Climate change intensifies plant-pollinator mismatch and increases secondary extinction risk for plants in northern latitudes

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ABSTRACT

Climate change can lead to “secondary extinction risks” for plants owing to the decoupling of life-cycle events of plants and their pollinators (i.e., phenological mismatch). However, forecasting secondary extinction risk under future climate change remains challenging. We developed a new framework to quantify plants’ secondary extinction risk associated with phenological mismatch with bees using *ca.* 15,000 crowdsourced specimen records of *Viola* species and their solitary bee pollinators spanning 120 years across the eastern United States. We further examined latitudinal patterns in secondary extinction risk and explored how latitudinal variation in plant-pollinator specialization influence this risk. Secondary extinction risk of *Viola* spp. increases with latitude, indicating that future climate change likely will pose a greater threat to plant-bee pollinator networks at northern latitudes. Additionally, the sensitivity of secondary extinction risk to phenological mismatch with both generalist and specialist bee pollinators decreases with latitude: specialist bees display a sharper decrease at higher latitudes. Our findings demonstrate that existing conservation priorities identified solely based on primary extinction risk directly caused by climate change may not be sufficient to support self-sustaining populations of plants. Incorporating secondary extinction risk resulting from ecological mismatches between plants and pollinators into future global conservation frameworks should be carefully considered.

SIGNIFICANCE

Climate change can directly contribute to primary extinction and indirectly lead to secondary extinction risks for plants if it decouples the timing of when plants flower and their pollinators are active. However, secondary extinction risk remains understudied. Using specimen records of *Viola* species and the bees that pollinate them, we demonstrate an increased secondary extinction risk with increasing latitude, indicating that climate change is expected to disrupt plant-bee pollinator networks more severely in northern latitudes; latitudinal variations in plant-pollinator specialization would significantly affect secondary extinction risk. Conservation plans should thus account for both primary and secondary extinction risks that can be anticipated in the face of future climate change.

Introduction

Predicting species responses to rapid climate change is of tremendous importance for understanding future biodiversity loss and directing limited resources toward the most effective conservation strategies (1-3). However, most research focuses on direct ("primary") extinction risks resulting from climate change rather than indirect ("secondary") extinction risks that arise for example from disrupted biotic interactions with key partners (i.e., ecological mismatch) (4-6). For example, phenological mismatches can occur when responses to climatic change cause plant flowering time and coincident pollinator activity to become temporally or spatially decoupled (7-9). Such decoupling may lead to reduced plant reproduction (i.e., fitness) via pollen limitation and possibly contribute to a "secondary extinction vortex" (5, 10) (Fig. 1A). However, the effects of climate change on secondary extinction risk in plants remain challenging to quantify and predict (5). Furthermore, the impacts of phenological mismatch on plants vary depending on the type of pollinators they interact with. For example, generalist pollinators can obtain floral resources from multiple plant taxa and thus could more effectively alleviate the negative impacts of climatically-driven phenological shifts in flowering. In contrast, plants that depend on specialist pollinators are more likely to be negatively impacted by phenological mismatch than those with generalist pollinators (11).

Most studies involving natural variation in phenological mismatch and its consequences on plant fitness have focused on temporal trends (12) at one or only a few locations, rather than on its spatial variability. However, phenological responses of both plants and pollinators to climatic change may vary spatially across latitudes (13-15). For example, Weaver and Mallinger (16) identified that the rate at which *Vaccinium* flowering phenology is advancing (i.e., flowering earlier in the year) is highest in southern populations. In contrast, bee activity is occurring significantly earlier in more northern populations (16). Such differential variation in the phenological responses of plants and bees along latitudinal gradients may lead to unexpected and undetected latitudinal patterns of phenological mismatch and associated secondary extinction risks for plants, especially if there are also latitudinal differences in pollinator specialization (17-20) (Fig. 1B).

Here, using crowdsourced historical specimen records of *Viola* species and their solitary bee pollinators spanning 120 years across the eastern United States (Fig. 2; *SI Appendix*, Tables S1-S2), we develop a new framework (Eqns. 1-6 in Methods) to quantify the risks of secondary extinctions for plants by integrating plant-pollinator interactions into species distribution models (SDMs). We then use this framework to assess (1) the latitudinal relationship between phenological mismatch and secondary extinction risk (Fig. 1A) and (2) document how latitudinal differences in plant-pollinator specialization versus generalization affects the risk of secondary extinction (Fig. 1B). Finally, we discuss how we can use our forecasts of geographical patterns in primary and secondary extinctions of plants to provide new directions for conservation planning under future climate change.

Results

Phenological responses of plants and bees along climatic gradients

The flowering times of *Viola* species were significantly affected by both the long-term average and anomaly of mean annual temperature (MAT), and by the interaction between MAT and annual precipitation anomalies (*SI Appendix*, Table S3). Holding other variables constant, an increase of 1 standard deviation (SD) in long-term average of MAT (i.e., 4°C) advanced flowering by 18 ± 0.3 d across all species. An increase of 1 SD in MAT anomaly (i.e., 0.7°C) advanced flowering by 2 ± 0.2 d on average. Our model also identified a positive interaction between MAT and annual precipitation anomalies: increased annual precipitation was associated with delayed flowering during warmer years.

The mean activity time of pollinator bees for *Viola* also was influenced by climate (*SI Appendix*, Tables S4-S5). For generalist bees, their mean activity time was affected only by long-term averages of MAT and annual precipitation, but not by any climate-anomaly variable (*SI Appendix*, Table S5). An increase of 1 SD in long-term average of MAT (i.e., 3.2°C) advanced the mean activity time of generalist bees by 17 ± 0.4 d, whereas an increase of 1 SD of long-term average of annual precipitation (i.e., 113.8 mm) delayed their mean activity time by 1.7 ± 0.3 d across all species. For the specialist bee, the long-term average of MAT and anomaly in annual precipitation strongly affected its mean activity time (*SI Appendix*, Table S4). An increase of 1 SD in long-term average of MAT (i.e., 1.7°C) advanced activity time by 10 ± 0.9 d on average. An increase of 1 SD in the annual precipitation anomaly (i.e., 20% of the long-term average) advanced mean activity time by 3 ± 1.0 d.

Species distribution models and primary extinction risk

Most of the SDMs for *Viola* had high predictive power, with mean TSS values of 0.7, 0.73 and 0.76 for GAM, GLM and MaxEnt, respectively. Fewer than 5% of models were excluded due to insufficient predictive power ($TSS < 0.5$). The probabilities of occurrence calculated by the three different SDM algorithms were highly correlated under both current and most future conditions estimated by three GCMs (mean $r > 0.7$ across all species; *SI Appendix*, Table S6–S7), suggesting that our results were not affected by the choice of SDM algorithm or GCM. Therefore, in the following sections we present only our results from MaxEnt and the median of all GCMs.

Secondary extinction risk

The secondary extinction risk of *Viola* spp. was estimated to monotonically increase with phenological mismatch with their bee pollinators. Approximately 52% (i.e., 84 out of 161) of plant- pollinator pairs had R^2 values > 0.2, and 30% (i.e., 47 out of 161) of pairs had R^2 values > 0.5 (*SI Appendix*, Table S8). The geographic patterns in secondary extinction risks forecast for the 2070s significantly differed from the patterns in primary extinction risk (i.e., mean Pearson's *r* between primary and secondary extinction risks across all plant-pollinator pairs < 0.4; Fig. 3; *SI Appendix*, Table S9).

In general, secondary extinction risk of *Viola* spp. increased with latitude as a function of their phenological mismatch with both specialist and generalist bees (Fig. 4). The phenological mismatch with generalist bees contributed more markedly to secondary extinction risk in high-latitude regions than did the mismatch with the specialist bee (Figs.

4B, 4C; Table 1). The sensitivity of secondary extinction risk to phenological mismatch was significantly lower for mismatches with generalist bees than for the specialist bee (Fig. 5). However, the sensitivity to phenological mismatch with the specialist bee showed a sharper decrease in high-latitude regions relative to the mismatch with generalist bees (Table 2). These results were qualitatively consistent across different R^2 thresholds used for filtering plant-bee pairs with low model fit (*SI Appendix*, Tables S10–S13) and across different GCMs (*SI Appendix*, Figs. S1-S2).

After accounting for potential buffering effects using the minimum phenological mismatch value across all generalist bees for each plant species at each grid cell, we still observed a significant increase in the secondary extinction risk of *Viola* spp. with latitude (*SI Appendix,* Table S14). The sensitivity of plants' secondary extinction risk to phenological mismatch also decreased with latitude for interactions with both generalist and specialist bees. Likewise, the sensitivity to phenological mismatch with the specialist bee showed a sharper decrease in high-latitude regions relative to the mismatch with generalist bees (*SI Appendix*, Table S15). These results suggest that generalist bees have no significant buffering effects on our conclusions.

Discussion

Although many plant species are being negatively affected by climate change (3, 21), their potential extinction risk might be especially severe for those that have lost their principle pollinators or for species whose phenological responses to climate change cause temporal mismatches with their pollinators (22-26), effectively creating a “secondary extinction vortex” (5). However, less is known about the latitudinal patterns in the risks of secondary extinctions for plants. Our results demonstrate increased risks of secondary extinctions for *Viola* species with latitude and reveal how latitudinal variations in plant-pollinator specialization influence plants' secondary extinction risk. These findings will help to inform conservation policies to mitigate further extinction risks resulting from disrupted plant-pollinator interactions under future climate change.

Secondary extinction risk increases with latitude

We identified a significant increase in secondary extinction risk of *Viola* spp. with increasing latitude that appears to be driven by phenological mismatch with generalist bees (Fig. 4). This result is likely attributable to the accelerated warming and the high climatic variability observed at higher latitudes (27, 28), and to differing phenological sensitivities of plants and pollinators to climate change. A high degree of warming and high climatic variability often lead to an acceleration in phenology (21). The different sensitivities of plants and pollinators results in increasing asymmetry in phenological responses between these groups, thereby increasing the likelihood of phenological mismatch (29, 30). Our results demonstrate that the flowering time of *Viola* spp. is predominantly influenced by temperature, but the activity time of a specialist bee is more strongly shaped by precipitation (*SI Appendix*, Table S4). However, generalist bees were not significantly affected by any climate factor we included in our models (*SI Appendix*, Table S5). This indicates that phenological (mis)matches between plants and pollinators likely depend on species (or taxa)-specific responses to climatic cues that vary with latitude.

It is worth noting that plants' secondary extinction risks may be ameliorated by reproductive strategies or other traits that may buffer against phenological mismatches with pollinators or their loss altogether. Relevant here is that the self-pollinating cleistogamous flowers of *Viola* spp. ensure reproductive success especially in environments such as high-latitude regions with unstable conditions, short growing seasons, and a lack of pollinators, albeit with a potential cost of inbreeding depression resulting from reduced genetic variability and fitness of offspring (31, 32). Other species may also evolve the ability for self-pollination, thereby achieving self-sustaining or even short-term expansion of plant populations during periods of rapid climate change (e.g., *Capsella rubella*) (33). However, inbreeding depression associated with self-pollination may severely limit the ability of plants to adapt to novel environmental conditions expected to occur under different climate change scenarios, ultimately increasing both primary and secondary extinction risks. Limited detection by crowd-sourcers precluded our ability to include cleistogamous flowers in our analysis, but future research should assess whether there is any association between the relative proportion of cleistogamous and chasmogamous flowers, climate change, and phenological mismatch.

More generally, our results indicate that climate change likely will pose a more serious threat to plant-pollinator networks at higher latitudes, with expected negative effects on ecosystem stability, gene flow, population maintenance, and biodiversity in these areas (34, 35). Future studies should explore mechanisms behind the different phenological responses of plants and insects to climate change, such as phenotypic plasticity, local adaptation, and evolutionary change in reaction norms (36), and assess the potential impacts of these changes on other important ecological interactions (e.g., seed dispersal, vegetation regeneration).

The sensitivity of secondary extinction risks to phenological mismatch

The degree of specialization in plant-pollinator interactions is not spatially uniform. Several studies have reported increasing biotic specialization in the tropics toward the equator. Because resources are divided more finely among a large number of plant species at lower latitudes, each species tends to rely on specific resources to promote niche differentiation and reduce interspecific competition (37). Therefore, plants at lower latitudes may be more ecologically specialized than those at high latitudes (38, 39). Based on this, we hypothesized that plant secondary extinction risks should have been more sensitive to phenological mismatches with specialist bees at lower latitudes. However, we identified that the sensitivity of *Viola* to phenological mismatch decreased with latitude for both specialist and generalist bees, with specialist bees showing a sharper decrease at higher latitudes (Fig. 5). This result suggests that plants rely on both specialists and generalists for pollination at low latitudes but may depend more heavily on generalist bees at high latitudes, partially supporting the arguments of previous studies (38, 39).

This may reflect the strategies of plant individuals at different latitudes to mitigate the risks of secondary extinction. Although higher diversity at low latitudes leads to greater specialization among individuals (18), the high resource diversity at low latitudes also requires pollinator bees to generalize their diet (40). Pollinator bees must thus diversify their food sources to meet the needs of various plants (41). Thus, it appears the high dependency of *Viola* on both specialist and generalist bees increases pollination efficiency and reduces secondary extinction risk in environments with high competition at low latitudes.

At high latitudes, in contrast, shorter growing seasons require plants to maximize pollination efficiency to ensure successful reproduction (42). Generalists associated with many partners may have distinct selective advantages compared to specialists because of sampling, complementarity, and portfolio effects, enabling these generalists to visit a variety of plants within a short time frame (43). Generalized pollination at higher latitudes is likely to increase functional redundancy and resistance of plant communities to secondary extinctions (7, 34).

Because our study incorporates only one plant taxon, our understanding of the evolutionary strategies behind variations in the sensitivity of secondary extinction risk to phenological mismatch with generalist and specialist bees across latitudes remains somewhat limited. Future research should seek to include multiple plant taxa at larger spatial and phylogenetic scales to gain a deeper insight into mechanisms driving these variations and make the relevant conclusions more broadly applicable.

Implications for future conservation planning

Although the protection of individual species remains the fundamental premise of conservation biology, conservation biologists (or ecologists) have evolved from their early emphasis on preserving individual species to a broader remit that now includes the maintenance of interaction networks and ecosystem services that improve human well-being (e.g., food and fruit supply) (44). For example, it is estimated that 5–8% of global crop production would be lost without effective pollination, especially by insects (45, 46).

We identified that the geographical patterns in primary extinction risk of *Viola* spp.

resulting from climate change were not strongly correlated with secondary extinction risk associated with phenological mismatch with bees (Fig. 3; *SI Appendix*, Table S9). This suggests that conservation priorities identified based solely on primary extinction risks as is commonly done is likely insufficient to address the multivalent complexities of extinction risk and could fail to support self-sustaining populations of either the plants or their mutualist bee pollinators (47). Our results underscore the importance for future conservation planning of addressing secondary extinction risks caused by asynchrony between plants and pollinators. These conservation strategies may include establishing effective pollinator corridors, long-term phenological monitoring of both plants and pollinators, protecting climate-sensitive pollinator habitats, and integrating secondary extinction risk into global conservation frameworks. Of course, continued basic science research into the nature, timing, and disposition of plants and associated pollinator interactions needs greater focus and emphasis in the coming years. Natural history collections remain an essential resource for such efforts (48-50).

Our study assumed that all bee species occurred across the entire eastern United States and focused only on the risks of secondary extinctions for plants. However, reduced floral resources could also cause the activity periods of bees to decline when no food plants are available, leading to decreased diet breadth and subsequent secondary extinction risks for pollinators (23). Future studies should further develop modeling frameworks to comprehensively consider the impacts of climate change on the secondary extinction of both plants and pollinators.

Materials and Methods

Study system

We focused on 23 *Viola* plant species and 7 solitary bee species (*SI Appendix*, Tables S1-S2) in the eastern United States (i.e., coastal states from Maine in the north to Florida, and also westward to West Virginia). We chose this system for several reasons. First, there is reliable, published information on generalist versus specialist bees that pollinate *Viola* spp. in this large geographic region. Second, both *Viola* and its pollinators have been widely collected, and abundant historical specimen records are available and digitized as part of the global metamuseum (50, 51). Herbarium specimens for *Viola spp.* have easily identifiable chasmogamous flowers, enabling us to estimate their corresponding flowering times using previously published methods (52-54). Finally, both *Viola* and its pollinators have large geographic ranges across the eastern United States (Fig. 2), which allow us to examine phenological shifts across latitudes.

Viola is a large genus of flowering plants in the family Violaceae. Many species of *Viola* have a dual reproductive strategy, which involves two distinct flower types: the early-stage floral bud is predetermined to develop into either a chasmogamous (open and outcrossing) or cleistogamous (closed, and self-pollinating) flower (55). Among the 73 species of *Viola* in the US north of Mexico, 60 are reported to have cleistogamous flowers (https://beta.floranorthamerica.org/Viola).

Solitary bees in the families Andrenidae, Anthophoridae, and Megachilidae are among the most important pollinators of chasmogamous *Viola* flowers (56). *Andrena violae* (Andrenidae) is a genus-level specialist pollinator of *Viola* in the eastern United States (https://jarrodfowler.com/specialist_bees.html). The other six bee species we studied are genus-level generalist pollinators with contrasting natural histories (solitary ground nesting *Andrena* spp. [Andrenidae] and solitary above-ground cavity nesting *Osmia* spp. [Megachilidae]; *SI Appendix*, Table S2). These generalist bee species are reported to pollinate plants belonging to genera other than *Viola*. For example, *Andrena carlini* is known to visit flowers from genera such as *Taraxacum*, *Rubus*, and *Vaccinium*. We follow the coding by Bartomeus et al. (7) of pollination relationships between *Viola* and these generalist bees. These bee species are known to emerge in early spring, which is a particularly important time period in terms of organismal response to climate change (57) and can be compared with early-flowering plants such as *Viola* spp.

Viola occurrence data

We derived occurrence and phenology data for *Viola* spp. from herbarium specimens digitized by the Consortium of Northeastern Herbaria (CNH;

https://portal.neherbaria.org/portal/) and the Southeast Regional Network of Expertise and Collections (SERNEC; https://sernecportal.org/portal/index.php). The 23 *Viola* species we used for our study (*SI Appendix*, Table S1) met the following criteria: 1) there were at least 50 unique specimens per species across the eastern United States; 2) the specimens had both an exact collection date (which we expressed as day of year [DOY] ranging from 1–365) and either exact or county-level location information (the latter were georeferenced to the geographic centroid of the county); and 3) the specimens included reproductive structures that were easily identifiable and countable (i.e., buds, flowers, and fruits).

For species distribution modeling, we augmented the occurrence data of the 23 *Viola* species from CNH and SERNEC with county-level distribution data (i.e., presence/absence data) from the Biota of North America Program's (BONAP; http://www.bonap.org/) North America Plant Atlas (NAPA) (58) and from the Global Biodiversity Information Facility (GBIF; https://gbif.org/). County-level occurrence data were then overlaid onto 40×40-km grid cells (Mercator projection EPSG 3857) covering the eastern United States. As some grid cells were located on the borders and along the coasts of the eastern United States, we removed incomplete grid cells (i.e., those with an area smaller than 800 km^2). If more than half of a grid cell was covered by one or several counties with the occurrence of a species, it was considered to be present in that grid cell (59).

***Viola* phenology**

We hired crowdsourcers through Amazon's Mechanical Turk service (MTURK; https://www.mturk.com/) to count the number of buds, flowers, and fruits of each digitized herbarium specimen from CNH and SERNEC using the CROWDCURIO platform (53) following well established protocols (54, 60, 61). Each specimen was independently evaluated by three individuals (on average), and we used a reliability score to assess the reliability of each individual and their data (54, 59). Specifically, each 10-image set, in random order and scored by one person, included nine unique images and one duplicate image. This duplicate image was randomly selected from the remaining nine images. The reliability scores were calculated as the absolute difference in counts of buds, flowers, and fruits between the two duplicate specimens, divided by the total counts for each phenological state separately, and then subtracted from 1 (62). Specimen observations scored by crowdsourcers with a reliability score of zero were excluded from our analyses following Park et al. (54). If an individual received a reliability score of zero for one organ, all organs would be assigned a score of zero and thus excluded from our analyses.

In total, the crowdsourcers provided reliable data on *Viola* flowering phenology from 8,200 specimens spanning 124 years (i.e., 1895–2018). Of these, ≈50% (4,075 specimens) were considered to represent the main flowering period following (61) of the *Viola* spp. and were used in subsequent analyses. That is, each of these 4,075 specimens: i) contained at least one open flower; ii) contained more open flowers than the combined number of buds and fruits; iii) contained a number of flowers representing at least 5% of the maximum number of flowers observed on a given species; and iv) had collection dates between the 5% and 95% quantile of observed flowering dates (DOYs).

Bee occurrence data

Approximately 80% of the occurrence records of the seven bee pollinator species (henceforth “bees”) were gathered from the online Symbiota Collections of Arthropods Network (SCAN) (63); additional collection records of preserved specimens were obtained from GBIF. Only SCAN and GBIF records that included an exact date and location (i.e., latitude and longitude or county information that could be assigned to its centroid) were included in our analysis. To prevent bias introduced by different collection efforts, only one record representing a certain collection event was retained; occurrence records were considered to be from the same collection event if they had both the same date and coordinates of collection (16). The 6,714 bee occurrence records that met the above criteria were collected in the eastern United States between 1900 and 2022. Bee occurrence data were mapped onto the same 40×40-km grid cells covering the eastern United States that we used to map the *Viola* occurrence data.

Bee phenology

We used DOY of the collection date of each unique bee occurrence record as a proxy for a day on which it was actively foraging. To estimate the main occurrence time of each bee species, we used MaxLike, a formal likelihood model that explicitly estimates the probability of species occurrence given presence-only data and a set of environmental covariates (64). Here, we assume that specimen records with a high probability of occurrence, generated by MaxLike, represent those periods when the focal bee species is most likely active. This is because MaxLike models are trained on presence-only data and can capture the temporal and environmental preferences associated with species' activity patterns. Although this approach ignores finer-scale behavioral variations, it provides a relatively reliable proxy for the main activity periods in MaxLike. We initially chose six bioclimatic variables relevant to bee distributions to build the models: temperature seasonality, mean temperature of the warmest quarter, mean temperature of the coldest quarter, precipitation seasonality, precipitation of the wettest quarter and precipitation of the driest quarter. We excluded mean temperature of the coldest quarter due to its high correlation with other variables ($r > 0.7$). Climatic data for each year-locality combination were obtained from PRISM database (*see* the detailed description in **Environmental data**, below).

For each bee species, we randomly selected 80% of the occurrence data as the training dataset, and this process was replicated 100 times. To evaluate the predictive accuracy of each model, we first identified the minimum predicted area (MPA) (65), which is the proportion of the study area predicted as present based on the probability threshold that ensures a user-defined percentage of the test data is correctly classified as present. Here, we set this proportion to 90%. Models yielding a low MPA are considered superior and models with an MPA larger than 0.7 were excluded, following Engler et al. (65). We calculated the median probability of occurrence value from the remaining models for each species and extracted the 95% confidence interval to present their main activity periods. However, it is worth noting that these data points do not fully represent the complete grid-cell level distributions of bee species when we overlap data points with 40km grid cells. Therefore, we did not construct SDMs for bee species and assumed that all bee species occurred over the entire eastern United States when calculating the secondary extinction risk of plant species.

Environmental data

Climatic and geographic data of specimen localities for plants and bees

We used estimates of historic (1895–2022) average monthly air temperature and precipitation data at a 4-km resolution from PRISM (product AN81m; https://prism.oregonstate.edu/). For each specimen with a specific location and year combination, we estimated the mean annual temperature (MAT) and annual precipitation and assigned these values to the corresponding specimens for both plants and bees. For each collection site, we first calculated the long-term mean temperature and precipitation conditions separately for plants and for bees. We then calculated the temperature and precipitation anomalies, which we defined as the difference in climatic conditions between the year of collection and the long-term mean for the entire collection range (i.e., 1895-2018 for plants and 1900-2022 for bees), for each record location, following Munson and Long (66) and Pearson et al. (67). Climatic anomalies have been shown to be associated with plant flowering time and are used widely in modeling phenology-climate relationships (30, 66).

Environmental data used for modeling plant distributions

Recent (1970–2000) and future forecasted (2061–2080; henceforth referred to as "2070s") climatic data at a resolution of 2.5-arc-minute were obtained from WorldClim (https://www.worldclim.org/, *ver.* 2.1; all 19 climatic variables, bio1–bio19) and climate values assigned to each grid cell were the means of all data points within it. We also included five soil variables (i.e., sand content, clay content, silt content, bulk density, and coarse fragments) in the SDMs for plants (68). We assumed these soil variables were constant through time, and calculated their within-grid-cell mean values at two soil depths (0–5 cm and 5–15 cm) using data from the SoilGrid250m database (https://www.soilgrids.org/).

We reduced the number of environmental and climatic variables using principal component analysis (PCA) on 24 soil and climatic variables. The eigenvectors were used to calculate the scores of the first seven derived principal components, which represent 97.3% of the total variance and were used as new predictors for creating the SDMs for all *Viola* species. The same eigenvectors were used to calculate the scores of the principal components for future environmental scenarios.

Future climatic projections were derived from three General Circulation Models (GCMs)—GISS-E2-1-G, HadGEM3, and INM-CM4-8—run for the most extreme Shared Socio-economic Pathways (SSPs)—SSP5-8.5 (69).

Statistical modeling

Relationships between climate and phenology

We applied linear mixed-effect models (LMMs) to examine the phenological sensitivities of plants and occurrence of bees to current and future climates across the eastern United States. This model framework allowed us to hierarchically incorporate the variation in phenological responses to climate across multiple species (70). The same structure and predictor variables were used for separate models fitted for *Viola*, the six generalist bees, and the specialist bee. All predictor variables were centered and scaled to a mean value of 0 and a SD of 1 to avoid introducing bias. Correlation coefficients among all selected predictor variables were < 0.1, limiting the effects of collinearity.

For the full *Viola* model, the response variable was the DOY for each specimen (as representing its mean flowering time). Predictor variables included as fixed effects the long-term average (1895-2018) and inter-annual anomalies of mean temperature and total precipitation, the interaction between temperature and precipitation anomalies. The full model also included a random-intercept term for species and random slopes for species responses to temperature and precipitation anomalies. The random slopes are interpreted as species-specific phenological sensitivities to inter-annual climate change.

For the full model for generalist bees, the response variable was the DOY for each collection record (as representing its mean activity time). Fixed predictor variables included long-term average (1900-2022) and inter-annual anomalies of mean temperature and total precipitation, the interaction between temperature and precipitation anomalies. The full model also included a random-intercept term for generalist bee species and random slopes for the responses of generalist bee species to temperature and precipitation anomalies. Since the dataset included only one specialist bee, the model for the specialist bee did not incorporate any random components.

All models were fitted using the "lmer" function in the "lmerTest" package (*ver.* 3.1-2) (71) of the R software system (*ver.* 4.2.1). The significance of the effects of the predictor variables was evaluated using the z-distribution to obtain p-values from the Wald t-values provided by the model output (72). We also applied the "fitme" function in the "spaMM" package to check whether our results were affected by spatial autocorrelation of response variables (ver. 4.5.0) (73). We found no substantial differences in the results of the LMMs that either included or excluded potential autocorrelation structures for both plants and bees, so we report only the results of the models without spatial autocorrelation included.

To predict the mean flowering time for each plant species in each grid cell and mean activity time for each bee species in each grid cell under recent (i.e., 1970–2000) and future (2070s) conditions, we applied the "predict" function in the "stats" package (ver. 4.0.0) to the fits of the LMMs. Recent and future temperature anomalies for each grid cell were estimated as differences from its long-term mean temperature, whereas precipitation anomalies were calculated as a proportion of its long-term mean precipitation.

Species distribution models to estimate the primary extinction risk of Viola species

As our *Viola* dataset lacked true absences, we generated random pseudo-absences (i.e., randomly selected grid cells that were considered as species absences) (74). For each species, twice as many pseudo-absences as real presences were generated; the entire procedure was repeated 10 times, each time with a new set of pseudo-absences. SDMs were calibrated for each species using three algorithms: generalized linear models (GLMs), generalized additive models (GAMs) and MaxEnt. We used a repeated data-splitting procedure to evaluate the predictive power of each individual model. Each model was trained on 80% of the distribution data of each species before being assessed against the remaining 20% using the true skill statistic (TSS) (75). This data-splitting procedure was also repeated 10 times (resulting in a total of 100 individual models for each algorithm and species). Those models with TSS > 0.5 were adopted to project the probability of occurrence of each plant species per grid cell under both current and future conditions. Model projections were carried out over the entire eastern United States and all models were projected at the same spatial resolution as the distribution data (i.e., 40-km). We used the median value of all model predictions as the final forecast of occurrence probability for each *Viola* species. In the context of extinction, the primary extinction risk (P_e) of plant species within each grid cell was simply calculated as 1−the probability of occurrence (76).

Calculation of secondary extinction risk of Viola species

We inferred the secondary extinction risk of *Viola* spp. based on their predicted probability of occurrence from SDMs and their predicted phenological mismatch with bees. The temporal gap (i.e., phenological mismatch) between the mean flowering DOY and the mean activity DOY of bees for each plant-bee species pair was calculated at each grid cell under both recent (1970–200) and future (2070s) climatic conditions. Since phenological events are cyclical and repeat annually, the entire year can be represented as a 360-degree circle. We first converted DOY to angles to directly compare the mean flowering time of plants and the activities of bees on a standardized circular scale (Eqns. 1 and 2). We then calculated the absolute value of the difference between $Angle_{plant}$ and $Angle_{bee}$ of each plant-bee pair (Eqn. 3). To ensure the difference accounted for the cyclical nature of angles, we adjusted the difference so it would not exceed 180° (Eqn. 4).

$$Angle_{plant} = \left(\frac{DOY_{plant}}{365}\right) * 360° \qquad \text{(Eqn. 1)}$$

$$Angle_{bee} = \left(\frac{DOY_{bee}}{365}\right) * 360° \qquad \text{(Eqn. 2)}$$

$$diff = \left|Angle_{plant} - Angle_{bee}\right| \qquad \text{(Eqn. 3)}$$

$$Phenological\ mismatch = \min\ (diff\ , 360° - diff) \qquad \text{(Eqn. 4)}$$

We assumed that the phenological mismatch between plants and bees would lead to a proportional decrease in probability of occurrence of plant species (as in Fig. 1A). Therefore, the secondary extinction risk of each plant species at each grid cell could be estimated approximately as follows (Equations 5 and 6):

$$P(\text{mismatch}) = P(\text{occurrence}) * (1 - \frac{Phenological\ mismatch}{180\,°}\ ) \qquad \text{(Eqn. 5)}$$

$$S_e = P(\text{occurrence}) - \ P(\text{mismatch}) \qquad \text{(Eqn. 6)}$$

where $P(\text{mismatch})$ represents the adjusted probability of occurrence of each *Viola* species in each grid cell after accounting for the phenological mismatch with each bee species; $P(\text{occurrence})$ was the original probability of occurrence of each *Viola* species in each grid cell inferred from the SDMs; and S_e was the estimated secondary extinction risk (i.e., decreases in probability of occurrence) of each *Viola* species at each grid cell. As the phenological mismatch increases, the adjusted probability of occurrence decreases, leading to a greater increase in potential secondary extinction risk.

Because estimated phenological mismatches between *Viola* and the bees usually spanned at most a few dozen days, dividing them by 180° resulted in relatively small values. Consequently, the final value of S_e may be predominantly influenced by the $P(\text{occurrence})$. To more intuitively describe the relationship between the S_e of each *Viola* species and its phenological mismatch with the bees, we fitted S_e to the phenological mismatch estimated under current conditions using a power function (Fig. 1A). We used three different R-squared thresholds (i.e., $R^2 = 0.2, 0.3, 0.5$) to exclude plant-bee pairs with poor model fit.

The phenological mismatch for the 2070s of each plant-bee pair with a high model fit was then substituted into the power function to obtain future S_e of each *Viola* species. Since we used the median value of the species' probability of occurrence at each grid cell across three GCMs, we extracted the median phenological mismatch across these GCMs to minimize the impact of extreme climate values on the mismatch and used it to estimate the future S_e. This approach allowed for a more robust representation of climate conditions by mitigating the influences of outliers that may skew the phenological mismatch estimates. We also reported the results from each GCM for the subsequent analysis (*SI Appendix*, Figs. S1-S2).

Finally, we compared the geographical patterns in P_e and S_e of each plant species. We then compared S_e of plant species resulting from its phenological mismatch with generalists and specialist bee species across latitudes. We used mixed-effects beta regression for this comparison; latitude, pollinator type (generalists vs. specialist), and their interactions were considered to be fixed factors, and individual plant and bee species were treated as separate random factors. To further examine how plant S_e changed with latitude under equivalent phenological mismatches and how this relationship varied between plants pollinated by generalists and aa specialist, we fitted two additional models: the first was a mixed-effects beta-regression model with S_e as the response variable, latitude and phenological mismatch of each plant-generalist pair as fixed factors, and plant and bee species as separate random factors. For the second one, we did not include a random component for bees because we only had a single specialist species. Since beta regression is designed for continuous outcomes strictly within the (0, 1) interval, it is not suitable when the response variable includes exact zero values. Therefore, we used alternative zero-inflated beta regression models to account for the presence of zeros in the S_e data. All these analyses were carried out using the “glmmTMB” function in the “glmmTMB” package (*ver.* 1.1.9) (77).

Because plant species depend on multiple generalist pollinators, they may still be able to rely on the most temporally aligned pollinator even in the presence of significant phenological mismatches with other generalist bees. To account for this buffering effect, we extracted the minimum phenological mismatch value across all generalist bees for each plant species at each grid cell and built two beta-regression models to separately explore the latitudinal patterns in S_e of plant species and the sensitivity of plant S_e to phenological mismatch across latitude. The beta-regression model structure is the same as the model described above, which included all generalist bee species within each grid cell (*SI Appendix*, Tables S14-S15).

Data availability

All codes and data used in the analyses are currently deposited on Github (https://github.com/Shijia818/Plant-bee-interactions) and will be available on Zenodo once accepted.

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Author contributions

SP conceived the idea and designed the project with support from CCD and AE. SP collected, analyzed and visualized the data under the supervision of AME and CCD. SP drafted initial manuscript. AME and CCD reviewed and edited the manuscript.

Competing interests

The authors declare no competing interest.

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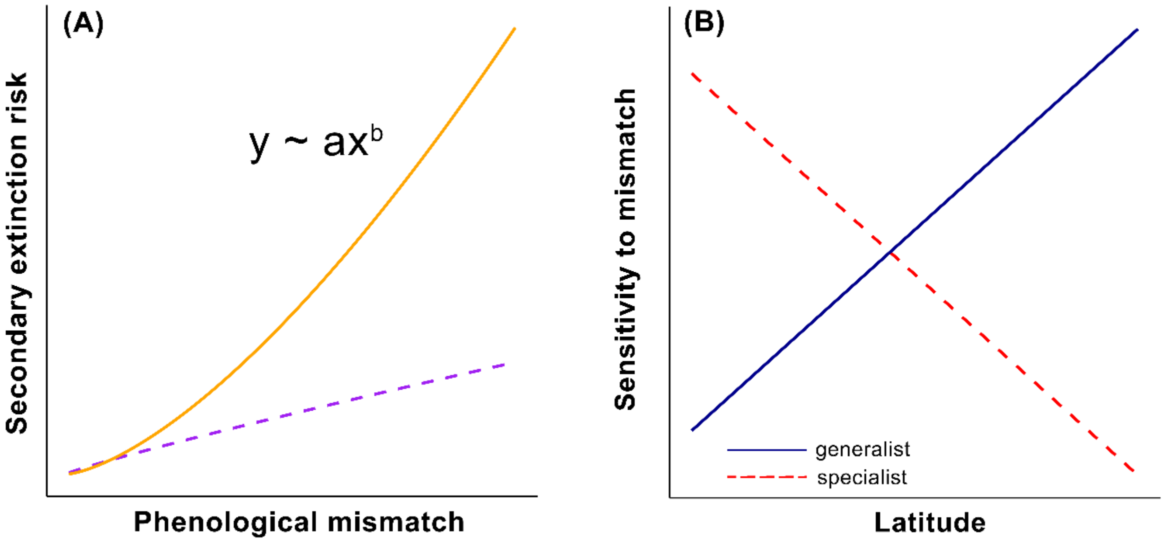

Fig. 1. Conceptual diagram showing hypothesized effects of the impacts of phenological mismatch between plants and pollinator bees on plants' secondary extinction risk. Standard models of how secondary extinction risk of plants is related monotonically to phenological mismatch with pollinator bees (A). The relationship can be fit with a general exponential model ($y \sim ax^b$; dashed purple line: $0 < b < 1$; orange line: $b > 1$). If the plants located in low-latitude regions are more dependent on specialist bees for pollination due to high specialization at low-latitudes whereas plants at high latitudes rely more on generalist bees, the sensitivity of secondary extinction risk to phenological mismatch could either decrease from low to high latitudes for plants interacting with specialist pollinators (dashed red line) or increase for plants interacting with generalist pollinators (dark blue line) (B).

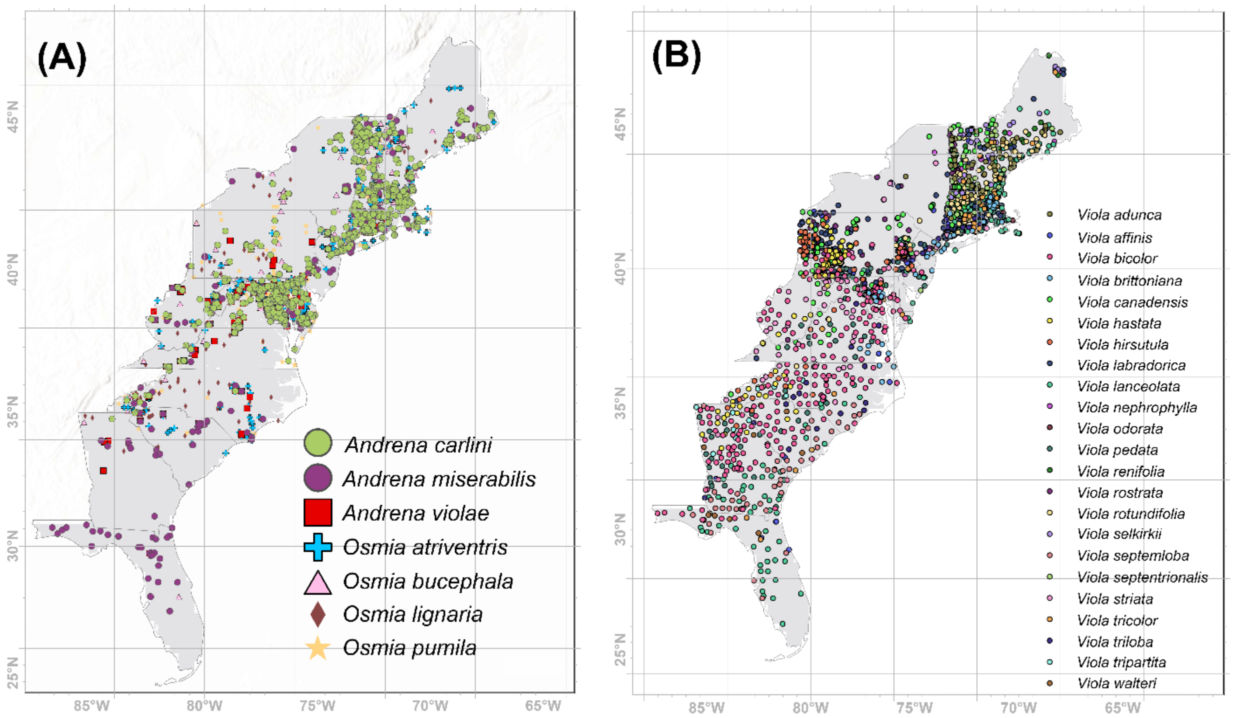

Fig. 2. Occurrence records and herbarium specimens separately collected for seven pollinator bee species (A) for the *Viola* genus and 23 *Viola* plant species (B) used in our study across the eastern United States.

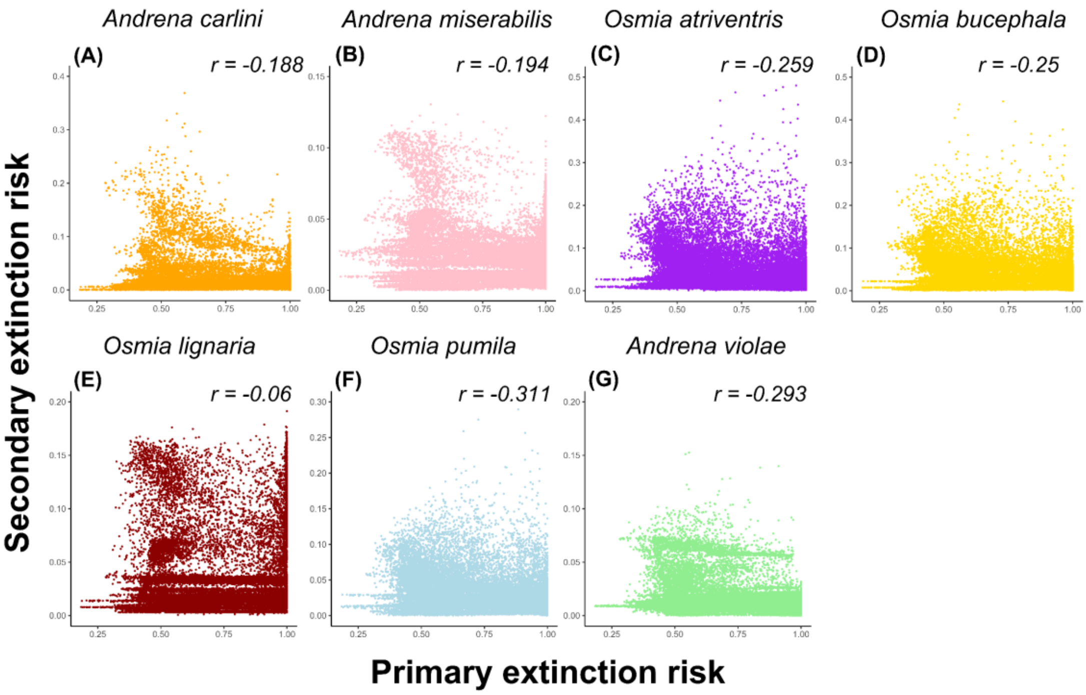

Fig. 3. The relationship between the primary extinction risk directly caused by climate change and the secondary extinction risk indirectly resulting from phenological mismatch with pollinator bees for *Viola* spp. Pearson's correlation coefficients (r) are shown for each panel, with each panel representing one of the seven bee species (generalists, A-F; specialist, G) in interaction with all *Viola* species across all grid cells.

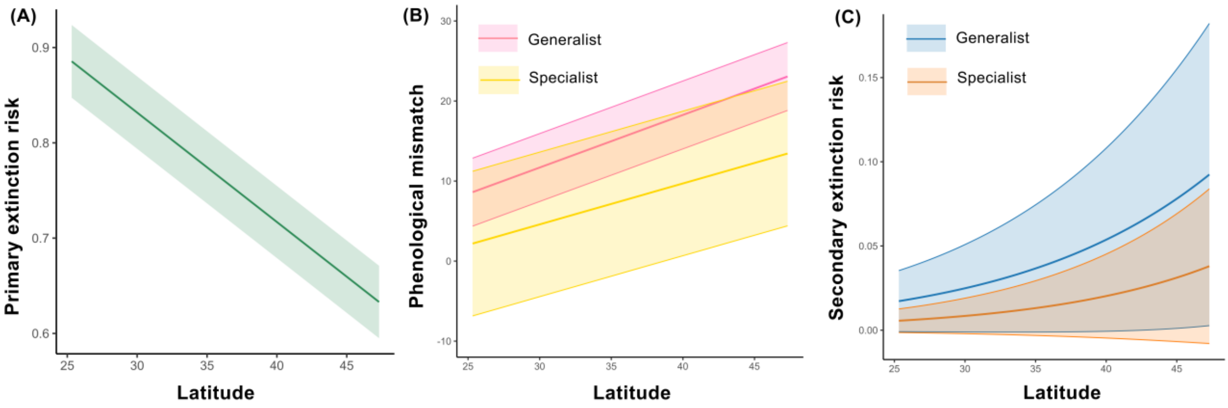

Fig. 4. The relationship between latitude and (A) primary extinction risk of plants; (B), phenological mismatch (separately for interactions with generalist and specialist pollinator bees); and (C) secondary extinction risks of plants (separately due to phenological mismatch with generalist and specialist pollinator bees) in the 2070s. The risk of secondary extinction for plants is calculated based on both species' probability of occurrence and phenological mismatch with pollinator bees. The median values of species' probability of occurrence and phenological mismatch at each grid cell are calculated across three General Circulation Models (GCMs, GISS-E2-1-G, HadGEM3, INM-CM4-8).

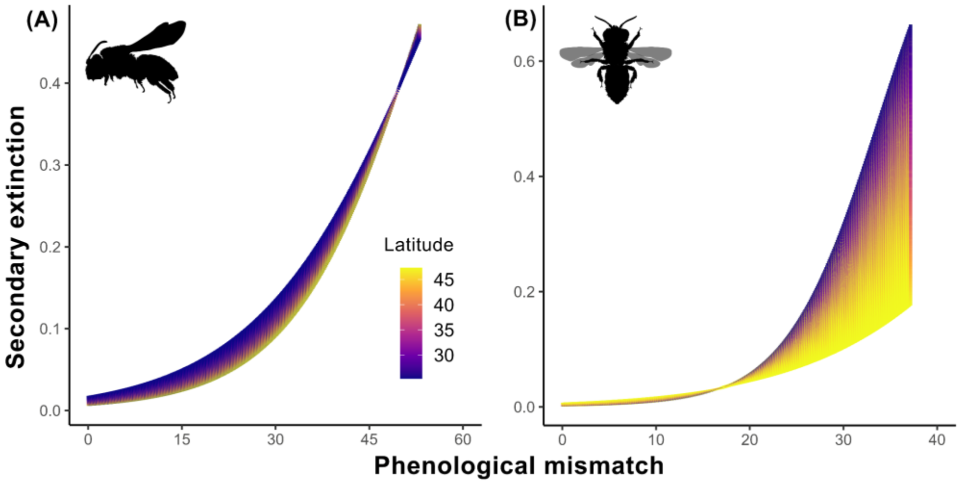

Fig. 5. Sensitivity of secondary extinction risk for *Viola* spp. to phenological mismatch with (A) generalist bees and (B) a specialist bee across latitudes in the 2070s. The median values of species' probability of occurrence and phenological mismatch at each grid cell are calculated across three GCMs (as in Fig. 4).

Table 1 Summary of mixed-effects beta regression for secondary extinction risk of *Viola* associated with phenological mismatch with six generalist and a specialist pollinator bee across latitudes. We included 47 plant-bee pairs with a model fit greater than 0.5 for the regression of secondary extinction risk on phenological mismatch. The median phenological mismatch value derived from three GCM climate scenarios was used to calculate the secondary extinction risk of plants for each grid cell.

Variable	**Estimate ± SE**	***Z*-value**	***P*-value**
Intercept	−3.028 ± 0.183	−16.58	< 0.001***
Scale(latitude)	0.413 ± 0.003	131.61	< 0.001***
Specialist	−1.027 ± 0.398	−2.58	0.009**
Scale(latitude): Specialist	−0.044 ± 0.011	3.91	< 0.001***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 2 Summary of mixed-effects beta regression for the sensitivity of secondary extinction risk of *Viola* to phenological mismatch across latitudes. Beta regressions were constructed separately for plants pollinated by generalist bees and those pollinated by a specialist bee. We included 47 plant-bee pairs with a model fit greater than 0.5 for the regression of secondary extinction risk on phenological mismatch. The median phenological mismatch value derived from three GCM climate scenarios was used to calculate the secondary extinction risk of plants for each grid cell.

	Variable	**Estimate ± SE**	***Z*-value**	***P*-value**
Generalists	Intercept	−3.007 ± 0.122	−24.7	< 0.001***
	Phenological mismatch	0.761 ± 0.002	382.4	< 0.001***
	Latitude	0.174 ± 0.002	95.8	< 0.001***
	Phenological mismatch: Latitude	−0.051 ± 0.002	−34.1	< 0.001***
Specialist	Intercept	−4.149 ± 0.137	−30.33	< 0.001***
	Phenological mismatch	1.073 ± 0.006	179.64	< 0.001***
	Latitude	0.155 ± 0.006	25.63	< 0.001***
	Phenological mismatch: Latitude	−0.205 ± 0.006	−36.22	< 0.001***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$