

Supplementary Materials for

Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence and Function

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Materials and Methods

Field methods: 1890s vs. 2009/2010. In 2009 and 2010, during March through May, L.A.B, T.M.K., and field assistants resampled plant-pollinator interactions and compared historic interactions networks with those that persist today to understand how plant-pollinator interactions are changing over time. Like Robertson, we sampled plantpollinator interactions and their phenologies in woodlands within ten miles of the town center of Carlinville, Illinois. Most of the natural areas that Robertson sampled have been converted to agricultural, commercial, or residential uses since then, so we were able to systematically investigate the remaining woodlands near Carlinville for populations of appropriate plant species (14 total sites). In temperate ecosystems, the majority of phenological shifts have been observed to occur in early season species (6-7). and the spring season itself is undergoing the greatest alterations in climate relative to the other seasons (33). Thus, we concentrated our sampling efforts to target 26 springblooming woodland forb species and their floral visitors. We observed these forb species in conditions and hours of peak insect activity (c. 0900 – 1500) and hand-netted floral visitors observed contacting the reproductive parts of flowers. Over 2009 and 2010, we spent 447 person hours of observation in the field, capturing 3,620 floral visitors, of which 2,778 were bees. We used these data to determine current plant-pollinator visitation networks across all sites.

What were the overall shifts in network structure? We calculated the number of interactions lost and gained. We further calculated the number of interactions lost due to bee extirpations.

Comparison of sampling effort: 1890s vs. 2009/2010. We investigated potential differences in sampling between historic and contemporary datasets to ensure that sampling effort did not contribute to the observed bee extirpations. The majority of Robertson's sampling of plant-pollinator interactions was between 1887 and 1899, though he performed additional, targeted investigations until c. 1916. No detailed methodological descriptions were available for the specific locations of Robertson's collections within the ten mile radius surrounding Carlinville town center. Based on Robertson's accession books, 13 of the 26 spring-blooming forbs were sampled for one or two consecutive years each, and are thus are comparable to our sampling efforts, given similar degrees of interannual variation in bee communities for both time periods. The remaining forbs appear to have been sampled intensively in one or two consecutive years with limited sampling in subsequent years (one to five), or were not recorded in these books. Based on our observations of extirpation of 50% of bee species sampled by Robertson (see Results), if anything, we would be concerned that our sampling was not adequate. To address this concern, we compared extrapolated, "true" bee species richness with that observed (Table S1). For each forb species, a collector's curve was calculated to show the relationship between the number of bee species observed, S(n), and the number of bee individuals collected, n. This collectors curve was calculated for each forb species by randomly sampling the observed individuals without replacement and calculating the number of species seen with each additional individual; this is replicated 1000 times, and an average collector's curve was calculated. The estimated number of species that visits each forb species and a constant, K, was quantified by fitting the resulting mean values for S(n) and n to the asymptotic, negative exponential

function, $S(n)=S_{max}(1-e^{-Kn})$. The asymptote, S_{max} , is the estimated "true" bee species richness (17). The observed bee species richness was equivalent to extrapolated, "true" species richness for 92% of the focal forb species (Table S1), indicating the absence of biases and confirming our observation methods as adequate. No detailed community-level plant-pollinator interaction data were available from the 1970s, though intensive, spatially-explicit pollinator collections were made on *Claytonia virginica* (see below).

Which bee traits, if any, help explain observed patterns in extirpation? To help determine why particular bee species went locally extinct, we used a multiple logistic regression to test the degree to which historic diet breadth (log-transformed), nesting habit (cavity vs. other: hive, soil, and wood/stem), life-style (parasitic vs. other: eusocial and solitary), and historic interaction strength (i.e., mean phenological overlap, 23) explained extirpation (Table S2, whole model: $\chi^2 = 21.79$, df = 5, P = 0.0002). With this analysis, we tested four hypotheses: 1) bee species with historically narrow diet breadths (specialists) were more likely to be extirpated than those with wider diet breadths, 2) bee species at higher trophic levels (parasitic species) were more likely to be extirpated than eusocial or solitary species, 3) bee species that nest in cavities were more likely to be extirpated than other nesting habits (hives, soil, wood/stem), and 4) bee species participating in historically weak interactions (shorter period of temporal overlap) were more likely to be extirpated than those participating in stronger interactions (longer period of temporal overlap) (Table S2).

The above statistics on non-random bee extinctions ignore the evolutionary relationships among taxa. Phylogenetically independent contrasts (PICs) correct for the lack of independence among related taxa (34). We created a taxonomic phylogeny of Robertson's 109 bee species. Taxonomic information available includes family, subfamily/tribe, genus, subgenus (when available) and species. In addition, topologies at the family and subfamily/tribe levels are known from molecular phylogenies (35), which provided a reference onto which taxonomy was superimposed. We used the Analysis of Traits module in Phylocom v4.2 (36) to calculate PICs and to test for evolutionary relationships between bee traits and extinction. We calculated PICs in four separate analyses and conducted subsequent Wilcoxon rank tests on the data to test whether each independent variable is associated with extinction.

Was the disproportionate extirpation of specialist compared to generalist bee species due to random chance? Robertson observed 109 bee species visiting 26 spring-blooming woodland forbs; 29 of those bee species were monolectic (i.e., they historically visited only one forb species each). Of the original 109 bee species, 55 are now locally extinct (50.5%). Of those 55 local species extinctions, 24 species were monolectic. Because these monolectic bee species made up a large proportion of the species pool, we constructed a model to test whether the level of monolectic extirpation that we observed could be random (i.e., due to chance). With each run of the model, we selected 55 of the original 109 bee species at random to go "extinct", and counted the number of monolects in the random sample. With 10,000,000 trials, the mean and standard deviation of the number of monolects among the selected 55 was 15.6 +/- 2.37. The observed 24 monolect extinctions was >3 standard deviations above the number expected by random chance (> 99.7% confidence; P < 0.003). Thus, we concluded that the observed loss of monolectic bee species was not due to random chance, and other factors (see main paper) contributed to their local extinction.

To what degree did loss of temporal and spatial co-occurrence contribute to interaction losses? We quantified floral phenology by patrolling our woodland sites in early spring to record the onset of flowering for each species. Once flowering began, we recorded floral densities of each forb species in a band transect (50 m by 2 m) on each sampling day at each site as well as documenting rare species in flower that fell outside of the transect. We used these data to determine current phenologies at each site and summed across all sites. We investigated the number of interactions that were lost because of loss of temporal co-occurrence of species at each site and across all sites. We further investigated the degree to which spatial mismatches (i.e., loss of spatial co-occurrence at our sampling sites between historically interacting forbs and bees) contributed to interaction loss by calculating the number of interactions that can no longer occur due to spatial mismatches. Spatial overlap between forb and bee species was deemed to occur if they co-occurred in at least one of our 14 study sites. We assume that individual bees are not regularly flying to forage and pollinate among our woodland study sites, which ranged from c. 2.5 to 16.1 km apart.

Was there evidence for differential phenological change that could lead to loss of temporal co-occurrence? Phenologies of forbs and bees, including peak bloom, peak bee activity and duration of bloom and activity, were compared between Robertson's time (1890s) and current data (2009 and 2010) using paired t-tests. In this way, we investigated phenologies of the subset of species that were present in both time periods. Peak bloom and peak bee activity were calculated as the middle day between onset and completion. These estimates of peak bloom and peak bee activity qualitatively matched peak floral densities and peak bee captures because of normal distributions of flowering and flight periods. No rigorous phenological data were available from the 1970s. We investigated relationships between current day of first (or peak) activity for bees and forbs with the change in day of activity compared to Robertson's phenologies. This analysis (Fig. S3) allowed us to determine whether there were differential patterns of phenological shifts in bee compared to forb species that may indicate the potential for interaction mismatches. We determined whether mean interaction strength (i.e., mean phenological overlap, 23) declined over time using a t-test.

What are the null-expected bee extinctions and interaction losses, based on phenology alone? We created a null model to quantify how many bee species would be expected to go extinct from Robertson's time until 2009-2010 based on changes in phenology alone. Our null model thus aids in the separation of diversity and interaction changes due to phenological shifts from other anthropogenic changes in this system. A null model approach is the only way to look at the role of phenology in extinctions, since other observations of phenological change and lack of temporal overlap between species can only consider extant species. This model is based on 109 species of bees and 26 species of spring ephemeral forbs and their interactions. Input data include the phenology of each species during Robertson's time (activity dates for bees and blooming times for forbs), the phenology of each extant species in 2009-2010, and whether or not bees and forbs interacted historically.

In each run of the model, we examined whether or not bees and forbs that interacted historically would interact after phenological change. For forbs, we used the real changes in phenology (change in day of first activity) observed from Robertson's time until 2009-2010, which is known for all 26 extant species. Because current

phenology is not known for all bee species due to extirpations, the model assigns each bee species a change in phenology based on patterns observed in extant species. For each bee species, the new start date of activity was based on a linear relationship between the date of first activity and the change in start date of activity (y=65.99-0.677x) and from picking randomly from a normal distribution around y with a standard deviation of 13.1, which represents the mean and standard deviation for observed change in start date of activity for extant bees. The percent change in duration of bee activity was determined by choosing randomly from a normal distribution with a mean of -41.1 and a standard deviation of 33.0, which is based on the distribution observed for extant bees. Both the observed change in start date and observed change in duration of bee activity were normally distributed. Interactions that were present in Robertson's time were assumed to still be present if the forb and bee still overlap in phenology. Interactions that were present in Robertson's time were lost if the forb and bee no longer overlap in flowering and flight phenology.

We assumed that it is possible for forbs and bees to acquire new interactions, based on how generalized each species was historically. For each forb and bee species, we calculated how generalized the species is based on the quotient of number of species it interacted with and number of species it could interact with based on phenology overlap in the historic data set. Interactions were gained with a probability that was the product of the forb and bee generalizations.

Bees go extinct in the model if they no longer interact with any forbs or if they go 10 days without food (i.e., the presence of a forb they visit for pollen or nectar) early in the season. Exceptions for the latter were made for seven of the 109 bee species (Andrena illinoiensis, Habropoda laboriosa, Hylaeus brevicornis, Lasioglossum laevissimum, L. pruinosum, L. tegulare and Nomada denticulata) in which bee activity preceded the blooming of spring ephemeral forbs during Robertson's time. These species interacted with flowering trees and shrubs (e.g., Salix sp. and Prunus sp.). We assume that these bee species always persist in the null model due to their ability to use early flowering plants that are not part of this forest understory community.

We examined how important the ability of forbs and bees to gain interactions was to the model results by running the model without allowing interaction gains to occur. We further quantified the effect of assuming a much wider distribution surrounding the means for both the change in the start date of activity and duration of activity of bees from Robertson's time to the present. It is possible that locally extinct bees experienced more dramatic changes in start date and duration of activity than that observed for extant species, and it is therefore interesting to examine a broader distribution of phenological changes. We varied the change in standard deviation from 0 (which assumes that extant and extinct bees experienced similar changes in phenology) to 100% at 10% increments (Fig. S5).

In each run of the model, we calculated whether or not each bee species went extinct (Fig. S6), the total number of bee extinctions, the number and identity of interactions that were lost (Fig. S4), the number and identity of interactions that were gained (Fig. S8), and whether interaction loss was associated with a bee extinction. We performed 1000 replicate runs to get means (and 95% confidence intervals) of these calculations. We tested associations between model predictions and the observed patterns in species extirpations and interactions. In particular, we determined the

relationships between bee diet breadth (log-transformed), nesting habit, historic interaction strength, and sociality with null-expected extinction probability (log-transformed for normality) using multiple linear regression (Fig. S6).

Why did certain bee species acquire new interactions while others did not? Interestingly, we did not find the expected relationship between the number of interactions acquired and historic diet breadth (Fig. S8), and we hypothesized that historically rare species that have persisted and increased in abundance might be most likely to acquire new interactions. Robertson categorized each of the plant-bee interactions that he observed as "present", "frequent" or "abundant". Using these categories, we estimated the historic abundance (present, frequent, or abundant) of each bee species based on their average historic interactions. We also categorized the current abundance of each bee species using the log-normal distribution of abundances across the 54 persisting bee species. A current bee species was categorized as "present" if the log(number of individuals captured) fell between 0 and 0.5 (25% quartile), "frequent" between 0.5 and 1.75, and "abundant" between 1.75 and 2.75 (75% quartile). The change in abundance of each bee species was then categorized as "increasing", "same", or "decreasing". We investigated the relationships between bee species traits (diet breadth, sociality and family) and change in abundance using nominal logistic regression to determine if bees with certain traits were more likely to increase or decrease in abundance. Next, we tested whether change in abundance affected change in diet breadth (i.e., acquisition of new interactions or loss of historic interactions) using an ANOVA. We note that Roberston's categories were not as rigorously justified as ours, and that these analyses should be considered exploratory rather than a rigorous test of our hypothesis.

How and why did the structure of the plant-pollinator interaction network change over time? We compared the nestedness of the forb-bee interaction networks observed historically and currently as a measure of how the structure of the network has changed over time using the vegan package in R 2.12.1 (nestedtemp, oecosimu, and nestedchecker with quasiswap). Since interaction rewiring contributed so greatly to changes in the interaction network, we determined which family of bees experienced the greatest loss of diet breadth and contributed most greatly to interaction loss throughout the network using ANOVA.

How did the richness of bees visiting *Claytonia virginica* change over the last century? *Claytonia virginica* is one of the dominant forbs in this system, averaging 5.4 flowers/m² compared to 1.0 flowers/m² across the other forb species. We compared bee species richness of visitors to *C. virginica* in 1916, 1971, 2009, and 2010 using standardized sampling effort with rarefaction. Rarefaction was possible because Robertson (13) recorded the abundances and species identity of the first 100 bee individuals to visit *C. virginica* on May 5, 6, 9, and 10, 1916. Because *C. virginica* no longer blooms in any abundance on those later dates in May, we used the same methodology, but utilized data from April 24 and 25 and May 1, 1971 (collected from 3 of the 12 sites), April 17 and 23 and May 5, 2009, and April 20, 21, and 27, 2010 (collected from the same 3 sites as in 1971). Rarefaction was performed using Ecosim (Gotelli and Entsminger, 2004).

How did the richness, visitation rates and community structure (i.e., species nestedness across sites) of bees visiting *Claytonia virginica* change between 1971 and

2010, and how did landscape modification contribute to these shifts? The 1971 data set collected by J.C.M. is powerful because it provides information on specific (spatiallyexplicit) sampling sites and sampling effort, which were not available for Robertson's collections. J.C.M specifically targeted the bee fauna visiting C. virginica, and thus community-level forb-bee interaction networks are not available for this time period. Methods for the 1971 data are detailed in Marlin and LaBerge (16). We returned to and sampled 12 sites that were sampled in 1971 in order to control for sampling location and to investigate the effects of land-use change on the species and interactions over time. Land use within 500 m of these sites was determined from aerial photos from 1968 (acquired from Historical Information Gatherers, Minneapolis, MN) and 2005 (available at http://www.isgs.illinois.edu/nsdihome/) and categorized as forest, agriculture, old field (not under active cultivation), water, or development. For each of the 12 sites, we calculated the change in forested land area (which was negatively correlated with developed land area) and the change in richness of bee species visitors (rarefied) to C. virginica between 1971 and 2010. We then used linear regression to evaluate the relationship between these variables across sites. We determined whether the geographic distance between sites influenced similarity in species composition – it did not (Mantel test: r = -0.25, P = 0.81). We calculated and compared be visitation rates to C. virginica (i.e., number of bee individuals captured per minute observing C. virginica) at each site in 1971 and 2010 using a paired t-test. Finally, we tested the degree to which C. virginica bee community composition was nested across sites in 1971 and 2010 (nestedtemp, oecosimu, and nestedchecker with quasiswap using the vegan package in R 2.12.1). Finding changes in the nestedness of the bee community over time would indicate shifts in the redundancy of bee species across sites that is characteristic of intact communities. Loss of bee community nestedness would suggest loss of redundancy and increased susceptibility to perturbations.

The degree of fidelity has implications for pollinator efficiency and pollination probability (37). To quantify bee fidelity to C. virginica, we selected six bee species in the genus Andrena (A. carlini, A. cressonii, A. erigeniae, A. forbesii, A. imitatrix, and A. miserabilis) that visit C. virginica for which we had access to specimens (housed at the Illinois Natural History Survey) collected by Robertson, J.C.M (1970's), and in the present study (2009, 2010). These species spanned a gradient from specialist to generalist. Across the three time periods, 538 total specimens were used. Each bee was washed twice in 70% ethanol to remove all pollen grains. Subsequent washes and inspection of the specimens under a microscope showed no additional pollen grains adhering to the bees. We were unable to use cubes of fuchsin dye to remove and sample pollen from the bees due to the fragility of Robertson's 120 year old specimens. Thus, our sampling scheme may have included some pollen grains that would not have been available for pollination, however, we avoided the scopae to limit that possibility. Pollen grains were fixed to microscope slides using fuchsin dye and identified to species using a reference collection. We calculated the proportion of *C. virginica* pollen grains on each specimen and compared these values (arcsin square-root transformed for normality) from the three sampling periods using a ANCOVA with bee species included as a covariate. All assumptions of statistical tests were met unless otherwise noted.

Author contributions: L.A.B., T.M.K., (2009, 2010) and J.C.M. (1971) designed the studies and collected the data; T.M.K. performed modeling; L.A.B. performed statistical analyses; L.A.B and T.M.K wrote the paper. All authors read and discussed the manuscript.

Supplementary Text

Null model code

```
%MATLAB code for calculated null-expected bee extinctions and
interaction
%losses, based on phenology alone.
clear
nn=0;
resulttable=zeros(5,4);
for nnn=1:5
nbees=109;
numberplants=26;
numreps=1000;
saveextinction=zeros(nbees,numreps);
savenumextinctions=zeros(1,numreps);
interlost1=zeros(numreps,2);
for xm=1:numreps
data
%INSERT INPUT DATA HERE (4 matrices)
%PART 1: matrix
*matrix contains three columns that gives phenology data for each bee
%species in Robertson's time.
%column #1 is the bee id (a number between 1 and 109),
%column #2 is the julian start date of activity,
%column #3 is the julian end date of activity.
%PART 2: matrixplants
%matrixplants contains three columns that gives phenology data for each
%plant species in Robertson's time
%column #1 is the plant id (a number between 1 and 26),
%column #2 is the julian start date of blooming,
%column #3 is the julian end date of blooming.
%PART 3: matrix2
%matrix2 contains two columns that show which plants and pollinators
%interacted historically.
%the first column is the pollinator ID and the
%second column is the Plant ID.
The numbers for pollinator id and plant id
%correspond to the numbers used in matrix and matrixplants
%PART 4: matrixplantsnow
%matrixplantsnow contains three columns that gives phenology data for
each
%plant species in the contemporary dataset.
%column #1 is the plant id (a number between 1 and 26),
%column #2 is the julian start date of blooming,
```

```
%column #3 is the julian end date of blooming.
%create a matrix (called pollinatorphen) that shows whether or not the
pollinator is active on each
%julian date. Each column shows the julian day, each row is a
different
%pollinator species
pollinatorphen=zeros(nbees,365);
for h=1:nbees
for g=1:365
    if g>=matrix(h,2)
        pollinatorphen(h,g)=1;
    end
    if g>matrix(h,3)
        pollinatorphen(h,g)=0;
    end
end
end
%create a matrix (plantphen) that shows whether or not the plant is
%blooming on each julian date
plantphen=zeros(26,365);
for h=1:26
    minn=matrixplants(h,2);
    maxx=matrixplants(h,3);
for g=1:365
if g>=minn && g<=maxx</pre>
    plantphen(h,g)=1;
end
end
end
%fill in matrix of possible interactions based on overlap in phenology
possible=zeros(numberplants, nbees);
for pt=1:numberplants
for pol=1:nbees
plant1=plantphen(pt,:);
poll1=plant1+pollinatorphen(pol,:);
pick=find(poll1(:,:)==2);
n=length(pick);
if n>0
    possible(pt,pol)=1;
end
end
end
%calculate whether or not the species actually interact
actual=zeros(numberplants, nbees);
for p=1:nbees
pick=find(matrix2(:,1)==p);
poll88=matrix2(pick,:);
n1=length(pick);
for pp=1:n1
    actual(poll88(pp,2),p)=1;
end
end
%save the data
savers=zeros(nbees,18);
matrixfood=zeros(nbees,170);
for go2=1:nbees
```

```
%save the pollinator number
savers(go2,1)=go2;
    %save the start date of activity
savers(go2,2) = matrix(go2,2);
    %save the end date of activity
savers(qo2,3) = matrix(qo2,3);
    %save the duration of activity
savers(go2,4) = matrix(go2,3) - matrix(go2,2);
%look at each pollinator
polnum1=actual(:,go2);
%pick plants that it interacts with now
pick=find(polnum1==1);
np=length(pick);
%save the number of plants each bee interacts with historically
savers(go2,5)=np;
%look at the days in which these plants flowers
%create a vector that has 0's
%where no plants are flowering that this pollintor can visit and 1s
where
%at least one plant species is flowering
julianmax=170; %170 is end of spring wildflower season
plantphen2=plantphen(:,1:julianmax);
saveit=plantphen2;
ub1=plantphen2(pick,:);
%sums up the number of plant species each pollinator is interacting
with on
%each julian date
if np>1
ub1=sum(ub1);
end
if np > = 1
for go1=1:julianmax
if ub1(go1)>0
    ub1(go1) = 5;
else
    ub1(go1)=3;
end
end
end
*look at the days in which the pollinator is active from julian day 1
%julian date 170
pollinatorphen2=pollinatorphen(:,1:julianmax);
ub2=pollinatorphen2(go2,:);
%sum up the days that both are open and active
ub3=ub1+ub2;
pick=find(ub3==2);
overlap=length(pick);
numberdayswithoutfood=ub2-ub1;
matrixfood(go2,:)=numberdayswithoutfood;
matrixfood=matrixfood';
*pollinators that use tree/shrub flowers and do not starve early in the
season
matrixfood(77:125,16)=1;
matrixfood(77:125,51)=1;
matrixfood(77:125,58)=1;
```

```
matrixfood(77:125,70)=1;
matrixfood(77:125,75)=1;
matrixfood(77:125,76)=1;
matrixfood(77:125,87)=1;
%for each pollinator, is it starving early, late or both
starvers=zeros(nbees,3);
for qo4=1:nbees
if matrixfood(170,go4) == -2
    starvers(go4,2)=1;
end
if matrixfood(matrix(go4,2),go4)==-2
    starvers(go4,1)=1;
starvers(go4,3)=starvers(go4,1)+starvers(go4,2);
%for each pollinator, compute number of days without food early in the
%season
for go5=1:nbees
    if starvers(go5,1)==1
        pick=find(matrixfood(:,go5)==-2);
        numberdayswithoutfood=length(pick);
        savers(go5,7)=numberdayswithoutfood;
    elseif savers(go5,5)==0;
        savers(go5,7) = savers(go5,4);
    else
        savers(go5,7)=0;
    end
end
%save the data for plants
savers2=zeros(numberplants,14);
for go20=1:numberplants
    %save the plant number
savers2(go20,1)=go20;
    %save the bloom start date
savers2(go20,2)=matrixplants(go20,2);
    %save the bloom end date
savers2(go20,3)=matrixplants(go20,3);
    %save the duration
savers2(go20,4)=matrixplants(go20,3)-matrixplants(go20,2);
%look at each plant
plnum1=actual(go20,:);
%pick pollinators that it interacts with now
pick=find(plnum1==1);
np1=length(pick);
savers2(go20,5)=np1;
end
%calculate the number of species of plants each pollinator interacts
pollinterbefore=sum(actual,1);
%calculate the number of species of pollinators each plant interacts
pltinterbefore=sum(actual,2);
%calculate generalization of each plant species
plantgen=zeros(numberplants,1);
for pt=1:numberplants
plant1=sum(actual(pt,:))/sum(possible(pt,:));
plantgen(pt,1)=plant1;
```

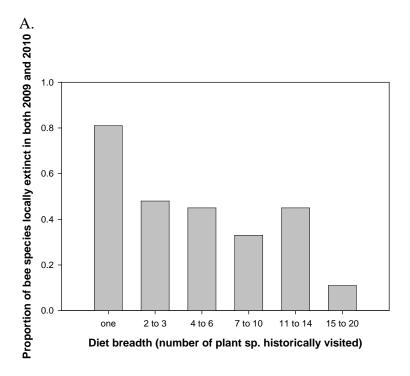
```
savers2(pt,6)=plant1;
end
%calculate generalization of each pollinator species
pollgen=zeros(nbees,1);
for pol=1:nbees
poll1=sum(actual(:,pol))/sum(possible(:,pol));
pollgen(pol,1)=poll1;
savers(pol,6)=poll1;
end
%shift plants based on observed changes in phenology
newmatrixplants=zeros(numberplants,3);
newmatrixplants(:,1)=matrixplants(:,1);
newmatrixplants(:,2)=matrixplantsnow(:,2);
newmatrixplants(:,3)=matrixplantsnow(:,3);
savers2(:,8)=newmatrixplants(:,2); %new start date for plant bloom
savers2(:,9)=newmatrixplants(:,3); %new end date for plant bloom
savers2(:,10)=savers2(:,9)-savers2(:,8); %new duration
plantphen3=zeros(26,365);
for h=1:26
   minn=newmatrixplants(h,2);
   maxx=newmatrixplants(h,3);
for q=1:365
if g>=minn && g<=maxx</pre>
    plantphen3(h,g)=1;
end
end
end
*shift pollinators on average 7.8 days earlier (SD=13.1 days).
Regression line with date of first activity used: Y = 65.99 - 0.677x
pollinatorphen3=zeros(nbees,365);
newmatrix=zeros(nbees,3);
newmatrix(:,1)=matrix(:,1);
for qo7=1:nbees
julch=65.99-(0.677*savers(go7,2));
Y = round(julch+(13.1+(13.1*nn)).*randn(1,1));
pollinatorphen3(go7,:) = circshift(pollinatorphen(go7,:), [0, Y]);
newmatrix(go7,2)=matrix(go7,2)+Y;
savers(go7,8)=newmatrix(go7,2);
if savers(go7,8)<30
    savers(go7, 8) = 30;
   newmatrix(go7,2)=30;
end
newmatrix(qo7,3) = matrix(qo7,3) + Y;
savers(go7,9)=newmatrix(go7,3);
savers(qo7,10) = savers(qo7,9) - savers(qo7,8);
%duration of activity is reduced on average by 41.1% (SD=33%)
for go9=1:nbees
y1=-41.1+((33+(33*nn)).*randn(1,1));
if y1>90
   y1=90;
elseif y1<-90
   y1 = -90;
end
if y1<0</pre>
```

```
Y=savers(go9,8)+round(savers(go9,4)*(1+(y1/100)));
newmatrix(go9,3)=Y;
pollinatorphen3(go9,(Y+1):365)=0;
savers(go9,9) = newmatrix(go9,3);
savers(go9,10)=savers(go9,9)-savers(go9,8);
else
Y=round(savers(go9,4)*(1-(y1/100)));
Y=min(savers(go9,8)+savers(go9,4)+(savers(go9,4)-Y),365);
newmatrix(go9,3)=Y;
pollinatorphen3(go9,newmatrix(go9,2):newmatrix(go9,3))=1;
savers(go9,9) = newmatrix(go9,3);
savers(go9,10) = savers(go9,9) - savers(go9,8);
end
end
%fill in matrix of possbile interactions based on overlap in phenology
newpossible=zeros(numberplants, nbees);
for pt=1:numberplants
for pol=1:nbees
plant1=plantphen3(pt,:);
poll1=plant1+pollinatorphen3(pol,:);
pick=find(poll1(:,:)==2);
n=length(pick);
if n>0
   newpossible(pt,pol)=1;
end
end
end
%take away interactions from the actual matrix that are no longer
%possible due to lack of phenology overlap,
newactual=zeros(numberplants, nbees);
for pt=1:numberplants
for pol=1:nbees
if newpossible(pt,pol)+actual(pt,pol)==2
    newactual(pt,pol)=1;
end
end
end
%make a matrix that shows the interactions that are lost because there
%no longer phenology overlap
interlost=zeros(numberplants, nbees);
for pt=1:numberplants
for pol=1:nbees
if actual(pt,pol)-newpossible(pt,pol)==1
    interlost(pt,pol)=1;
end
end
savers(:,15)=(sum(interlost))';
pollinterafter=sum(newactual,1);
pltinterafter=sum(newactual,2);
%add interactions that occur because plants and pollinators pick up
%new interactions based on their generalization
newpicks=zeros(numberplants,nbees);
for pt=1:numberplants
for pol=1:nbees
gen1=plantgen(pt)*pollgen(pol);
```

```
rand1=rand(1);
if (newpossible(pt,pol)==1) && (rand1<gen1);</pre>
    newactual(pt,pol)=1;
    newpicks(pt,pol)=1;
end
end
end
sm=sum(sum(newpicks))-sum(newpicks(:,65));
savers(:,18)=sum(newpicks)';
matrixfood2=zeros(nbees,170);
for go3=1:nbees
%look at each pollinator
polnum1=newactual(:,go3);
%pick plants that it interacts with now
pick=find(polnum1==1);
np=length(pick);
savers(go3,11)=np;
%look at the days in which these plants flowers
%create a vector that has 0's
%where no plants are flowering that this pollintor can visit and 1s
where
%at least one plant species is flowering
plantphen4=plantphen3(:,1:julianmax);
ub1=plantphen4(pick,:);
%sums up the number of plant species each pollinator is interacting
with on
%each julian date
if np>1
ub1=sum(ub1);
end
if np > = 1
for go1=1:julianmax
if ub1(go1)>0
    ub1(go1) = 5;
else
    ub1(qo1)=3;
end
end
end
if np==0
ub1=zeros(1,170);
%look at the days in which the pollinator is active from julian day 1
%julianmax (end of spring wildflower season)
pollinatorphen4=pollinatorphen3(:,1:julianmax);
ub2=pollinatorphen4(go3,:);
%sum up the days that both are open and active
numberdayswithoutfood=ub2-ub1;
matrixfood2(go3,:)=numberdayswithoutfood;
matrixfood2=matrixfood2';
%pollinators that use tree/shrub flowers and do not starve early in the
season
matrixfood2(77:125,16)=1;
matrixfood2(77:125,51)=1;
matrixfood2(77:125,58)=1;
```

```
matrixfood2(77:125,70)=1;
matrixfood2(77:125,75)=1;
matrixfood2(77:125,76)=1;
matrixfood2(77:125,87)=1;
%for each pollinator, is it starving early, late or both
starvers=zeros(nbees,3);
for qo4=1:nbees
if matrixfood2(170,go4)==-2
    starvers(qo4,2)=1;
end
if
   newmatrix(go4,2)<170 \&\& matrixfood2(newmatrix(go4,2),go4)==-2
    starvers(go4,1)=1;
end
starvers(go4,3)=starvers(go4,1)+starvers(go4,2);
%for each pollinator, compute number of days without food early in the
%season
for go5=1:nbees
    if starvers(go5,1)==1
        pick=find(matrixfood2(:,go5)==-2);
        numberdayswithoutfood=length(pick);
        savers(go5,12) = numberdayswithoutfood;
    elseif savers(go5,11)==0;
        savers(go5, 12) = min(savers(go5, 9), 170) - savers(go5, 8);
    else
        savers(go5,12)=0;
    end
end
matrixbees2=zeros(numberplants,170);
%create column 13 in savers that tells whether or not the bee goes
%extinct(0=no extinction, 1=extinction)
%bee goes extinct if it is starving for more than 10 days early in the
%season
for qo5=1:nbees
    if savers(go5,12)>10
    savers(go5, 13) = 1;
end
%bee goes extinct if it no longer interacts with any plants
for go5=1:nbees
    if savers(go5,11)==0
    savers(go5, 13) = 1;
    end
end
%if the bee goes extinct, then it does not interact with any plants
savers(:,14)=savers(:,11);
for go1=1:nbees
    if savers(go1,13)==1
        savers(go1,14)=0;
    end
end
%interactions that are lost when bee is also lost (savers 16) and not
lost
%(Savers 17)
for go5=1:nbees
    if savers(go5,13)==1
```

```
savers(go5,16)=savers(go5,5);
   else savers(go5,17) = savers(go5,15);
end
saveextinction(:,xm)=savers(:,13);
savenumextinctions(1,xm)=sum(savers(:,13));
interlost1(xm,1)=sum(savers(:,16));
interlost1(xm,2)=sum(savers(:,17));
interlost2=mean(interlost1);
interlostbeelost=interlost2(1);
interlostbeeok=interlost2(2);
resulttable(nnn,1)=nn;
resulttable(nnn,2)=interlostbeelost;
resulttable(nnn,3)=interlostbeeok;
resulttable(nnn,4)=mean(savenumextinctions,2);
nn=nn+.25;
end
```



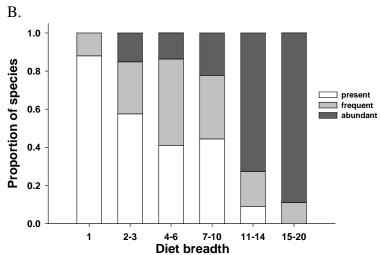


Fig. S1. We observed a disproportionate loss of specialist bee species from the late 1800s to 2009/2010 (A). We cannot, however, distinguish between diet breadth and rarity (B). Bee species with narrow diet breadths in the late 1800s were also rare species (categorized as participating in "present" interactions by Robertson rather than "frequent" or "abundant" interactions; ANOVA: $F_{2,106} = 41.40$, P < 0.0001). These species were more prone to extirpation because they were specialized, rare, or both.

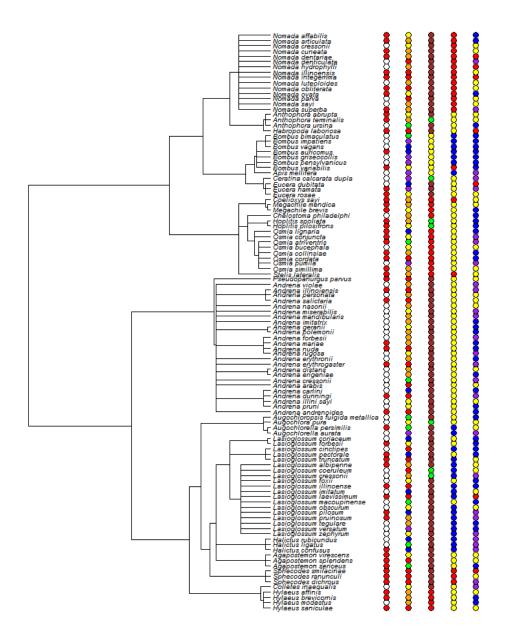
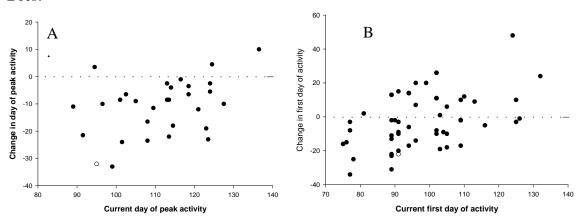


Fig. S2

Cladogram of Robertson's 109 bee species based on taxonomy (branch lengths are arbitrarily scaled; data were not available to calculate branch lengths, so all branches were considered equal in analyses). Discrete data for each species is presented in the circles to the right of the species name. Column 1 shows whether or not the species is locally extinct (red) or extant (white) in 2009-2010, column 2 shows the historic diet breadth of the bee species (red 1, orange 2-3, yellow 4-6, green 7-10, blue 11-14, purple 15-20), column 3 shows the nesting habit (red=cavity, yellow=hive, brown=soil, green=wood/stem), column 4 shows the sociality of the bee (blue=eusocial, red=parasitic, yellow=solitary) and column 5 shows the strength of historic interactions based on the average number of days the bee overlapped with plant resources (red=less than 20 days, yellow 20-30 days, blue 30-40 days, purple greater than 40 days).





Spring-blooming forbs:

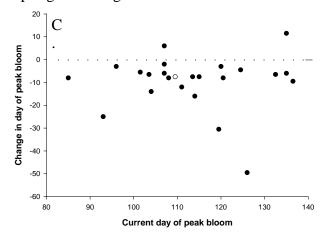


Fig. S3

Bee species that are currently active earliest in the season have experienced the greatest degree of phenological advancement, and they were formerly (in the 1880s) active later in the season. These figures involve 30 of the 54 bee species that we still observe today, for which we have estimates of their peak activity (A). The other 24 bee species occurred too rarely to make valid estimates of their peak activity. Thus, we also included figures illustrating the relationships between first day of activity for all 54 bee species and the change in first day of activity (B). Both representations of these data showed the same patterns. Correlations reported in the main paper were performed in JMP (version 4.0.4). Day of peak bloom for spring-flowering forbs has advanced similarly for all species across the flowering season (C).

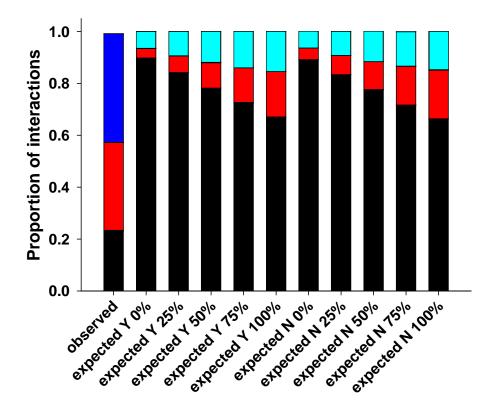


Fig. S4

Fate of the 532 interactions observed in Robertson's historic data set in the observed data and 10 different null model scenarios. In the observed data, 125 interactions persisted (black), 183 were lost because the bee went extinct (red), and the rest of the lost interactions are lost despite bee persistence (blue). In the null model scenarios, most interactions persisted (black), and phenological mismatches that result in interaction loss are sometimes associated with bees that go extinct in the same run of the model (red) and sometimes not (turquoise). For the null model, each bar represents averages observed across 1000 replicate simulations. Scenarios include whether or not bees are able to acquire new interactions across time (yes[Y] or no [N]), and the percent increase in standard deviation (0%, 25%, 50%, 75%, or 100%) from the mean change in bee phenology (first date of activity). In cases for which the percent increase in standard deviation is 0%, it is assumed that extant and extinct bees experienced similar changes in phenology, whereas increases in the standard deviation examine the possibility that extinct bees might have experienced wider shifts in their phenology.

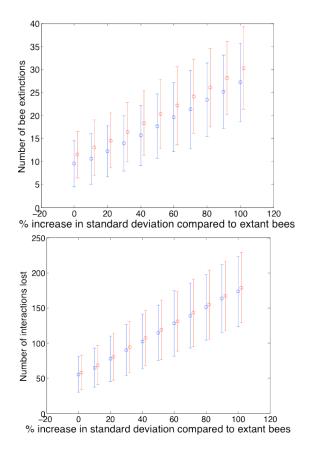
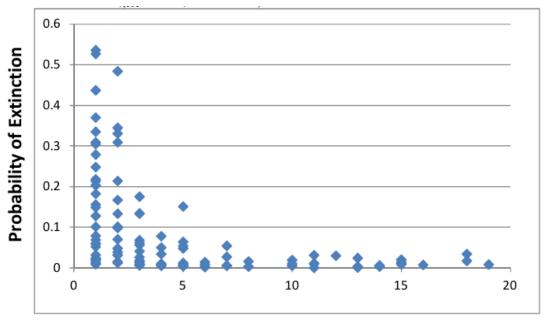


Fig. S5

Null model results. The number of bees that are projected to go extinct (top figure) and number of interactions lost (bottom figure) as a function of the percent increase in the standard deviation surrounding the mean change in date of first activity and duration of activity of all bees compared to the standard deviation observed for extant bees. When the x-axis is 0, the model assumes that all bees (extinct and extant) experience similar changes in phenology. As the x-axis increases, the model assumes that extinct bees had more dramatic changes in phenology than those observed for extant bees, which would increase the standard deviation of all bees. We examined percent changes in standard deviation from 0-100% at 10% intervals; 100% represents an extreme case in which the change in phenology of extinct bees diverges so much from extant bees that it doubles the standard deviation surrounding the mean change in date of first activity and duration of activity of all bees. Error bars are 95% confidence intervals from 1000 replicate runs of the model. Blue colors represent the scenario in which forbs and pollinators have the opportunity to gain new interactions and red represents the scenario in which forbs and pollinators do not have this ability; values of red are offset from blue on graph for visualization purposes. We found that preventing forbs and bees from acquiring new interactions did not significantly influence model results. However, when we allowed wider changes in the activity start time and duration of bees, we project an increase in the number of bee extinctions and in the number of interactions lost. However, even in our most extreme cases, the modeled values are significantly lower than the observed extinctions (55 bees) and interaction losses (407 lost interactions).



Historic diet breadth

Fig. S6

Relationship between the probability that a bee goes extinct (average from 1000 runs of model) and historic diet breadth of the bee. Specialist bees are more likely to go extinct. In the null model, extinction probability (log-transformed) was not associated with nesting habit ($F_{3,101} = 1.21$, P = 0.31), historic interaction strength ($F_{1,101} = 0.15$, P = 0.70), or sociality ($F_{2,101} = 1.74$, P = 0.18); whole model with diet breadth: $F_{7,101} = 5.83$, P < 0.0001).

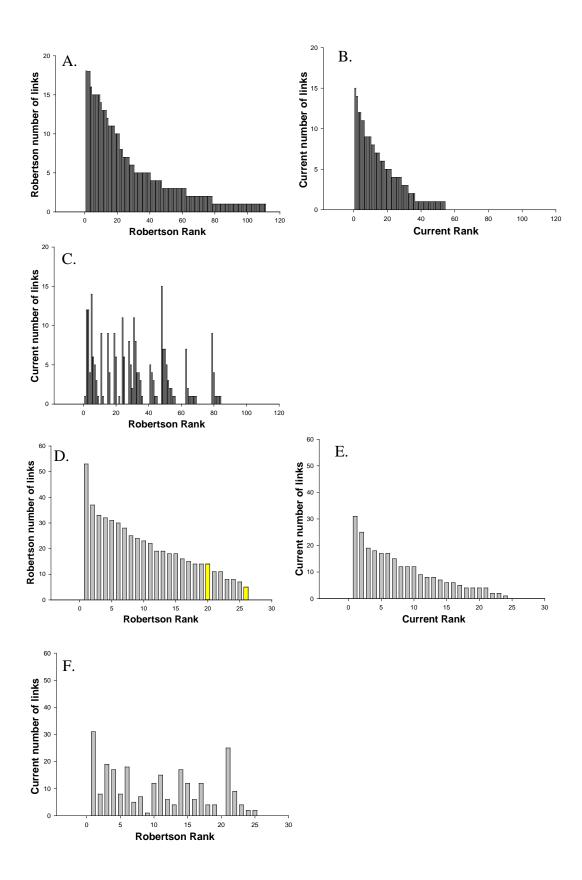
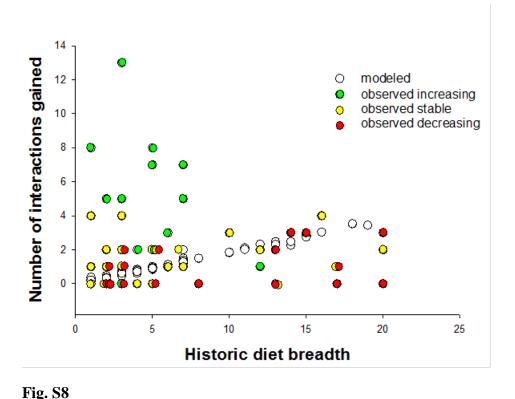


Fig. S7

Rank order of the number of links (interactions) to forb species observed by Robertson (A) and this study (B and C) for 109 bee species, illustrating changes in the degree distribution (loss of links per species) among bee species as well as interaction reorganization and flexibility over time. Although persisting bee species maintain 2.3 fewer interactions on average now than historically (paired t-test: t = 2.96, df = 53, P = 0.0046), an analysis comparing all historic bee species (109) to those that persisted (54) showed that mean links per species did not change through time (t-test: t = 0.98, df = 162, P = 0.33). These patters result from the combined effects of extirpations of specialist species, losses of interactions by generalist species, and interaction rewiring (primarily historic specialists acquiring new interactions). Historically, there were few bee species with many interactions and many bee species with few interactions. Over time, this distribution was truncated at both ends, such that both highly generalized and highly specialized species have declined.

Rank order of the number of links (interactions) to bee species observed by Robertson (D) and this study (E and F) for 26 spring-blooming forb species. Two of the forb species were present in the system, but we did not observe any bee visitors (highlighted in yellow). Across forb species, all of which persisted, links per species declined by 8.7 (paired t-test: t = 4.54, df = 25, P < 0.0001).

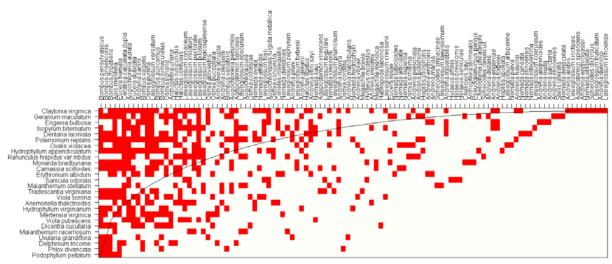


The relationship between average number of interactions gained per bee species (observed data or mean of 1000 runs of model) and historic diet breadth (historic number of forbs each bee interacted with). In the model, the probability of acquiring new interactions was a function of how generalized the bee species was historically, and thus bees with historically high diet breadth acquired more new interactions than more specialized bees. In reality, the majority of new interactions are gained by bee species with relatively narrow historic diet breadths. In particular, bee species whose abundances were estimated to increase (green) acquired the majority of new interactions, while

species whose abundances were estimated to be decreasing (red) rarely gained any new

interactions (ANOVA: $F_{2,51} = 17.47$, P < 0.0001).





B.

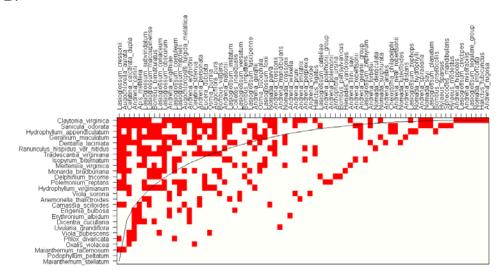
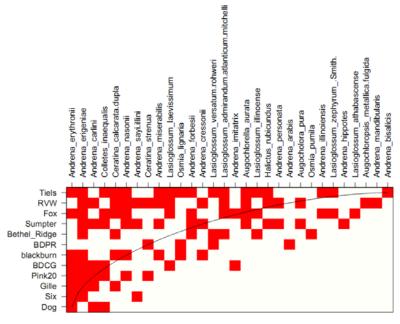


Fig. S9

There has been a loss of nestedness of the spring forb-bee interaction network between Robertson's time (A) and now (B). Robertson's network (A; 26 spring-blooming woodland forb species, 109 bee species) is significantly nested (p = 0.01). The forb-bee interaction network of the subset of Robertson's interactions that we observed in 2009/2010 (B; 26 spring-blooming woodland forbs and 54 bee species) was not significantly nested (P = 0.43). The observed loss of specialist bee species cannot account for the loss of interaction network nestedness, given that maintenance of nestedness is robust to loss of specialists. Thus, we concluded that the loss of non-specialist bee species and the reorganization of interactions that has occurred contributed to the loss of interaction redundancy and structure that is inherent in a loss of nestedness. Interaction rewiring may be due to a wide variety of factors, including loss of species, changes in the relative abundance of remaining species, different biotic and abiotic filters influencing species persistence at each site, and evolutionary responses.







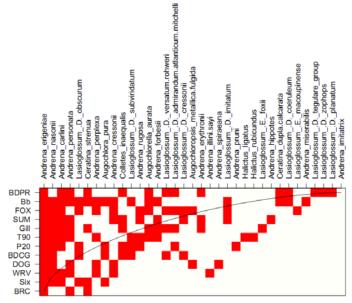


Fig. S10

Nestedness in bee community composition visiting *Claytonia virginica* in 1971 (A) and 2010 (B). Bee species sampled from *C. virginica* at 12 different sites in 1971 and 2010 were significantly nested 1971, but not significantly nested in 2010. This change in community structure over time (i.e., decline in nestedness) suggests the presence of different biotic and abiotic filters influencing species persistence at each site and suggests a loss of redundancy in species across space that is characteristic of more intact communities.

Table S1.

For each forb species, we show the number of individual bees collected and number of bee species observed in the most recent time period (2009-2010). Observed and estimated bee species are similar for all forb species except two, *Maianthemum racemosum* and *Phlox divaricata*. *Maianthemum racemosum* was sparse in 2009-2010 and bloomed for a short period of time; these factors might influence both the true diversity of visitors as well as our ability to observe diversity. *Phlox divaricata* was common in 2009-2010. We spent 12 hours observing this species and only observed four visitors. Overall, we found that our observed richness was close to 'true richness' and thus increasing our sampling effort would not have significantly increased observed bee species richness.

| Forb species | Number of | Observed | Estimated bee |
|--------------------------|-----------------|--------------|---------------------------|
| | individual bees | bee species, | species, S _{max} |
| | collected, n | S(n) | |
| Anemonella thalictroides | 11 | 6 | 7.84 |
| Camassia scilloides | 33 | 7 | 7.13 |
| Claytonia virginica | 923 | 33 | 31.2 |
| Delphinium tricorne | 35 | 9 | 10.69 |
| Dentaria laciniata | 101 | 16 | 16.76 |
| Dicentra cucullaria | 52 | 4 | 3.72 |
| Erigenia bulbosa | 206 | 5 | 4.89 |
| Erythronium albidum | 43 | 4 | 3.67 |
| Geranium maculatum | 129 | 20 | 20.01 |
| Hydrophyllum | 366 | 18 | 17.14 |
| appendiculatum | | | |
| Hydrophyllum virginianum | 56 | 12 | 11.84 |
| Isopyrum biternatum | 18 | 8 | 9.01 |
| Maianthemum racemosum | 2 | 2 | 7* |
| Mertensia virginica | 90 | 12 | 12.16 |
| Monarda bradburiana | 46 | 12 | 12.29 |
| Oxalis violacea | 11 | 1 | 1.00 |
| Phlox divaricata | 4 | 4 | 8* |
| Polemonium reptans | 15 | 8 | 11.10 |
| Ranunculus hispidus var | 70 | 15 | 14.77 |
| nitidus | | | |
| Sanicula odorata | 480 | 25 | 23.04 |
| Tradescantia virginiana | 63 | 17 | 17.48 |
| Uvularia grandiflora | 3 | 2 | 2.58 |
| Viola pubescens | 6 | 4 | 6.06 |
| Viola sororia | 14 | 6 | 8.80 |

^{*} The richness observed in the historical data set is shown rather than the estimated richness in the contemporary data because the sample size is too low for true richness to be estimated in the contemporary data.

Table S2.

Hypotheses tested, P-values from logistic regression or χ^2 -test for which all bees are treated as independent samples, P-values from phylogenetically independent contrasts (PICs), and outcome of statistical tests for the role of diet breadth, sociality, nesting habitat, and historic interaction strength (temporal overlap) on extirpation across 109 bee species, which provide 19 pairs of contrasts for PICs.

| Hypothesis tested | P-value logistic | P- | Support |
|--|--------------------------|-------|-------------|
| | regression or χ^2 - | value | hypothesis? |
| | test | PICs | |
| Bees with historically narrow diet breadths | 0.0003^{a} | 0.003 | Yes, Yes |
| were more likely to be extirpated than those | | | |
| with wider diet breadths. | | | |
| Bees at higher trophic levels (parasitic bees) | 0.032 | 0.173 | Yes, No |
| were more likely to be extirpated than | | | |
| eusocial and solitary bees. | | | |
| Bees that nest in cavities were more likely | 0.020 | 0.500 | Yes, No |
| to be extirpated than bees that nest in hives, | | | |
| soil, and wood/stem. | | | |
| Bees that had weaker historical interaction | 0.017 | 0.198 | Yes, No |
| strengths were more likely to be extirpated | | | |
| than those with stronger historic interaction | | | |
| strengths. | | | |

a. P=0.003 after correcting for potential observation bias.

Table S3.

In the last 40 years, rarefied species richness of bees visiting *C. virginica* declined by over half (Figure 3). This pattern held when we accounted for sampling site, sampling effort, and bee visitation rates – i.e., rarefied bee species richness declined significantly at 9 out of 12 sites that were sampled in both 1971 and 2010. For 2010, we reported the mean rarefied number of bee species and the range. For 1971, we reported observed richness because the number of bee individuals collected and total sampling effort per site was always lower compared with that in 2010. Analyses were done with EcoSim 3.0.3.37.

| Site | # bee species in | Rarefied # of bee species in |
|------|------------------|------------------------------|
| | 1971 | 2010 |
| BDCG | 6 | 4.79 (3-7) |
| BDPR | 4 | 1.85 (1-2) |
| BRC | 7 | 1.48 (1-3) |
| BB | 7 | 4.48 (2-6) |
| DOG | 3 | 6.52 (5-7) |
| FOX | 14 | 4.59 (3-7) |
| GILL | 4 | 7.85 (5-10) |
| P20 | 6 | 6.64 (4-9) |
| SUM | 13 | 4.82 (3-7) |
| SIX | 3 | 1.44 (1-2) |
| T90 | 17 | 3.86 (2-6) |
| WRV | 16 | 5.69 (4-7) |

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