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Short Communication

Long-Term Persistence of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome, in the Absence of Bats

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Abstract: Wildlife diseases have been implicated in the declines and extinctions of several species. The ability of a pathogen to persist outside its host, existing as an ''environmental reservoir'', can exacerbate the impact of a disease and increase the likelihood of host extinction. *Pseudogymnoascus destructans*, the fungal pathogen that causes white-nose syndrome in bats, has been found in cave soil during the summer when hibernating bats had likely been absent for several months. However, whether the pathogen can persist over multiple years in the absence of bats is unknown, and long-term persistence of the pathogen can influence whether hibernacula where bats have been locally extirpated due to disease can be subsequently recolonized. Here, we show that *P. destructans* is capable of long-term persistence in the laboratory in the absence of bats. We cultured *P. destructans* from dried agar plates that had been kept at 5°C and low humidity conditions (30–40% RH) for more than 5 years. This suggests that *P. destructans* can persist in the absence of bats for long periods which may prevent the recolonization of hibernation, sites where bat populations were extirpated. This increases the extinction risk of bats affected by this disease.

Keywords: white-nose syndrome, *Pseudogymnoascus destructans*, environmental reservoir, fungal pathogen, pathogen persistence, wildlife disease

Emerging infectious diseases present a serious threat to wildlife (Daszak et al. 2000). One factor that influences whether a disease will drive a host extinct is the ability of a pathogen to persist in the environment outside its host (de Castro and Bolker 2005). The presence of environmental reservoirs can reduce the likelihood of pathogen fadeout if host populations reach low levels, and may prevent the recolonization of hibernacula where host populations were extirpated, which increases the risk of extinction (Mitchell et al. 2008; McCallum 2012).

The recently emerged fungal disease of hibernating bats, white-nose syndrome (WNS), has caused precipitous declines and local extirpations of bat populations (Frick et al. 2010; Turner et al. 2011), and now threatens several species with extinction (Langwig et al. 2012). White-nose syndrome is caused by the fungal pathogen *Pseudogymnoascus destructans* (formerly classified in the genus *Geomyces*) (Lorch et al. 2011; Warnecke et al. 2012; Minnis and Lindner 2013). The disease affects bats in the winter

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Figure 1. Locations of isolations (origins) of *P. destructans* isolates from New York State tested for viable conidia are shown by the *symbols* on the map. The *triangles* indicate the isolates that still contained viable conidia, and the *circles* indicate isolates that did not grow. All isolates shown were originally cultured from bats during the winter of 2008/2009, and then subcultured in 2013.

during their annual hibernation period when the pathogen, *P. destructans*, invades bats' epidermal tissue and appears to disrupt homeostatic processes and alters normal hibernation behavior (Reeder et al. 2012; Warnecke et al. 2012; Wilcox et al. 2014).

Pseudogymnoascus destructans grows optimally between 8 and 14°C, which is within the range of temperatures in areas frequently used by hibernating bats (Webb et al. 1996; Langwig et al. 2012; Verant et al. 2012) and reproduces asexually, forming conidia at the end of long, branched conidiophores (Gargas et al. 2009). Although transmission mechanisms are not completely known, these conidia can either reinfect the same host, be transmitted to a new host (Lorch et al. 2011), or are shed into the environment. Viable fungal material has been obtained from soil samples taken during the summer from infected hibernacula, when bats are usually absent (Lorch et al. 2013). This suggests that *P. destructans* that is shed into the environment from hibernating bats may persist on substrates where bats roost and may serve as a reservoir and source of new infection. However, the duration that conidia can persist on hibernacula surfaces in the absence of bats is unknown, and persistence over multiple years could prevent recolonization of a hibernaculum where bats were extirpated.

In the winter of 2008/2009, when *P. destructans* was rapidly spreading throughout New York and much of the Northeastern United States (Wilder et al. 2011), we isolated *P. destructans* from affected bats by smearing their plagiopatagium and/or uropatagium on Sabouraud's dextrose agar (SDA). Bats were collected from within hibernacula (caves and mines) and from areas near hibernacula across New York and the affected region. Pure isolates of *P. destructans* were grown and stored in an incubator (Fisher Scientific Isotemp Model 304) 5°C in the laboratory of the Wildlife Pathology Unit of the New York State Department of Environmental Conservation in Delmar, NY for subsequent experiments and genetic studies.

All isolates remained in the incubator under low humidity conditions [30–40% relative humidity (RH)] over the next 5–6 years. However, during initial culturing humidity was likely higher (>70% RH) on the surface of the plates as the media still contained moisture. In December 2013, more than 5 years after the samples were originally plated and stored, we sampled nine plates containing *P. destructans* isolates from different locations around New York State (Fig. 1). None of the nine plates had any signs of contamination, and the dehydrated colonies of each isolate were examined microscopically prior to plating to ensure that conidia were present (Fig. 2a, b). We spread conidia from the dehydrated plates (Fig. 2a) across a fresh SDA plate using a sterile inoculating loop, replicating this three times for each isolate. We also included control plates of a *P. destructans* isolate from 2012 that was isolated from a bat in Virginia. This control isolate was plated from a purified culture that had been growing for 32 days at 10°C. The conidia were harvested and spread using the same technique as the dehydrated plates. All plates were sealed with parafilm to reduce contamination, which also increased RH inside the plate to >90%. We allowed the subcultured plates to incubate at 10°C and visually inspected the plates every 2 days for 8 days and weekly thereafter for 90 days.

Approximately 30 days after plate inoculation, colonies of *P. destructans* from six of the nine isolates grew to cover

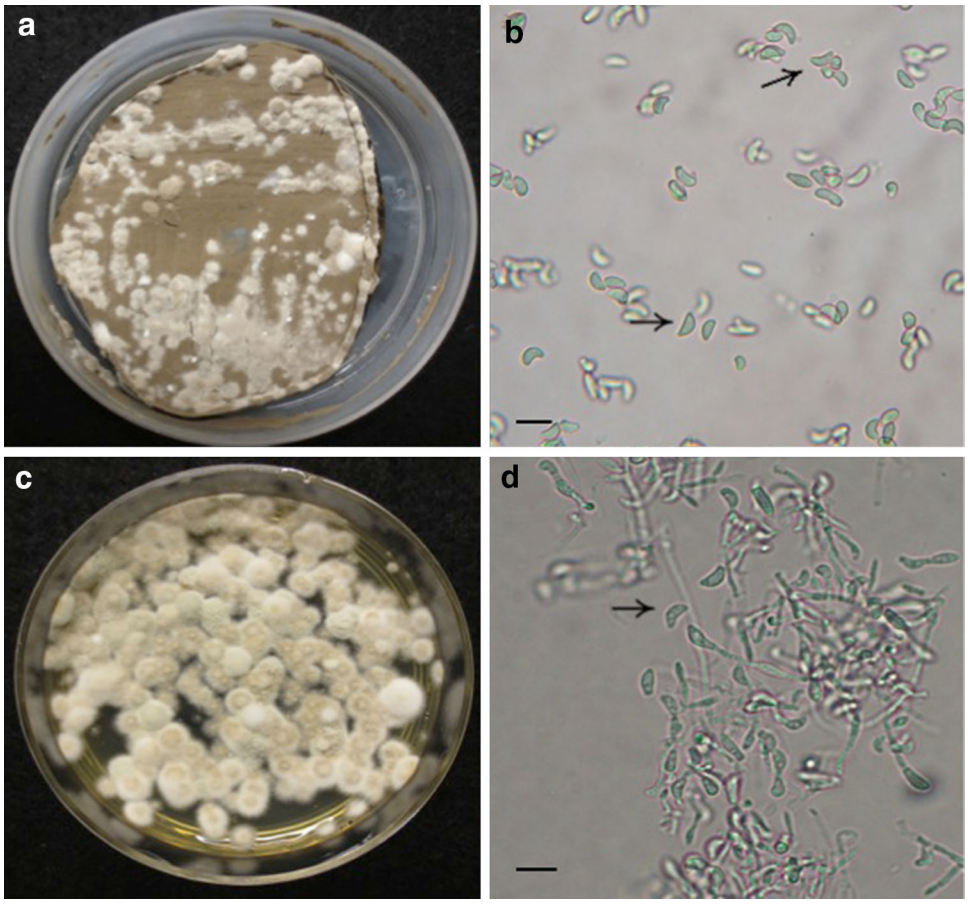

Figure 2. a Culture plate of *P. destructans* that was incubated at 5°C and 30–40% RH for over five years. The media dried to a thin wafer and contracted away from the edge. **b** Characteristically curved conidia (*arrow*) present on the plate in (**a**). **c** Subculture of plate in (**a**) was incubated for 30 days on fresh media and *P. destructans* colonies covered the new plate. **d** Newly formed conidia (*arrow*) present on conidiophores after 30 days of growth at 10°C. Scale bars, 10 μm.

the plate indicating there was a large quantity of viable conidia present on the dehydrated plate (Fig. 2c). There was no delayed germination or growth compared to our control plates observed during this period. Colony growth and morphology was consistent with previous studies showing long slender hyphae with characteristically curved conidia being produced on conidiophores (Fig. 2c, d) (Gargas et al. 2009).

The long-term persistence of *P. destructans* under laboratory conditions in the absence of bats suggests that environments contaminated with *P. destructans* may serve as long-term environmental reservoirs. *Pseudogymnoascus destructans* is capable of growing saprophytically on a wide variety of substrates (Raudabaugh and Miller 2013; Reynolds and Barton 2014). Our results also suggest that *P. destructans* may be able to survive outside hibernacula on equipment or clothing (e.g., caving gear) if they are stored in cool dry conditions, and this could increase inadvertent spread to new locations (Shelley et al. 2013). However, several differences between the conditions in our study and natural conditions should be noted and could influence the ability of *P. destructans* to persist. First, although many hibernacula also have nearly constant year-round temperatures of 1–10°C, they have higher humidity (>70%) than in our study (Langwig et al. 2012). Second, in nature there are other microbiota (e.g., bacteria, fungi, viruses) that might influence *P. destructans* survival. Future studies should determine the influence of these factors on *P. destructans* survival.

If *P. destructans* can persist on hibernacula surfaces as it did under laboratory conditions, this could lead to infection of bats attempting to recolonize sites where bat extirpation has previously occurred. This could impede recovery of highly impacted species such as *Myotis septentrionalis*, *M. lucifugus*, and *Perimyotis subflavus* (Langwig et al. 2012). The ability of fungal pathogens like *P. destructans* to persist outside their host, likely increases their impact on populations and increases the risk of extinction (Fisher et al. 2012).

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References

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287:443

de Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. *Ecology Letters* 8:117–126

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, et al. (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, et al. (2010) An emerging disease causes regional population collapse of a common north american bat species. *Science* 329:679–682

Gargas A, Trest MT, Christensen M, Volk TJ, Bleher DS (2009) *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon* 108:147–154

Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Marm Kilpatrick A (2012) Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15:1050–1057

Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, et al. (2011) Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480:376–378

Lorch JM, Muller LK, Russell RE, O'Connor M, Lindner DL, Blehert DS (2013) Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the Eastern United States. *Applied and Environmental Microbiology* 79:1293–1301

McCallum H (2012) Disease and the dynamics of extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2828–2839

Minnis AM, Lindner DL (2013) Phylogenetic evaluation of Geomyces and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* 117:638–649

Mitchell KM, Churcher TS, Garner TWJ, Fisher MC (2008) Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. *Proceedings of the Royal Society B: Biological Sciences* 275:329–334

Raudabaugh DB, Miller AN (2013) Nutritional capability of and substrate suitability for pseudogymnoascus destructans, the causal agent of bat white-nose syndrome. *PLoS ONE* **8**:e78300.

Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, Britzke ER, et al. (2012) Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE* 7:e38920

Reynolds HT, Barton HA (2014) Comparison of the white-nose syndrome agent pseudogymnoascus destructans to cave-dwelling relatives suggests reduced saprotrophic enzyme activity. *PLoS ONE* **9**:e86437.

Shelley V, Kaiser S, Shelley E, Williams T, Kramer M, Haman K, et al. (2013) Evaluation of strategies for the decontamination of equipment for Geomyces destructans, the causative agent of white-nose syndrome (WNS). *Journal of Cave and Karst Studies* **75**:1–10.

Turner GG, Reeder DM, Coleman JTH (2011) A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and look to the future. *Bat Reseach News* 52:13–27

Verant ML, Boyles JG, Waldrep W, Wibbelt G, Blehert DS (2012) Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. *PLoS ONE* 7:e46280

Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, et al. (2012) Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 109:6999–7003

Webb PI, Speakman JR, Racey PA (1996) How hot is a hibernaculum? A review of the temperatures at which bats hibernate *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 74:761–765

Wilcox A, Warnecke L, Turner JM, McGuire LP, Jameson JW, Misra V, et al. (2014) Behaviour of hibernating little brown bats experimentally inoculated with the pathogen that causes white-nose syndrome. *Animal Behaviour* 88:157–164

Wilder AP, Frick WF, Langwig KE, Kunz TH (2011) Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. *Biology Letters* 7:950–953