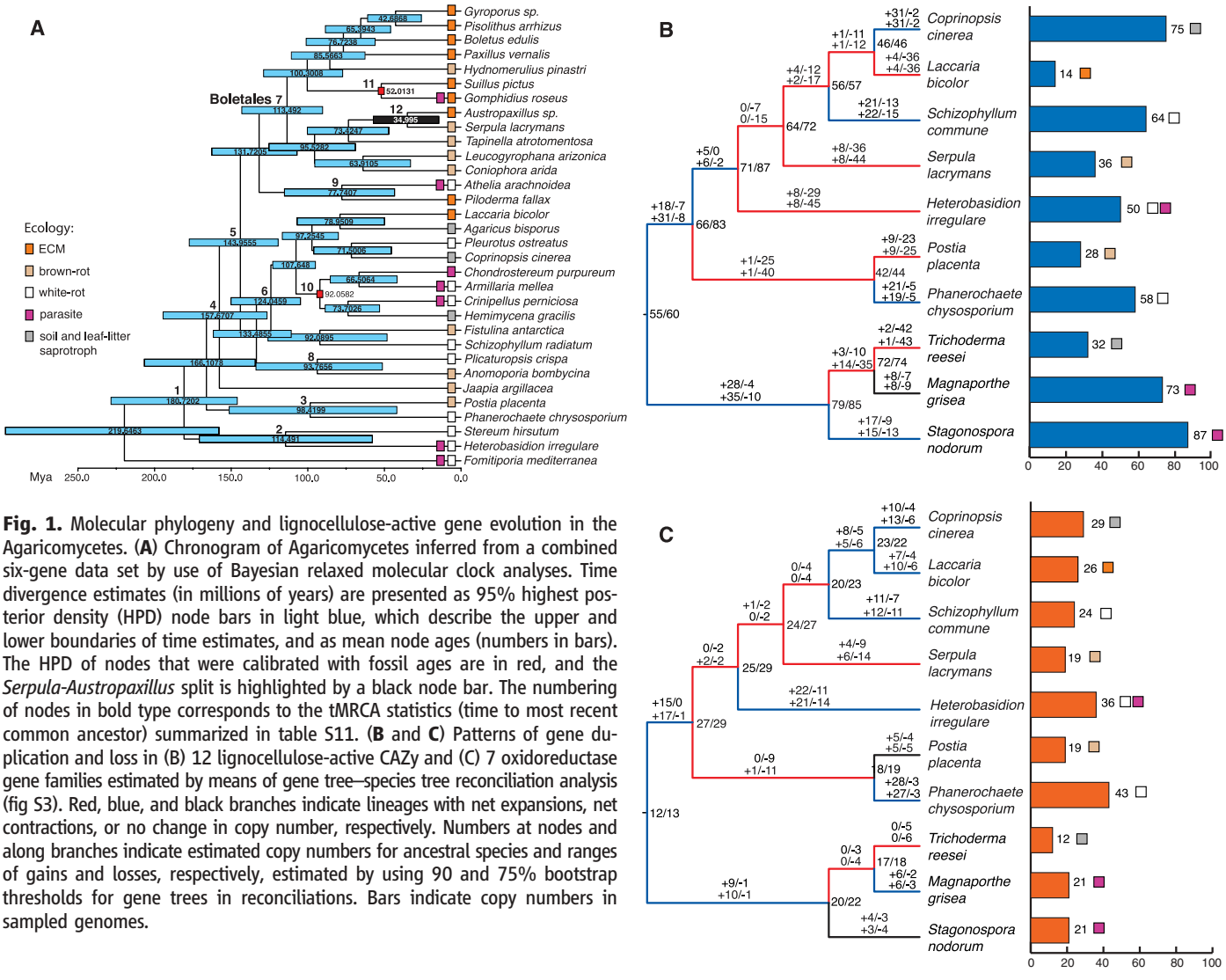

Fig. 1. Molecular phylogeny and lignocellulose-active gene evolution in the Agaricomycetes. (**A**) Chronogram of Agaricomycetes inferred from a combined six-gene data set by use of Bayesian relaxed molecular clock analyses. Time divergence estimates (in millions of years) are presented as 95% highest posterior density (HPD) node bars in light blue, which describe the upper and lower boundaries of time estimates, and as mean node ages (numbers in bars). The HPD of nodes that were calibrated with fossil ages are in red, and the *Serpula-Austropaxillus* split is highlighted by a black node bar. The numbering of nodes in bold type corresponds to the tMRCA statistics (time to most recent common ancestor) summarized in table S11. (**B** and **C**) Patterns of gene duplication and loss in (B) 12 lignocellulose-active CAZy and (C) 7 oxidoreductase gene families estimated by means of gene tree–species tree reconciliation analysis (fig S3). Red, blue, and black branches indicate lineages with net expansions, net contractions, or no change in copy number, respectively. Numbers at nodes and along branches indicate estimated copy numbers for ancestral species and ranges of gains and losses, respectively, estimated by using 90 and 75% bootstrap thresholds for gene trees in reconciliations. Bars indicate copy numbers in sampled genomes.

Only 6% of wood-decay species are brown rots (*3*), but being associated with conifer wood (*4*), they dominate decomposition in boreal forests. Their lignin residues contribute up to 30% of carbon in the organic soil horizons (*5*). Long-lived (*6*) and with capacity to bind nitrogen and cations (*7*), these phenolic polymers condition the nutrient-poor acidic soils of northern conifer forests.

Brown rot wood decay involves an initial non-enzymic attack on the wood cell wall (*8*), generating hydroxyl radicals (·OH) extracellularly via the Fenton reaction:

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {\cdot}OH + H_2O$$

Hydrogen peroxide is metabolically generated by oxidase enzymes such as glyoxal oxidases and copper radical oxidases. The hydroxyl radical has a half-life of nanoseconds (*8*) and is the most powerful oxidizing agent of living cells. However, we do not know how it is spatially and temporally targeted to wood cell wall components. Divalent iron is scarce in aerobic environments, where the fungus is obligate and the trivalent ion is energetically favored. Phenolates synthesized by brown rot fungi, including *S. lacrymans* (*9*), can reduce Fe^{3+} to Fe^{2+}. Such phenolates may be modified lignin derivatives or fungal metabolites (*10*). After initial bond breakages in the cellulose chain, side chain hemicelluloses (arabinan and galactan) are removed, followed by main chains [xylan and mannan (*11*)], with subsequent hydrolysis of cellulose by synergistic glycoside hydrolases

[1]College of Science, University of Swansea, Singleton Park, Swansea SA2 8PP, UK. [2]Department of Biology, Clark University, Worcester, MA 01610, USA. [3]Georg-August-University Göttingen, Büsgen-Institute, Büsgenweg 2, 37077 Göttingen, Germany. [4]Friedrich-Schiller-Universität, Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany. [5]U.S. Department of Energy Joint Genome Institute, Walnut Creek, CA 94598, USA. [6]Department of Forest Sciences, Box 27, University of Helsinki, Helsinki 00014, Finland. [7]Pacific Northwest National Laboratory, 902 Battelle Boulevard, Post Office Box 999, MSIN P8-60, Richland, WA 99352, USA. [8]Natural History Museum, University of Oslo, Post Office Box 1172, Blindern, NO-0138, Norway. [9]Wood Science and Technology, University of Maine, Orono, ME 04469–5755, USA. [10]UMR 6098 CNRS–Universités Aix-Marseille I and II, 13288 Marseille Cedex 9, France. [11]Forest Products Laboratory, Madison, WI 53726, USA. [12]Centraalbureau voor Schimmelcultures–Royal Netherlands Academy of Arts and Sciences Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, Netherlands. [13]Department of Biology, Southeast Missouri State University, Cape Girardeau, MO 63701, USA. [14]Department of Wood Science and Forest Products, 230 Cheatham Hall, Virginia Tech, Blacksburg, VA 24061, USA. [15]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden. [16]Department of Biology, University of Oslo, Post Office Box 1066 Blindern, N-0316 Oslo, Norway. [17]UMR 1136, Institut National de la Recherche Agronomique (INRA)–Nancy Université, Interactions Arbres/Microorganismes, INRA-Nancy, 54280 Champenoux, France. [18]Department of Agrarian Production, Public University of Navarre, 31006 Pamplona, Spain. [19]Department of Agricultural Biotechnology, Seoul National University, Seoul 151*921, Korea. [20]Department of Wood Biology, University of Hamburg, Leuschnerstrasse 91, D-21031 Hamburg, Germany. [21]Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, UK.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: d.c.eastwood@swansea.ac.uk

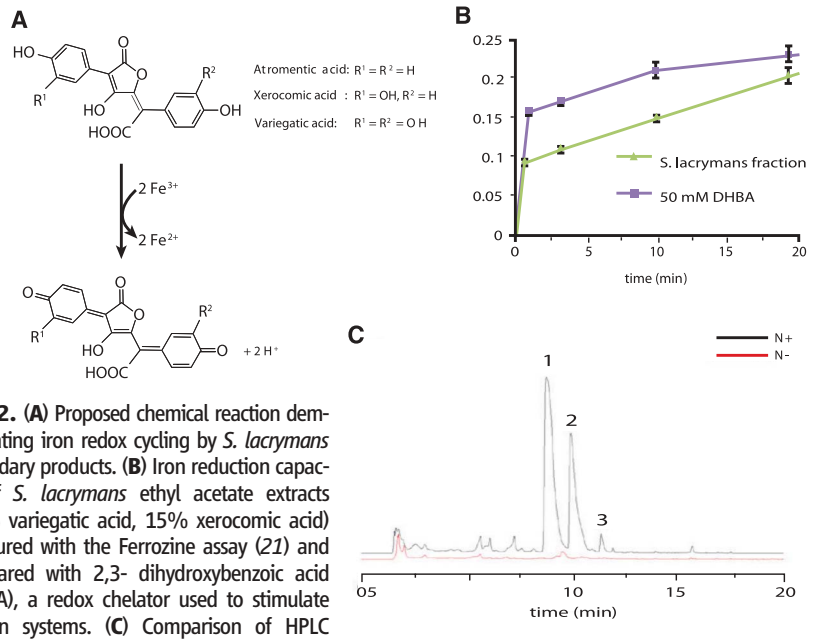

Fig. 2. (**A**) Proposed chemical reaction demonstrating iron redox cycling by *S. lacrymans* secondary products. (**B**) Iron reduction capacity of *S. lacrymans* ethyl acetate extracts (60% variegatic acid, 15% xerocomic acid) measured with the Ferrozine assay (*21*) and compared with 2,3- dihydroxybenzoic acid (DHBA), a redox chelator used to stimulate Fenton systems. (**C**) Comparison of HPLC chromatograms of *S. lacrymans* ethyl acetate extracts as a function of nitrogen supply. Red trace, nitrogen rich medium (+N); black trace, nitrogen-depleted minimal medium (–N). The identity of the compounds was confirmed with mass spectrometry and by their ultraviolet-visual spectrum (1, variegatic acid; 2, xerocomic acid; 3, atromentic acid).

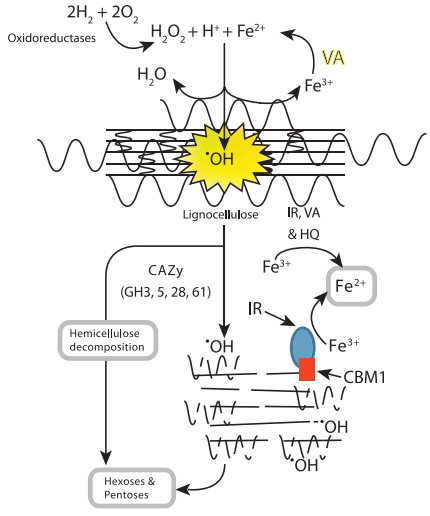

Fig. 3. Schematic overview of the proposed mechanism of wood decay by *S. lacrymans*. Scavenging mycelium colonizes a new food source, inducing VA production and expression of oxidoreductase enzymes, which drive hydroxyl radical attack on the lignocellulose composite. CAZy gain access to the weakened composite structure and break down accessable carbohydrates. Cellulose-binding iron reductase targets ·OH-generating Fenton's reaction on cellulose chains, releasing chain ends for hydrolysis and assimilation. IR, iron reductase; HQ, hydroxyquinones; CBM, cellulose-binding module.

(GHs). Residual lignin is demethylated. In contrast, white rot fungi decompose both cellulose and lignin, with free radical attack theorized to break a variety of bonds in the lignin phenylpropanoid heteropolymer.

S. lacrymans is in the Boletales, along with several ectomycorrhizal lineages (Fig. 1A) (*12*). *S. lacrymans* is thus phylogenetically distant from brown rot *Postia placenta* (*Polyporales*) (*13*), as well as other sequenced ectomycorrhizal fungi (*14*, *15*), parasites, and white rot wood decomposers (*16*). We estimated divergence dates in fungal phylogeny using the data set of Binder *et al.* [supporting online material (SOM), molecular clock analyses] (*17*), with two well-characterized fungal fossils that were used to calibrate the minimum ages of the marasmioid (Fig. 1A, node 10) and suilloid clades (Fig. 1A, node 11). The estimated age of the split between *Serpula* and its ectomycorrhizal sister-group *Austropaxillus* (53.1 to 15 million years ago) (Fig. 1A and table S11) suggests that transition from brown rot saprotrophy to mutualistic symbiosis occurred after rosids (Eurosids I) became widespread (Fig. 1A) (*18*). Diversification in fungal nutritional modes occurred alongside diversification of angiosperms and gymnosperms, as these fungi are currently associated with members of both gymnosperms (Pinaceae) and angiosperms (*18*).

S. lacrymans comprises two subgroups that diverged in historic time (*19*), *S. lacrymans* var. *shastensis*, which is found in montane conifer forest, and *S. lacrymans* var. *lacrymans*, which is a cause of building dry rot. Two *S. lacrymans* var. *lacrymans* complementary monokaryons (haploids of strain S7), S7.9 (A2B2) and S7.3 (A1B1) (*20*), were sequenced via Sanger and 454 pyrosequencing, respectively. The genome of *S. lacrymans* S7.9 was 42.8 megabase pairs (Mbp), containing 12,917 gene predictions (*21*).

We analyzed 19 gene families of enzymes for lignocellulose breakdown: carbohydrate active enzymes (CAZys; www.cazy.org) (*22*) (GHs and carbohydrate esterases) and oxidoreductases (table S9). Losses and expansions in these families were compared across 10 fungi, including Agaricomycetes, with a range of nutritional modes (Fig. 1, B and C, and table S9). Convergent changes in enzyme complement were found in the two independently evolved brown rot species, with parallels in the ectomycorrhizal *Laccaria bicolor* (fig. S3 and table S9). The inferred most recent common ancestor of the Agaricales, Boletales, and Polyporales is predicted to be a white rot with 66 to 83 hydrolytic CAZy genes and 27 to 29 oxidoreductases (Fig. 1, B and C). Brown rot and ectomycorrhizal fungi have the fewest hydrolytic CAZy genes. Brown rot fungi have the fewest oxidoreductases, not because of gene losses but because of gene duplications in white rot species.

Both brown rot and ectomycorrhizal fungi lacked class II peroxidases, which are used by white rot fungi in depolymerizing the lignin matrix of wood and unmasking usable cellulose embedded within it. This family was expanded in the white rots *Coprinopsis cinerea*, *Phanerochaete chrysosporium*, and *Schizophyllum commune*, with 29, 43, and 24 genes, respectively, with only 19 each in *S. lacrymans* and *P. placenta*. Oxidoreductases conserved in brown rot fungi included iron and quinone reductases and multicopper oxidases (fig. S3 and table S8). Absence of ligninolysis in brown rots raises the question of how they achieve pervasive cellulolysis in wood with the lignin matrix intact.

GH gene families had parallel patterns of losses and expansion in both brown rots and ectomycorrhizas. CAZy families GH5 (endoglucanases, hydrolyzing cellulose) and GH28 (pectinases, hydrolyzing intercellular cohesive polysaccharides in plant tissues) were expanded in both brown rot species, in which they might facilitate intercellular enzyme diffusion, and retained in *L. bicolor*, in which they might facilitate intercellular penetration of living roots. Both brown rot species lacked GH7 (endoglucanase/cellobiohydrolase CBHI), and GH61 genes—with unknown function but recently implicated in oxidative attack on polysaccharides (*23*)—were reduced. GH6 (cellobiohydrolase CBHII) and cellulose-binding modules (CBM1), which were absent from *P. placenta* (*13*), were present in *S. lacrymans*. One CBM was associated with an iron reductase in a gene (*S. lacrymans* S7.9 database protein ID, 452187) originally derived from a cellobiose dehydrogenase (fig. S5).

The general utility of the conserved suite of GH genes in wood decay by *S. lacrymans* was supported through transcriptomic and proteomic analysis. Carbohydrate-active enzymes accounted for 50% of proteins identified (table S14), and 33.9% of transcripts regulated greater than 20-fold by *S. lacrymans* growing on pine wood as compared with glucose medium (fig. S4).

Cellulose-, pectin-, and hemicellulose-degrading enzymes (GH families 5, 61, 3, and 28) were prominent, and GH5 endoglucanase (*S. lacrymans* S7.9 database protein ID, 433209) and GH74 endoglucanase/xyloglucanase (*S. lacrymans* S7.9 database protein ID, 453342) were up-regulated greater than 100-fold.

We conclude that brown rot fungi have cast off the energetically expensive apparatus of ligninolysis and acquired alternative mechanisms of initial attack. Wood decomposition by *S. lacrymans* may involve metabolically driven nonenzymatic disruption of lignocellulose with internal breakage of cellulose chains by highly localized ·OH radical action. Mycelia in split plates mimicking realistic nutrient heterogeneity (fig. S1) produced variegatic acid (VA), an iron-reducing phenolate (Fig. 2, A to C), via the Boletales atromentin pathway, which was recruited in *S. lacrymans* for the Fenton's reaction. The genome was rich in secondary metabolism genes (table S15), including a putative atromentin locus (*24*). Mycelium imports amino acids to sites of wood colonization (*25*), which is consistent with observed up-regulation of oligopeptide transporters on wood (table S12). Localizing variegatic acid production to well-resourced parts of the mycelium could enhance Fenton's chemistry in contact with wood.

Wood colonization is presumably followed by coordinated induction of the decay machinery revealed in the wood-induced transcriptome (Fig. 3 and fig. S4). GHs and oxidoreductases accounted for 20.7% of transcripts, accumulating greater than fourfold on wood relative to glucose medium (fig. S4 and table S12). Iron reduction mechanisms included an enzyme harboring a C terminal cellulose-binding module (*S. lacrymans* S7.9 database protein ID, 452187) (fig. S5) that is up-regulated 122-fold on wood substrate (fig. S4 and table S12). This enzyme, which is present in *Ph. chrysosporium* but absent from *P. placenta* (*26*), is a potential docking mechanism for localizing iron reductase activity, and hence ·OH generation, on the surface of microcrystalline cellulose. Cellulose-targeted iron reduction, combined with substrate induction of variegatic acid biosynthesis, might explain the particular ability of brown rot fungi in Boletales to degrade unassociated microcrystalline cellulose without the presence of lignin (*27*).

Thus, comparative genomics helps us understand the molecular processes of forest soil fungi that drive the element cycles of forest biomes (*28*). Sequenced forest Agaricomycetes revealed shared patterns of gene family contractions and expansions associated with emergences of both brown rot saprotrophy and ectomycorrhizal symbiosis. In Boletales, loss of aggressive ligninolysis might have permitted brown rot transitions to biotrophic ectomycorrhiza, which is promoted in soils impoverished in nitrogen by brown rot residues, and by the nutritional advantage conferred by the connection to a mycorrhizal network. *S. lacrymans* and other fungi cultured with conifer roots (*29*) ensheath *Pinus sylvestris* roots with a mantle-like layer (fig. S6), suggesting nutrient exchange.

The chronology of divergences in extant fungal nutritional mode (Fig. 1A) matches the predicted major diversification in conifers (*18*), suggesting that the boreal forest biome may have originated via genetic coevolution of above- and belowground biota.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S6
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References (*30*–*89*)

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The Leukemogenicity of AML1-ETO Is Dependent on Site-Specific Lysine Acetylation

Lan Wang,[1] Alexander Gural,[1] Xiao-Jian Sun,[2] Xinyang Zhao,[1] Fabiana Perna,[1] Gang Huang,[1] Megan A. Hatlen,[1] Ly Vu,[1] Fan Liu,[1] Haiming Xu,[1] Takashi Asai,[1] Hao Xu,[1] Tony Deblasio,[1] Silvia Menendez,[1] Francesca Voza,[1] Yanwen Jiang,[3] Philip A. Cole,[4] Jinsong Zhang,[5] Ari Melnick,[3] Robert G. Roeder,[2] Stephen D. Nimer[1]*

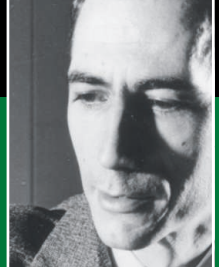
The chromosomal translocations found in acute myelogenous leukemia (AML) generate oncogenic fusion transcription factors with aberrant transcriptional regulatory properties. Although therapeutic targeting of most leukemia fusion proteins remains elusive, the posttranslational modifications that control their function could be targetable. We found that AML1-ETO, the fusion protein generated by the t(8;21) translocation, is acetylated by the transcriptional coactivator p300 in leukemia cells isolated from t(8;21) AML patients, and that this acetylation is essential for its self-renewal–promoting effects in human cord blood CD34$^+$ cells and its leukemogenicity in mouse models. Inhibition of p300 abrogates the acetylation of AML1-ETO and impairs its ability to promote leukemic transformation. Thus, lysine acetyltransferases represent a potential therapeutic target in AML.

Histone-modifying enzymes can regulate the binding of specific chromatin-binding proteins to histone marks and can change the affinity of the histones for DNA (*1*, *2*). These enzymes also affect nonhistone proteins, and posttranslational modifications of transcription factors such as p53 or AML1 (which is required for definitive hematopoietic development) can

ERRATUM

Post date 30 September 2011

Reports: "The plant cell wall–decomposing machinery underlies the functional diversity of forest fungi" by D. C. Eastwood *et al.* (5 August, p. 762). The second sentence of the caption for Fig. 2C should read, "Black trace, nitrogen-rich medium (+N); red trace, nitrogen-depleted minimal medium (–N)."



LETTERS

edited by Jennifer Sills

Retraction

IN OUR 2006 REPORT, "DESORPTION OF H FROM SI(111) BY RESONANT EXCITATION OF THE Si-H vibrational stretch mode" (*1*), we reported resonant photodesorption of hydrogen from a Si(111) surface using tunable infrared radiation that corresponded to the Si-H vibrational stretch mode. Our recent attempts to reproduce these experiments have been unsuccessful, and the free electron laser facility at Vanderbilt, a unique light source for this experiment, has shut down, prohibiting further research. Because our conclusions are now in question, we retract the Report.

ZHIHENG LIU,[1,2]* L. C. FELDMAN,[2,3]† N. H. TOLK,[2] ZHENYU ZHANG,[3,4] P. I. COHEN[1]‡

[1]Department of Electrical and Computer Engineering, University of Minnesota, Minneapolis, MN 55455, USA. [2]Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37235, USA. [3]Materials Science and Technology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA. [4]Department of Physics and Astronomy, University of Tennessee, Knoxville, TN 37996, USA.

*Present address: Department of Physics, The City University of New York, Brooklyn, NY 11210, USA.

†Present address: Institute for Advanced Materials, Devices, and Nanotechnology, Rutgers University, Piscataway, NJ 08854–8065, USA.

‡To whom correspondence should be addressed. E-mail: picohen@umn.edu

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Editor's Note

IN THE REVIEW "CHINA'S DEMOGRAPHIC history and future challenges" in the 29 July special section on Population (*1*), Fig. 1 showed a map of the South China Sea. We have become aware that some readers are interpreting the publication of this map as a statement by *Science* on the maritime borders marked in the image. This is not the case.

Science's policy, found on the masthead page of each issue, states that "all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by AAAS or the institutions with which the authors are affiliated." *Science* does not have a position with regard to jurisdictional claims in the area of water included in the map. We are reviewing our map acceptance procedures to ensure that in the future *Science* does not appear to endorse or take a position on territorial/jurisdictional disputes.

MONICA BRADFORD

Executive Editor

Reference

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Tiger Conservation: Trust Tradition

IN THEIR LETTER "RESTORING TIGERS TO THE Caspian region" (12 August, p. 822), C. A. Driscoll *et al.* propose the reintroduction of tigers into the historic range of the extinct Caspian tiger. Driscoll *et al.* assert that new approaches such as this one are needed because "traditional conservation approaches are proving insufficient." We disagree.

Tiger biologists and conservationists have shown how to save tigers. So-called traditional approaches—including law enforcement, scientific assessments, monitoring of tiger and prey populations, and community outreach—are demonstrably effective in reversing tiger declines when properly implemented by conservation nongovernmental organizations (NGOs) and government agencies (*1*–*6*). New approaches should always be considered in our efforts to save the tiger, but the focus must be on addressing the most critical threats to those remaining tigers that survive in little more than four dozen source populations throughout their range (*7*). The immediate solution lies in convincing NGOs, conservationists, donor agencies, and government authorities to properly implement the proven best practices of tiger conservation: the traditional approaches. If we are considering reconstructive surgery for the tiger, then let's stop the bleeding first.

ALAN RABINOWITZ,* LUKE HUNTER, JOSEPH SMITH

Panthera, 8 West 40th Street, 18th Floor, New York, NY 10018, USA.

*To whom correspondence should be addressed. E-mail: arabinowitz@panthera.org

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The Ant Who Learned to Be an Elephant

IN 1998, THE EUROPEAN AND DEVELOPING Countries Clinical Trials Partnership established regional networks of excellence in sub-Saharan Africa to strengthen research capacity for clinical trials on tuberculosis, HIV/AIDS, and malaria (*1*). Through this program, the Faculty of Health Sciences of the University Marien Ngouabi of Brazzaville, like a poor, tiny Ant in an African tale, prepared to partner with a magnificent Elephant: the University of Tübingen in Germany.

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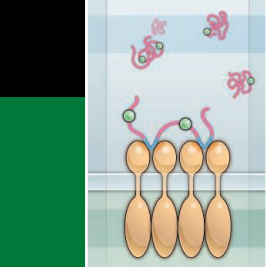
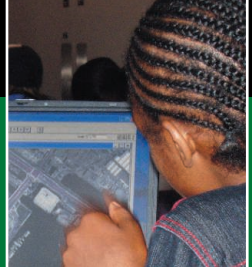


Stress sensor engagement
1830



SPORE Prize Essay
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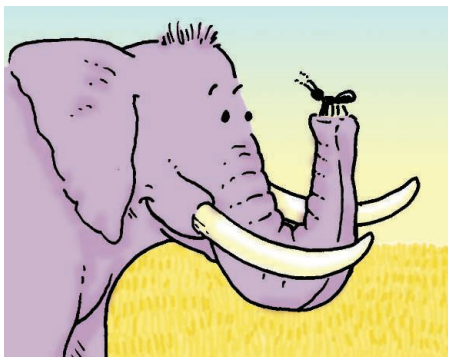

The Elephant is beautiful, muscular, and respected by all the animals in the jungle. The Ant is small and ignored. Could an Ant possibly build a strong relationship with an Elephant? This Ant was going to try.

First, the Ant sought national authorization to conduct a clinical research project that would develop baseline studies and collect baseline data necessary for future clinical trials. After submitting the research protocol, the Ant waited 15 long months for approval by Congo's only Institutional Ethics Committee, and two more months for authorization from the Ministry of Health. A 17-month delay could compromise the rest of the project, thought the Ant with alarm. The work plan was often misunderstood, and as the Ant explained again and again how the money would be used to address specific challenges, she worried that the Elephant would move on and leave her behind.

The Ant realized that a good research team must be multidisciplinary, consisting of junior and seniors scientists selected by an experienced panel from a list of qualified applicants. This would be a challenge in a place with limited postgraduate academic opportunities. To overcome this limitation, the Ant launched an open call for applications. The other animals in the jungle viewed the Ant's new approach with suspicion.

To invest in infrastructure, the Ant renovated an abandoned facility into the first molecular biology laboratory of the Faculty of Health Sciences, and then equipped it. Now the other animals started to appreciate the Ant's hard work. They congratulated her for the change and encouraged her to maintain the spirit.

To create a culture of research, the Ant had to be thoughtful and innovative. She stimulated scientific discussions by implementing regular scientific meetings. But how would she attract students and scientists to these meetings and foster interest and loyalty? The Ant formed brigades of students to urge others to participate. A year later, the seminar room was always filled with an enthusiastic audience.

Once she had met these challenges, the Ant invited the Elephant to her home to share a cup of tea. She told him about all of her accomplishments, and showed him the new facilities. When the Elephant returned home, he was smiling and convinced. And he wondered, "What kind of Ant is this, this Ant who acts like an Elephant?"

It was just as the Ant had hoped. Next, the Ant hopes to sustain positive momentum and establish stable local research teams that will regularly publish in international scientific journals (*2*). The moral of the story: For young Congolese scientists wondering how to contribute scientifically to their country, the metamorphosis from tiny Ant to majestic Elephant is possible, but it will require time, cunning, and determination.

FRANCINE NTOUMI

Congolese Foundation for Medical Research/Faculty of Health Sciences, University Marien Ngouabi, Brazzaville, Congo, and Institute for Tropical Medicine, University of Tübingen, Germany. E-mail: fntoumi@fcrm-congo.com

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

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CORRECTIONS AND CLARIFICATIONS

Editors' Choice: "Loud enough?" by J. S. Yeston (12 August, p. 803). Dalian's location should have been specified as Northeast, rather than Northwest, China.

News Focus: "Climate change sparks battles in classroom" by S. Reardon (5 August, p. 688). The credit for the second image (bottom of page 688) was incorrect. The correct credit is Morgan Heim, Cooperative Institute for Research in Environmental Sciences. The credit has been corrected in the HTML version online.

Reports: "The plant cell wall–decomposing machinery underlies the functional diversity of forest fungi" by D. C. Eastwood *et al.* (5 August, p. 762). The second sentence of the caption for Fig. 2C should read, "Black trace, nitrogen-rich medium (+N); red trace, nitrogen-depleted minimal medium (–N)."

Perspectives: "Sentence and word complexity" by J. Heinz and W. Idsardi (15 July, p. 295). Due to a production error, the inner region in the figure was mislabeled "Context-sensitive." It should read "Context-free."

TECHNICAL COMMENT ABSTRACTS

Comment on "The Response of Vegetation on the Andean Flank in Western Amazonia to Pleistocene Climate Change"

Surangi W. Punyasena, James W. Dalling, Carlos Jaramillo, Benjamin L. Turner

Cárdenas *et al.* (Reports, 25 February 2011, p. 1055) used the presence of *Podocarpus* pollen and wood to infer ≥5°C cooling of Andean forests during Quaternary glacial periods. We show that (i) *Podocarpus* has a wide elevation range in the Neotropics and (ii) edaphic factors cannot be discounted as a factor governing its distribution. Paleoecologists should therefore reevaluate *Podocarpus* as a cool-temperature proxy.

Full text at www.sciencemag.org/cgi/content/full/333/6051/1825-b

Response to Comment on "The Response of Vegetation on the Andean Flank in Western Amazonia to Pleistocene Climate Change"

Macarena L. Cárdenas, William D. Gosling, Sarah C. Sherlock, Imogen Poole, R. Toby Pennington, Patricia Mothes

Punyasena *et al.* question our interpretation of climate-driven vegetation change on the Andean flank in western Amazonia during the middle Pleistocene and suggest that the use of *Podocarpus* spp. as a proxy of past climate change should be reassessed. We defend our assertion that vegetation change at the Erazo study site was predominantly driven by climate change due to concomitant changes recorded by multiple taxa in the fossil record.

Full text at www.sciencemag.org/cgi/content/full/333/6051/1825-c