Patterns of widespread decline in North American bumble bees

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Bumble bees (Bombus) are vitally important pollinators of wild plants and agricultural crops worldwide. Fragmentary observations, however, have suggested population declines in several North American species. Despite rising concern over these observations in the United States, highlighted in a recent National Academy of Sciences report, a national assessment of the geographic scope and possible causal factors of bumble bee decline is lacking. Here, we report results of a 3-y interdisciplinary study of changing distributions, population genetic structure, and levels of pathogen infection in bumble bee populations across the United States. We compare current and historical distributions of eight species, compiling a database of >73,000 museum records for comparison with data from intensive nationwide surveys of >16,000 specimens. We show that the relative abundances of four species have declined by up to 96% and that their surveyed geographic ranges have contracted by 23–87%, some within the last 20 y. We also show that declining populations have significantly higher infection levels of the microsporidian pathogen Nosema bombi and lower genetic diversity compared with co-occurring populations of the stable (nondeclining) species. Higher pathogen prevalence and reduced genetic diversity are, thus, realistic predictors of these alarming patterns of decline in North America, although cause and effect remain uncertain.

Bumble bees (Bombus) are integral wild pollinators within native plant communities throughout temperate ecosystems (1–5), and recent domestication has boosted their economic importance in crop pollination to a level surpassed only by the honey bee (6). Their robust size, long tongues, and buzz-pollination behavior (high-frequency buzzing to release pollen from flowers) significantly increase the efficiency of pollen transfer in multibillion dollar crops such as tomatoes and berries. Disturbing reports of bumble bee population declines in Europe have recently spilled over into North America, fueling environmental and economic concerns of global decline (7–9). However, the evidence for large-scale range reductions across North America is lacking. Many reports of decline are unpublished, and the few published studies are limited to independent local surveys in northern California/southern Oregon (10), Ontario, Canada (11), and Illinois (12).

Furthermore, causal factors leading to the alleged decline of bumble bee populations in North America remain speculative. One compelling but untested hypothesis for the cause of decline in the United States (10) entails the spread of a putatively introduced pathogen, Nosema bombi, which is an obligate intracellular microsporidian parasite found commonly in bumble bees throughout Europe (13–16) but largely unstudied in North America. Pathogenic effects of N. bombi may vary depending on the host species and reproductive caste and include reductions in colony growth and individual life span and fitness (15, 16). Population genetic factors could also play a role in Bombus population decline (8). For instance, small effective population sizes and reduced gene flow among fragmented habitats can result in losses of genetic diversity with negative consequences (17), and the detrimental impacts of these genetic factors can be especially intensified in bees (18). Population genetic studies of Bombus are rare worldwide. A single study in the United States identified lower genetic diversity and elevated genetic differentiation ($F_{ST}$) among Illinois populations of the putatively declining B. pensylvanicus relative to those of a codistributed stable species (19). Similar patterns have been observed in comparative studies of some European species (8), but most investigations have been geographically restricted and based on limited sampling within and among populations.

Although the investigations to date have provided important information on the increasing rarity of some bumble bee species in local populations, the different survey protocols and limited geographic scope of these studies cannot fully capture the general patterns necessary to evaluate the underlying processes or overall gravity of declines. Furthermore, valid testing of the N. bombi hypothesis and its risk to populations across North America call for data on its geographic distribution and infection prevalence among species. Likewise, testing the general importance of population genetic factors in bumble bee decline requires genetic comparisons derived from sampling of multiple stable and declining populations on a large geographic scale. From such range-wide comparisons, we provide incontrovertible evidence that multiple Bombus species have experienced sharp population declines at the national level. We also show that declining populations are associated with both high N. bombi infection levels and low genetic diversity.

Results

Geographic Range Analysis. To assess large-scale geographic range reductions and changes in relative abundance (RA), we compared historical collection records with those from current field surveys. Current data are based on surveys (details provided in SI Methods, Contemporary Field Surveys of US Bumble Bees) conducted at 382 sites throughout the United States between 2007 and 2009 (Fig. S1A and Table S1). We netted and identified a total of 16,788 bumble bees, including four focal target species suspected of recent population declines (west: B. occidentalis, N = 129; east: B. affinis, N = 22; B. pensylvanicus, N = 532; B. terricola, N = 31) (10, 12, 20) and four thought to have relatively stable populations (west: B. bifarius, N = 2,760; B. vosnesenskii, N = 902; east: B. bimaculatus, N = 1,033; B. impatiens, N = 3,128) (11, 12, 21). Historical data are based on the assembly of a 73,759-specimen database (SI Methods, US Bumble Bee Natural History Collection Database) of the eight target species recorded from natural history...
museum collections throughout the United States (Fig. S1B and Table S2). Comparisons of the historical and current data revealed extensive range reductions (Fig. 1A, D, G, and H) and significant decreases in RA in all four species suspected of population decline (all \( P < 0.001 \)) (Fig. 2); each was absent from significantly more sites predicted to have high occurrence probabilities than were stable species (Fisher’s exact tests; all \( P < 0.001 \)) (Table S4). Declines in RA appear only within the last 20–30 y, with RA values from current surveys lower than in any decade of the last century (Fig. S1C). The four allegedly stable species showed no clear patterns of range reduction (Fig. 1B, C, E, and F and Tables S2, S4, and S5) or consistent declines in RA.

Historically, *B. occidentalis* and *B. pensylvanicus* had among the broadest geographic ranges of any bumble bee species in North America (Fig. 1 and Table S5). However, the current surveys detected *B. occidentalis* only throughout the intermountain west and Rocky Mountains; it was largely absent from the western portion of its range (Figs. 1A and 2) (detected range-area re-

![Fig. 1. Summary of *Bombus* individuals surveyed from 382 collection locations for eight target species, including historical range maps (grayscale shading) with current sightings (pie charts) and associated photographs of hypothesized declining western *B. occidentalis* (A) and eastern *B. pensylvanicus* (D), *B. affinis* (G), and *B. terricola* (H); stable species are represented by the western *B. bifarius* (B) and *B. vosnesenskii* (E), and the eastern *B. bimaculatus* (E), and *B. impatiens* (F). Sizes of the pie charts indicate total number of individuals surveyed at each location; size of the orange segment indicates the fraction of the respective target species collected at that site (some locations are pooled across sites for visual clarity; for detailed data, refer to Table S1). Underlying grayscale shading represents the modeled distribution of each target species from unique presence localities obtained from natural history collections (SI Methods, Statistical Niche Models). Photograph A (B. occidentalis) taken by D. Ditchburn, B (B. bifarius) by L. Solter, C (B. vosnesenskii) by M. Layne, D (B. pensylvanicus) by T. Wilson, E (B. bimaculatus) by J. Whitfield, F (B. impatiens) by J. Lucier, G (B. affinis) by J. James-Heinz, and H (B. terricola) by J. Whitfield.](image-url)
produced relative abundances (2007–2009; gray bars) for six North American bumble bee species using z tests of equal proportions. Methods has a description of the four following geographic regions used in comparisons of relative abundance. (A) Global west, AZ, CA, CO, ID, MT, NM, NV, OR, SD, UT, WA, and WY; (B) Pacific west, CA, OR, and WA; (C) B. bifarius: z = −15.09, P < 0.001; B. occidentalis: z = 56.26, P < 0.001; B. vosnesenskii: z = 10.40, P < 0.001. (D) Northern/coastal east, CT, GA, IL, IN, IA, ME, MA, MN, MO, NE, NY, NC, ND, OH, PA, SC, SD, TN, TX, VA, and WI; B. bimaculatus: z = −15.70, P < 0.001; B. impatiens: z = −31.27, P < 0.001; B. pensylvanicus: z = −56.57, P < 0.001. All have df = 1.

Host Pathogen Infection. We also investigated the relationship between patterns of decline and levels of pathogen infection. To quantify the prevalence of N. bombi in the target species (SI Methods, Pathogen Screening), we examined midgut tissues from 6,708 specimens for presence of the microsporidian spores using phase-contrast microscopy. We confirmed the identity of N. bombi by sequencing a ~600-bp fragment, including the internal transcribed spacer and parts of the large and small rRNA genes (13). We found significantly higher prevalence of N. bombi in declining B. occidentalis (37% of individuals surveyed) and B. pensylvanicus (15.2%) than in the stable species [binomial generalized linear models (GLM); P < 0.001] (Fig. 3A and Table S6). B. affinis and B. terricola were excluded from statistical analyses because of small sample sizes, but the available data show that B. affinis followed the infection trend of the other declining species with infected individuals collected at four of five sites (7 of 14 total individuals infected). The trend for B. terricola was less strong, although the proportion of infected individuals was nonetheless greater than that of any stable species (two of nine sites and 3 of 32 individuals infected). The infection intensities were also highest within B. occidentalis and B. pensylvanicus individuals (SI Methods, Pathogen Screening). All sequenced North American N. bombi isolates were genetically identical to European isolates (Table S7).

Genetic Diversity. We tested whether population genetic diversity and structure are related to the observed patterns of population decline and stability by genotyping 8–11 microsatellite loci in six of the target species (insufficient samples were available for B. affinis and B. terricola (Table S8). Declining populations had significantly reduced gene diversity (Hf) relative to species with stable populations (Fig. 3B and Table 1). Also, foragers of the declining B. pensylvanicus and to a lesser extent, B. occidentalis (relative to B. bifarius but not B. vosnesenskii) originated from significantly fewer colonies at survey sites than foragers of stable species (Tables S8 and S9). Contrary to expectations from an earlier local study of B. pensylvanicus in Illinois (19), there was no evidence that declining populations had significantly elevated range-wide population structure relative to stable species. Estimates of genetic differentiation (FST and D) were low for all taxa (Table 1). FST ranged from 0.004 to 0.007, and D ranged from 0.026 to 0.042 for most species (Table 1); however, both were slightly higher in B. bifarius (FST = 0.026; D = 0.140) and B. occidentalis (declining; FST = 0.032; D = 0.124). Only B. bifarius exhibited intraspecific clustering (Fig. S1D) when species were analyzed with the Bayesian genotype clustering algorithm STRUCTURE (23). Overall, these species seem genetically cohesive, and it seems...
probable that populations experience substantial gene flow, even at large geographic scales.

Discussion

From a large-scale interdisciplinary study of Bombus species across the United States, we have quantified dramatic range-wide population declines in B. occidentalis, B. pensylvanicus, B. affinis, and B. terricola that have occurred over the last few decades. Our data show that these species are significantly less abundant and absent from many more localities than would be predicted from natural history collections, providing a broad-scale geographic perspective of decline (Fig. 1). Although these species have become rare or absent throughout large areas of their historical ranges, co-occurring species, such as B. bifarius, B. vosnesenskii, B. impatiens, and B. bimaculatus, remain relatively abundant and widespread.

The wide-scale reductions in range and abundance of North American species, which also confirm earlier studies of decline at local levels, are striking and cause for concern. However, it is unlikely that species have become fully extirpated from regions where we did not detect them. Although we surveyed the majority of geographic regions multiple times over multiple years, establishing local extinction would require more intensive sampling than was possible within the constraints of a 3-y nationwide study. Our conservative interpretation of the data is that, based on historical information and the large number of sites and specimens surveyed, declining species have become sufficiently rare in parts of their ranges to be difficult to detect. The persistence of residual populations beyond the ranges detected in our surveys is fully expected under the emerging pattern of changing bumble bee diversity in both North America and Europe, where global extinction of species has been rare to date. Rather, both continents are witnessing major reductions in the range and abundance of multiple species. In Europe, accumulating evidence suggests that narrow climatic niche breadth combined with reductions in food and nesting resources are responsible for the gradual declines observed in many Bombus since the 1950s. These declines seem to occur more rapidly near range margins (9), which may also be the case in the United States (e.g., greater losses of B. occidentalis west of the Cascade–Sierra crest and declines of B. pensylvanicus in the north and northeast). However, contrary to a developing consensus in Europe that bumble bees with narrower climatic ranges are most susceptible to decline (9), population declines in the United States can occur in some of the most previously abundant species that formerly occupied broad climatic ranges. Additional causes of decline, thus, seem to be at play in North America.

Before this study, circumstantial evidence linking the timing of Bombus population declines in the Pacific west to the collapse of commercial bumble bee production in California after N. bombi infection (24) led to the hypothesis that N. bombi had escaped into wild populations and was responsible for the declines (10). This temporal correlation was not verified by collection of N. bombi infection data in wild bees. Nevertheless, the hypothesis became widely reported (7, 9, 25, 26). The significantly elevated N. bombi prevalence in declining Bombus populations detected in our study is consistent with the hypothesis that this pathogen could be adversely affecting some species. These observations are reminiscent of reports of other introduced fungal pathogens that pose widespread threats to some taxa, including frogs (Batrachochytrium dendrobatidis) and bats (Geomyces destructans) (27, 28), but confirming a direct link between N. bombi and North American bumble bee decline will require further research. Comparative studies of susceptibility in declining and stable species will reveal whether the increased prevalence in declining species is the result of higher susceptibility to the pathogen or if N. bombi is simply more common in declining species for other reasons. Regarding the geographic origin of N. bombi, the identical ribosomal RNA (rRNA) sequence in North American and European isolates is consistent with the hypothesis of a recent introduction, but in-depth sampling and genetic screening are needed to determine whether N. bombi is invasive or a distinct North American strain. There is additional need to study other known bumble bee pathogens, such as Crithidia bombi (29, 30), and possible viruses that could contribute to the observed species declines.

Estimates of lower range-wide genetic diversity suggest that B. occidentalis and B. pensylvanicus may also have smaller effective population sizes than stable co-occurring Bombus species, and this may play a role in bumble bee decline. The increased potential for inbreeding and genetic drift in small effective populations could lead to increased susceptibility to environmental pressures (17, 18, 31), including N. bombi. On the positive side, high rates of gene flow, inferred from the low levels of genetic structure in both declining and stable species, suggest that diversity lost through drift in small effective populations could be replenished by dispersal. However, high dispersal rates could also facilitate the spread of

Fig. 3. Nosema bombi infection prevalence (A) and microsatellite gene diversity (B). Average N. bombi prevalence (A) for B. vosnesenskii was 1.33% across all sites (n = 103, detected at 10 of 28 sites); B. bifarius was 0.57% (n = 2096, 7 of 88 sites); B. occidentalis was 37.2% (n = 172, 18 of 39 sites); B. impatiens was 0.73% (n = 2864; 10 of 131 sites); B. bimaculatus was 0.28% (n = 1070, three of 95 sites); and B. pensylvanicus was 15.2% (n = 545; 29 of 64 sites). Each circle represents a collecting site; its size indicates the number of individuals screened. Letters above each species plot indicate pairs with significantly different prevalence (P < 0.001) assessed by binomial GLMs (Table S6). (A) Average Hs (± SE) per subpopulation. Letters indicate species pairs with significantly different Hs (P = 0.001) as determined by 1,000 subpopulation permutations. In both A and B, statistical comparisons were conducted separately for western (no †) and eastern (†) species.
Table 1. Gene diversity (total \( H_t \)) and measures of among-subpopulation genetic structure \( (F_{ST} \) and \( D) \) for target Bombus species

<table>
<thead>
<tr>
<th>Species</th>
<th>( N )</th>
<th>Loci</th>
<th>Total ( H_t ) (interlocus SE)*</th>
<th>( F_{ST} ) (95% CI)</th>
<th>( D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. bimaculatus</td>
<td>472</td>
<td>11</td>
<td>0.693 (0.027)</td>
<td>0.005 (0.002–0.007)†</td>
<td>0.026</td>
</tr>
<tr>
<td>B. impatiens</td>
<td>622</td>
<td>10</td>
<td>0.692 (0.020)</td>
<td>0.004 (0.002–0.007)†</td>
<td>0.034</td>
</tr>
<tr>
<td>B. pensylvanicus</td>
<td>342</td>
<td>11</td>
<td>0.577 (0.030)</td>
<td>0.007 (0.003–0.011)†</td>
<td>0.036</td>
</tr>
<tr>
<td>B. vosnesenskii</td>
<td>364</td>
<td>8</td>
<td>0.676 (0.013)</td>
<td>0.005 (0.000–0.010)†</td>
<td>0.042</td>
</tr>
<tr>
<td>B. bifarius</td>
<td>587</td>
<td>8</td>
<td>0.700 (0.043)</td>
<td>0.026 (0.019–0.034)†</td>
<td>0.140</td>
</tr>
<tr>
<td>B. occidentalis</td>
<td>93</td>
<td>8</td>
<td>0.584 (0.037)</td>
<td>0.032 (0.014–0.053)†</td>
<td>0.124</td>
</tr>
</tbody>
</table>

*Total \( H_t \) calculated by pooling all individuals in a species.
†\( F_{ST} > 0 \) at \( P < 0.01 \).

Infectious agents like \( N. \) bombi. Bumble bees are known to pick up certain pathogens while foraging on flowers (32), although there is no empirical evidence to indicate that \( N. \) bombi is transmitted in this fashion. Nonetheless, if infected reproductive disperses relatively long distances for mating or colony-founding, this could facilitate \( N. \) bombi transmission among populations. Our inference of high dispersal could, however, be reflecting past gene flow if habitat fragmentation has been too recent for migration and drift to reach equilibrium at the broad geographic scale presented here. Intensive genetic analyses of individuals and populations at a local level across a fragmented landscape could provide information about barriers to dispersal at a finer scale. Behavioral studies of dispersal distances of reproductive would further elucidate the potential for gene flow.

Understanding the link between pathogen infection levels and population genetic parameters is a promising avenue for future research, and exploring species- and population-specific genetic differences in susceptibility to \( N. \) bombi infection would provide an important test of the pathogen hypothesis of decline. In this context, phylogenetic relationships may also be important in susceptibility to \( N. \) bombi or more generally, to population decline. Three of four seriously declining species in the United States are close relatives (\( B. \) affinis, \( B. \) terricola, and \( B. \) occidentalis) within the subgenus \( Bombus \) sensu stricto (22). Only two other \( Bombus \) s. s. species occur in North America. One of these is critically imperiled or possibly extinct (\( B. \) franklini) and therefore, could not be included in this study. The other occurs in Alaska (\( B. \) moderatus) and has yet to be fully assayed. \( B. \) pensylvanicus (subgenus Thoracobombus) is not closely related to \( Bombus \) s. s. species, but given the pattern of decline among North American \( Bombus \) s. s. relatives, we suspect other \( Thoracobombus \) (\( B. \) sonorus, \( B. \) californicus, and \( B. \) fervidus) may be at risk and deserve future monitoring.

Pollinator decline has become a worldwide issue (9, 34), raising increasing concerns over impacts on global food production (35), risk and deserve future monitoring. Such efforts to elucidate the causes and ecological impacts of bumble bee biodiversity. In agricultural systems, where a heterogeneous community of native species can help buffer against the decline of managed species (5). Large-scale coordinated efforts to address the status of native pollinators in North America are, however, in their infancy, and bumble bee research is at the forefront. Future research on the complex interactions of habitat fragmentation, loss of floral and nesting resources, disease, and climate is needed to identify the major factors that lead to decline in bumble bee biodiversity. In accordance with the goals of the United Nations Convention on Biological Diversity to reduce the rate of species loss by 2010 (37), such efforts to elucidate the causes and ecological impacts of bumble bee decline, in coordination with informed conservation strategies, will go a long way to mitigating further losses.

Methods

Study Species. We selected eight historically abundant North American \( Bombus \) as focal taxa, because preliminary observations suggested that these species have experienced recent demographic trajectories ranging from population declines to possible expansions. In the western United States, we focused on \( B. \) occidentalis (declining), \( B. \) vosnesenskii (stable), and \( B. \) bimaculatus (stable), and \( B. \) impatiens (stable). All statistical analyses are presented separately for western and eastern taxa.

Distribution and Relative Abundance Comparisons (SI Methods). To determine contemporary distributions and relative abundances, between 2007 and 2009, we surveyed all bumble bee species present at 382 sites in 40 US states for a period of -1 ± 0.5 SD person-h. Only target species were killed; other sampled species were released at the end of each survey. To determine historical distributions and relative abundances, we compiled a 73,759-specimen natural history collection database. The current iteration of the \( Bombus \) database is available on request (from S.A.C.) and on completion, will be hosted on the Global Biodiversity Information Facility (GBIF). We predicted potential historical ranges of each species with the statistical niche modeling algorithm MaxEnt v3.3 (38). We used z tests of equal proportions (Eq. 1) to compare relative abundances of target species between contemporary and historical collections (1900–1999) across four geographic categories: global west, \( B. \) bifarius and \( B. \) occidentalis; Pacific west, \( B. \) bifarius, \( B. \) occidentalis, and \( B. \) vosnesenskii; global east, \( B. \) bimaculatus, \( B. \) impatiens, and \( B. \) pensylvanicus; and northern coastal east, \( B. \) affinis, \( B. \) bimaculatus, \( B. \) impatiens, and \( B. \) pensylvanicus, and \( B. \) terricola (Fig. 2 has the states included) (Eq. 1).

\[
z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}} \tag{1}
\]

where \( \hat{p}_1 = \) estimated historic relative abundance, \( \hat{p}_2 = \) estimated current relative abundance, \( n_h = \) total historic abundance across all target bumble bee species, and \( n_c = \) total current abundance of all target bumble bee species. A similar approach to determine changes in relative abundance of bumble bee communities has been applied previously (11). Nonstatistical comparisons of relative abundance were also made for each decade (Fig. 51C). We partitioned the relative abundance analysis into these four regional categories, because \( B. \) vosnesenskii, \( B. \) affinis, and \( B. \) terricola are more restricted in geographic range than the other target species. The more restricted regional categories, Pacific west and northern/coastal east, allowed a more direct geographic comparison of these species.

We used predictions from our statistical niche models (Fig. 1) in two additional assessments of decline patterns. We created binary presence–absence rasters from the continuous MaxEnt models (logistic threshold = 0.20), which reproduced conservative (i.e., omitted several actual survey observations) but reasonably realistic distribution maps for the eight target species. For each species, survey sites within the presence distribution were scored as an expected occurrence (any omitted actual occurrences caused by the conservative threshold were added to this presence class), and we calculated the fraction of expected sites where the species was observed; differences among species were tested with Fisher’s exact tests (Table S4). To obtain estimates of range-area losses for declining species, we then calculated the areas of minimum convex polygons, constructed in ArcView 9.2, for species occurrences in historical records and contemporary surveys, constraining areas to environments classified as suitable in the binary MaxEnt rasters (Table S5) and adjusting.
estimates downward to compensate for range loss overprediction caused by sampling error (SI Methods, Comparisons of Historical and Contemporary Collections). These niche model-based approaches are only approximations of range loss for the declining Bombus species, because they do not account for differences in abundance across the species’ ranges and assume occupancy of all environmentally suitable sites; however, given the broad distributions of North American Bombus and presently available data, they provide a useful initial approximation to be refined with future survey efforts.

Pathogen Analyses (SI Methods, Pathogen Screening). We determined the prevalence (individuals per species per site) and intensity (spores per microliter) of infection with Nosema bombi by phase-contrast microscopy. Differences in prevalence were tested using binomial GLMs. Species identity of Nosema bombi was confirmed by DNA sequencing of small and large rRNA subunits and internal transcribed spacer (ITS) region (GenBank accession nos. HM142724–HM142729 and HM173334–HM173341 (Table S7).

Genetic Analyses (SI Methods, Genetic Analysis). Six species were genotyped at 8–11 microsatellite loci. Full sibs collected at each site were determined using COLONY 2.0 (39), and a single genotype per colony was retained for analysis. Differences among species in the proportion of unique colonies per site were tested using GLMs with quasibinomial errors. We calculated Nei’s measure of gene diversity (Hs) and interlocus SE (40), and differences among species were tested by 1,000 randomizations of subpopulation estimates of Hs (using only loci successfully genotyped in all species within each region). Intraspecific genetic differentiation was estimated using Fst (41), actual differentiation (D) (42), and the computer program STRUCTURE v.2.3.3 (23).

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SI Methods
Contemporary Field Surveys of US Bumble Bees. During spring to fall seasons (April to October) of 2007–2009, we conducted intensive nationwide surveys of US bumble bee populations. In total, we sampled from 382 sites in 40 states (Fig. S1A and Table S1) and netted and identified a total of 9,006 bumble bees in the west and 7,832 in the east. Because it is difficult to predict areas that will have abundant bumble bees a priori, most survey sites were chosen opportunistically along roadsides by identifying areas with abundant floral resources, although site selection was also guided by historical specimen data and species distribution models (SI Methods, Statistical Niche Models). We divided the United States into western and eastern study regions, because the distribution of Bombus species is roughly split along the 104th western longitude, with the exception of certain nontarget species, such as B. fervidus, B. griseocollis, and B. nevadensis, that appear in both regions. All statistical analyses presented in this study were conducted separately for each region. After identifying a site and conducting a brief informal observation to confirm the presence of bumble bees, the site was typically surveyed for at least 0.5 person-h, with an average of ±0.5 SD survey h per site. Surveys were conducted by walking back and forth through floral patches and collecting all observed bumble bees without consideration of species identity. Specimens were collected with aerial nets while in flight or while foraging at flowers; then, they were placed in vials and chilled on ice until the end of the collection period. Chilled specimens were identified to species using several identification tools, including color pattern guides, dichotomous keys (1–8), and other identification resources (http://www.life.illinois.edu/scameron/research/images/BumbleBeeFieldguide2010.pdf, http://beespotter.mste.uiuc.edu/topics/key/, http://www.nhm.ac.uk/research-curation/research/projects/bombus/key_colour_world/Colour%20key%20to%20species%20for%20male%20bumblebees.html, and pinned reference collections). Unless identifications could not be determined in the field, specimens not belonging to the target species were generally released after identification to limit the impact of collecting on wild populations. In some cases, we collected additional specimens of abundant target species to increase sample sizes for genetic and pathogen analyses (SI Methods, Pathogen Screening and SI Methods, Genetic Analysis). Retained specimens from the eastern region were given unique identification codes and frozen in liquid nitrogen until returned to the University of Illinois, where they were maintained in liquid nitrogen or at −80 °C for genetic and pathogen screening. Western specimens were first killed in cyanide vials, dissected to remove gut tissue, and preserved dry until returned to the United States Department of Agriculture-Agricultural Research Service Pollinating Insect Research Unit in Logan, UT. Dissected gut tissue was frozen immediately in liquid nitrogen for pathogen analysis.

US Bumble Bee Natural History Collection Database. To understand the general patterns of historical relative abundance and distributions of the target species, we made use of the extensive natural history collections (NHC) of Bombus available at academic and government institutions throughout the United States. In some cases, records were obtained as electronic files directly from institutions, but most specimen data presented here are the result of our large-scale databasing effort between 2008 and 2010. Specimens were obtained through loans of all target species (including previously underdetermined Bombus) from the institutions listed in Table S2. Species identity was confirmed with dichotomous keys (1–8) for all specimens by one of the authors or a trained technician. Collection-label data were entered into an electronic file. Specimens missing global positioning system (GPS) coordinate data were georeferenced using the digital tools Google Earth (http://earth.google.com/), TopoQuest (http://www.topoquest.com/), Earth Point (http://www.earthpoint.us), and Lat-Long.com (http://lat-long.com). Locations of some reserves and parks were obtained from relevant government agency websites. Placement of coordinates for each specimen depended on the specificity of the collection-label data (e.g., county-level vs. populated place or other named geographic feature vs. distance and direction along a road from a populated place), and each record was given a qualitative accuracy score to aid in filtering for subsequent usage in distribution modeling. In total, we constructed a database of 77,991 Bombus specimens from 47 institutions, 73,759 of which were specimens of the eight target species. The specimens in this database represent much of the known continental US distributions of the target species as well as the temporal extent of each species’ collection history (Fig. S1B and Table S2).

Statistical Niche Models. We predicted the potential ranges of the target species using the statistical niche modeling algorithm in the program MaxEnt v3.3 (9). MaxEnt uses presence-only locality data and random background points sampled from a study area to estimate the species distribution that is closest to uniform (subject to limited information on the true distribution and environmental conditions). Occurrence data were obtained from the US bumble bee database described above. We examined and filtered records, using only specimens identified by ourselves or a trusted expert and for which we could confidently specify geographical coordinates. This resulted in 2,063 locality records for B. affinis, 2,546 for B. bimaculatus, 6,822 for B. impatiens, 5,903 for B. pensylvanicus, 3,667 for B. terricola, 4,262 for B. bifarius, 3,302 for B. occidentalis, and 1,960 for B. vosnesenskii, which covered most of the known US range of each species. If multiple records occurred within the same environmental grid cell (5-min resolution) or fell outside of the geographic extent considered for this study, they were excluded during analysis. Environmental data were obtained from a set of 19 bioclimatic variables in the WorldClim 1.3 dataset (10). These variables summarize annual averages, seasonality, and extremes of temperature and precipitation that have been interpolated from global weather stations and are averaged over a period ranging from ∼1950 to 2000. To limit the number of variables used for modeling, we calculated Pearson’s correlation coefficient (r) between each pair of the 19 WorldClim variables for 1,000 randomly selected points from the geographic extent used for modeling. Correlations were assessed separately for the east and west. For each comparison with r ≥ 0.90, we selected one variable for modeling. For the eastern species, selected variables were annual mean temperature, mean diurnal range, isothermality, maximum temperature of the warmest month, minimum temperature of the warmest quarter, median temperature of the driest quarter, annual precipitation, precipitation of the wettest month, precipitation of the driest month, precipitation seasonality, and precipitation of the warmest quarter. However, it should be noted that the variables selected made little difference in the resulting maps. MaxEnt
models were trained separately for the eastern and western regions. We averaged models over 50 replicates using a random 80% subset of localities to train the model and 20% reserved for testing using the area under the receiver operating curve (AUC) statistic.

Overall, the niche models (Fig. 1) produced by MaxEnt generally reflect what is known of the historical range of these species (4, 8, 11, 12). AUC values generally indicated good model performance (all test AUC values > 0.80 averaged over 50 sub-sampled MaxEnt runs) except for B. pensylvanicus (AUC = 0.731 ± 0.015 SD) (Table S3). However, the distribution of B. pensylvanicus covers the vast majority of the geographic extent used for modeling. Because MaxEnt is a presence-only modeling method, true absences are not used to estimate commission errors when plotting the receiver operating characteristic curve, but rather, pseudoabsence points are randomly sampled from the predicted distribution (9). When a species has a large area of occurrence, the maximum achievable AUC will be well below unity (in the case of B. pensylvanicus, this expected maximum value is 0.699 ± 0.003 SD, although this can be exceeded in practice). Thus, the observed AUC for B. pensylvanicus likely reflects the very large area of occurrence for this bumble bee, as observed in the NHC data (Fig. S1B), rather than poor model performance. Our models should, therefore, represent good approximations for areas where we would expect the target species to occur given historical information about their occurrences.

Comparisons of Historical and Contemporary Collections. When inferring temporal changes in abundance and distribution of most organisms, biases exist in the collection records. Nonetheless, we wanted to compare the relative abundance of the eight target species in our standardized surveys (2007–2009) to their historical relative abundances in the NHC database. To minimize bias, we analyzed the data at broad geographic and temporal scales. Only target species were used in the relative abundance estimates (i.e., nontarget species identified from surveys and NHC were not included in the total species counts).

We pooled specimen data from 1900 to 1999 from the US bumble bee NHC database to represent historical abundances for the six target species. We excluded data from 2000 to 2006 because of generally low collection efforts documented in NHC (Table S2) during this time frame. It should also be noted that, between 2000 and 2006, declines of some western bumble bees were being documented in North America (13), and therefore, including data from this period could confound our calculations of historical abundances. We excluded specimen data before the 1900s because of spotty collection histories and overly generalized locality information (e.g., Utah and Northwest Territory). Considering the temporal depth (100 y) (Table S2) and geographic breadth (~10,000 historic collection locations) (Fig. S1B and Table S2) representing the abundance of each target species, we make the assumption that the collection history of each NHC is not strongly biased to any one species. In our analysis, we excluded historical records from states in which we did not conduct standardized surveys (Delaware, Florida, Maryland, Michigan, New Hampshire, New Jersey, Rhode Island, and West Virginia).

We partitioned the relative abundance analysis into four regional categories, because three of eight target bumble bee species have restricted geographic distributions: global west, B. bifarius and B. occidentalis; Pacific west, B. bifarius, B. occidentalis, and B. vosnesenskii; global east, B. bimaculatus, B. impatiens, and B. pensylvanicus; northern/coastal east, B. affinis, B. bimaculatus, B. impatiens, B. pensylvanicus, and B. terricola (Fig. 2 lists all states included). We calculated relative abundance for each regional category as the number of individuals collected for each target species divided by the total number of respective targets collected in a given region. We used z tests of equal proportions (Methods) to compare relative abundances.

We also used predictions from our MaxEnt models (Fig. 1) in an additional assessment of decline. We set a relatively strict logistic probability presence threshold of 0.20 to create binary presence–absence raster layers, which produced conservative (i.e., omitted a number of actual survey observations for each species) but reasonably realistic distribution maps for the eight target species. For each species, if a current survey site fell within the threshold distribution, we specified that locality as an expected presence (any actual occurrences omitted because of the conservative threshold were added to this presence class) and calculated the fraction of those sites where we actually observed the species (Table S4).

Finally, to obtain estimates of range losses for declining species within our surveyed study areas, we used MaxEnt niche models together with minimum convex polygons (MCPs) constructed for species occurrences in historical records and contemporary surveys. First, to approximate our areas of study for both east and west regions, we used the ArcView 9.2 (ESRI) extension Hawth’s Tools (14) to calculate MCPs around all 2007–2009 survey sites. For each target species, we then extracted all historical records from the NHC database that fell within the boundaries of these polygons and constructed MCPs for these historical localities as well as for occurrences within our contemporary surveys. Although it would be possible to analyze these MCPs alone to determine percent range loss observed for our surveys, in most cases, their boundaries covered geographic regions unlikely to be inhabited by a given species. To improve our estimates, we, thus, excluded regions with low probability of species occurrence by overlaying the historical and contemporary MCPs with the binary presence–absence MaxEnt maps (see above). This niche model-based constraint improved overestimates of range loss compared with the use of uncorrected MCPs (see below) (Table S5). The percent range remaining for each species was estimated by dividing the areas (total number of 5-min resolution pixels) of the niche model-constrained contemporary and historical MCPs. We observed some degree of range loss for all species (Table S3), not only for the hypothesized declining species (loss ranging from 34% to 98%) but also for those that our surveys indicate are abundant and widespread (loss of 5–11%). We expect that this result reflects both the sparser survey densities at the edges of our study areas and the much more numerous historical localities available for each species. We, thus, used the most severe range loss (11%) observed for a stable species (B. bifarius) to approximate the degree to which range loss is overestimated in our analysis because of sampling effects (Table S5).

Pathogen Screening. We examined dissected midgut tissues of target Bombus species to determine the presence of infection by Nosema bombi. Infections were recognized by presence of mature infective spores that develop in the cytoplasm of cells in the tissues. The spores are oval, ~2 × 4 μm in size, and brightly refractive under phase-contrast microscopy. We dissected western Bombus specimens in the field or in the laboratory in Logan, UT, and shipped the digestive tracts on dry ice to the insect pathogen laboratory at the Illinois Natural History Survey. We placed the collected eastern Bombus specimens on ice or directly into liquid nitrogen shipping tanks and stored the samples in the laboratory at ~80 °C. Specimens were thawed and dissected immediately before screening for N. bombi.

Microscopic examination of pathogen prevalence. We removed midgut tissues from the abdomens of collected bees, smeared fresh tissue samples on glass microscope slides, and screened for pathogens using phase-contrast microscopy at 400x magnification. Dissection tools were sterilized between samples. We determined presence/absence of N. bombi spores by inspecting an area of 4 × 5 visual fields (20 visual fields per tissue smear) on a slide. For light infections or smears that were difficult to evaluate, additional tissue was prepared for repeated screenings. We determined prevalence of infection for each host population (individual hosts infected per
**Bombus** species per site). To assess significant among-sites differences in the proportion of infected individuals per collection site, we used generalized linear models for weighted binomial proportion data (Table S6) implemented in R v2.10.1 (15).

**Pathogen infection intensity.** To determine total production of mature *N. bombi* spores in the midgut of a host, we homogenized the tissues in a tissue grinder with 100 μL water and determined the spore count per microliter suspension using a Petroff-Hauser hemocytometer. High-intensity infections were visible under the microscope as very dense layers of spores, whereas light infections often were detected at less than 20 spores per slide. We recorded the average number of spores per visual field to represent infection intensity in the entire gut of each individual. Based on repeated spore counts of low-, medium-, and high-infected gut tissues determined by visual inspection, we defined the levels of infection intensity as follows: low infection, an average of <2 spores per visual field = 1–1,000 spores/μL; moderate infection, 2–20 spores/visual field = 1,000–100,000 spores/μL; high infection, >20 spores/visual field ≥ 100,000 spores/μL. We did not perform statistical analysis of infection intensity because of the small number of infections outside of *B. pensylvanicus* and *B. occidentalis*, and high-intensity infections were much more common in these two species: spore counts for 38.6% and 64% of all infections, respectively, were higher than 100,000 spores/μL. With the exception of *B. vosnesenskii*, in which 33.3% of infections (although in only 4 out of 12) were heavy, all other species had less than 20% heavy infections, and there were no high-level infections in *B. bimaculatus*.

**Genetic assessment.** We determined the species identity of observed microsporidian infections using DNA sequencing. To extract DNA from infected midgut tissues of a set of representative *Bombus* individuals, we added tissue samples to 150 μL 5% Chelex 100 resin (Bio-Rad) and 5 μL proteinase K (20 mg/mL). The sample was incubated at 55 °C for 1 h followed by 95 °C for 15 min to denature the enzyme. DNA was available in the supernatant after a short centrifugation. Samples were stored at −20 °C until needed. PCR was carried out using the oligonucleotide primers 1061f (16), 2282r, 530f, r17 (17), SSUres-f1t1 (18), 18f, 1492r, and 1047r (19) to amplify portions of the small subunit, large subunit, or internal transcribed spacer regions of the ribosomal DNA. Reactions were generally performed with 25-μL samples containing 4 μL DNA, 5 μL 5x Promega GoTaq flexi buffer, 2 μL 25 mM MgCl2, 0.5 μL dNTPs (10 mM each), 0.125 μL 5 U GoTaq polymerase (Promega), and 2.5 μL of each forward and reverse primer (2.5 μM). The PCR parameters were initial DNA template denaturing at 95 °C for 3 min and 35 cycles of denaturation at 95 °C for 45 s; primer annealing temperature ranged from 48 °C to 56 °C for 30 s, and primer extension was at 72 °C for 30 s followed by an extension cycle of 72 °C for 45 min, with adjustments made depending on the quality of initial amplification results. Electrophoresis for eastern species was performed on ABI 3730xl capillary DNA sequencers (Applied Biosystems) at the high-throughput DNA facility at the University of Illinois W. M. Keck Center for Comparative and Functional Genomics, and electrophoresis for western species was performed on ABI 3730xl capillary DNA sequencers at the Utah State University Center for Integrated BioSystems core facility. Alleles were scored manually using GENEMAPPER 4.3 (Applied Biosystems) with separate bin sets for each species. There was no evidence of amplification or scoring error based on repeat genotyping of subsets of individuals for each species.

**Genetic data analysis.** Bumble bees live socially in colonies, and when collecting workers at a given site, it is possible to sample multiple sisters from the same colony (full sibs), potentially affecting estimates of population genetic parameters (25). For each species, we identified sisters using a full-maximum likelihood approach for monogamous haplodiploids implemented in COLONY 2.0 (26). Population allele frequencies for each species were estimated from the pooled sample data from the 38 sample sites, and 20% were considered as potential sibs. Because no evidence of error was detected from replicate genotyping of individuals and we excluded all problematic markers (see below), we specified a relatively low probability of null alleles and other errors (0.5% per locus). In practice, the precise value of these probabilities did not alter results in preliminary assessments. For each full-sib group, one individual was randomly chosen to represent the colony in subsequent genetic analyses.

The final datasets were tested for deviations from Hardy–Weinberg and linkage equilibria using GENEPOP v4.0 (27). Deviations from Hardy–Weinberg equilibrium (HWE) were tested with a Markov chain approximation to an exact (significance) test, whereas significant linkage disequilibrium (LD) was assessed with likelihood ratio tests; in both cases, Bonferroni corrections were applied. Only two loci by subpopulation comparisons were significant for deviations from HWE for the eastern *Bombus* species: locus BL15 in impNCb (Mt. Mitchell, NC) in *B. impatiens* and B121 in penMOKS (a pool of three sites in southwest Missouri and southeast Kansas) in *B. pensylvanicus*. *B. bimaculatus* exhibited significant LD for BL13 + BT10 in bimSD and for B121 + BT10 and BL15 + BT10 in bimWlc. For *B. impatiens*, LD was significant for BL15 + BT10 in impALA, B10 + BL15 in impOH, and B10 + BL15 in impWla. There was no LD detected for *B. pensylvanicus*. In the west, *B. bifarius* showed highly significant deviations from HWE at B119 in all subpopulations, and therefore, this locus was excluded from further analysis. There were no
HWE deviations in *B. vosnesenskii* or *B. occidentalis* and no significant LD in any western species. Overall, the low number of significant values across all species suggests that HWE deviations and LD are not a major problem for this dataset.

We used FSTAT v2.9.3.2 (28) to estimate global genetic structure among populations using *F* _ST_ (29), with 95% confidence intervals estimated by bootstrapping loci and significance of genotypic differentiation among populations estimated using 1,000 permutations of genotypes among populations. We also estimated Jost’s *D* (30), a statistic that provides a true measure of differentiation for highly variable markers, such as microsatellites, using the software SMOGD v2.6 (31). Finally, in an attempt to identify well-defined genetic groups that might be useful for diagnosing evolutionarily significant management units (32), we examined population structure with the Bayesian clustering algorithm implemented in STRUCTURE v2.3.3 (33). The STRUCTURE model assumes that a sample of individuals comprises *K* potential populations, to which individual genotypes (or fractional genotypes) can be assigned. We used default parameter settings to assign individuals to populations (allowing for correlated allele frequencies and admixture), with 20,000 burn-in steps followed by 50,000 samples, and evaluated results over a range of *K* values. For the purposes of this study, we limit results to *K* = 3 to illustrate an overall presence or lack of genetic structure; detailed results will be presented in a companion paper. All results were stable across multiple runs.

For each species, we estimated average expected genetic variation and interlocus SE per subpopulation and in total (i.e., all individuals pooled) using Nei’s measure of gene diversity (*H* *) (34). Significance levels of pair-wise differences in *H* _E_ among species were estimated using 1,000 subpopulation-level randomizations in FSTAT v2.9.3.2 (only loci shared by all species within each region were included in the test). We also tested for differences in the proportion of unique colonies to total specimens collected per site (as identified by COLONY; excluding sites with only one individual of a given species) among species in each region using generalized linear models with quasibinomial errors and a logit link function in R v2.10.1 (Table S9).

2. Stephen WP (1957) *Bumble Bees of Western America* (Oregon State College, Agricultural Experiment Station, Corvallis, OR), Technical Bulletin.
3. LaBerge WE, Webb MC (1962) *The Bumblebees of Nebraska* (University of Nebraska College of Agriculture, Agricultural Experiment Station, Lincoln, NE), Research Bulletin.
Fig. S1. (A) Map of the 382 sites surveyed for Bombus from 2007 to 2009 (see Table S1 for details). (B) Digitized natural history collection records for the eight target Bombus species (see Table S2 for a detailed summary). (C) Temporal trends in relative abundance for each target Bombus species in four regional comparisons (Fig. 2). Data for 1900–1999 (black axis labels; specimens pooled by decade) were taken from the Bombus natural history collections database (B and Table S2) and for 2007–2009 (red axis labels), from field surveys (A) (Table S1). Plots of historical and contemporary relative abundances are consistent with recent declines for the less abundant bumble bee species over the last 20–30 y, with our 2007–2009 surveys recovering proportionally fewer specimens of B. affinis, B. occidentalis, B. pensylvanicus, and B. terricola than in any decade of the 20th century. (D) Selected results from STRUCTURE analyses of six genotyped Bombus species. Each vertical bar represents a single sample taken from throughout the range of each species (x axis) (Table S8). The y axis indicates the proportion of an individual’s genotype assigned to a particular genetic cluster (each cluster shown as a unique color). Only B. bifarius shows any evidence of genetic structure. All individuals in each of the other species are assigned equally to the three clusters, indicating a lack of subspecies or other major genetic subdivisions. For simplicity, only K = 3 is shown; the same results seem to hold for any specified K. Detailed results will be the subject of a companion paper.
Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)
Table S5 (DOCX)
Table S6 (DOC)
Table S7 (DOC)
Table S8 (DOCX)
Table S9 (DOC)