We have shown that contemporary evolution of local adaptation to climatic conditions strongly influences seed production of a globally important invasive species—as much as or more so than biotic factors such as enemy release or the evolution of increased competitive ability, which have up to now dominated the literature on plant invasions. Local adaptation can evolve rapidly in outbreeding invaders like *L. salicaria* if multiple introductions from diverse native sources (*6*, *14*, *15*) contribute substantial standing genetic variation (*5*). In such cases, higher recombination rates increase the efficiency of natural selection in invasive populations of outcrossing relative to selfing species. Management efforts and comparative studies of native and introduced populations could be improved by explicitly considering that invasive species are not static entities, but can evolve rapidly, with important implications for future spread.

References and Notes

1. R. B. Huey, G. W. Gilchrist, M. L. Carlson, D. Berrigan, L. Serra, *Science* **287**, 308–309 (2000).
2. J. L. Maron, M. Vilà, R. Bommarco, S. Elmendorf, P. Beardsley, *Ecol. Monogr.* **74**, 261–280 (2004).
3. J. M. Alexander, P. J. Edwards, M. Poll, C. G. Parks, H. Dietz, *Ecology* **90**, 612–622 (2009).
4. R. I. Colautti, C. G. Eckert, S. C. H. Barrett, *Proc. R. Soc. B* **277**, 1799–1806 (2010).
5. R. I. Colautti, S. C. H. Barrett, *Evolution* **65**, 2514–2529 (2011).
6. D. Q. Thompson, R. L. Stuckey, E. B. Thompson, *Spread, impact, and control of purple loosestrife* (Lythrum salicaria) *in North American wetlands* (U.S. Fish and Wildlife Service, 1987).
7. S. Lowe, M. Brown, S. Boudjelas, M. DePoorter, in *100 of the world's worst invasive alien species: A selection from the Global Invasive Species Database*, ISSG, Ed. (IUCN, 2004), pp. 1–12.
8. K. Olsson, J. Ågren, *J. Evol. Biol.* **15**, 983–996 (2002).
9. J. L. Montague, S. C. H. Barrett, C. G. Eckert, *J. Evol. Biol.* **21**, 234–245 (2008).
10. R. I. Colautti, S. C. H. Barrett, *Int. J. Plant Sci.* **171**, 960–971 (2010).
11. Supplementary materials are available on *Science* Online.
12. R. Leimu, M. Fischer, *PLOS ONE* **3**, e4010 (2008).
13. J. Hereford, *Am. Nat.* **173**, 579–588 (2009).
14. J. Houghton-Thompson, H. H. Prince, J. J. Smith, J. F. Hancock, *Ann. Bot.* **96**, 877–885 (2005).
15. Y. J. Chun, J. D. Nason, K. A. Moloney, *Mol. Ecol.* **18**, 3020–3035 (2009).
16. R. M. Keane, M. J. Crawley, *Trends Ecol. Evol.* **17**, 164–170 (2002).
17. B. Blossey, R. Nötzold, *J. Ecol.* **83**, 887–889 (1995).
18. T. R. Hunt-Joshi, B. Blossey, R. B. Root, *Ecol. Appl.* **14**, 1574–1589 (2004).
19. M. Schat, B. Blossey, *Environ. Entomol.* **34**, 906–914 (2005).
20. A. J. Willis, B. Blossey, *Biocontrol Sci. Technol.* **9**, 567–577 (1999).
21. R. I. Colautti, J. L. Maron, S. C. H. Barrett, *Evol. Appl.* **2**, 187–199 (2009).
22. G. D. Clewley, R. Eschen, R. H. Shaw, D. J. Wright, *J. Appl. Ecol.* **49**, 1287–1295 (2012).

Acknowledgments: We thank D. Carr and A. Weis for assistance at BEF and KSR, respectively, and K. Pols (Millson Forestry Service) and G. Paquette for assistance in Timmins. We also thank S. Yakimowski, M. Johnson, J. Alexander, J. Stinchcombe, K. Rice, K. Dlugosch, and L. Rieseberg for helpful comments. This work was supported by Ontario Government and University of Toronto graduate scholarships (to R.I.C.), the Canada Research Chair's program and an Ontario Premier's Discovery Award for funding (to S.C.H.B.), and the Natural Sciences and Engineering Research Council of Canada for graduate and postdoctoral funding to R.I.C. and Discovery Grants to S.C.H.B. The Original data and analysis are available from the Dryad Data Repository (DOI:10.5061/dryad.878m3).

Supplementary Materials

www.sciencemag.org/content/342/6156/364/suppl/DC1
Materials and Methods
Figs. S1 to S3
References (*23*–*36*)

19 June 2013; accepted 16 September 2013
10.1126/science.1242121

The Invasive Chytrid Fungus of Amphibians Paralyzes Lymphocyte Responses

J. Scott Fites,[1] Jeremy P. Ramsey,[2]* Whitney M. Holden,[2] Sarah P. Collier,[2] Danica M. Sutherland,[2] Laura K. Reinert,[2] A. Sophia Gayek,[2] Terence S. Dermody,[2,3] Thomas M. Aune,[2,4] Kyra Oswald-Richter,[2] Louise A. Rollins-Smith[1,2,3]†

The chytrid fungus, *Batrachochytrium dendrobatidis*, causes chytridiomycosis and is a major contributor to global amphibian declines. Although amphibians have robust immune defenses, clearance of this pathogen is impaired. Because inhibition of host immunity is a common survival strategy of pathogenic fungi, we hypothesized that *B. dendrobatidis* evades clearance by inhibiting immune functions. We found that *B. dendrobatidis* cells and supernatants impaired lymphocyte proliferation and induced apoptosis; however, fungal recognition and phagocytosis by macrophages and neutrophils was not impaired. Fungal inhibitory factors were resistant to heat, acid, and protease. Their production was absent in zoospores and reduced by nikkomycin Z, suggesting that they may be components of the cell wall. Evasion of host immunity may explain why this pathogen has devastated amphibian populations worldwide.

Although causes of global amphibian declines are complex (*1*), the chytrid fungus, *Batrachochytrium dendrobatidis* (*2*, *3*), is now recognized as a leading contributor (*1*, *4*). Previous studies in *Xenopus laevis* suggest that both innate immune defenses, such as antimicrobial peptides in the mucus, and adaptive immunity contribute to resistance against *B. dendrobatidis* infection (*5*). However, lack of extensive lymphocyte infiltration in diseased skin (*2*, *6*) suggests an impaired immune response (*7*–*9*). Because evasion of host immunity is a common strategy employed by pathogenic fungi (*10*, *11*), we hypothesized that *B. dendrobatidis* avoids clearance by inhibiting critical immune functions. To test this hypothesis, we examined the effects of *B. dendrobatidis* on peritoneal leukocytes enriched for macrophages, and we cultured *X. laevis* splenocytes stimulated with T lymphocyte–specific activators (*12*) or heat-killed bacteria to stimulate B lymphocytes (*13*) in the presence of *B. dendrobatidis* [see materials and methods in supplementary materials (*14*)]. Viability and functions of peritoneal phagocytes were not impaired by *B. dendrobatidis* (figs. S1 and S2). However, when splenic lymphocytes were cultured with either live or heat-killed *B. dendrobatidis*, T cell proliferation was reduced (Fig. 1, A and B, and fig. S3, C and E). Live *B. dendrobatidis* cells also inhibited B cell proliferation (fig. S3A). When lymphocytes were separated from *B. dendrobatidis* by a cell-impermeable membrane in a transwell culture system, the fungal cells inhibited lymphocyte proliferation, but less effectively than in coculture (Fig. 1C). The inhibitory effects of *B. dendrobatidis* were replicated in *X. laevis* T and B cell populations enriched by magnetic sorting (fig. S4). Inhibition of T and B lymphocyte proliferation by *B. dendrobatidis* was also observed when the splenocytes were isolated from another frog, *Rana pipiens* (fig. S5). Induced T and B cell proliferation was inhibited in a dose-dependent manner by 24-hour supernatants derived from *B. dendrobatidis* incubated in water (Fig. 1D and fig. S3, B, D, and F). Proliferation of mouse and human lymphocytes was also inhibited by *B. dendrobatidis* supernatants (fig. S6). Frog splenocytes pretreated with *B. dendrobatidis* supernatants for 48 hours had reduced proliferative capacity in response to phytohemagglutinin (PHA), and delayed addition of supernatants at 24 hours after PHA stimulation still inhibited proliferation (fig. S7). Thus, *B. dendrobatidis* can prevent activation and interfere with proliferation after lymphocyte activation has been induced. Further, *B. dendrobatidis* supernatants derived from killed cells inhibited proliferation

[1]Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA. [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [3]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [4]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232, USA.

*Present address: Department of Microbial Pathogenesis, University of Maryland Dental School, Baltimore, MD 21021, USA.

†Corresponding author. E-mail: louise.rollins-smith@vanderbilt.edu

(fig. S8). Cells and supernatants from the closely related nonpathogenic chytrid, *Homolaphlyctis polyrhiza* (*15*), inhibited splenocytes poorly in comparison with those from *B. dendrobatidis* (fig. S9). Proliferation of mammalian epithelial cell lines [HeLa and Chinese hamster ovary (CHO)] was also inhibited by *B. dendrobatidis* supernatant (fig. S10). These data suggest that *B. dendrobatidis* releases a soluble factor that prevents lymphocytes and other cell types from proliferating and renders lymphocytes incapable of normal functions. Thus, immune paralysis is not due to defects in the initial innate immune response of macrophages and neutrophils. Instead, it is due to defects in the lymphocyte-mediated effector arm of the response.

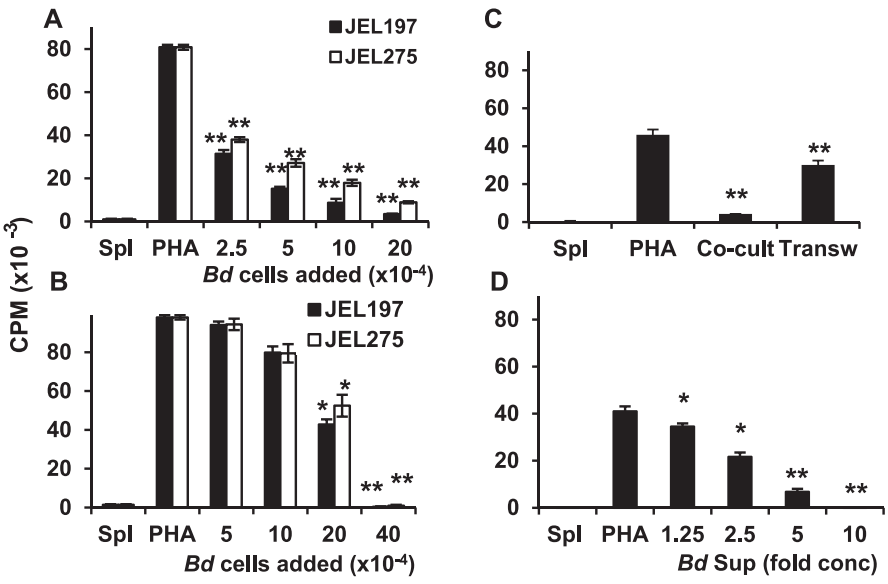
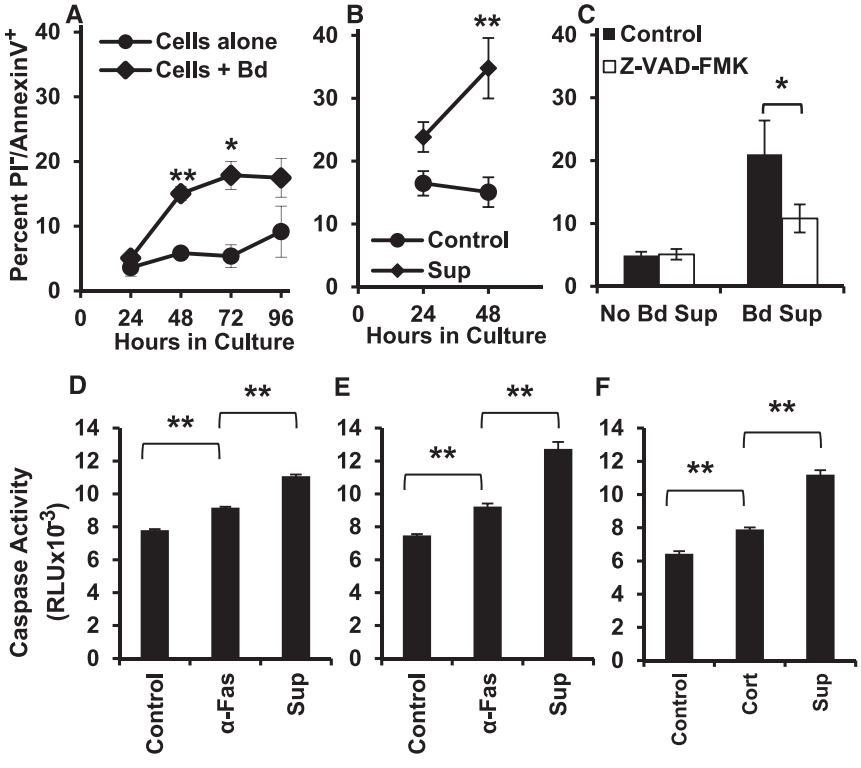


Fig. 1. Inhibition of lymphocyte proliferation by *B. dendrobatidis* (*Bd*). Splenocytes (Spl) from *X. laevis* were cultured alone or with phytohemagglutinin (PHA). PHA-stimulated Spl were cultured alone or with increasing numbers of live (**A**) or heat-killed (**B**) *Bd* cells from two pathogenic isolates, JEL197 or JEL275. (**C**) Spl were cultured as in (A), and PHA-stimulated Spl were cocultured (Co-cult) with or separated from live *Bd* cells by a 0.4-μm pore filter in transwell (Transw). (**D**) Lymphocytes were cultured as in (A) except that live *Bd* cells were replaced by *Bd* supernatants (Sup) at increasing concentrations. Significantly reduced ^{3}H-thymidine uptake detected as counts per minute (CPM) using a scintillation counter compared to the control treatment, $*P < 0.05$, $**P < 0.01$ [analysis of variance (ANOVA) with post hoc test]. CPM data in each panel are means ± SEM of five or more replicate wells and represent three or more similar experiments. Unless noted, JEL197 was the *Bd* isolate used for all cell culture and supernatant experiments.

Pathogenic fungi, including *Cryptococcus neoformans* (*16*), *Aspergillus fumigatus* (*17*), and *Paracoccidioides brasiliensis* (*18*), inhibit immune defenses by activating apoptosis signaling pathways. Some fungal products directly induce lymphocyte apoptosis (*19*). Thus, we investigated whether *B. dendrobatidis* induces splenocyte apoptosis. Resting *X. laevis* splenocytes were cultured across a cell-impermeable membrane from live *B. dendrobatidis* cells in transwells and analyzed for apoptosis by flow cytometry with propidium iodide and annexin V staining (*20*). Splenocytes exposed to *B. dendrobatidis* in transwell cultures showed significantly increased percentages of apoptotic cells at 48 and 72 hours in comparison with controls (Fig. 2A and fig. S11A). When splenocytes were gated to distinguish T and B cells, apoptosis appeared to be preferentially induced in T cells, but some B cells were also apoptotic (fig. S11B). Concentrated *B. dendrobatidis* supernatants also induced splenocyte apoptosis at 48 hours (Fig. 2B and fig. S12), which was significantly decreased by the pan-caspase inhibitor Z-VAD-FMK (Fig. 2C and fig. S13A) but not by necrostatin-1, an inhibitor of programmed necrosis (*21*) (fig. S13, B and C). The *B. dendrobatidis* supernatants appeared to activate both the intrinsic and extrinsic

Fig. 2. Lymphocyte apoptosis induced by *B. dendrobatidis* (*Bd*). Mean percent apoptosis ± SEM of splenocytes (**A**) cultured with or without *Bd* cells separated by a 0.4-μm filter (20:1 *Bd* to splenocytes) in transwell (three experiments); (**B**) cultured with or without *Bd* supernatant (Sup) (four experiments); and (**C**) cultured for 48 hours with or without *Bd* Sup and with or without Z-VAD-FMK (five experiments) and quantified by flow cytometry. (**D** to **F**) Caspase activity assays for caspase-3 and -7 (D), caspase-8 (E), and caspase-9 (F) induced by *Bd* Sup, anti-Fas (α-Fas) monoclonal antibody, or corticosterone (Cort) (representative of four experiments). For (A) and (B), percent apoptosis in the presence of *Bd* cells or Sup was significantly greater than that observed for splenocytes alone by a paired Student's *t* test; $*P < 0.05$, $**P < 0.01$. For (C), percent apoptosis induced by *Bd* Sup alone was significantly greater than that for splenocytes with no *Bd* Sup or splenocytes treated with both *Bd* Sup and Z-VAD-FMK by a paired Student's *t* test; $*P < 0.05$. For (D) to (F), Sup treatments induced significantly greater caspase activity than that of splenocytes alone and of positive controls; $**P < 0.01$ (ANOVA with post hoc test).



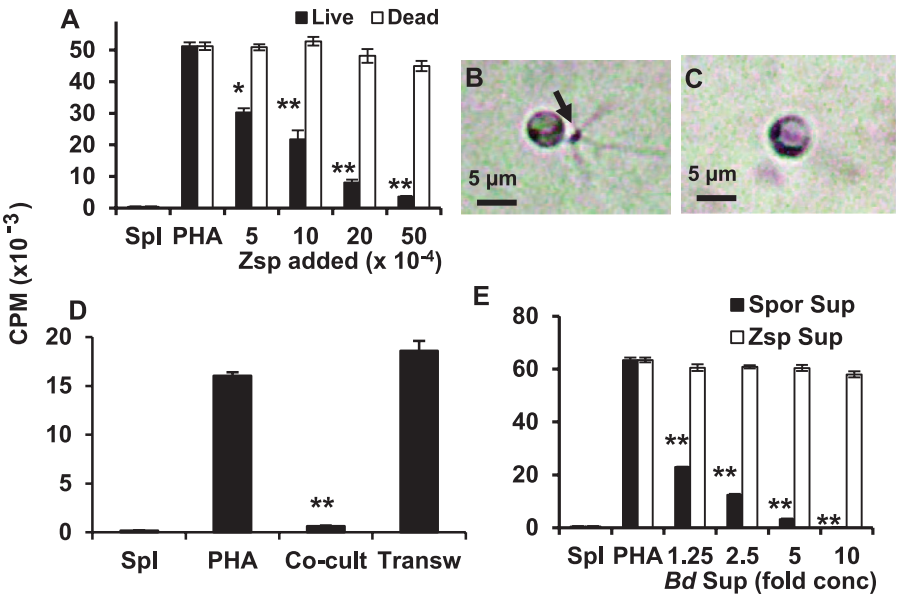


Fig. 3. Effects of *B. dendrobatidis* (*Bd*) zoospores (Zsp) or zoospore supernatants on lymphocyte proliferation. (**A**) Splenocytes (Spl) were cultured alone or with PHA. PHA-stimulated Spl were incubated with increasing numbers of live or heat-killed *Bd* Zsp. Live Zsp (**B**) developed into germlings during the 3-day culture as shown by the formation of rhizoids (arrow); dead Zsp (**C**) failed to develop (bar: 5 µm). (**D**) Spl were cultured as in (A), and PHA-stimulated Spl were cocultured (Co-cult) with or separated from live Zsp by a 0.4-µm filter in transwell (Transw). (**E**) Supernatant from whole *Bd* cultures containing both zoospores and sporangia (Spor Sup) or from zoospores alone (Zsp Sup) were concentrated and incubated with PHA-stimulated Spl. Significantly reduced ^{3}H-thymidine uptake compared to the control treatment, $*P < 0.05$, $**P < 0.01$ (ANOVA with post hoc test). CPM in each panel are means ± SEM of five or more replicate wells and represent three similar experiments.

caspase signaling pathways in splenocytes, as shown by increased activity of caspase-3 and -7 (Fig. 2D), caspase-8 (Fig. 2E), and caspase-9 (Fig. 2F). These results indicate that *B. dendrobatidis* inhibits splenocyte function by activating apoptotic signaling pathways.

B. dendrobatidis has two discernible life stages: the zoospore, which lacks a cell wall, and the mature sporangium (*3*). To identify the life stages that inhibit lymphocytes, we purified zoospores (*22*) and mixed them directly with PHA-stimulated splenocytes (Fig. 3A). During 3 days of incubation, zoospores matured (Fig. 3B) and inhibited proliferation (Fig. 3A, solid bars, and fig. S14). Because zoospores can mature rapidly at 26°C, we tested whether heat-killed zoospores (Fig. 3C) also inhibit splenocytes. In contrast to live zoospores or freshly killed mature cells, heat-killed zoospores could not inhibit lymphocyte proliferation (Fig. 3A, open bars). Live zoospores did not inhibit lymphocyte proliferation when physically separated from lymphocytes in a transwell, but reduced proliferation when in direct coculture with lymphocytes (Fig. 3D). Even when zoospores were placed with splenocytes in the top chamber of a transwell, proliferation was not inhibited in the bottom chamber (fig. S14). Furthermore, supernatants from zoospores were not inhibitory (Fig. 3E). These results show that zoospores do not release splenocyte inhibitory factors until they mature.

To biochemically characterize the lymphocyte inhibitory factors released by *B. dendrobatidis*, we subjected concentrated *B. dendrobatidis* supernatants to heat, proteinase K, or acid. The supernatants retained the capacity to inhibit splenocyte proliferation after incubation at 100°C for 30 min (Fig. 4A) or treatment with either proteinase K (Fig. 4B) or trifluoroacetic acid (fig. S15). Proteinase K digested the protein present in the supernatant (fig. S15A), yet the supernatants retained inhibitory activity (Fig. 4B). These results suggest that the inhibitory factors are not proteins.

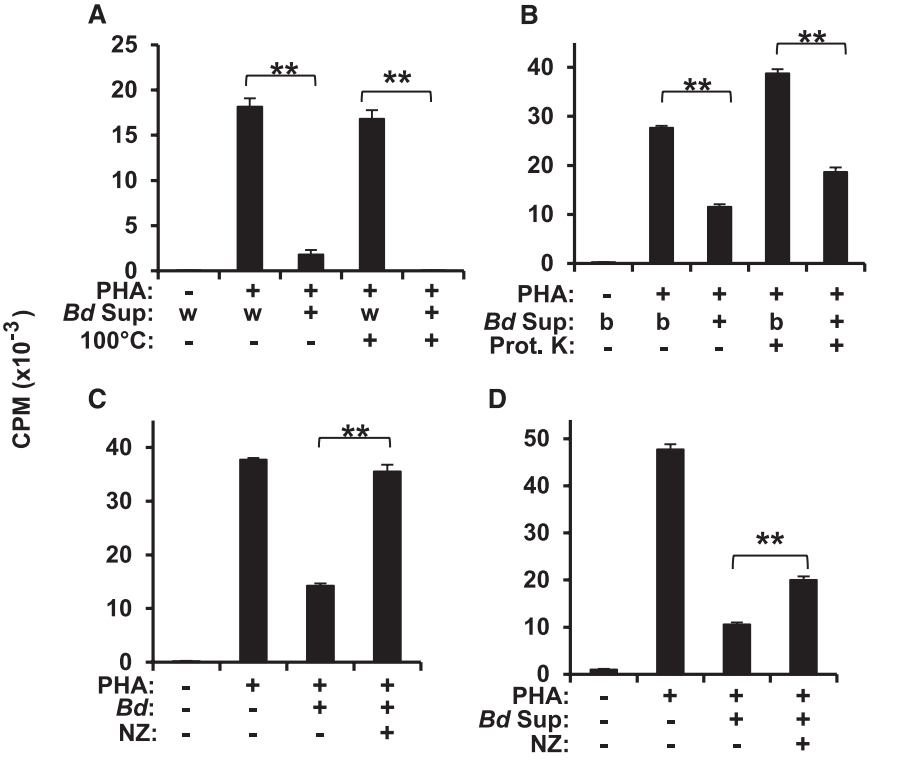


Fig. 4. Partial characterization of *B. dendrobatidis* (*Bd*) inhibitory factors. (**A**) Splenocytes were cultured alone or stimulated with PHA. PHA-stimulated splenocytes were exposed to water only (w) or concentrated *Bd* supernatants (Sup) (5×) that had (+) or had not (–) been incubated at 100°C. (**B**) Splenocytes were cultured as in (A), and PHA-stimulated splenocytes were incubated with control buffer (b) or with *Bd* Sup (5×) previously treated with (+) or without (–) proteinase K–conjugated agarose beads (Prot. K). (**C** and **D**) Splenocytes were treated as in (A), and PHA-stimulated splenocytes were mixed with (+) or without (–) 10^{5} *Bd* cells (C) or 5× *Bd* Sup (D) that had (+) or had not (–) been pretreated with 10 µg/ml (C) or 5 µg/ml (D) nikkomycin Z (NZ). For (A) to (D), proliferation was monitored by ^{3}H-thymidine uptake and CPM were means ± SEM of five or more replicate wells per treatment. Significant differences between treatments grouped by bars using a Student's *t* test (with correction for multiple tests in the same experiment), $**P < 0.01$. Panels are representative of three similar experiments.

Some inhibitory factors produced by other fungi are cell-wall components (*23*); therefore, factors produced by *B. dendrobatidis* may also be located in the cell wall. This idea is consistent with the failure of zoospores, which lack a cell wall, to inhibit. To determine whether inhibitory factors are derived from the cell wall, we interfered with cell-wall synthesis using nikkomycin Z (NZ), a chitin synthase inhibitor (*24*). Preculturing *B. dendrobatidis* with NZ significantly decreased inhibition by both *B. dendrobatidis* cells and supernatants (Figs. 4, C and D). These experiments, along with the observation that noninhibitory zoospores lack cell walls, suggest that the inhibitory factors produced by *B. dendrobatidis* are cell-wall components. Chitin and β-1,3-glucan are the main structural cell-wall components of many fungi (*25*). Therefore, we conducted experiments to determine whether they might be inhibitory. Treatment of *B. dendrobatidis* supernatants with β-glucanases and chitinases did not affect the inhibitory activity (fig. S16). Furthermore, treatment of proliferating lymphocytes with a soluble β-glucan (laminarin) did not inhibit function (fig. S16). Thus, the inhibitory factor does not appear to be a β-glucan or chitin.

We conclude that *B. dendrobatidis*, like other pathogenic fungi, produces toxic factors that inhibit potentially protective host immune responses and likely impair the function of other cells in close proximity. Soluble molecules released by *B. dendrobatidis* inhibited proliferation of amphibian and mammalian lymphocytes and induced apoptosis of target cells by activating both intrinsic and extrinsic pathways. The role of phagocytic cells (macrophages and neutrophils) in controlling chytridiomycosis is not yet well understood. These cells can engulf *B. dendrobatidis*, and accessory functions do not appear to be impaired by *B. dendrobatidis* supernatants. Because these soluble mycotoxins inhibited proliferation and caused death of nonlymphoid cell lines, they are more broadly cytotoxic and could be responsible for other symptoms of chytridiomycosis including disruption of the skin (*2*, *7*, *26*) and behavioral changes, such as lethargy and loss of righting reflex (*6*, *7*). One or more of the factors produced by *B. dendrobatidis* may be derived from the cell wall. The capacity of *B. dendrobatidis* to evade protective immune responses helps to explain how this fungus can be so lethal to amphibians lacking effective innate defenses (*27*) and why some amphibian species with more robust innate responses persist with mild infections as *B. dendrobatidis* reservoirs (*28*–*30*).

References and Notes

1. J. P. Collins, *Dis. Aquat. Organ.* **92**, 93–99 (2010).
2. L. Berger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9031–9036 (1998).
3. J. E. Longcore, A. P. Pessier, D. K. Nichols, *Mycologia* **91**, 219 (1999).
4. L. F. Skerratt *et al.*, *EcoHealth* **4**, 125–134 (2007).
5. J. P. Ramsey, L. K. Reinert, L. K. Harper, D. C. Woodhams, L. A. Rollins-Smith, *Infect. Immun.* **78**, 3981–3992 (2010).
6. A. P. Pessier, D. K. Nichols, J. E. Longcore, M. S. Fuller, *J. Vet. Diagn. Invest.* **11**, 194–199 (1999).
7. J. Voyles, E. B. Rosenblum, L. Berger, *Microbes Infect.* **13**, 25–32 (2011).
8. E. B. Rosenblum *et al.*, *PLoS ONE* **4**, e6494 (2009).
9. L. Ribas *et al.*, *PLoS ONE* **4**, e8408 (2009).
10. J. R. Collette, M. C. Lorenz, *Curr. Opin. Microbiol.* **14**, 668–675 (2011).
11. G. D. Brown, *Annu. Rev. Immunol.* **29**, 1–21 (2011).
12. L. A. Rollins-Smith, S. C. V. Parsons, N. Cohen, *Immunology* **52**, 491–500 (1984).
13. H. Morales, A. Muharemagic, J. Gantress, N. Cohen, J. Robert, *Cell Stress Chaperones* **8**, 265–271 (2003).
14. Detailed materials and methods are available as supplementary materials on *Science* Online.
15. S. Joneson, J. E. Stajich, S.-H. Shiu, E. B. Rosenblum, *PLoS Pathog.* **7**, e1002338 (2011).
16. A. Vecchiarelli, C. Monari, *Mycopathologia* **173**, 375–386 (2012).
17. R. Ben-Ami, R. E. Lewis, D. P. Kontoyiannis, *Br. J. Haematol.* **150**, 406–417 (2010).
18. A. P. Campanelli *et al.*, *J. Infect. Dis.* **187**, 1496–1505 (2003).
19. E. Pericolini *et al.*, *J. Immunol.* **182**, 6003–6010 (2009).
20. I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutellingsperger, *J. Immunol. Methods* **184**, 39–51 (1995).
21. A. Degterev *et al.*, *Nat. Chem. Biol.* **1**, 112–119 (2005).
22. L. A. Rollins-Smith *et al.*, *Dev. Comp. Immunol.* **26**, 471–479 (2002).
23. C. A. Rappleye, L. G. Eissenberg, W. E. Goldman, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1366–1370 (2007).
24. J. P. Gaughran, M. H. Lai, D. R. Kirsch, S. J. Silverman, *J. Bacteriol.* **176**, 5857–5860 (1994).
25. F. M. Klis, P. Mol, K. Hellingwerf, S. Brul, *FEMS Microbiol. Rev.* **26**, 239–256 (2002).
26. J. Voyles *et al.*, *Science* **326**, 582–585 (2009).
27. L. A. Rollins-Smith, J. P. Ramsey, J. D. Pask, L. K. Reinert, D. C. Woodhams, *Integr. Comp. Biol.* **51**, 552–562 (2011).
28. R. Mazzoni *et al.*, *Emerg. Infect. Dis.* **9**, 995–998 (2003).
29. C. Weldon, L. H. du Preez, A. D. Hyatt, R. Muller, R. Speare, *Emerg. Infect. Dis.* **10**, 2100–2105 (2004).
30. D. C. Woodhams, A. D. Hyatt, D. G. Boyle, L. A. Rollins-Smith, *Herpetol. Rev.* **39**, 66 (2008).

Acknowledgments: This research was supported by NSF grants IOS-0619536, IOS-0843207, and IOS-1121758 (to L.A.R-S.); NIH grants AI044924 (to T.M.A.), AI007281 (to D.M.S.), AI038296 (to T.S.D.), 1K01HL103179-01, The Foundation for Sarcoidosis Research (to K.O-R.) and a dissertation enhancement grant from Vanderbilt University (to J.S.F.). W.M.H. was supported by an individual NSF Graduate Research Fellowship. We thank J. Galgiani, director of the Valley Fever Center for Excellence of the University of Arizona, for the gift of nikkomycin Z under a Materials Transfer Agreement. Anti-*Xenopus* monoclonal antibodies were provided by the University of Rochester *Xenopus laevis* Research Resource for Immunobiology. All data necessary to understand this manuscript are presented in the main text or supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6156/366/suppl/DC1
Materials and Methods
Figs. S1 to S16
References (*31*–*66*)

16 July 2013; accepted 6 September 2013
10.1126/science.1243316

Measuring Chromatin Interaction Dynamics on the Second Time Scale at Single-Copy Genes

Kunal Poorey,[1]* Ramya Viswanathan,[1]* Melissa N. Carver,[1] Tatiana S. Karpova,[2] Shana M. Cirimotich,[1] James G. McNally,[2] Stefan Bekiranov,[1]† David T. Auble[1]†

The chromatin immunoprecipitation (ChIP) assay is widely used to capture interactions between chromatin and regulatory proteins, but it is unknown how stable most native interactions are. Although live-cell imaging suggests short-lived interactions at tandem gene arrays, current methods cannot measure rapid binding dynamics at single-copy genes. We show, by using a modified ChIP assay with subsecond temporal resolution, that the time dependence of formaldehyde cross-linking can be used to extract in vivo on and off rates for site-specific chromatin interactions varying over a ~100-fold dynamic range. By using the method, we show that a regulatory process can shift weakly bound TATA-binding protein to stable promoter interactions, thereby facilitating transcription complex formation. This assay provides an approach for systematic, quantitative analyses of chromatin binding dynamics in vivo.

The chromatin immunoprecipitation (ChIP) assay is an approach for determining where chromatin-binding factors interact with DNA sequences and as such has provided fundamental insight into where and how gene regulatory processes occur in cells. In the ChIP assay, cellular constituents are cross-linked with formaldehyde, the isolated chromatin is fragmented, and protein-DNA complexes are then recovered by immunoprecipitation using an antibody that detects a chromatin-associated protein of interest. DNA sequences in the immunoprecipitate are then inventoried by polymerase chain reaction. The assay accurately defines where proteins bind (*1*), but it provides limited information about how stable the interactions are. For example, a relatively high ChIP signal could reflect high-occupancy stable binding or that a low-occupancy dynamic interaction was trapped owing to the long formaldehyde incubation period used in standard assays. In fact, live-cell imaging approaches indicate that many chromatin interactions are exceedingly short-lived (*2*, *3*), although such techniques do not provide high-resolution data regarding chromatin binding location. Precise chromatin

[1]Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, VA 22908, USA. [2]Center for Cancer Research Core Fluorescence Imaging Facility, Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

*These authors contributed equally to this work.
†Corresponding author. E-mail: auble@virginia.edu (D.T.A.); sb3de@virginia.edu (S.B.)

Science
AAAS

The Invasive Chytrid Fungus of Amphibians Paralyzes Lymphocyte Responses

J. Scott Fites *et al.*

Science **342**, 366 (2013);
DOI: 10.1126/science.1243316