PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Hoyt JR *et al*. 2016 Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions. *Proc. R. Soc. B* **283**: 20152861.
http://dx.doi.org/10.1098/rspb.2015.2861

Received: 30 November 2015
Accepted: 11 February 2016

Subject Areas:
ecology

Keywords:
White-nose syndrome, resistance, tolerance, *Geomyces destructans*, emerging infectious disease, *Pseudogymnoascus destructans*

Authors for correspondence:
Joseph R. Hoyt
e-mail: hoytjosephr@gmail.com
Jiang Feng
e-mail: fengj@nenu.edu.cn

†These authors contributed equally to this study.
‡Present address: Department of Molecular, Cellular and Biomedical Science, University of New Hampshire, Durham NH 03824, USA.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.2861 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions

Joseph R. Hoyt[1,†], Kate E. Langwig[1,†], Keping Sun[2], Guanjun Lu[2,3], Katy L. Parise[4], Tinglei Jiang[2], Winifred F. Frick[1], Jeffrey T. Foster[4,‡], Jiang Feng[2] and A. Marm Kilpatrick[1]

[1]Department of Ecology and Evolutionary Biology, University of California, EE Biology/EMS, Santa Cruz, CA 95064, USA
[2]Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, Northeast Normal University, Changchun 130117, People's Republic of China
[3]Urban and Environmental Science College, Changchun Normal University, Changchun 130032, People's Republic of China
[4]Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ 86011, USA

WFF, 0000-0002-9469-1839

Predicting species' fates following the introduction of a novel pathogen is a significant and growing problem in conservation. Comparing disease dynamics between introduced and endemic regions can offer insight into which naive hosts will persist or go extinct, with disease acting as a filter on host communities. We examined four hypothesized mechanisms for host–pathogen persistence by comparing host infection patterns and environmental reservoirs for *Pseudogymnoascus destructans* (the causative agent of white-nose syndrome) in Asia, an endemic region, and North America, where the pathogen has recently invaded. Although colony sizes of bats and hibernacula temperatures were very similar, both infection prevalence and fungal loads were much lower on bats and in the environment in Asia than North America. These results indicate that transmission intensity and pathogen growth are lower in Asia, likely due to higher host resistance to pathogen growth in this endemic region, and not due to host tolerance, lower transmission due to smaller populations, or lower environmentally driven pathogen growth rate. Disease filtering also appears to be favouring initially resistant species in North America. More broadly, determining the mechanisms allowing species persistence in endemic regions can help identify species at greater risk of extinction in introduced regions, and determine the consequences for disease dynamics and host–pathogen coevolution.

1. Introduction

A major outstanding question in evolution and ecology in the Anthropocene is predicting the state that ecosystems will reach after the introduction of invasive species, and in particular, novel pathogens [1]. The introduction of novel pathogens can have lasting effects on species, communities and ecosystems, but impacts are extremely variable with some species suffering little mortality from disease, while others are driven towards extinction [2–6]. Hosts in endemic regions can coexist with these same pathogens through at least four possible mechanisms: reduced transmission, resistance (host defences that reduce pathogen growth), tolerance (host defences that reduce damage experienced by the host without reducing pathogen growth) and/or demographic compensation [7–10]. Inherent host traits, pathogen evolution and the response of species to the selective pressures of mortality from disease determine the

outcome of pathogen introductions. Understanding host–pathogen interactions in endemic regions can offer insight into both the long-term impacts and potential persistence mechanisms of naive hosts [11]. For example, finding that hosts in disease-endemic areas are resistant to a disease would suggest that phylogenetically and ecologically similar hosts in invading regions might also persist by evolving resistance. However, few, if any, studies exist that have explicitly compared disease dynamics in introduced and endemic regions.

The mechanisms of host persistence with novel pathogens also determine, in large part, the intensity of transmission. Two mechanisms, reduced host density for pathogens in which transmission is density-dependent, and poor environmental conditions for pathogen survival or replication outside hosts, will result in lower pathogen transmission [7,12]. Similarly, if hosts in the introduced region are inherently resistant to the pathogen or evolve to become resistant, then this will limit transmission [9,13]. By contrast, if hosts are tolerant (or evolve tolerance) then transmission of the pathogen will be maintained at a much higher intensity, with correspondingly large impacts on intolerant, non-resistant species. In this way, diseases 'filter' communities [14], allowing resistant or tolerant species to persist while more susceptible species are driven extinct.

White-nose syndrome (WNS), a recently emerged disease of hibernating bats, was first detected in North America in 2006 [15] and has caused precipitous declines in temperate bat populations across eastern and midwestern North America [16–18]. The pathogen that causes WNS, *Pseudogymnoascus destructans*, is a cold growing fungus that infects bats' skin during their hibernation period [19–21], and can persist in the environment for long periods of time in the absence of bats [22,23]. The resulting infections lead to the disruption of homeostatic processes and ultimately mortality [24,25].

Pseudogymnoascus destructans has been documented widely across Europe and Asia on multiple species of bats [26–28]. European isolates of *P. destructans* are at least as virulent as the North American strain for North American bats [20], but the virulence of *P. destructans* from Asia for North American bats is unknown. The widespread declines observed in North America have not been observed in Asia or Europe [29] and genetic data suggest that *P. destructans* has been present in the Old World for millennia [30]. By understanding how bats persist with *P. destructans* infections in endemic regions, we may be able to predict the long-term disease dynamics in North America. Here, we present the first comparison of *P. destructans* infection patterns in introduced and endemic regions and the first data on infection prevalence and intensity using molecular methods from a WNS-endemic region.

2. Material and methods

(a) Sample collection and testing

We sampled bats and hibernacula (caves and mines) substrate for *P. destructans* at five sites across northeastern Asia and five sites in North America between 2012 and 2015. The North American sites were sampled during the first 3 years of pathogen invasion to each site where populations were experiencing severe declines [16,18].

We selected sites in Asia and North America at similar latitudes to control for climate and winter severity. The average daily above-ground temperature at the sites in Asia during winter when sampling was conducted (1 October–30 April 2015) was 0.35°C (s.d. = 9.67°C) and the average at the North American sites was 0.53°C (s.d. = 10.33°C). The number of days during this period when the temperature was below 10°C (an approximate threshold for flying insect and bat foraging activity [31]) was similar between regions, averaging 200 days at Asian sites and 198 at North American sites (an alternate threshold of 5°C produced equivalent results—175 days in Asia versus 172 days in North America).

Samples were collected using identical methods and during the same time period (March) in the two regions to make the comparison as similar as possible. Past studies indicate that prevalence and infection intensities (measured as the amount of fungal DNA detected in a sample) and WNS lesions on bats throughout North America increased over the winter, and were the highest in late winter [19,21], making March an ideal sampling time to examine and compare infection prevalence and fungal growth. In both regions, we estimated fungal prevalence and infection intensity (based on the quantity of fungus on the surface of the skin) by rubbing sterile swabs on bats' wings and muzzles as previously described [19,32,33]. We also swabbed areas of cave/mine surfaces directly under and 10 cm from the swabbed bat to determine the extent of the environmental reservoir. We stored samples in RNAlater prior to testing. We extracted DNA from samples using a modified Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) [19] and tested for the presence of *P. destructans* using quantitative PCR [34]. All samples were run in duplicate, with quantification standards on each plate and 16 negative controls per plate. All quantification standards were within a consistent range and all negative controls had no fungal detection. We also surveyed a subset of bats' wing and tail membranes for orange fluorescence using an ultraviolet (UV) flashlight (395 nm; Hayward, CA, USA). The presence of orange fluorescence on wing and tail membranes under UV light has been shown to be correlated with the presence of lesions in infected bats [35].

We measured the temperature next to the swabbed hibernating bat using a laser temperature thermometer (Fluke 62 MAX Plus Infrared Thermometer) that was calibrated across a range of surfaces and temperatures from 0°C to 20°C using a factory calibrated HOBO temperature logger (model U23–001). We confirmed that these single time point measurements of temperatures next to hibernating bats accurately represent winter hibernacula temperatures; point estimates of temperature (mean per site = 41; range 24–105) at 11 sites in the Midwestern USA were very tightly correlated with winter temperatures measured every 3 h from 1 December–31 March using HOBO temperature loggers ($r = 0.95$; $n = 11$; $p < 0.00001$).

At each site, we estimated colony size by counting all individuals of each species. Hibernating bats roosted on cave and mine surfaces making near complete counts of individuals possible.

(b) Statistical analysis

We used generalized linear mixed-effects models with continent as a fixed effect, and species and site as random effects to make comparisons of *P. destructans* prevalence and loads on bats and in the environment using binomial and Gaussian distributions, respectively. We controlled for temperature and colony size by including these variables as fixed effects in the models. We also examined variation in prevalence and load among species by modelling species as a fixed effect. We used the R package brglm to compare prevalence among species with species, temperature and $\log_{10}$ colony size as fixed effects. We used Tukey's post-hoc HSD test to compare pairs of species. To compare environmental prevalence and loads, we used generalized linear mixed-effects models with sample type (under the bat or

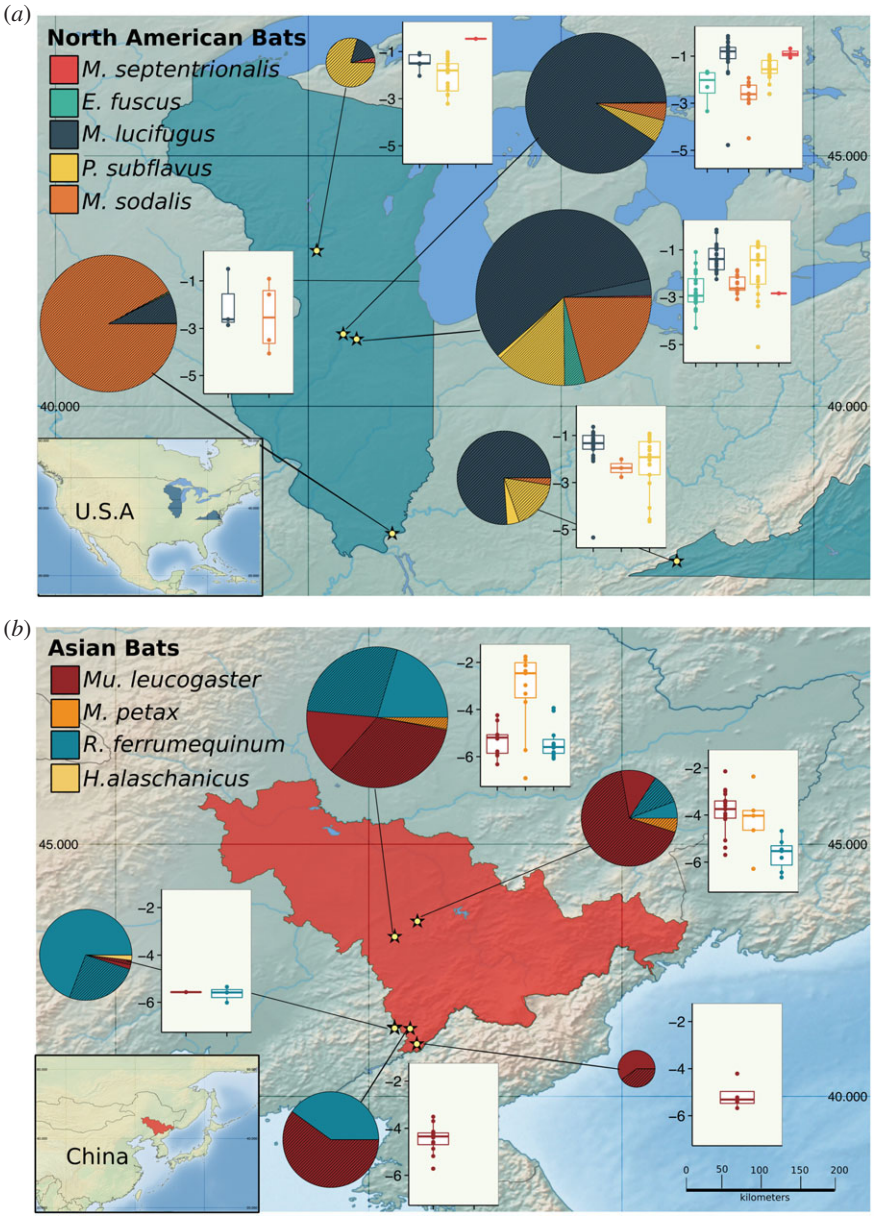

Figure 1. (*a*,*b*) Geographical variation in bat colony size, community composition and *Pseudogymnoascus destructans* prevalence and infection intensity in Asia and North America. Maps show the study regions and study sites (stars) in China and the USA. The pie charts show the total colony size (pie size, on a log scale, range 20–5158) and community composition of bats at each site. The fraction of each species that were tested positive for *P. destructans* is shown by the hatched or darker portion of each pie slice. Boxplots show the infection intensity for each species at the same sites on a log scale (ng) (note different scales for Asia and North America). Pie charts and boxplots use the same colour scheme for species, which is indicated by the legend. (Online version in colour.)

10 cm from the bat) as a fixed effect, and site as a random effect. We standardized roosting temperatures by fitting a mixed-effects model with site as a random effect, and season (early or late hibernation) as a categorical fixed effect because temperatures of hibernation sites were colder as winter progressed and data at all sites was not collected at identical times. We then compared adjusted mean late hibernation site temperatures using a *t*-test. We compared colony sizes among continents using a generalized linear mixed-effects model with Poisson distribution and log link, with site and species as random effects and continent as a fixed effect. All analyses were run in R, v. 3.0.2, and mixed-effects models were fit using the lme4 package.

3. Results

Colony sizes and hibernacula environments were very similar between the two continents (electronic supplementary material, table S1). Colony sizes for individual species ranged from 19 to 3154 bats per hibernaculum and were not significantly different among continents (figure 1; coeff: 0.64 ± 1.54, $Z = 0.42$, $p = 0.68$). Hibernacula temperatures were also very similar in Asia and North America (figure 2*a*; *t*-test of site means: $t = -0.76$, d.f. $= 8$, $p = 0.47$).

Prevalence of *P. destructans* on bats across all sites and species was significantly lower in Asia than on the invasion front in North America (figures 2*a* and 3; $N = 337$, continent coeff.: -4.70 ± 1.44, $t = 3.3$, $p < 0.001$) and neither temperature nor colony size were significant predictors of prevalence in this analysis (temperature coeff.: 0.13 ± 0.14; $t = 0.93$; $p = 0.36$; $\log_{10}$ colony size coeff.: -0.17 ± 0.51, $t = 0.33$, $p = 0.74$). Prevalence in late winter on all five species of bats in North America was more than 95%, whereas prevalence in late winter on four species of bats in Asia averaged 51% (figure 3*a*). Infection intensity, measured as fungal loads on bats' wings and muzzles, was significantly lower

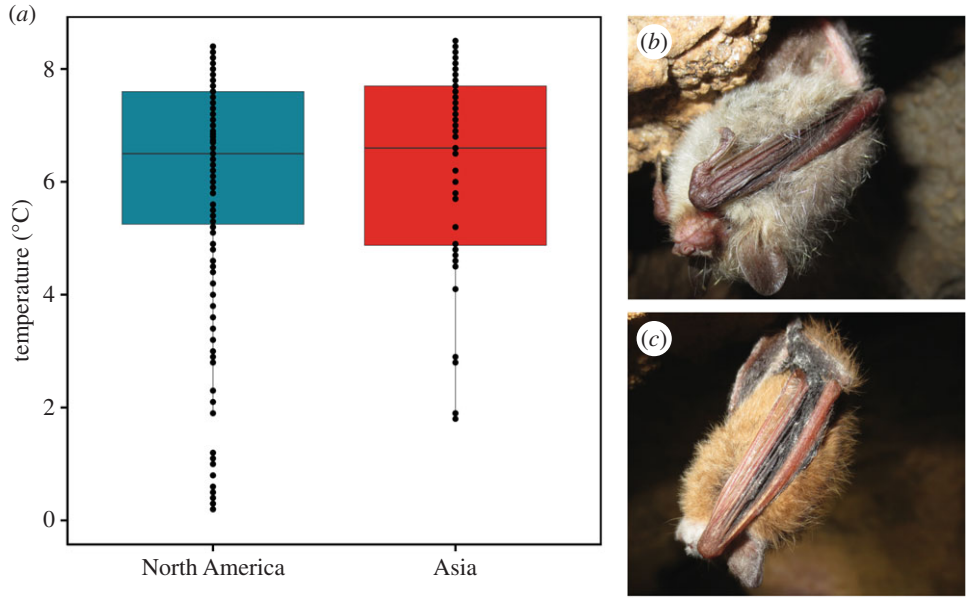

Figure 2. Hibernacula temperatures and visual evidence of WNS in Asia and North America. (*a*) Late winter (March) hibernacula temperatures for individual bats at five sites in each region. Analyses were performed on site average temperatures as described in the methods. (*b*) A bat (*Mu. leucogaster*) in Asia that tested positive for *P. destructans*, but shows no signs of visual infection. (*c*) A bat in North America (*P. subflavus*) that also tested positive, but shows intense visual evidence of fungal growth. Lower visual evidence has been correlated with lower fungal loads [36] among bats. (Online version in colour.)

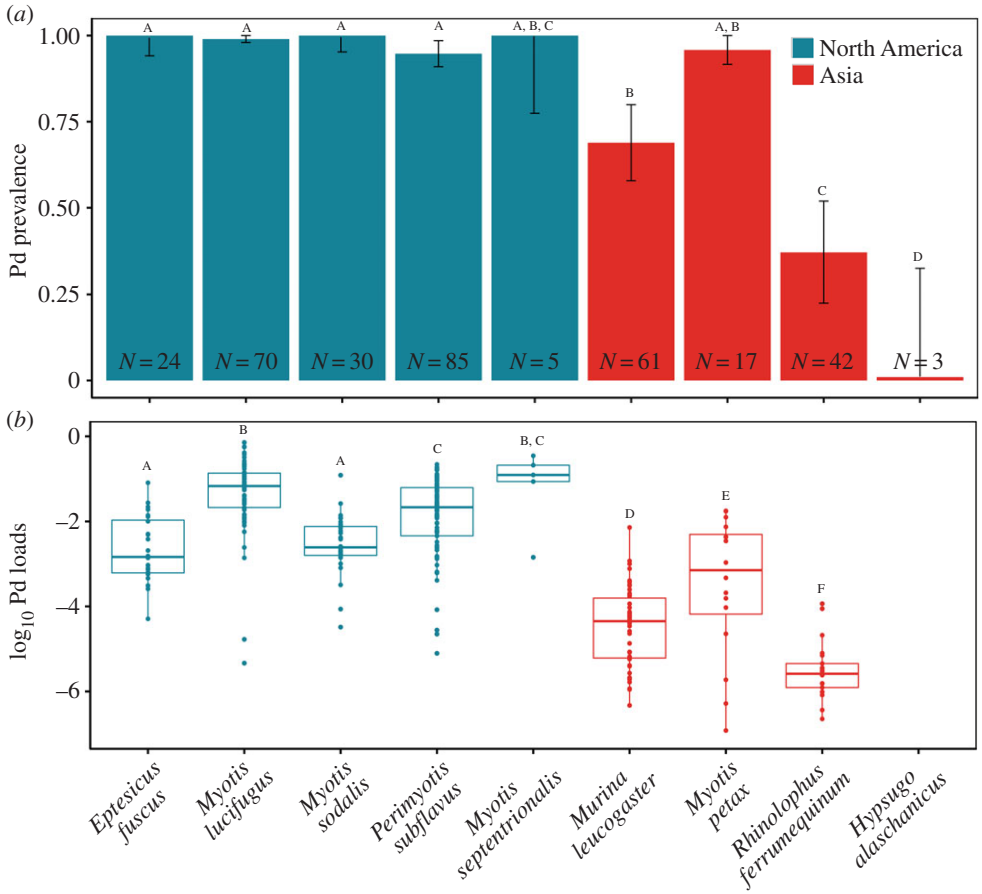

Figure 3. Prevalence and infection intensity of *P. destructans* (Pd) on five species of bats in North America and four species of bats in Asia. (*a*) Fraction of bats testing positive for *P. destructans* at five sites in each region (site random effect s.d.: 1.02, species random effect s.d.: 0.92, intercept: 4.52 $\pm$ 0.9). (*b*) Infection intensity, measured as fungal load, on a $\log_{10}$ scale in nanograms at the same sites as in (*a*) for *P. destructans* positive bats only (site random effect s.d.: 0.15, species random effect s.d.: 0.76, intercept: -1.98 ± 0.36). In both panels shared letters indicate species that do not differ significantly by Tukey's HSD tests. (Online version in colour.)

on Asian bats than North American bats (figure 3*b*; $N = 291$, continent coeff: -2.57 ± 0.62, $t = -4.12$, $p < 0.00001$). As with prevalence, neither temperature nor colony size were significant predictors of infection intensity in this analysis (temperature coeff.: -0.026 ± 0.03, $t = -0.76$, $p = 0.30$; $\log_{10}$ colony size coeff.: 0.03 ± 0.12, $t = 0.24$, $p = 0.39$).

There were significant differences in fungal loads among species, but all species in Asia were statistically significantly lower than all North American species (figure 3*b*). The most heavily infected species in Asia, *Myotis petax*, had loads that were twofold lower than the species with the lowest infection intensity in North America, *Eptesicus fuscus* (figure 3*b*). At the extreme, the species with the lowest infection intensity, *Rhinolophus ferrumequinum*, had loads that were 1000-fold lower than the average load of North American species.

We found that, on average, 28% of 124 bats in China (range among species: 2–73%), showed orange UV fluorescence consistent with the presence of skin lesions due to *P. destructans* infection, whereas the prevalence of UV orange fluorescence in North America was higher (75% of 127 bats; range 38–100%; species random effect s.d.: 2.2; continent coeff: -4.5 ± 1.8; $Z = -2.5$; $p = 0.01$). This difference was likely partly related to the differences in fungal load (figure 3*b*), because the probability of UV orange fluorescence increased with fungal infection intensity (generalized linear model with binomial distribution and logit link: coeff: 0.93 ± 0.28, d.f. $= 262$; $p = 0.01$), and the difference in orange UV fluorescence among continents was not significant after accounting for differences in fungal loads (coeff: -0.79 ± 0.51, d.f. $= 262$; $p = 0.13$).

Spatial variation in prevalence within each continent was much higher in Asia than North America (figure 1; Kruskal–Wallis $p = 0.001$), despite sampling from a narrower geographical region. For all five species sampled in North America, prevalence was uniformly high (95–100% for all species at all sites). By contrast, prevalence varied from 40 to 100% in *Murina leucogaster*, and 0–67% in *R. ferrumequinum* across sites (figure 1). Similarly, median fungal loads were almost uniformly high across sites in North American bats, including *Myotis lucifugus* ($10^{-1.5}$–$10^{-0.5}$ ng), and *P. subflavus* (10^{-2}–$10^{-1.5}$ ng), whereas loads were highly variable for bats in Asia, with median loads for both *Mu. leucogaster*, and *R. ferrumequinum* spanning two orders of magnitude (figure 3).

Finally, environmental prevalence, while highly variable among sites in Asia, was significantly lower in Asia than North America (figure 4*a*; $N = 198$, coeff: -1.58 ± 0.79, $Z = -2.0$, $p = 0.045$). *Pseudogymnoascus destructans* fungal loads on substrates were also significantly lower in Asia (figure 4*b*; $N = 131$, coeff: -1.4 ± 0.62, $t = -2.24$, $p = 0.01$).

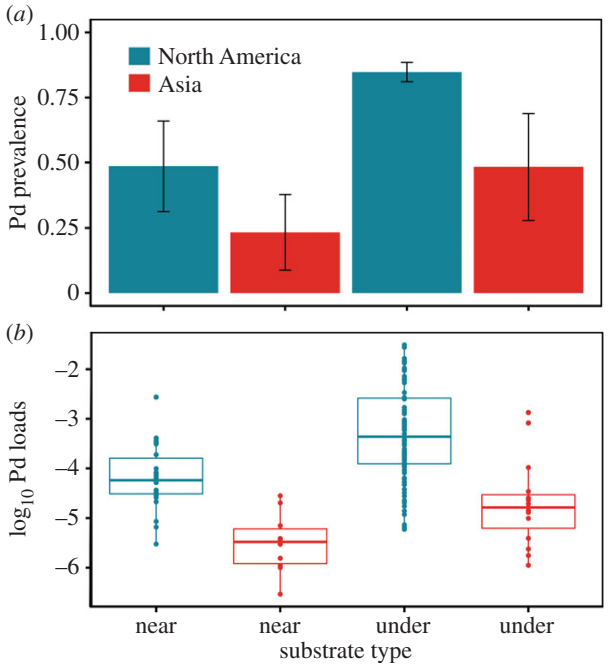

Figure 4. Extent of environmental reservoir for *P. destructans* (Pd) in hibernacula in North America and Asia. (*a*) Fraction of hibernacula substrate samples from under and near (10 cm from bats) testing positive for *P. destructans* at five sites in each region (site random effect s.d.: 0.44, substrate type random effect s.d.: 0.18, intercept: 0.32 ± 0.56). (*b*) Fungal loads on a $\log_{10}$ scale in nanograms at the same sites as in (*a*) for *P. destructans* positive samples only (site random effect s.d.: 0.1, substrate type random effect s.d.: 0.52, intercept: -3.96 ± 0.38). (Online version in colour.)

4. Discussion

Disease can drive population cycles, cause extinctions that re-structure communities, and create continental-scale differences in abundance [17,37,38]. WNS has had devastating effects on bat populations across eastern and midwestern North America, with dozens of populations being extirpated and several species predicted to be driven extinct [16–18]. By contrast, although *P. destructans* is widespread in Europe and Asia, bat species have likely persisted with this pathogen for millennia [26,28,30].

We found that prevalence, the presence of UV orange fluorescence, and infection intensity of *P. destructans* was much lower on bats in Asia than on bats at invasion sites in North America, and that the environmental reservoir was less extensive in Asia as well. These data are consistent with Asian bats having higher resistance to pathogen growth, and are inconsistent with three other mechanisms that can lead to coexistence of hosts with an initially virulent pathogen: tolerance, reduced transmission because of smaller population size and lower pathogen growth rate because of lower temperatures. If tolerance, alone, was the mechanism allowing persistence of Asian bat populations with *P. destructans*, then prevalence and infection intensity would be similar on bats in both continents [9]. Colony sizes in Asia and North America were broadly overlapping, and differences among continents in prevalence and infection intensity were still highly significant in analyses that included colony size. Thus, the data are inconsistent with reduced transmission in Asia being due to smaller colony sizes and reduced density-dependent transmission. Similarly, hibernacula temperatures (as well as winter severity) were very similar between sites in Asia and North America and, as analyses indicated that the lower fungal loads of *P. destructans* on bats and hibernacula substrate in Asia cannot be attributed to cooler environmental conditions.

We found that the environmental reservoir was lower in Asia than North America, which is consistent with overall lower transmission intensity and reduced shedding of *P. destructans* by resistant bats in Asia. Lower transmission intensity, as well as differences in hibernation behaviour and phenology may have contributed to the higher variability in prevalence and infection intensity in Asian bats compared with North American bats. In addition, the higher prevalence and infection intensity in North American bats is not simply a transient phenomenon associated with the spreading wavefront of *P. destructans*, because both prevalence and infection intensity has been shown to remain high

at sites several years after populations had declined [19]. Fungal loads were much lower on Asian bats, resulting in a lower probability of WNS lesions, measured by UV florescence. For some bat species in Asia with especially low fungal loads (e.g. *R. ferrumequinum*), the fungus may be present only on the surface of their skin and only rarely causing invasion and pathology. However, the absence of *P. destructans* detection on non-hibernating *R. ferrumequinum* bats during the summer at some of these same sites [28] suggests that our winter estimates of prevalence and infection intensity do not simply represent surface contamination, and the presence of detectable amounts of fungus suggests that these bats are likely infectious to other individuals and the environment.

Our data show that while there are significant differences in infection intensity between North America and Asia, there is also substantial variation in infection intensity among individuals within most species in North America, and these data may offer insight into which species are at greater risk of extinction than others. *Eptesicus fuscus* and *Myotis sodalis* have a relatively large fraction of individuals with fungal loads similar to Asian bats, and thus are unlikely to be driven extinct by WNS. By contrast, loads on *Myotis septentrionalis* were high, with most individuals having loads higher than bats in Asia. Two other species, *My. lucifugus* and *P. subflavus*, have populations that include many individuals with high loads, but a small fraction of these species at some sites have lower infection intensities, similar to those of bats in Asia. Thus, these species would be predicted to suffer large declines from WNS, but some individuals should be able to persist with the fungus.

These predictions are consistent with patterns of initial declines of these species reported previously [17,18]. Specifically, *My. septentrionalis* populations have been extirpated from most sites where WNS has been present for 3 or more years [17,18] whereas, *My. lucifugus* and *P. subflavus* populations have declined more than 90%, but have stabilized at much smaller sizes in some colonies [18]. Finally, as would be predicted based on their low infection intensity, colonies of *My. sodalis* and *E. fuscus* suffered much lower declines from WNS.

The traits conferring resistance in Asian bats and some species and individuals of North American bats are yet to be identified. They may include differences in microbial communities on bats [39], or increased immune function, although differences in antibodies do not appear to be important [40]. The smaller environmental reservoir in Asia could be due to the presence of natural enemies (e.g. mycoviruses or nematodes) that are not present or less abundant in North America.

The extent of declines in multiple species and the presence of both environmental and biological reservoirs of *P. destructans* [16,19], result in intense and persistent selective pressure on North American bats. It remains to be seen if the speed of evolution will be rapid enough to prevent extinctions of the most heavily impacted species [2,6,8,38,41,42]. Management actions to conserve these species face the challenge of reducing disease impacts to prevent extinction without compromising species' evolutionary response to this and other diseases.

Ethics. All sampling was conducted under UCSC IACUC protocol FrickW1106 and National Animal Research Authority in Northeast Normal University, China, approval number: NENU-20080416.

Authors' contributions. J.R.H., K.E.L., K.S., J.T.F., W.F.F., J.F. and A.M.K. designed the study. J.R.H., K.E.L., K.S., G.L., K.L.P., T.J. and A.M.K. collected the data. J.R.H., K.E.L. and A.M.K. analysed the data. J.F. and J.T.F. provided materials, and J.R.H., K.E.L. and A.M.K. wrote the manuscript with input from all authors.

Competing interests. We declare we have no competing interests.

Funding. Financial support was provided by the National Science Foundation (NSF) East Asian Pacific Summer Institute programme IIA-1415092, NSF grant DEB-1115895 and DEB-1336290, National Speleological Society Rapid Response Fund, US Fish and Wildlife Service, China National Science and Technology Foundation 2013FY113600, The Robert and Patricia Switzer Foundation and Experiment.com's crowdfunding platform and all of the people who contributed to the project.

Acknowledgements. We thank the members of Jiang Feng's lab at Northeast Normal University for all their help and support. We also thank Wisconsin Department of Natural Resources (J. P. White, H. Kaarakka, J. Redell, G. Emerson), Illinois Department of Natural Resources (J. Kath, D. Kirk), Virginia Department of Game and Inland Fisheries (R. Reynolds, W. Orndorf), H. Guedi and N. Tran for assistance with sample collection and hibernacula census data.

References

1. Strayer DL, Eviner VT, Jeschke JM, Pace ML. 2006 Understanding the long-term effects of species invasions. *Trends Ecol. Evol.* **21**, 645–651. (doi:10.1016/j.tree.2006.07.007)
2. Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007 Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Ecohealth* **4**, 125–134. (doi:10.1007/s10393-007-0093-5)
3. LaDeau SL, Kilpatrick AM, Marra PP. 2007 West Nile virus emergence and large-scale declines of North American bird populations. *Nature* **447**, 710–713. (doi:10.1038/nature05829)
4. Holdo RM, Sinclair ARE, Dobson AP, Metzger KL, Bolker BM, Ritchie ME, Holt RD. 2009 A disease-mediated trophic cascade in the Serengeti and its implications for ecosystem C. *PLoS Biol.* **7**, e1000210. (doi:10.1371/journal.pbio.1000210)
5. McCallum H, Jones M, Hawkins C, Hamede R, Lachish S, Sinn DL, Beeton N, Lazenby B. 2009 Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction. *Ecology* **90**, 3379–3392. (doi:10.1890/08-1763.1)
6. de Castro F, Bolker B. 2005 Mechanisms of disease-induced extinction. *Ecol. Lett.* **8**, 117–126. (doi:10.1111/j.1461-0248.2004.00693.x)
7. Scholthof KBG. 2007 The disease triangle: pathogens, the environment and society. *Nat. Rev. Microbiol.* **5**, 152–156. (doi:10.1038/nrmicro1596)
8. Kilpatrick AM. 2006 Facilitating the evolution of resistance to avian malaria in Hawaiian birds. *Biol. Conserv.* **128**, 475–485.
9. Roy BA, Kirchner JW. 2000 Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**, 51–63. (doi:10.1111/j.0014-3820.2000.tb00007.x)
10. Gandon S, Mackinnon MJ, Nee S, Read AF. 2001 Imperfect vaccines and the evolution of pathogen virulence. *Nature* **414**, 751–756. (doi:10.1038/414751a)
11. Biggins DE, Kosoy MY. 2001 Influences of introduced plague on North American mammals: implications from ecology of plague in Asia. *J. Mammal.* **82**, 906–916. (doi:10.1644/1545-1542(2001)082<0906:ioipon>2.0.co;2)
12. Anderson RM, May RM. 1979 Population biology of infectious diseases I. *Nature* **280**, 361–367. (doi:10.1038/280361a0)
13. Boots M, Best A, Miller MR, White A. 2009 The role of ecological feedbacks in the evolution of host defence:

what does theory tell us? *Phil. Trans. R. Soc. B* **364**, 27–36. (doi:10.1098/rstb.2008.0160)

14. Brashares JS. 2010 Filtering wildlife. *Science* **329**, 402–403. (doi:10.1126/science.1190095)
15. Blehert DS *et al.* 2009 Bat white-nose syndrome: an emerging fungal pathogen? *Science* **323**, 227. (doi:10.1126/science.1163874)
16. Langwig KE, Hoyt JR, Parise KL, Kath J, Kirk D, Frick WF, Foster JT, Kilpatrick AM. 2015 Disease dynamics of white-nose syndrome invasion, Midwestern United States, 2012-2014. *Emerg. Infect. Dis.* **21**, 1023–1026. (doi:10.3201/eid2106.150123)
17. Frick WF *et al.* 2015 Disease alters macroecological patterns of North American bats. *Glob. Ecol. Biogeogr.* **24**, 741–749. (doi:10.1111/geb.12290)
18. 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. *Ecol. Lett.* **15**, 1050–1057. (doi:10.1111/j.1461-0248.2012.01829.x)
19. Langwig KE *et al.* 2015 Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proc. R. Soc. B* **282**, 20142335. (doi:10.1098/rspb.2014.2335)
20. Warnecke L *et al.* 2012 Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proc. Natl Acad. Sci. USA* **109**, 6999–7003. (doi:10.1073/pnas.1200374109)
21. Lorch JM *et al.* 2011 Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* **480**, 376–378. (doi:10.1038/nature10590)
22. Hoyt JR, Langwig KE, Okoniewski J, Frick WF, Stone WB, Kilpatrick AM. 2014 Long-term persistence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, in the absence of bats. *EcoHealth* **12**, 330–333. (doi:10.1007/s10393-014-0981-4)
23. 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. *Appl. Environ. Microbiol.* **79**, 1293–1301. (doi:10.1128/aem.02939-12)
24. Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G, Willis CKR. 2013 Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. *Biol. Lett.* **9**, 20130177. (doi:10.1098/rsbl.2013.0177)
25. Verant ML, Carol MU, Speakman JR, Cryan PM, Lorch JM, Blehert DS. 2014 White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. *BMC Physiol.* **14**, 10. (doi:10.1186/s12899-014-0010-4)
26. Puechmaille SJ *et al.* 2011 Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. *PLoS ONE* **6**, e19167. (doi:10.1371/journal.pone.0019167)
27. Zukal J *et al.* 2014 White-nose syndrome fungus: a generalist pathogen of hibernating bats. *PLoS ONE* **9**, e97224. (doi:10.1371/journal.pone.0097224)
28. Hoyt JR *et al.* 2016 Widespread occurrence of *Pseudogymnoascus destructans* in northeast China. *Emerg. Infect. Dis.* **22**, 140–142. (doi:10.3201/eid2201.151314)
29. Martinkova N *et al.* 2010 Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. *PLoS ONE* **5**, e13853. (doi:10.1371/journal.pone.0013853)
30. Leopardi S, Blake D, Puechmaille SJ. 2015 White-nose syndrome fungus introduced from Europe to North America. *Curr. Biol.* **25**, R217–R219. (doi:10.1016/j.cub.2015.01.047)
31. Langwig KE *et al.* 2015 Context dependent conservation responses to wildlife disease. *Front. Ecol. Environ.* **13**, 195–202. (doi:10.1890/140241)
32. Hunt A, Collins J, Langwig KE. 2013 *Swabbing protocol for* Geomyces destructans. See https://www.youtube.com/watch?v=KU1EJPJXNPk.
33. Haas CN, Rose JB, Gerba CP. 1999 *Quantitative microbial risk assessment*, 1st edn. New York, NY: John Wiley & Sons, Inc.
34. Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, Blehert DS. 2013 Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. *Mycologia* **105**, 253–259. (doi:10.3852/12-242)
35. Turner GG *et al.* 2014 Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *J. Wildl. Dis.* **50**, 566–573. (doi:10.7589/2014-03-058)
36. Janicki AF, Frick WF, Kilpatrick AM, Parise KL, Foster JT, McCracken GF. 2015 Efficacy of visual surveys for white-nose syndrome at bat hibernacula. *PLoS ONE* **10**, e0133390. (doi:10.1371/journal.pone.0133390)
37. Hudson PJ, Dobson AP, Newborn D. 1998 Prevention of population cycles by parasite removal. *Science* **282**, 2256–2258. (doi:10.1126/science.282.5397.2256)
38. van Riper CIII, van Riper SG, Goff ML, Laird M. 1986 The epizootiology and ecological significance of malaria in Hawaiian (USA) land birds. *Ecol. Monogr.* **56**, 327–344. (doi:10.2307/1942550)
39. Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF, Kilpatrick AM. 2015 Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS ONE* **10**, e0121329. (doi:10.1371/journal.pone.0121329)
40. Johnson JS *et al.* 2015 Antibodies to *Pseudogymnoascus destructans* are not sufficient for protection against white-nose syndrome. *Ecol. Evol.* **5**, 2203–2214. (doi:10.1002/ece3.1502)
41. Daszak P, Cunningham AA. 1999 Extinction by infection. *Trends Ecol. Evol.* **14**, 279. (doi:10.1016/S0169-5347(99)01665-1)
42. Gomulkiewicz R, Holt RD. 1995 When does evolution by natural-selection prevent extinction. *Evolution* **49**, 201–207. (doi:10.2307/2410305)