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# Dominant bee species and floral abundance drive parasite temporal dynamics in plant-pollinator communities

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**Pollinator reductions can leave communities less diverse and potentially at increased risk of infectious diseases. Species-rich plant and bee communities have high species turnover, making the study of disease dynamics challenging. To address how temporal dynamics shape parasite prevalence in plant and bee communities, we screened >5,000 bees and flowers over an entire growing season for five common bee microparasites (*Nosema ceranae*, *Nosema bombi*, *Crithidia bombi*, *Crithidia expoeki* and neogregarines). Over 110 bee species and 89 flower species were screened, revealing that 42% of bee species (12.2% individual bees) and 70% of flower species (8.7% individual flowers) had at least one parasite in or on them, respectively. Some common flowers (for example, *Lychnis flos-cuculi*) harboured multiple parasite species whilst others (for example, *Lythrum salicaria*) had few. Significant temporal variation of parasite prevalence in bees was linked to bee diversity, bee and flower abundance and community composition. Specifically, we found that bee communities had the highest prevalence late in the season, when social bees (*Bombus* spp. and *Apis mellifera*) were dominant and bee diversity was lowest. Conversely, prevalence on flowers was lowest late in the season when floral abundance was highest. Thus turnover in the bee community impacted community-wide prevalence, and turnover in the plant community impacted when parasite transmission was likely to occur at flowers. These results imply that efforts to improve bee health will benefit from the promotion of high floral numbers to reduce transmission risk, maintaining bee diversity to dilute parasites and monitoring the abundance of dominant competent hosts.**

As the world experiences its sixth mass extinction event, communities are becoming less diverse and increasingly fragmented, and the dynamics of disease spread within these communities is being transformed[1–4]. Despite a growing urgency to understand these dynamics, effective management of disease spread in wildlife is hampered by a poor understanding of parasite dynamics in species-rich communities[5–7]. This is especially important for the conservation of complex pollinator communities which, in addition to suffering from disease-linked declines and extinctions, have outstanding value to the environment and economy[8–11].

Temporal change in multi-species communities presents a major challenge to our understanding of disease ecology due to the turnover of hosts, transmission sites and parasite species over time[7,12], and ignoring these dynamics can lead to incorrect conclusions about disease risk. Three key parameters that can shape disease prevalence and spread in multi-host communities are host contact rates, density of disease spreaders and frequency of transmission sites. The contact rate between competent hosts will change over time due to species turnover influencing host diversity and abundance, driving so-called dilution and amplification effects[13–19]. To our knowledge, few studies have addressed temporal dynamics of amplification/dilution in species-rich communities. Heterogeneity in species' ability to harbour and transmit parasites is common[12], so community changes over time can alter the relative abundance of disease spreaders. Indeed, the identification and targeting of such super-spreaders often determines the success of disease control programmes[20]. Finally, the presence and location of transmission hotspots such as shared food resources can change over time, influencing the risk of disease spread[12].

Plant and bee communities are complex multi-host, multi-parasite systems where solitary and social bees experience differing levels of host–host contact rates, and where flowers can act as microbial transmission and dispersal hubs[21–24]. Plant and bee communities also exhibit a high degree of species turnover throughout the year, with overlapping periods of floral blooms and bee activities[25]. With so many factors changing over time, it is difficult to understand parasite dynamics without temporal sampling. Nevertheless, to our knowledge, no studies have quantified parasite temporal dynamics in natural, species-rich pollinator communities, or on flowers acting as parasite transmission/dispersal hubs. This dearth of data prevents the development of targeted management strategies that could reduce parasite spread and increase host and ecosystem health[6,26].

Here, we screened >5,000 samples of flowers and bees over a 26-week period at three old-field meadow sites. All samples were screened for five common multi-host parasite species or groups using PCR assays. Using these data of parasite prevalence on flowers and in bees, we address two main questions: (1) how does parasite prevalence change over time in species-rich plant–bee communities; and (2) are changes in plant and bee species abundance, diversity and/or composition associated with changes in parasite prevalence? Overall, our goal was to view in high resolution the

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temporal dynamics of multiple parasites across entire plant and bee communities. With these data, we gain further insight into the epidemiology of species-rich communities.

## Results

**Parasite prevalence on flowers and in bees.** In total, we screened 2,624 flowers from 89 species and 2,672 bees from at least 110 species for parasites. Overall, we found an unexpectedly high proportion (8.7%) of individual flowers and 53.8% of flower genera to be positive for at least one of the five parasite species (see Fig. 1b and Supplementary Table 2), and 12.5 and 72.3% prevalence, respectively, for at least one of the two broad parasite groups (trypanosomes or microsporidians). Broken down by parasite species, 2.0% of flowers were positive for *Nosema bombi*, 0.7% were positive for *Nosinia ceranae*, 3.2% were positive for *Crithidia bombi*, 1.2% were positive for *Crithidia expoeki* and 2.9% were positive for neogregarines. From 89 flower species, 16 were sampled over 5 times, representing >60% of all flower samples. Of these, *Lychnis flos-cuculi* (ragged robin) had the highest prevalence of *N. bombi* (8%), *N. ceranae* (2%), *C. bombi* (10%) and neogregarines (6%), whilst *Leucanthemum vulgare* (ox-eye daisy) had the highest prevalence of *C. expoeki* (5%). Whilst it is known that parasites can be horizontally transmitted between bees at flowers, these data confirm that bee parasites are present on a wide variety of wild flowers in natural communities.

For bees, we found that 12.2% of individual bees and 57.7% of bee genera were positive for at least one parasite (see Fig. 1a and Supplementary Table 3), with 12.5 and 65.4% prevalence, respectively, for the broad parasite groups (trypanosomes, neogregarines or microsporidians). Broken down by parasite species, 0.3% of bees were positive for *N. bombi*, 4.9% were positive for *N. ceranae*, 2.5% were positive for *C. bombi*, 1.0% were positive for *C. expoeki* and 4.0% were positive for neogregarines. From 110 bee species, 10 were sampled over 50 times, representing 70% of all bee samples. Of these, *Bombus bimaculatus* had the highest prevalence of *N. bombi* (1.8%) and *C. expoeki* (12.5%), *Apis mellifera* the highest *N. ceranae (18.8%)* and *Bombus impatiens* had the highest prevalence of *C. bombi* (11%) and neogregarines (9.6%). Although our understanding of these parasites is largely restricted to their presence in *Apis* or *Bombus* hosts, these data confirm their presence across a much broader range of bee species (30 non-*Apis* and non-*Bombus* species; Fig. 1a and Supplementary Table 3).

**Temporal trends of parasite prevalence in bee and floral communities.** Temporal trends of parasite prevalence were evaluated using generalized linear mixed models (GLMMs), with parasite status as a binomial response, week number within the field season as the explanatory variable and site as a random factor. Analyses were conducted separately for each broad parasite group, each parasite species, neogregarines and a combined group comprising all four species together with neogregarines. We used a Bonferroni-corrected significance level of $\alpha = 0.05\, n_p^{-1}$, where $n_p$ is the number of parasite species/groups considered in each analysis. We did not use autoregressive time-series models, since Durbin–Watson tests for temporal autocorrelation using scaled residuals were not significant. *N. bombi* was excluded from the bee analysis and *N. ceranae* from the flower analysis, due to the small number of positives (<20).

Prevalence of all parasite species/groups showed positive temporal trends in the bee community, with all except the trend in *C. expoeki* being statistically significant (Fig. 2a; $\chi^2_1 = 2.9$, $P = 0.087$ for *C. expoeki*; $\chi^2_1 = 7.6$, $P = 0.006$ for neogregarines; and $\chi^2_1 \geq 14$, $P < 0.001$ for the remainder, likelihood ratio test, $n = 2{,}672$; see Supplementary Table 4). In contrast, prevalence of all parasite species/groups showed negative temporal trends in the floral community although only the trend in the combined parasite group was significant (Fig. 2b; $\chi^2_1 = 8.3$, $P = 0.004$ for the combined group, likelihood ratio test, $n = 2{,}624$; see Supplementary Table 5 for the remainder). We also noted that whilst prevalence tended to be at a comparable level between bee and flower communities for most parasite species/groups, *N. ceranae* was much less prevalent on flowers than in bees whereas the reverse was true for *N. bombi*.

**Associations between bee community composition and parasite prevalence.** The four most common bee genera—*Apis*, *Bombus*, *Ceratina* and *Lasioglossum*—comprised 76% of all surveyed and screened bees. We found a gradual turnover in the bee community across the season for all sites combined (Fig. 3a), initially comprised mostly of *Ceratina*, *Lasioglossum* and other genera, while *Apis* and *Bombus* became dominant later in the season. Results of exact multinomial ($P < 0.001$ at every site; see Supplementary Fig. 1 legend for $n$ at each site and window) and post hoc binomial tests (Supplementary Fig. 1), evaluated at three different temporal windows, confirmed this turnover in species.

We explored parasite heterogeneity across species and sites using binomial GLMMs fitted for each parasite, with genus and its interaction with week number as additional explanatory variables. Bee genus was found to be a significant predictor of prevalence for all parasite species/groups ($\chi^2_1 \geq 28$, $P < 0.001$, likelihood ratio test, $n = 2{,}672$; see Supplementary Table 6 and Supplementary Fig. 2). Post hoc pairwise contrasts (Supplementary Table 7) indicated significantly higher prevalence in *Apis* and *Bombus* than in other genera, with prevalence in the former mostly associated with microsporidians and *N. ceranae*, and prevalence in the latter associated with trypanosomatids, *C. bombi* and neogregarines. Most temporal trends of parasite prevalence in individual bee genera were not significant (Supplementary Table 8), with a notable exception being that of *N. ceranae* showing a negative trend in *Apis* ($z = -4.1$, $P < 0.001$, $z$-test).

In the early period of the season, solitary bee genera, particularly *Ceratina* and *Lasioglossum*, were the dominant bees yet contributed minimally to community-wide parasite prevalence (Fig. 3b). By the late period of the field season, *Apis* and *Bombus* were the dominant bees and contributed substantially to overall parasite prevalence (Fig. 3b; see Supplementary Fig. 3 for individual parasite species/groups). These results support findings that increased abundance of bumblebees and honeybees in an area is linked to increased parasite prevalence[27–30].

**Associations between bee diversity and parasite prevalence.** To assess how bee diversity varied across the season we calculated the Shannon index for each week at each site, based on collected bee samples, and assessed the temporal trend using a linear mixed model. Effects of rarefaction on Shannon index of the bee community are shown in Supplementary Fig. 4. Decreasing subsample size increased the range of weeks covered by the data (we excluded samples smaller than the subsample size), but at the cost of reducing the strength of any temporal trends. In subsequent analyses, we chose a subsample size of 36 because this affected Shannon indices by no more than 13% yet allowed >70% of the site/week samples to be retained. No significant differences between sites were observed ($F_{2,21} = 1.2$, $P = 0.32$, one-way analysis of variance (ANOVA), $n = 35$; Supplementary Fig. 5). Shannon index showed a significant decrease from weeks 6 to 23 ($\chi^2_1 = 42$, $P < 0.001$, likelihood ratio test, $n = 35$; Fig. 3c); this decrease is consistent with Fig. 3a, suggesting a shift in the community from one with comparable proportions among all four major genera in mid-season to one mostly of *Apis* and *Bombus* in late season. Shannon indices without rarefaction also suggested an initial increase in bee diversity early in the season (black line in Fig. 3c), but this trend was not robust as it could have been influenced by the much smaller bee samples in the first few weeks.

Negative associations were detected between parasite prevalence and the Shannon diversity index of bees for all parasite species/groups, with all except the associations in *C. expoeki* and

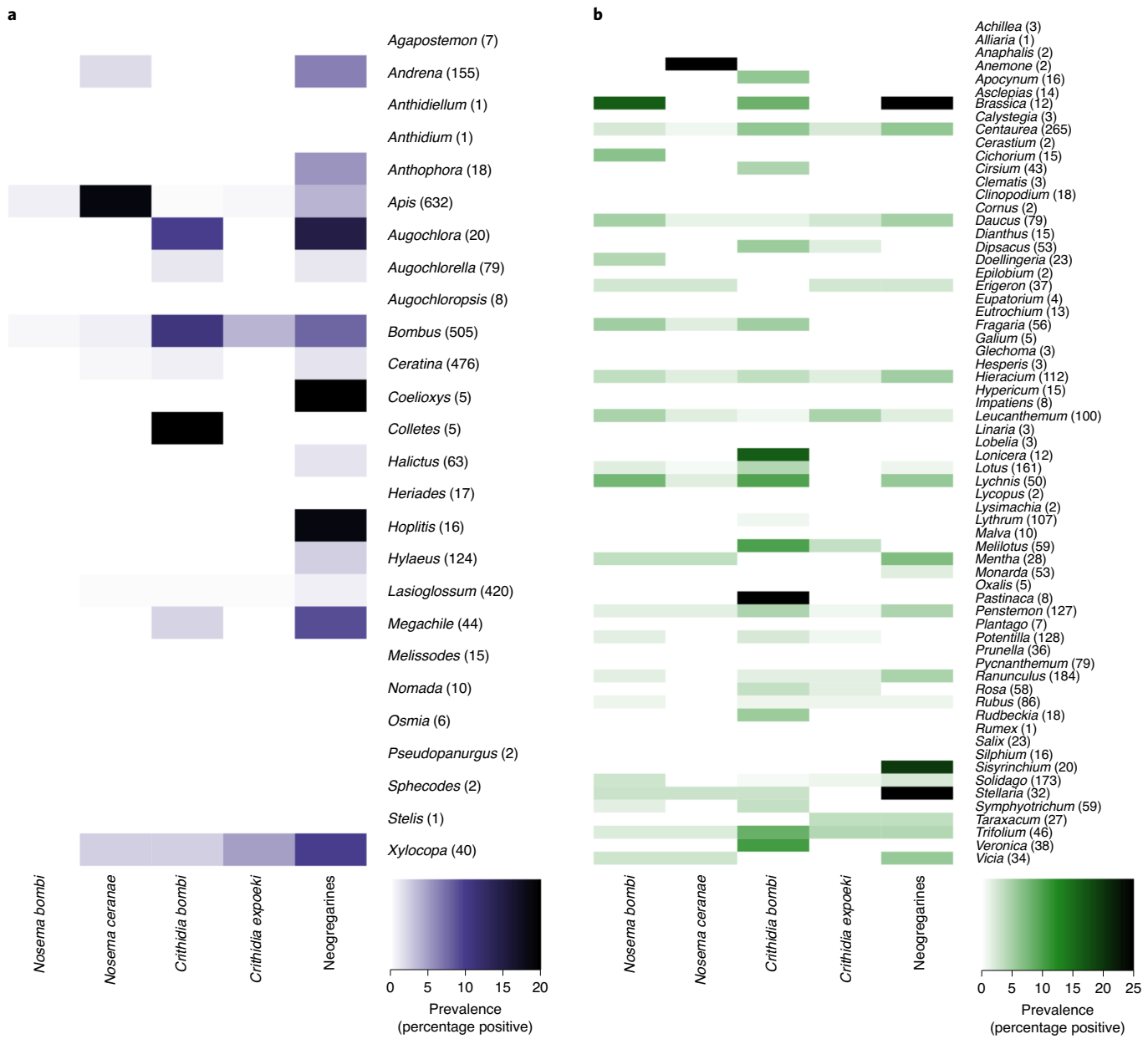


**Fig. 1 | Parasite prevalence in bee and on flower genera across three old-field communities. a**,**b**, Screenings of 2,672 bees representing 26 genera (at least 110 species) and of 2,624 flowers representing 65 genera (89 species). Rows represent bee (**a**) or plant (**b**) genera, with sample numbers in parenthesis. Details of PCR protocols for screenings of parasites (*N. bombi*, *N. ceranae*, *C. bombi*, *C. expoeki* and neogregarines) are outlined in Methods. Full parasite information listed by species is shown in Supplementary Tables 2 and 3.

neogregarines being statistically significant (Fig. 3d; $\chi^2_1 = 7.4$, $P = 0.007$ for microsporidians; $\chi^2_1 < 0.001$, $P = 0.99$ for *C. expoeki*; $\chi^2_1 = 3.6$, $P = 0.060$ for neogregarines; and $\chi^2_1 \geq 13$, $P < 0.001$ for the remainder, likelihood ratio test, $n = 2{,}446$; see Supplementary Table 9). Together with the negative trend of Shannon diversity index in Fig. 3c, these results are consistent with increase in parasite prevalence over time in the bee community, as shown in Fig. 2a.

To explore whether these negative associations were driven entirely by the greater abundance of the high-prevalence genera *Apis* and *Bombus* later in the season, we repeated the same analysis but this time without *Apis* and *Bombus* samples when calculating parasite prevalence. We found that, among the four parasite groups with a sufficient number of positives for analysis, trypanosomatids retained a significant negative association but the remaining parasites did not ($\chi^2_1 = 0.54$, $P = 0.46$ for microsporidians; $\chi^2_1 = 22$, $P < 0.001$ for trypanosomatids; $\chi^2_1 = 0.001$, $P = 0.97$ for neogregarines; and $\chi^2_1 = 2.6$, $P = 0.11$ for the combined group, likelihood ratio test, $n = 1{,}390$; see Supplementary Table 9 and Supplementary Fig. 7). These results indicate that *Apis* and *Bombus* were responsible for some, but not all, associations between parasite prevalence and Shannon diversity. Focusing on the prevalence of two parasites in their known narrow host ranges, we found that *N. ceranae* in *Apis* hosts decreased over the year (Supplementary Table 8) and was positively correlated with Shannon diversity ($\chi^2_1 = 8.2$, $P = 0.0041$, likelihood ratio test, $n = 586$), whereas *C. bombi* in *Bombus* hosts increased over the year (Supplementary Table 8) and was negatively correlated with Shannon diversity ($\chi^2_1 = 4.32$, $P = 0.038$, likelihood ratio test, $n = 470$).

**Associations between floral abundance and parasite prevalence.** Floral abundance was measured at each site in randomly placed

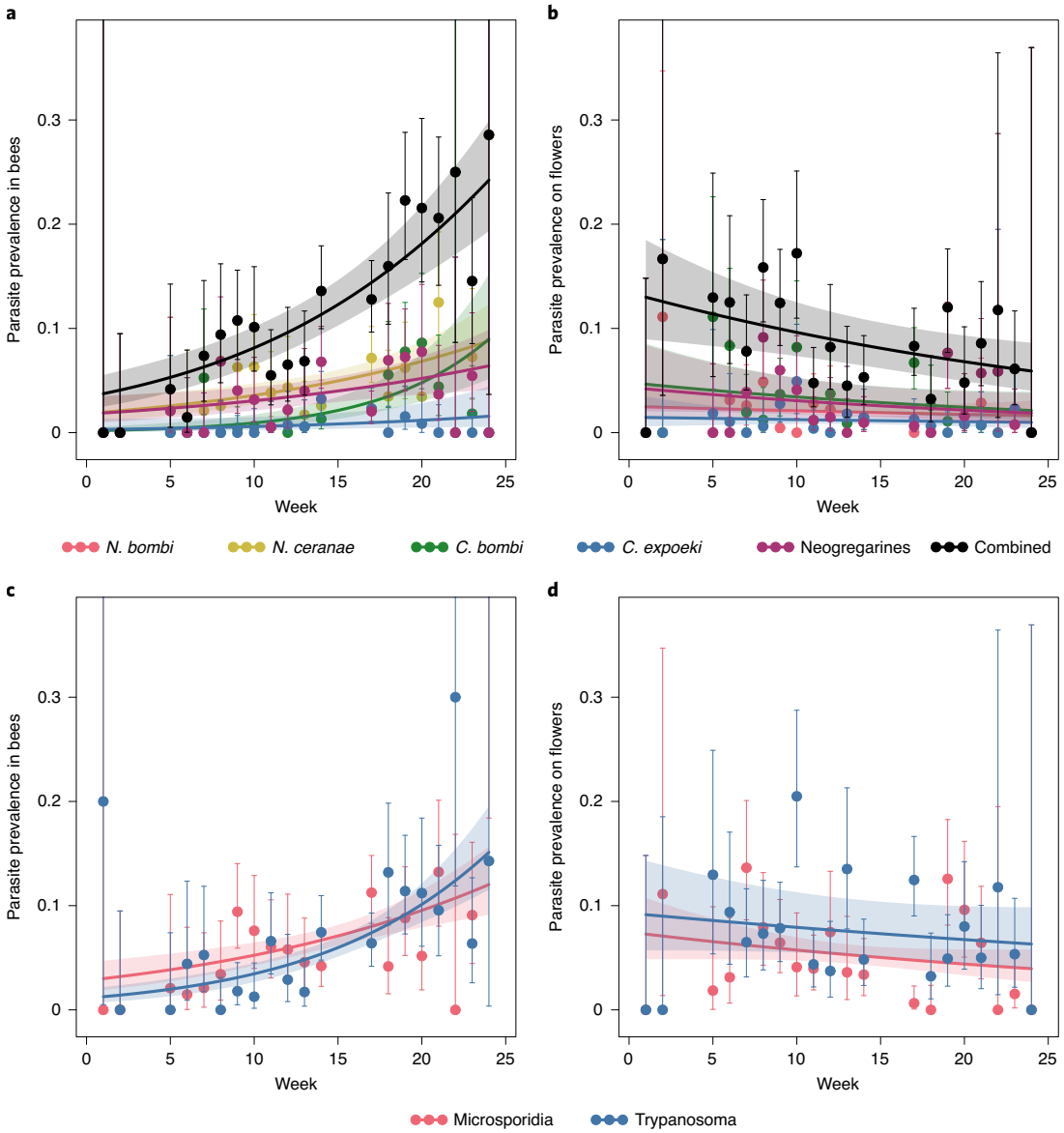


**Fig. 2 | Parasite prevalence increased throughout the season in the bee community while it decreased or remained constant in the floral community.** **a**,**b**, Prevalence of specific parasite species or groups (*N. bombi*, *N. ceranae*, *C. bombi*, *C. expoeki* and neogregarines) in bees (**a**) and on flowers (**b**). **c**,**d**, Prevalence of broad groups (microsporidians, trypanosomatids) in bees (**c**) and on flowers (**d**). The *x*-axes correspond to week number in the field season (week 1 starting 18 April, week 24 ending 22 September). Error bars, 95% Clopper–Pearson confidence intervals. To reduce plot clutter, points and error bars are based on pooled data from all three sites each week; while statistical tests, prediction curves and confidence bands are based on GLMMs with site as a random factor.

10-m$^2$ quadrats and found to increase across the season ($\chi^2_1 = 132$, $P < 0.001$, likelihood ratio test, $n = 147$; Fig. 4a). Prevalence of all parasite species/groups showed negative associations with floral abundance, although only the association in the combined parasite group was significant (Fig. 4b; $\chi^2_1 = 8.0$, $P = 0.005$ for the combined group, likelihood ratio test, $n = 2{,}624$; see Supplementary Table 11 and Supplementary Fig. 10 for the remainder). These results hint at the possibility that parasite dispersal amongst flowers was diluted by increasing floral abundance as the year progressed. Although we do not have absolute abundance data for bees throughout the season, we postulate that bee numbers were stable or decreased over the later period of the season, reducing overall bee visitation per flower whilst at the same time parasite prevalence reduced on flowers. This positive relationship between floral visitation and the prevalence of parasites on flowers is partially supported by recent work showing that flowers in high-visitation areas (apiaries) have a higher prevalence of bee viruses[31].

The diversity of flowers surveyed within the quadrats did not differ between the field sites ($F_{2,31} = 1.1$, $P = 0.36$, one-way ANOVA, $n = 49$; Supplementary Fig. 5), nor was there any significant temporal trend ($\chi^2_1 = 0.035$, $P = 0.85$, likelihood ratio test, $n = 49$). Low Shannon indices were consistent with observations that floral abundance was dominated at any point in time by a small number of species (Supplementary Fig. 8). No significant relationships were found between parasite prevalence on flowers and floral diversity for all parasite species/groups (Supplementary Table 10 and Supplementary Fig. 9).

## Discussion

In this study, we found that parasite prevalence in plant and bee communities varies over the season and is linked to bee diversity,

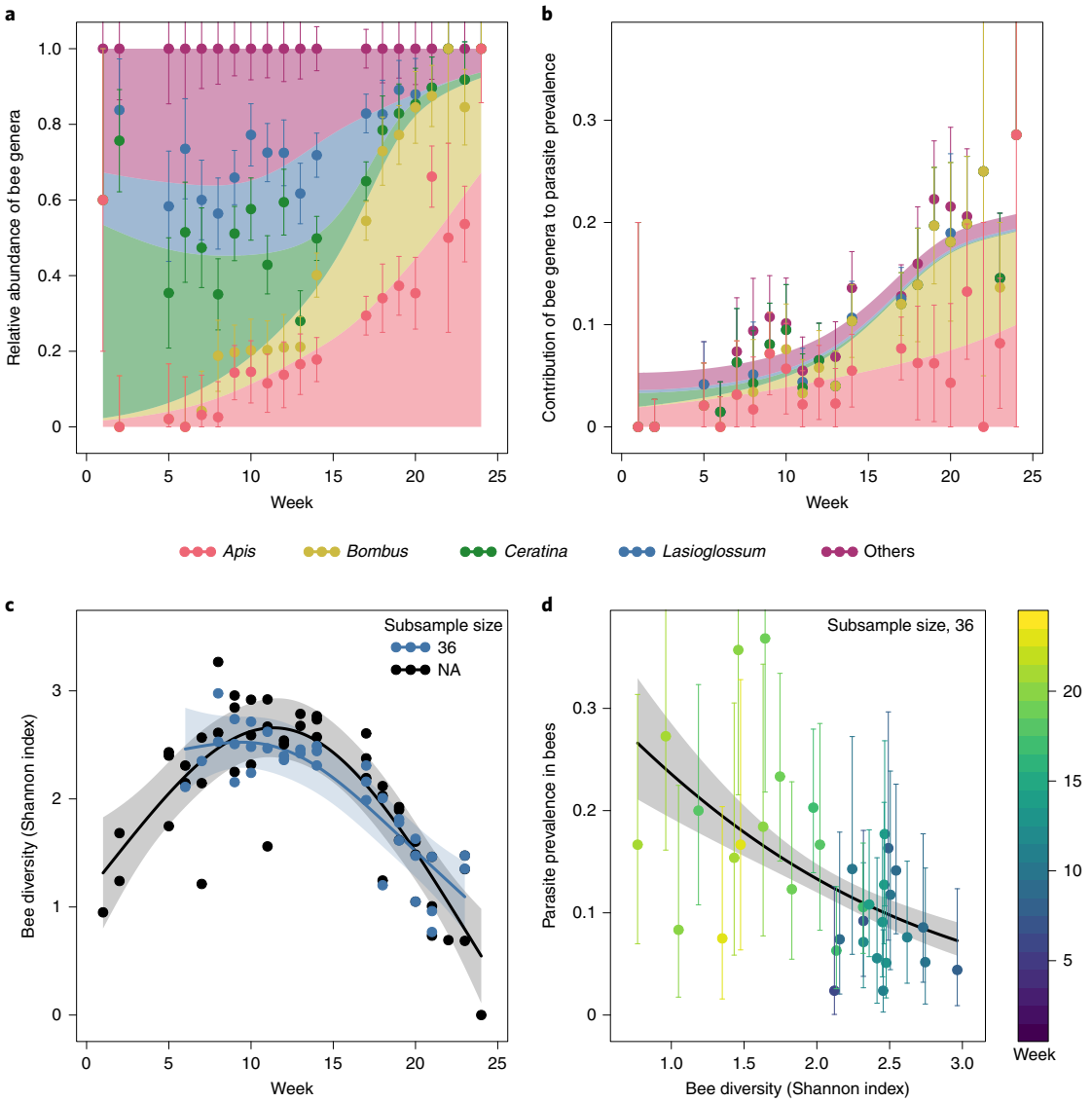


**Fig. 3 | Associations between bee community composition, diversity and parasite prevalence over time. a**,**b**, Relative abundance of each bee genus (**a**) and contributions from each genus to overall parasite prevalence in the community throughout the season (**b**). A gradual turnover in the community was observed for *Ceratina*, *Lasioglossum* and other unspecified genera early in the season and to *Apis* and *Bombus* later on. Because *Apis* and *Bombus* exhibited higher parasite prevalence than other genera, this drove the observed increase in overall prevalence over time. **c**,**d**, Temporal trends in bee diversity (Shannon index), with rarefaction (subsample size, $n = 36$, see Methods) and without rarefaction (NA) (**c**), and association between parasite prevalence and rarefied Shannon index (**d**). The rarefied Shannon index decreased from weeks 6 to 23, while parasite prevalence also showed a negative association with Shannon index. Both observations are consistent with increase in parasite prevalence over time. **b**,**d**, The prevalence shown is that of all four parasite species and neogregarines combined as a single parasite group. Error bars, 95% Sison–Glaz multinomial confidence intervals (**a**) and 95% Clopper–Pearson binomial confidence intervals (**b**,**d**).

bee and flower abundance and community composition. The three most common bee parasites were most prevalent late in the year, when bee communities were least diverse and social bees (*Bombus* spp. and *Apis*) were dominant. This pattern is consistent with an overall dilution effect and/or increased importance of within-colony transmission and dominance of social bee hosts. We also found that bee parasites are prevalent on numerous wildflower species and that the overall prevalence on flowers decreased with increasing floral abundance later in the season. These data suggest that risk of parasite transmission among bee species may be reduced when the abundance of *Apis* and *Bombus* bees is low (as in early season) and when floral abundance is high (as in late season). Thus, species turnover and abundance across the bee–flower community is important to consider when identifying super-spreaders, disease hotspots and key periods when transmission risk is likely to be high.

Over time, parasite prevalence in the community and within particular species changed, sometimes with contradictory trends, highlighting the importance of repeated temporal sampling to accurately determine disease risk. Temporally, diversity of the bee community exhibited a unimodal distribution and, as diversity declined from its peak, parasite prevalence increased. This coincided with the increasing dominance of two social host groups, *Apis* and *Bombus*. During this period, parasites may benefit from increased contact rates of competent hosts (driven by increased density and within-colony transmission) and reduced overall host diversity, both increasing transmission rates and driving an increase in parasite prevalence. The combined increases in parasite prevalence and

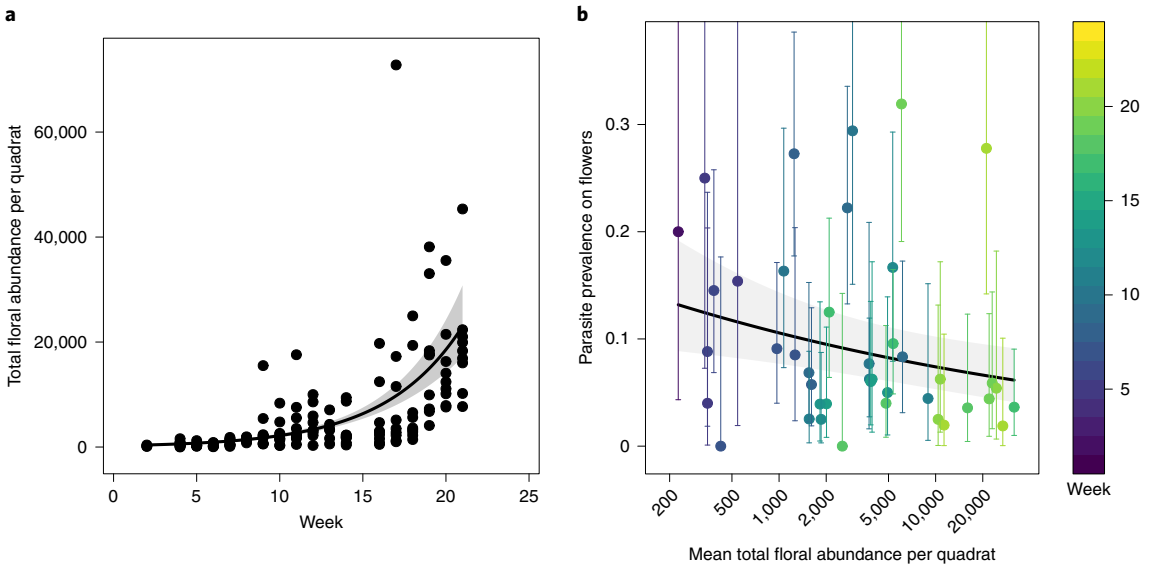


**Fig. 4 | Increase in floral abundance over time may dilute parasite prevalence on flowers. a**, Total floral abundance within each quadrat showed a significant positive temporal trend. **b**, Associations between parasite prevalence, floral abundance per quadrat and week number of samples. The prevalence shown in **b** is that of all four parasite species and neogregarines combined. Error bars, 95% Clopper–Pearson confidence intervals.

relative abundance of dominant, competent hosts suggest that the health of these two dominant social groups may play an influential role in driving parasite prevalence across the community. For example, *N. ceranae* prevalence over the year decreased in its primary host, *Apis*, although the increasing relative abundance of *Apis* in the community drove an overall increase of the parasite at the community level. Similarly, prevalence of *C. bombi* and neogregarines increased in *Bombus* throughout the year, coinciding with an increase in prevalence of these parasites at the community level. It is also notable that the diversity of parasites in wild solitary bees is understudied, and there may be additional parasite species in these bees that have dynamics less influenced by social bees.

Whilst the ubiquity of the dilution/amplification effect in nature is still debated[32], here we find evidence that these effects may be present in species-rich communities of bees. Various mechanisms, including changes in host density, contact rates or parasite contagiousness, have been proposed to cause dilution/amplification in several other systems[14,16,33–35]. At the community level, our data show a negative association between bee diversity and parasite prevalence—supportive of a dilution effect. Conversely, despite rarely being found on flowers, the prevalence of *N. ceranae* in its primary host, *A. mellifera*, was positively correlated with local bee diversity—supportive of an amplification effect. However, reductions in host diversity were intrinsically linked to increased densities of competent hosts, and the social nature of these hosts facilitates within-colony transmission as the season progresses[36,37]. These confounding factors prevent a conclusive determination of amplification/dilution effects. To determine whether these forces could be teased apart, we tested for associations between host diversity and parasite prevalence excluding *Apis* and *Bombus*; although this result could have been influenced by spillover, the absence of any associations in the remaining community would indicate that dilution is not a driver. We found that trypanosomes remained negatively associated with diversity (supportive of a dilution effect) although other parasites were not. Thus we find support for both dominant species and dilution influencing parasite prevalence in the community. Given these patterns, the foundations are laid for manipulative studies to elucidate the mechanisms involved.

We found a range of bee parasites on a high number and diversity of flowers. In other disease ecology systems, it is also common to find that shared feeding areas can be sites of disease spread (for example, watering holes)[12,38,39]. We found that parasite prevalence differed by flower species, supporting earlier manipulative studies that have identified transmission variation among flower species/traits[21,23,40,41]. This supports the prediction that risk of parasite transmission to bees will vary by flower availability and foraging choice. Furthermore, we found that parasite prevalence on transmission hubs (that is, flowers that can potentially transmit and disperse microbes) declined over time although, unlike in bee hosts, the diversity of transmission hub species remained constant whilst their abundance increased. This increase in transmission hub abundance may have led to dilution via density, where an increase in the ratio of parasite-free to contaminated hubs resulted from increases in floral abundance compared to bee abundance over time. Theory indicates that increasing flower numbers will reduce parasite transmission in plant-pollinator networks[42]. Whilst our data cannot directly support this finding, we did see that increasing flower numbers reduced the prevalence of parasites on flowers. Therefore, floral composition and abundance, in addition to the foraging patterns and abundance of bees, probaby play an important role in parasite transmission. More work is required to understand the underlying mechanics behind these complex interactions and, in particular, whether the introduction of managed honeybees or bumblebees to a landscape and the resulting change in bee/flower ratios influences parasite spread across wider geographic areas.

Species-rich bee and plant communities are complex, and our findings of multiple factors influencing parasite prevalence and transmission risk pose challenges for conservation and management. In various systems, the health of animals at risk from disease has been shown to improve following targeted disturbance of transmission pathways and direct removal/killing of parasites[5,6,26,43–46]. By performing this temporal study of multi-parasite prevalence across plant–bee communities, we identified patterns and species that may be targeted. An important conservation target is the maintenance of bee biodiversity, with reductions likely to exacerbate parasite threats to the community. Furthermore, the identification of honeybees and dominant bumblebee species as key drivers of parasite prevalence and transmission risk indicates that their health probably has knock-on effects to others. During times of pandemic threat, it is the control of such high dispersers that can be the key

to reducing disease spread[12,43,47–52]. Parasite management strategies targeting these host groups could greatly increase the efficiency of disease control efforts within local bee communities, allowing control efforts to be less intrusive and more cost effective than a system-wide approach[43]. Finally, to foster healthy bee communities, our data support the promotion of high floral numbers to reduce parasite prevalence and risk of transmission at flowers. Further work is now required to understand the role of foraging patterns and floral traits on parasite spread, and to determine the full host range and virulence of parasites across various bee species.

## Methods

**Bee and flower collections.** To determine parasite prevalence within bees and on the surfaces of flowers, we collected samples weekly across three old-field sites in upstate New York between 18 April 2017 and 22 September 2017. The sites were named Lansing (latitude: 42° 32' 24.493" N, longitude: 76° 29' 47.9076" W), McDaniels (latitude: 42° 32' 11.5872" N, longitude: 76° 25' 3.7668" W) and Whipple (latitude: 42° 29' 23.6328" N, longitude: 76° 25' 49.818" W). The McDaniels and Whipple sites are managed by Cornell University and no permits were required for their use. The Lansing site is privately owned, and we obtained permission to use the site. The distance between sites ranges from 5.3 to 7.5 km. Weather depending, we dedicated a day of collecting (~7 h) per site, every week. On a typical collection day, three members would collect samples from the same site (21 person-hours per site per week). In total, we spent 58 d collecting samples over the 5-month period (equivalent of ~1,200 person-hours). We collected bees in sterile tubes either directly or with the use of a net. Variety of floral form meant that 'flowers' were either single flowers or inflorescence depending on the plant species. The number of florets used per inflorescence was the same number that a bee would forage upon in a single interaction with that species. We placed flowers on which the bees were foraging directly into separate sterile tubes using sterilized forceps. Bee and flower samples were placed on dry ice immediately after collection, then transported to the laboratory and stored at −80 °C until we commenced DNA extraction.

**Floral abundance and diversity.** Each week throughout the growing season we randomly selected three quadrats (each $10 \times 10\,m^2$) at each site, within which we identified the floral species in bloom and counted the number of stems for each (two out of 26 weeks were not surveyed due to adverse weather). Per species, we estimated the number of floral units per stem and averaged this across the sites and weeks in which the species was in bloom (minimum of five measurements per species). We define a floral unit as being the typical unit (single flower or inflorescence) from which a bee typically foraged[53]. This definition of a floral unit is also consistent with the amount of plant material we used for each sample during parasite screening. We therefore define the floral abundance of each plant species within the quadrat as the product of stem counts and estimated number of floral units per stem. The raw floral survey data are held on Dryad (https://doi.org/10.6086/D1X09V).

**Plant and bee identification.** We identified plants either in the field or via specimens and/or photographs brought back to the laboratory and keyed out[54–57]. We confirmed the identity of each individual plant screened for parasites before DNA extraction. Overall, we screened 2,624 flowers from 89 species for parasites (Supplementary Table 2). We identified bees after performing gut dissection for each specimen, using reference materials in the Cornell University Insect Collection (CUIC: http://cuic.entomology.cornell.edu/) and published keys[58–61]. All identifications were conducted by P.A.M., taxa verifications were conducted by M. Arduser and all voucher specimens are housed in either the McArt laboratory or the CUIC. Overall, we screened 2,672 bees from at least 110 species for parasites (Supplementary Table 3).

**Bee parasites.** We selected to screen samples for parasites with known effects on bee health and links to population declines[62,63]. Historically, research has focused on parasites of honeybees and a small number of bumblebee species. This research bias has contributed to a fundamental lack in our understanding of the full host ranges of bee parasites. Because we were interested in screening across bee taxa, we specifically sought to screen for parasites that have been identified in multiple bee species. We considered common microparasites of bees as three groups: microsporidians, trypansomatids and neogregarines. The microsporidians are comprised mostly of *Nosema* spp. whose spores are transmitted via the faecal–oral route[64]. The effects of microsporidia infections in honeybees and bumblebees include wing deformity, reduced foraging efficiency, reduced colony fitness and increased mortality, with *N. bombi* being found in multiple species of bumblebees and *N. ceranae* in bumblebees and honeybees[65–71] In addition, *N. ceranae* may be able to infect the mason bee, *Osmia bicornis*[72]. While this study did not find impacts on survival, a similar study that assessed the impacts of *N. ceranae* on larval *O. bicornis* did find negative impacts on survival[73]. The trypanosomatids are mostly *Crithidia* spp. whose cells are transmitted via the faecal–oral route. The effects of trypanosomatid infection in bumblebees include reduced foraging efficiency, reduced queen fitness and increased mortality of infected bees. *Crithidia* spp. can infect bumblebees, honeybees and several solitary bee species[74–82]. The neogregarines are an understudied group with one described bee parasite, *Apicystis bombi*. This neogregarine has been detected in honeybees, a range of bumblebee species and solitary bees. Infected bumblebees have reduced fat bodies and increased mortality, and queen bumblebees are less likely to survive hibernation[30,83–88].

**Parasite DNA extraction from individual bees and inflorescences.** Parasite detection does not confirm an active infection and although we endeavoured to reduce the likelihood of reporting uninfected bees by eliminating parasites on the outer cuticle via surface sterilization, and report only parasites in tissues known to harbour the selected parasites, some of the detections may have been due to transient parasite material rather than active infection[89]. We performed dissections in bleached, ultraviolet-sterilized hoods and sterilized instruments between samples using a dry-bead sterilizer set to 250 °C. We carefully removed the alimentary canal from the mid-gut to the rectum using standard techniques[90]. If the gut broke apart inside the bee, in addition to dissecting out the torn gut we washed 10 µl of PBS in and out of the bee cavity with a pipette to recapture any spilled gut contents before adding it to the gut tissue for DNA extraction. We performed dissections with minimal destruction to the cuticle, to allow accurate species identification.

Similarly with parasite detection in bees, we do not know whether parasites molecularly detected on flowers are viable, or pathogenic to all foraging bees. We term flowers as potential 'transmission hubs' because of their role in providing a physical platform for microbes to be deposited by bees and then acquired by subsequent foraging bees[21,24,40]. Using the 96-well plate Qiagen DNeasy blood and tissue Extraction Kit protocol, we washed single flowers/inflorescences in 600 µl of ATL buffer by pulse vortexing for 30 s. We then transferred 450 µl of the wash to a 2-ml screwcap tube with ~100 µl of 0.10-mm zirconia beads and one 5-mm steel bead. The wash was then lysed for 30 s at $6.5\,m\,s^{-1}$ on an Omni Bead Ruptor 24 homogenizer. For bee guts, we added 180 µl of ATL buffer, ~100 µl of 0.1-mm zirconia beads and one 5-mm steel bead to each sample. We then homogenized the bee guts at 30 hz for 3 min on a Qiagen Tissue Lyser II. For both flower washes and bee gut homogenate, we next added 50 µl of Proteinase K before allowing the samples to incubate at 56 °C overnight. Following incubation, we followed the standard Quick Start protocol provided by Qiagen until DNA was eluted in 100 µl of AE buffer.

**Parasite screening.** We adopted a two-step approach to determine parasite prevalence on flowers and in bee guts. (1) We screened for the presence of two broad taxonomic groups (trypanosomatids and microsporidians) known to contain multiple parasite genera, before (2) screening these positively identified groups for probable species of bee parasites within those groups. In addition, multiplex PCR also identified samples containing neogregarine parasites. The broad multiplex panel was designed for efficient screening of flowers and insect guts for the most common bee-infecting parasites within a single reaction[91]. Samples positive for *Crithidia* or *Nosema* were then diagnosed with species-specific multiplex panels (*C. bombi*, *C. expoeki*, and *N. bombi*, *N. ceranae*, respectively). Primers were either newly designed (see Supplementary methods) or chosen from the existing literature[91–93]. The concentrations of each reagent and thermocycling conditions are given in Supplementary Table 1. PCR products were run alongside a size standard on a 2% agarose gel, stained with GelRed to visualize and confirm amplicon size. Each assay included a negative and a positive control.

**Statistical analysis.** *Parasite prevalence of the bee community over time.* For each of the broad taxonomic groups and parasite species for which we screened, temporal trends in the overall parasite prevalence of bee communities were evaluated using GLMMs, with parasite status of the samples as binomial response, week number within the field season as the explanatory variable and site as a random factor. All subsequent GLMMs also used site as the random factor. Many issues can potentially arise from the low parasite prevalence in the data (for example, prevalence <1% for some parasite species): for instance, maximum likelihood estimates are only asymptotically unbiased, meaning that they can be significantly biased if the number of positives is small[94]. Therefore, we excluded any parasite groups or species with <20 positives in the analysis, which in this case meant the omission of *N. bombi*. The same criterion was used in all subsequent analysis involving parasite status. In addition, we repeated the analysis for the four species and neogregarines combined as a single group of bee parasites. The broad groups microsporidia and trypanosomatids were not included in this combination since they also contain species that may not infect bees. To account for multiple testing, given the number of parasite species/groups under consideration, throughout this manuscript we use a Bonferroni-corrected significance level of $\alpha = 0.05\,n_p^{-1}$, where $n_p$ is the number of parasite species/groups considered in each analysis. To justify the use of GLMMs rather than autoregressive time-series models, we checked for temporal autocorrelation using Durbin–Watson tests on scaled residuals.

Next we investigated bee community composition and diversity as potential drivers of any observed temporal trends. These are now discussed in detail below.

Bee community composition. Should there be significant heterogeneity in host competence among taxa, community turnover may drive changes in parasite prevalence. Because the four most common bee genera—*Apis*, *Bombus*, *Ceratina* and *Lasioglossum*—comprised as much as 76% of the bee samples, we explored how the individual contributions of these genera (including all other genera as a single group) to the overall parasite prevalence of the community varied over time. First, temporal trends in the relative abundance of these genera were visualized with smoothing splines fitted using multinomial generalized additive models (GAMs) with site included as a random factor. Relative abundances were further quantified by partitioning the season into three equal windows each of 8 weeks, and performing for each site and window an exact multinomial goodness-of-fit test (against the hypothesis of equal multinomial proportions), followed by post hoc tests (with Bonferroni-corrected $\alpha$ given the number of genera) to identify the genera falling above or below the expected proportions.

Second, to evaluate heterogeneity in parasite prevalence across genera, binomial GLMMs were again fitted for each parasite but this time with genus and its interaction with week number as additional explanatory variables. Main effects of genus were evaluated regardless of whether the interactions were significant. Testing for main effects in the presence of significant interactions is known to violate the principle of marginality[95,96]. More specifically, the main effects of genera are given by the differences in fitted logit links at week 0 between genera (that is, differences in $y$-intercepts); because significant interactions imply different temporal trends between genera (different slopes), the main effects of genera become ambiguous since they are now dependent on what date we choose to assign as week 0. To address this, we defined week 0 for each genus as the median week number among all samples of that genus; week numbers of the samples were then shifted accordingly (the shifts are not shown in Supplementary Fig. 2 to avoid confusion). By doing so, the main effects could be unambiguously interpreted as the difference in parasite prevalence between genera, evaluated for each genus at a characteristic date of that genus. Post hoc tests of pairwise contrasts (with multiple testing corrections for the number of contrasts) were performed whenever the main effects of genus turned out to be significant. The significance of temporal trends in the parasite prevalence of each genus was also assessed (with multiple testing corrections for the number of genera).

Finally, temporal trends in the individual contributions of each genus to the overall parasite prevalence of the community (relative abundance × parasite prevalence of genus) were visualized using smoothing splines fitted using GAMs with site as a random factor.

Bee diversity. Should bee diversity affect parasite prevalence, temporal trends in the former may drive that of the latter. To assess how bee diversity varied across the season, we calculated the weekly Shannon index at each site based on the collected bee samples and assessed the temporal trend using a linear mixed model. Before fitting the model, rarefaction was implemented for the bee Shannon indices to address non-uniformity in bee collection efforts. Note that rarefaction tended to reduce larger indices more than smaller ones, hence potentially affecting the strength of any temporal trends. Therefore, we evaluated a range of subsample sizes rather than simply having the size determined by the smallest site/week sample. Each time, samples smaller than the size being considered were discarded. This allowed us to identify an optimal size sufficiently large for most indices to remain close to their non-rarefied values, but yet sufficiently small to minimize the number of discarded samples. All analyses were conducted using rarefied indices at this optimal size; robustness of any results to the choice of subsample size was also assessed, in addition to whether there were differences in bee diversity between sites using one-way ANOVA.

Next, we evaluated the relation between parasite prevalence and diversity using a GLMM with parasite status of the weekly bee samples as binomial response, and rarefied Shannon indices calculated using the samples as the explanatory variable. To explore whether any observed associations were entirely driven by temporal trends in the abundance of *Apis* and *Bombus*, we also repeated the analysis but this time without *Apis* and *Bombus* samples when calculating parasite prevalence.

*Parasite prevalence of the floral community over time.* As was done for bees, temporal trends in the overall parasite prevalence of the floral community were evaluated using GLMMs. Rather than being obtained at random, flower collections were directed by the co-collection of foraging bees upon them. Hence the floral community being screened should be thought of as being biased to some extent by bee foraging preferences—unlike the floral surveys used for abundance/diversity analyses. *N. ceranae* was excluded from the analysis because <20 flower samples tested positive.

Temporal trends in total floral abundance within quadrats were also evaluated using GLMMs, with counts as negative binomial response. The negative binomial was chosen to allow for over-dispersion due to clustering. To investigate whether floral abundance can dilute parasite prevalence on flowers, we evaluated the relation between parasite prevalence and floral abundance using a binomial GLMM, with parasite status on flower samples as binomial response and $\log_{10}$(mean total floral abundance per quadrat) as explanatory variable. We chose to take the logarithm of floral abundance, first because of model convergence issues and second because it was the more appropriate linear predictor for the logit link if we assumed prevalence to be inversely proportional to floral abundance.

Finally, temporal trends in floral diversity (Shannon index based on quadrat floral abundance surveys) were also evaluated using linear models, and the relation between parasite prevalence on flowers and floral diversity evaluated using a binomial GLMM.

**Software and packages used.** All analyses were performed in R v.3.5.1 (ref. [97]). Packages used were lme4 (ref. [98]) and glmmTMB[99] for fitting of GLMMs, DHARMa[100] for performing Durbin–Watson tests using scaled residuals, DescTools[101] for generating binomial and multinomial confidence intervals in the plots, mgcv[102] for fitting smoothing splines using GAM, XNomial[103] for exact multinomial tests, multcomp[104] for pairwise contrasts and Vegan 2.54 for calculating Shannon indices[105].

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

All raw data, including site surveys and screening results, are found on Dryad in addition to all analysis code used (https://doi.org/10.6086/D1X09V). Sequence data are also deposited in the NCBI database, with accession nos. MT212154, MT212155, MT212156, MT212157, MT212158, MT212159, MT296581, MT296582, MT296583, MT296584, MT296585, MT296586, MT302779, MT302780, MT302781, MT302782, MT302783, MT302784, MT359894–MT359896, MT366919, MT387450 and MT387451.

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## Author contributions

P.G., Q.S.M., C.R.M. and S.H.M. conceived the study. P.G., A.A.F., Q.S.M., C.R.M. and S.H.M. contributed to study design. P.G. and A.A.F. collected the field data. P.G., K.P. and Q.S.M. conducted the molecular work. A.D.T. developed molecular primers. P.A.M. identified and pinned the bee samples collected. W.H.N., P.G., C.R.M. and S.H.M. contributed to data analysis and wrote the first draft of the manuscript. All authors contributed substantially to the final draft.

## Competing interests

The authors declare no competing interests. A.D.T. contributed to this article in her personal capacity. The views expressed are her own and do not necessarily represent the views of the Agricultural Research Service or the United States Government.

## Additional information

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41559-020-1247-x.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No software was used to collect data |
| Data analysis | All analyses were performed in R v3.5.1. Packages used were lme4 and glmmTMB for fitting GLMMs, DHARMa for performing Durbin-Watson tests using scaled residuals, DescTools for generating binomial and multinomial confidence intervals in the plots, mgcv for fitting smoothing splines using GAM, XNomial for exact multinomial tests, multcomp for pairwise contrasts, and Vegan 2.54 for calculating Shannon indices. |

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# Field-specific reporting

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# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Study description | Bees, and the flowers they were foraging upon, were collected weekly from 3 sites over a 5 month period. We screened these samples for a range of bee parasites to determine how parasite prevalence changes over time and during periods of host turnover. |
| Research sample | We screened over 5,000 samples encompassing at least 89 flower and 110 bee species, respectively, from weekly collections over a 26-week period at 3 old-field meadow sites in upstate New York, USA. Collections over such a period captured the emergence and disappearance of different species over the sampling period which is typical across plant-pollinator communities. |
| Sampling strategy | Sampling was performed to maximize the number of bees collected. In early and late weeks these numbers were naturally low. To assess how the pollinator diversity varied across the season, we calculated the weekly Shannon index at each site, based on the collected bee samples, and assessed the temporal trend using a linear model. Before fitting the model, rarefaction was implemented for the bee Shannon indices to address non-uniformity in bee collection efforts. Note that rarefaction tended to reduce larger indices more than smaller ones, hence potentially affecting the strength of any temporal trends. Therefore, we evaluated a range of subsample sizes, rather than simply have the size be determined by the smallest site/week sample. Each time, samples smaller than the size being considered were discarded. This allowed us to identify an optimal size large enough for most indices to remain close to their non-rarefied values, but yet small enough to minimize the number of discarded samples. All analyses were conducted using rarefied indices at this optimal size; robustness of any results to the choice of subsample size were also assessed. To address statistical issues arising from the low levels of parasite prevalence, we also performed analysis for the four parasite species and neogregarines combined as a single group. |
| Data collection | We collected pollinators in sterile tubes either directly or with the use of a net. Flowers were collected with sterile forceps and placed into sterile tubes. Both bees and flowers were immediately placed on dry ice then stored at -80C until molecularly analysed. Sample details were recorded on the tubes and on collection sheets provided to each collector. New data sheets were provided daily and data was digitised and cross checked with the sample tubes during molecular processing. Initials of the collector of the samples is included in the sample ID. |
| Timing and spatial scale | We collected samples weekly across three old-field sites in upstate New York, between 18th April 2017 and 22nd September 2017. The sites were named Lansing (Lat: 42° 32' 24.4932'' N, Long: 76° 29' 47.9076'' W), McDaniels (Lat: 42° 32' 11.5872'' N, Long: 76° 25' 3.7668'' W), and Whipple (Lat: 42° 29' 23.6328'' N, Long: 76° 25' 49.818'' W). In total we spent 58 days collecting samples over the five-month period |
| Data exclusions | No data were excluded |
| Reproducibility | Analyses were performed for each replicate site separately and for the combined dataset. all results included in the ms |
| Randomization | When surveying floral abundance, sites were divided in to numbered grids and selected randomly using the phone app 'Random number generator' by UX Apps. We identified plants either in the field or via specimens and/or photos brought back to the lab and keyed out. We confirmed the identity of each individual plant screened for parasites before DNA extraction occurred. |
| Blinding | During DNA extraction, samples were identified only by their ID which did not relate to their species identification. Sample meta-data (including species) were not paired back with the raw data until final data analysis. |

Did the study involve field work? ☒ Yes ☐ No

## Field work, collection and transport

| | |
|---|---|
| Field conditions | Samples were only collected on fair weather days (bees rarely fly in windy/rainy days). |
| Location | We collected samples across three old-field sites in upstate New York. The sites were named Lansing (Lat: 42° 32' 24.4932'' N, Long: 76° 29' 47.9076'' W), McDaniels (Lat: 42° 32' 11.5872'' N, Long: 76° 25' 3.7668'' W), and Whipple (Lat: 42° 29' 23.6328'' N, Long: 76° 25' 49.818'' W). |
| Access and import/export | Sites McDaniels (Lat: 42° 32' 11.5872'' N, Long: 76° 25' 3.7668'' W) and Whipple (Lat: 42° 29' 23.6328'' N, Long: 76° 25' 49.818'' W) are managed by Cornell University and required no permit. The site named Lansing (Lat: 42° 32' 24.4932'' N, Long: 76° 29' 47.9076'' W) was privately owned and permission was granted to use the site. |
| Disturbance | Collectors entered sites on foot, causing minimal disturbance. Only bees within reach were collected - no trees were climbed, trenches dug etc. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| | |
|---|---|
| Laboratory animals | The study did not use laboratory animals |
| Wild animals | A wide variety of bee species were collected, a full list of which can be found in the supplementary data. To screen for parasite prevalence, bees were required to have their guts removed. Bees were placed on dry ice after capture which resulted in immediate death. |
| Field-collected samples | Bees were placed on dry ice after capture which resulted in immediate death. |
| Ethics oversight | No ethical approval was required for this study |

Note that full information on the approval of the study protocol must also be provided in the manuscript.