Mycobiome Traits Associated with Disease Tolerance Predict Many Western North American Bat Species Will Be Susceptible to White-Nose Syndrome

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ABSTRACT White-nose syndrome (WNS), a fungal disease that has caused catastrophic population declines of bats in eastern North America, is rapidly spreading across the continent and now threatens previously unexposed bat species in western North America. The causal agent of WNS, the fungus Pseudogymnoascus destructans, can infect many species of hibernating bats, but susceptibility to WNS varies by host species. We previously reported that certain traits of the skin microbiome, particularly yeast diversity and abundance, of bat species in eastern North America are strongly associated with resistance to WNS. Using these traits, we developed models to predict WNS susceptibility of 13 species of western North American bats. Based on models derived from yeast species diversity, only one bat species, Myotis velifer, was predicted to be WNS resistant (i.e., may develop the disease, but with low mortality rates). We also screened yeasts found on western bats for P. destructans-antagonistic properties by spore germination and growth inhibition/competition assays and found the ability of yeasts to inhibit P. destructans in vitro to be strain specific. Similar to results of inhibition assays performed with yeasts isolated from bats in eastern North America, few yeasts isolated from bats in western North America inhibited P. destructans in vitro. Continued monitoring of western bat populations will serve to validate the accuracy of the mycobiome analysis in predicting WNS susceptibility, document population and susceptibility trends, and identify additional predictors to assess the vulnerability of naive bat populations to WNS.

IMPORTANCE White-nose syndrome is one of the most devastating wildlife diseases ever documented. Some bat species are resistant to or tolerant of the disease, and we previously reported that certain traits of the skin mycobiome of bat species in eastern North America are strongly associated with resistance to WNS. Predicting which western bat species will be most susceptible to WNS would be of great value for establishing conservation priorities. Based on models derived from yeast species diversity, only one bat species was predicted to be WNS resistant. High susceptibility to WNS would pose a significant conservation threat to bats in western North America.

KEYWORDS bats, commensal yeast, fungal disease, microbiome, predictive modelling, white-nose syndrome, disease resistance, mycology, predictive modeling

White-nose syndrome (WNS) is a devastating disease that has killed millions of hibernating bats in eastern North America (1, 2). The causative fungal pathogen, Pseudogymnoascus destructans (3), was first detected in New York in 2006 and has spread rapidly across North America, threatening some bat species with extinction (4, 5). In 2016, P. destructans was documented in western North America for the first time (6), and several western bat species have subsequently been found with P. destructans on their skin or diagnosed with clinical WNS (7). Western North America has higher bat
biodiversity than the eastern portion of the continent, and several species are already designated “species of concern” by the U.S. Fish and Wildlife Service due to threats such as habitat loss (8, 9). Despite the substantial conservation impact that WNS has had in eastern North America, the nature and severity of WNS in western bat species remain unclear.

Although *P. destructans* can infect many species of hibernating bats, disease dynamics and population-level impacts of WNS vary by host species (4, 10). Specifically, some bat species in eastern North America are highly susceptible to WNS, others are resistant (develop WNS without experiencing mass mortality), and a few species appear to resist infection altogether (impervious) despite repeated exposure to *P. destructans* (4, 10). Identification of which western bat species are likely susceptible to WNS prior to the onset of disease would help management agencies set conservation priorities. However, mechanisms of resistance have not been elucidated, making such predictions difficult. Regardless of mechanism, we found in a previous study that certain traits of skin mycobiomes (the fungal component of the microbiome) of eastern bat species were strongly associated with resistance to WNS (11). WNS-resistant species of bats had higher abundance of fungi, especially yeasts, compared to WNS-susceptible species of bats, and some fungal taxa were enriched on WNS-resistant species of bats (11). Here, we describe skin yeast assemblages on bats in western North America and use data from our previous analyses with eastern bats to construct predictive models to forecast which western bat species may be susceptible or resistant to WNS.

RESULTS

We isolated a total of 98 yeast operational taxonomic units (OTUs) from 52 genera (see Table S1 in the supplemental material). We found that 57.7% of yeast OTUs were cultured from a single bat, and 86.6% of yeast OTUs were cultured from ≤5 individual bats each (Table S1). The most common yeast genera were *Debaryomyces* spp. (isolated from 27.7% of the 447 bats sampled), *Aureobasidium* spp. (17.2%), *Vishniacozyma* spp. (6.5%), *Filobasidium* spp. (3.4%), and *Holtermanniella* sp. (2.9%). Bats had a mean ± standard deviation (SD) of 0.9 ± 1.1 yeast OTUs and 41.4 ± 97.5 yeast colony forming units (CFU) per individual, not including singleton OTUs.

The Shannon diversity index and number of CFU (abundance) of yeast assemblages on bat wings varied among species, seasons, sampling locations, and whether bats were sampled at underground sites (Fig. 1; see Table S2 in the supplemental material). Shannon diversity decreased as the number of days samples were stored before processing increased, although this had no effect on yeast abundance (Table S2). Both Shannon diversity and yeast abundance were higher at underground sites compared to samples collected at other areas (e.g., forest sites, maternity roosts). Samples were collected at underground sites in all seasons, but winter samples were exclusively collected at underground sites. Shannon diversity and yeast abundance were highest in winter and lowest in summer. *Myotis velifer* and *Eptesicus fuscus* had the highest Shannon diversity and yeast abundance compared to the other bat species tested (Fig. 1; Table S2). *Corynorhinus townsendii* and *Myotis yumanensis* were more likely to have no yeast than the other tested bat species.

Skin fungal assemblage composition was significantly associated with bat species (pseudo-$F_{12,184} = 1.79$, $R^2 = 0.078$, $P = 0.001$), collection location (pseudo-$F_{11,184} = 2.58$, $R^2 = 0.103$, $P = 0.001$), season of collection (pseudo-$F_{3,184} = 2.35$, $R^2 = 0.026$, $P = 0.001$), and whether samples were collected at an underground site (pseudo-$F_{1,184} = 1.84$, $R^2 = 0.007$, $P = 0.008$), but not with the number of days swabs were stored before processing (pseudo-$F_{1,184} = 1.25$, $R^2 = 0.005$, $P = 0.187$).

Several isolates of *Aureobasidium* spp. showed weak inhibition of *P. destructans* growth under a variety of conditions (Table 1). Results varied depending on the medium type used, including results with the positive control voriconazole. *Holtermanniella takashimae* only inhibited *P. destructans* growth on Sabouraud dextrose (SD) medium (Table 1). Most of the tested yeast strains showed no inhibition of *P. destructans*. As demonstrated with
FIG 1 The Shannon diversity index (A and C) and number of yeast CFU (B and D) for each bat by species (A and B) and location of sample collection (C and D), colored by season of collection, are shown for western North America. Whether a sample was collected at an underground site (cave or mine) is indicated by “yes” and “no.” We defined winter as December through February, spring as April through May, summer as June through August, and fall as September through October.
The four yeast taxa that were enriched on WNS-resistant species of bats in eastern North America were not as abundant on western bats: *Cutaneotrichosporon moniliiforme* isolated from eastern bats inhibited *P. destructans* in vitro (11), strains of *Cu. moniliiforme* isolated from western bats showed no inhibition.

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**DISCUSSION**

Based on models of the presence of three yeast taxa enriched on resistant bat species in eastern North America, the only western bat species predicted to be WNS resistant was *Myotis velifer* (Fig. 2 and Table 2). The remaining 12 species sampled were predicted to be WNS susceptible, based on all criteria examined, although some species (e.g., *E. fuscus*) approached the percent cutoff for WNS resistance in some models (Fig. 2).

Aside from *E. fuscus*, predictions of susceptibility for western populations of species with continental distributions (e.g., *Myotis lucifugus*) were consistent with the known susceptibilities of their eastern counterparts. However, it is important to note that the mycobiome characteristics we used in our predictions do not distinguish WNS-susceptible from WNS-impervious bat species in eastern North America, as both have low yeast diversity and abundance on their skin. This means that a bat classified as “susceptible” by our model may be either susceptible or impervious. Western populations of *Co. townsendii* were classified as “susceptible” by our model, but they are likely to be

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### TABLE 1 Mean diameter of the zone of inhibition of *Pseudogymnoascus destructans* in the presence of the indicated yeast strains after 2 weeks of incubation at 7°C under various conditions in vitro

| Yeast strain or condition | Mean diam of zone of inhibition (mm) on medium*: | SD | BHI | BHB | pH 4.5 | pH 5.0 | pH 5.0 with 6% NaCl | pH 7.0 |
|---------------------------|-----------------------------------------------------|----|-----|------|--------|--------|-------------------|--------|
| *Aureobasidium pullulans* 46370-1185-2SD | 2* | 0 | 0 | 3* | 0 | 0 | 0 | 0 | 0
| *A. pullulans* 45699-972-4aSD | 2c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0
| *A. pullulans* 46379-835-2LNA | 2, 12c | 12c | 7c | 2, 16c | 7c | 5c | 0 | 4c | 0
| *Holtermanniella takashimae* 45701-673-1SD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0
| Voriconazole | 6c | 25 | 12c | 10 | 19 | 0 | 0 | 0 | 0
| PBST | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0

*Shown are the mean diameters of the zone of inhibition (n = 3 replicates for each condition except positive controls) of *P. destructans* in the presence of the indicated yeast strains after 2 weeks of incubation at 7°C under the following conditions in vitro: Sabouraud dextrose medium (SD), brain heart infusion medium (BHI), brain heart infusion agar with 10% sheep blood (BHIB), yeast morphology medium (YM). Voriconazole and phosphate-buffered saline with 0.5% Tween 20 (PBST) were used as positive and negative controls, respectively. Filobasidium magnun (46370-1167-0LNA), Filobasidium sp. (46379-1438-2LNA), Vishniaczyma sp. cluster 9 (46388-1843-1LNA), Blastobotrys buckinghamii (45699-84-1LNA), Vishniaczyma victoriae cluster 11 (45701-666-25D), Debaryomyces hansenii (45701-677-15D), Debaryomyces sp. 1 (45702-288-15D), Debaryomyces sp. 3 (45698-832-4SD), Trichosporon atae (44797-05-25D), and Cutaneotrichosporon moniliiforme (44797-11-1LNA) showed no inhibition under any conditions and thus do not appear in the table.

*Plates checked at 7 weeks due to slow growth of *P. destructans* on this medium.

*Inhibition weak, with some growth in the “inhibited” area.

*c, complete inhibition.
WNS impervious based on observations from eastern North America (12). Haase et al. (13) predicted that *Myotis velifer*, *E. fuscus*, and *Corynorhinus townsendii* would have a higher probability of survival when infected with *P. destructans* compared to other bat species in the western United States, such as *M. lucifugus* and *M. evotis*, based on models with body mass and hibernacula microclimate. Aside from bat species that do not hibernate for long periods, currently the only known WNS-impervious bat species are in the genus *Corynorhinus*. *Corynorhinus* is phylogenetically divergent from *Myotis*, among which there are no known impervious taxa. Thus, our predictions may be more accurate for species of *Myotis* and *Parastrellus* (the latter of which is a sister genus to *Perimyotis*), an eastern North American taxon that is highly susceptible to WNS (4, 8). Several western species included in our model do not fall into the WNS-impervious category because individuals of these species have been confirmed to develop WNS. For example, *M. velifer*, *M. evotis*, *Myotis thysanodes*, *Myotis volans*, and *M. yumanensis* have recently been confirmed to develop WNS, although population declines have not yet been observed (7), indicating these species are not WNS impervious. There is, however, more uncertainty in predictions for some other western bat species for which no close relatives occur in eastern North America (e.g., *Euderma maculatum*, *Antrozous pallidus*), and these species may be WNS impervious independent of their mycobiomes.

**FIG 2** The percentage of individuals of each bat species sampled in western North America predicted to be resistant or susceptible to white-nose syndrome based on the presence of *Debaryomyces hansenii* and overall yeast abundance (counts of CFU per bat) cultured from wing swabs. A species with >50% of individuals predicted as resistant (portion of blue bar above 50%) was considered resistant. Bat images used in this figure were free-for-use through a Creative Commons license (Attribution-NonCommercial 4.0 License [http://getdrawings.com/bat-silhouette-images]; Natasha Sinegina, Creative Commons Attribution-Share Alike 4.0 License [http://www.supercoloring.com/silhouettes/bat]).
If our model predictions are accurate, more western bat species in North America could be WNS susceptible than eastern bat species. However, there are several caveats. First, the mycobiome is but one of several factors that influence host susceptibility to WNS. Our models intentionally focused on the mycobiome because of its association with host susceptibility in our previous study (11), but we fully acknowledge the multifactorial nature of host susceptibility to fungal pathogens. Second, the traits used in our predictive models likely vary temporally and spatially beyond the parameter ranges that we measured and modeled. For instance, the bacterial microbiomes of several western bat species differed between individuals sampled in underground (i.e., caves and mines) versus surface sites (14). Many bats sampled for our project were captured from the surface and during the nonhibernation season (i.e., when the species would not be at risk of developing WNS) due to the difficulty of locating hibernacula for most western bat species, and we found that Shannon diversity and yeast abundance and composition differed between bats sampled at underground sites compared to surface sites.

Yeast assemblages on bat skin are believed to represent primarily commensal rather than transient inhabitants compared to filamentous fungi (11), and thus are more likely to be permanent residents of the skin. Although multiple yeast taxa were shared between bats in both eastern and western North America, several differed in abundance or were unique to the west. Of the five yeast taxa associated with WNS-resistant eastern bat species (11), one taxon was detected on a single bat, and the other four taxa were generally uncommon on western bats. Whether yeast taxa unique to the west are associated with WNS resistance is unknown, although our inhibition assays uncovered few yeasts that inhibit P. destructans growth in vitro. Potentially other microbes on the skin, such as bacteria, perform this role (15).

Several yeast strains isolated from bats in western North America inhibited P. destructans growth in vitro. Aureobasidium spp. were more common on bats in western North America than in the east, and several isolates of Aureobasidium showed weak inhibition of P. destructans growth under a variety of conditions (Table 1). Holtermanniella takashimae only inhibited P. destructans growth on Sabouraud dextrose medium (Table 1). As demonstrated with Aureobasidium spp., the ability of yeast to inhibit P. destructans growth in vitro is strain specific. Cutaneotrichosporon moniliiforme isolated from bats in the east inhibited P. destructans growth on two types of media (11), but Cu. moniliiforme isolated from bats in western North America did not inhibit P. destructans growth on any of the same types

### Table 2: Model predictions of which bat species in western North America will be resistant to white-nose syndrome based on different mycobiome characteristics

| Bat species (n) and parameter | Susceptibility or resistance based on yeast abundance | Cutaneotrichosporon moniliiforme | Debaryomyces Hansenii sp. 1 | Debaryomyces Hansenii sp. 3 | Debaryomyces Hansenii sp. 4 |
|------------------------------|-----------------------------------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Matthews correlation coefficient | 0.80 | 0.48 | 0.05 | 0.31 | 0.85 |
| Antrozous pallidus (39) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Corynorhinus townsendi (83) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Eptesicus fuscus (9) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Euderma maculatum (8) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis californicus (34) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis ciliolabrum (36) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis evotis (47) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis lucifugus (13) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis thysanodes (32) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis velifer (31) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis volans (43) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Myotis yumanensis (55) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |
| Parastrellus hesperus (17) | Susceptible | Susceptible | Susceptible | Susceptible | Susceptible |

*Shown are the results of our model predictions of which bat species in western North America will be resistant to white-nose syndrome (WNS) based on different mycobiome characteristics, including yeast abundance cultured from skin swabs (counts of CFU) and the presence of four yeast taxa that were differentially abundant on WNS-resistant bat species in eastern North America (as determined by Vanderwolf et al. [11]). A Matthews correlation coefficient of ±1 represents perfect prediction, 0 no better than random prediction, and −1 indicates total disagreement between prediction and observation for each model. *"n" indicates sample size.*
of media tested (Table 1). Similar to results of inhibition assays performed with yeasts isolated from bats in eastern North America, few yeasts isolated from bats in western North America inhibited *P. destructans* growth *in vitro*. It is unknown which yeasts could inhibit *P. destructans* on bat wings, given that *in vivo* conditions would differ from *in vitro* conditions and the chemical environment on bat wings is largely unknown. Finally, it is plausible that yeast assemblage characteristics common to resistant bat species do not directly modulate disease susceptibility but are rather a result of other behavioral or physiological processes that also influence resistance (11).

Yeast assemblage traits used in the predictive models could also exhibit variation due to geography and associated host genetics. Eastern and western populations of North American bat species with continent-wide distributions are genetically distinct, often exhibiting higher genetic diversity in the west (16, 17). As such, susceptibility to WNS could be different between eastern and western populations and even within western populations. There was greater intraspecific variation in yeast community traits observed in some western bat species compared to eastern species. For example, 7.7% of individuals from western populations of *Myotis lucifugus*, a WNS-susceptible species, were classified as resistant by the yeast abundance model, whereas 0% of individuals from eastern populations had yeast abundance traits consistent with WNS resistance. The disparity in yeast abundance profiles between susceptible and resistant species of bats in eastern North America creates uncertainty between predicted and observed disease susceptibilities.

Wildlife diseases such as WNS pose significant management challenges due to the difficulty of treating free-ranging populations, especially once a disease has become established. Thus, management strategies focusing on prevention or early intervention hold the most promise for controlling wildlife diseases before they impact new populations or emerge in new locations. Such proactive strategies, however, require intimate knowledge of a host’s susceptibility to a pathogen. The microbiome is increasingly recognized as an important moderator of disease (18, 19) but has rarely been used to predict host susceptibility. We have attempted to forecast disease impacts on novel host species using traits of the mycobiome. We emphasize that our predictions will likely change as new data on bat skin microbiomes are generated, such as the role of bacteria and viruses. More data will help assess the utility of skin microbiomes in forecasting disease susceptibility. However, despite uncertainties, our results provide information that may assist wildlife managers and strategic decision-makers. Specifically, our model predicts that WNS will likely pose a continent-wide conservation threat to *Myotis* spp., and the disease may have more severe ecological impacts in western North America due the greater number of potentially susceptible host species. Continued monitoring of western bat populations will serve to document population and susceptibility trends, determine the accuracy of the mycobiome in predicting WNS resistance, and identify additional predictors to assess the vulnerability of naive bat populations to WNS.

**MATERIALS AND METHODS**

**Sampling.** We collected swab samples from a total of 36 sites in 11 U.S. states and one Canadian province in western North America from 2015 to 2018. In all, we sampled 450 individual bats, representing 14 species (Table 3). We sampled only those western North American bat species known to hibernate (8, 9), because bats that do not hibernate are not known to be susceptible to WNS. Unlike areas of eastern North America where multiple species of bats form large hibernation aggregations (>100 individuals) in caves and mines during winter, hibernation locales for many western bat species are unknown or contain small numbers of bats (9, 20). Due to the difficulties of locating many western bat species during the hibernation period, we collected samples year-round, only some of which were at underground sites (caves or mines). Other sampling sites included maternity colonies (bat boxes, bat condos, buildings) and forest landscapes. We conducted sampling under U.S. Geological Survey—National Wildlife Health Center Institutional Animal Care and Use Committee Protocols EP140212 and EP081124-A2.

**Fungal culture and identification.** We collected and processed swabs of bat wings (each bat swabbed once) following the methods of Vanderwolf et al. (11), with some modifications. First, swabs were stored from 2 to 30 days (mean ± SD, 9.5 ± 7.8 days) before processing due to time variation in shipping samples to the laboratory. Second, we cultured fungi only on Sabouraud dextrose (SD) agar
plates with chloramphenicol and gentamicin and Leeming and Notman agar; we did not use dermatophyte test medium because in our previous study, this medium contributed little to the overall assessment of fungal abundance and diversity on bat skin (11). Finally, we selected only fungi with a yeast morphotype for identification because the abundance of yeasts (CFU) was the only component of the mycobiome strongly associated with WNS susceptibility (11).

Briefly, we streaked each swab five times, discretely, across the two medium types and incubated plates in darkness at 7°C (to approximate typical conditions in hibernacula) for 2 months. We checked plates weekly and isolated morphologically unique fungal colonies in pure culture. We counted the number of colonies of each morphotype on each plate weekly for 2 months or until growth had covered the entire agar surface. We measured P. destructans germination near the discs and then "m"l suspensions, each containing 150 μl of P. destructans conidial suspension containing 2 million conidia onto agar medium, placed six presterilized Whatman no. 1 filter paper discs equidistant from one another on each plate, and inoculated each disc with 8-μl suspensions, each containing 500,000 cells of different yeast strains. We tested each yeast strain in triplicate, incubating all plates in the dark at 7°C. Negative controls were discs treated with sterile phosphate-buffered saline only, and positive controls were discs containing voriconazole (30 μg [Sensi-disc; Becton Dickinson]). We checked plates daily for the first week to assess inhibition of P. destructans germination near the discs and then weekly for 2 months or until P. destructans growth had covered the entire agar surface. We measured zones of inhibition around the discs to the nearest mm after 2 weeks of incubation.

**Statistical analyses.** Singletons (fungal OTUs that were isolated from one bat) were removed prior to analysis. *Myotis septentrionalis* samples were removed before analysis because we collected samples from only three individuals. We performed all analyses in R (29). We calculated the Shannon diversity index of fungi on each bat using the diversity function in the vegan package (30). To assess which factors influence skin fungal assemblage structure, we constructed a Gaussian zero-inflated model with Shannon index as the response variable and bat species (13-level factor), state (12-level factor), the number of days swabs were stored, whether samples were collected at underground sites (caves or mines

### TABLE 3 Bat species, sample sizes, and locations sampled in western North America

| Bat species       | WNS susceptibility | Sample size (n) | State/province-no. of sites |
|-------------------|--------------------|-----------------|----------------------------|
| Antrozous pallidus| ?                  | 39              | AZ-1, CA-1, OR-1           |
| Corynorhinus townsendii| Impervious in East | 83              | CA-2, CO-1, ID-1, MT-1, NV-1, UT-1, WY-2 |
| Eptesicus fuscus  | Resistant in East  | 9               | AZ-1, MT-1, WA-1           |
| Euderma maculatum | ?                  | 8               | BC-1, NV-1                 |
| Myotis californicus| ?                 | 34              | AZ-1, CA-3, OR-2           |
| Myotis ciliolabrum| ?                 | 36              | AZ-1, BC-1, CA-2, ID-1, NV-2, UT-1, WY-2 |
| Myotis evotis     | 47                | BC-2, CA-1, ID-1, MT-1, NV-5, OR-2 |
| Myotis lucifugus  | Susceptible in East| 13              | BC-2, ID-1                |
| Myotis septentrionalis| Susceptible in East | 3             | BC-1                       |
| Myotis thyasnodes | ?                 | 32              | AZ-1, CA-3, OR-1, TX-1    |
| Myotis vellifer   | ?                 | 31              | TX-1                       |
| Myotis volans     | ?                 | 43              | AZ-1, BC-2, CA-1, MT-1, NV-4, OR-3 |
| Myotis yumanensis | ?                 | 55              | BC-1, CA-4, WA-2          |
| Parastreptus hesperus| ?                | 17              | AZ-1, NV-1                |

*The names and coordinates of the collection sites have been withheld due to the sensitive nature of bat hibernacula. “East” refers to eastern North America. AZ, Arizona; CA, California; CO, Colorado; BC, British Columbia; ID, Idaho; MT, Montana; NV, Nevada; OR, Oregon; TX, Texas; UT, Utah; WA, Washington; and WY, Wyoming. ? indicates unknown susceptibility to white-nose syndrome (WNS), and * indicates a species in which WNS has been confirmed, indicating the species is either susceptible or resistant (but not impervious) to the disease.
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[binary factor]), and season (4-level factor) as explanatory variables (package glmmTMB) (31). We defined
‘winter’ as December through February, ‘spring’ as April through May, ‘summer’ as June through August, and ‘fall’ as September through October. No samples were collected in March or November. A separate
model with yeast CFU (abundance) as the response variable and identical explanatory variables was also con-
structed. We used the function AICab (package bbml) (32) to compare model Akaike information criteria
(AIC) values. We determined the best data transformation with the transformTukey function (package compa-
panion) (33) for each response variable. Lambda values were 0.4 and 0.2 for Shannon diversity and yeast
abundance, respectively. We tested multicollinearity among the variables using the corvif function (34).

To determine if wing fungal assemblage composition varied among WNS susceptibility groups, bat
species, and sites, we implemented a nonparametric permutational multivariate analysis of variance
(PERMANOVA) on abundance-based (CFU) Bray-Curtis dissimilarity coefficients using the function
ADONIS (Vegan). As Bray-Curtis dissimilarity values cannot be calculated for samples that have no com-
position, we first removed individual bats with no cultured fungi and bats from which the only fungus cul-
tured was the single representative of that OTU within our data set (n = 17 for Antrozous pallidus, n = 57 for
Corynorhinus townsendii, n = 1 for E. fuscus, n = 6 for Eudema maculatum, n = 14 for Myotis californicus,
n = 16 for My. ciliabrum, n = 33 for My. evotis, n = 11 for My. lucifugus, n = 10 for My. thysanodes, n = 34 for
My. volans, n = 22 for My. yumanensis, and n = 12 for Parastrellus hesperus). We ran PERMANOVA for 1,000
iterations, and report R² values when the variable enters the model last.

To predict WNS susceptibility in western bat species, we constructed a logistic regression model. We
built our model using previously published data on the abundance of yeasts (CFU per bat) within skin
myocobiomes of bat species of known WNS susceptibility sampled in eastern North America (11). We fil-
tered the data set as previously described (11) and excluded the yeast Malassezia vespertilionis because
it was commonly found on both WNS-susceptible and WNS-resistant bat species in the eastern United
States (11) and thus is not a good predictor of WNS susceptibility status. We removed WNS-impervious
bat species (Corynorhinus townsendii and Corynorhinus rafinesquii) from the eastern North American data
set prior to constructing the model because WNS-susceptible and WNS-impervious bat species had simi-
lar yeast abundances on their skin (11) and were therefore uninformative for model purposes. We fitted
WNS susceptibility status (binomial: resistant or susceptible) as our response variable and yeast abun-
dance as the sole predictor variable. We assessed model performance with 5-fold cross validation using
the function cross_validate in the cvms package (35). We report the Matthews correlation coeffi-
cient because it is considered the best metric for establishing the quality of a binary classifier (36). The eastern
data set had unequal numbers of the two susceptibility groups, but the Matthews correlation coefficient
can be used even if classes are of very different sizes (37). A coefficient of +1 represents perfect predic-
tion, 0 indicates no better than random prediction, and −1 indicates total disagreement between pre-
diction and observation. We used the “predict” function of the stats package (29) to predict the suscepti-ilitiy of individuals of unknown susceptibility in the western North America data set. If >50% of indivi-
duals of a given western bat species were predicted as WNS resistant, we considered the species
WNS resistant; otherwise, the species was considered WNS susceptible. In addition to yeast abundance,
we constructed additional models that used the presence or absence of four yeast taxa that were
enriched on WNS-resistant species of bats in eastern North America as binary predictor variables of WNS
susceptibility for western bat species in separate binomial models constructed as described above. The four yeast taxa were Cutaneotrichosporon moniliiforme, Debaryomyces sp. 1, Debaryomyces sp. 3, and
Debaryomyces hansenii.

Data availability. Metadata associated with this project is available at https://www.sciencebase.gov/
catalog/item/5cf6c524e4b0d63728b9b463. The Fungal ITS sequences determined by Sanger sequencing
and submitted to GenBank as described above are available under GenBank accession no. MK782157
to MK782494.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.02 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.

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