Impact of climate change on parasite infection of an important pollinator depends on host genotypes

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Abstract

Climate change is predicted to affect host–parasite interactions, and for some hosts, parasite infection is expected to increase with rising temperatures. Global population declines of important pollinators already have been attributed to climate change and parasitism. However, the role of climate in driving parasite infection and the genetic basis for pollinator hosts to respond often remain obscure. Based on decade-long field data, we investigated the association between climate and Nosema bombi (Microsporidia) infection of buffed-tailed bumblebees (Bombus terrestris), and whether host genotypes play a role. For this, we genotyped 876 wild bumblebee queens and screened for N. bombi infection of those queens between 2000 and 2010. We recorded seven climate parameters during those 11 years and tested for correlations between climate and infection prevalence. Here we show that climatic factors drive N. bombi infection and that the impact of climate depends on mitochondrial DNA cytochrome oxidase I (COI) haplotypes of the host. Infection prevalence was correlated with climatic variables during the time when queens emerge from hibernation. Remarkably, COI haplotypes best predict this association between climatic factors and infection. In particular, two host haplotypes ("A" and "B") displayed phenotypic plasticity in response to climatic variation: Temperature was positively correlated with infection of host haplotype B, but not haplotype A. The likelihood of infection of haplotype A was associated with moisture, conferring greater resistance to parasite infection during wetter years. In contrast, infection of haplotype B was unrelated to moisture. To the best of our knowledge, this is the first study that identifies specific host genotypes that confer differential parasite resistance under variable climatic conditions. Our results underscore the importance of mitochondrial haplotypes to ward off parasites in a changing climate. More broadly, this also suggests that COI may play a pertinent role in climate change adaptations of insect pollinators.

KEYWORDS

Bombus, bumblebee, climate change, host–parasite interaction, mitochondrial DNA, mtDNA haplotypes, Nosema, parasitism, phenotypic plasticity, pollinator
INTRODUCTION

An important mechanism for organisms to respond to global change is phenotypic plasticity. Phenotypic plasticity is the capacity of genotypes to express alternative phenotypes under different environmental conditions (Bradshaw, 1965; West-Eberhard, 2003). A plastic response to global change, thus, entails producing different phenotypes that lead to differing morphology, phenology, or rate of activity (Bonamour et al., 2019; Sasaki & Dam, 2019; West-Eberhard, 2003). The accelerating rate of climate change and its large impact on many species provides not only a unique opportunity but also an urgent need to investigate the genetic basis of adaptation and phenotypic plasticity in natural populations (Franks & Hoffmann, 2012; Rodrigues & Beldade, 2020).

Several candidate genes, including mitochondrial DNA (mtDNA) genes, have been identified as important contributors to climate change adaptation across various taxa (Balloux et al., 2009; Cheng et al., 2013; Coskun et al., 2003; Fontanillas et al., 2005; Lamb et al., 2018; Mallard et al., 2020). Although mtDNA has long been characterized as an adaptively neutral, or near-neutral genomic region (Nachman et al., 1996; Weinreich & Rand, 2000), more recent studies have challenged this neutrality assumption (Bazin et al., 2006; Galtier et al., 2009; Manlik et al., 2017). Several observations underscore the adaptive patterns of mtDNA and its plasticity in response to climate change. Regions of mtDNA, such as cytochrome oxidase (COx), are important players in metabolic pathways, and certain mtDNA variants may confer a fitness advantage by inducing more efficient metabolic responses under variable climatic conditions. To illustrate the case, organisms with specific mtDNA haplotypes that generate more heat during oxidative phosphorylation may survive longer in cooler climates (Ruiz-Pesini et al., 2004). Indeed, survival of individuals that harbor specific mtDNA COX variants is temperature-dependent in Drosophila simulans (Ballard et al., 2007). In addition, thermogenesis in the greater white-toothed shrew (Crocidura russula) has been associated with specific mitochondrial DNA haplotypes, which show a clinal distribution associated with environmental and climatic conditions (Ehinger et al., 2002; Fontanillas et al., 2005).

Lamb et al. (2018) showed that climate was a significant predictor of mtDNA variation in eight Australian songbird species, allowing the authors to identify specific mitochondrial variants associated with climate-change adaptation.

Bumblebees (Bombus spp.) are important pollinators of crops and other flowering plants, making them indispensable for global food production, and providing crucial ecosystem services (Allen-Wardell et al., 1998; Klein et al., 2006; Michener, 2007). However, bumblebee populations have been declining worldwide, lowering pollination services and thereby threatening biodiversity and the security of food crop yields (Allen-Wardell et al., 1998; Arbetman et al., 2017; Cameron & Sadd, 2020; Goulson et al., 2008; Williams & Osborne, 2009). Population declines of bumblebees are partly attributed to climate change and parasite infection (Cameron et al., 2011, 2016; Cameron & Sadd, 2020; Kerr et al., 2015; Sirois-Delisle & Kerr, 2018)—and, very likely, the interaction of both climate and parasitism (Potts et al., 2010). In general, climate change can alter the response to parasite infection, and, vice versa, parasitism can disrupt the ability of hosts to cope with climate-change-induced stress (Víctor et al., 2019, 2021). In addition, the association between climate and bumblebee declines is to some degree due to a climate-facilitated change in land use (Marshall et al., 2018), as well as due to range loss or range shifts (Kerr et al., 2015). For example, Bombus species with southern geographical ranges have shifted to higher elevations across North America and Europe (Kerr et al., 2015). Such climate change-driven range losses are expected to accelerate (Sirois-Delisle & Kerr, 2018) and may, in some ecosystems, be facilitated by a climate-mediated reduction in floral resources (Aldridge et al., 2011).

Nevertheless, a recent study by Maebe et al. (2021) showed that bumblebee resilience to climate change is mediated through plastic and adaptive responses. Likewise, parasite infection is implicated in insect pollinator declines worldwide (Cameron et al., 2016; Cameron & Sadd, 2020). In particular, infection by microsporidian (fungal) parasites of the genus Nosema has been identified as a major threat to bumblebees and has been labeled an emerging infectious disease (Brown, 2017), partly because of reported spillovers across host species and between commercial and natural bumblebee populations (Brown, 2017; Colla et al., 2006). Nosema bombi (Fantham & Porter, 1914) infects the gut of bumblebees and can cause a creeping disease (MacFarlane et al., 1995; Schmid-Hempel, 1998, 2021) that is highly detrimental to the fitness of its host (Otti & Schmid-Hempel, 2007, 2008). Moreover, N. bombi infection has been associated with bumblebee population declines. For instance, infection prevalence of N. bombi was significantly greater in declining Bombus spp. populations than in stable ones (Cameron et al., 2011). Furthermore, an analysis of museum specimens showed that N. bombi infection prevalence was significantly greater in declining species in the 1990s, which coincided with N. bombi outbreaks in North America (Cameron et al., 2016). This association between N. bombi infection and population declines of this important pollinator may also be linked to climate. For instance, infection intensity, based on spore counts, of Nosema ceranae in honeybees is associated with temperature (Chen et al., 2012). A recent study by McNeil et al. (2020) showed a positive correlation between N. bombi infection loads in Bombus impatiens and precipitation in spring, across various landscapes in Pennsylvania.

In this study, we investigated the association between changing temperatures and prevalence of N. bombi infection and host mtDNA COX haplotypes in a natural population of Bombus terrestris in Switzerland. The two most common COX haplotypes of this population, “A” and “B,” differ by a single nonsynonymous nucleotide substitution within cytochrome oxidase I (COI; Manlik et al., 2017). These two COI haplotypes were shown to differ in their response to immune challenges (Johnson, 2007; Johnson et al., 2011). Moreover, N. bombi infection prevalence of this Swiss bumblebee population has been shown to fluctuate widely over time—ranging from 2% (2010) to 81% (2003) of spring queens being infected between 2000 and 2010 (Manlik et al., 2017). Likewise,
the COI haplotype frequencies showed temporal variation during the same time period. Moreover, *N. bombi* infection prevalence was associated with these COI host haplotypes. Notably, the highest recorded *N. bombi* infection prevalence of this population coincided with a heatwave in Europe in 2003 (Ciais et al., 2005; Manlik et al., 2017), suggesting that infection prevalence might be associated with climate.

With this current study, we addressed two major questions: Is *N. bombi* infection associated with a changing climate (e.g. change in temperature and precipitation)? If so, is the effect of climate change on infection prevalence dependent on the mtDNA haplotypes of the hosts? To address this, we screened infection by *N. bombi* queens and the corresponding host mtDNA-haplotype frequencies in 876 bumblebees between 2000 and 2010, based on Manlik et al. (2017). We also recorded publicly available climate parameters during those 11 years and tested for correlations between climate variables and infection prevalence of specific host genotypes.

## MATERIAL AND METHODS

We compared climate parameters and *N. bombi* infection prevalence of specific mtDNA haplotypes of the host, *B. terrestris* between 2000 and 2010. Our study focused on a *B. terrestris* population in Neunforn (Canton Thurgovia), northeastern Switzerland, which is situated within the core range of *B. terrestris* in mainland Europe (Manlik et al., 2017). Spring queens were always sampled from the same location, within an area of <1 km², each year, at the site “Neunforn” (47.6°N, 8.8°E).

### 2.1 Climate parameters

*B. terrestris* queens emerge from hibernation in spring (Alford, 1969; Makinson et al., 2019) and the emergence window of queens in Switzerland falls between March and April. If climatic factors play a role in *Nosema* infection prevalence of queens, we would expect this emergence window, characterized by resting periods with intermittent short flights, foraging, and nest searching (Makinson et al., 2019) to be critical. Therefore, our analyses included this time period, but we also considered time periods before emergence/hibernation: (a) March, (b) February (just before emergence), (c) August to February (hibernation period, before emergence), and (d) August to March (hibernation period, and emergence). We collected climate data from the European Climate Assessment & Dataset (ECAD&D) project (http://www.ecad.eu; Klein Tank et al., 2002), recording the following seven climate parameters between 2000 and 2010 (Zurich, Fluntern Station, ca. 40 km from Neunforn): (1) mean of daily maximum temperatures (°C; “max. Temp.”); (2) mean of daily average temperature (°C; “avg. temp.”); (3) mean of daily minimum temperature (°C; “min. temp.”); (4) mean of daily average relative humidity (%; “humidity”); (5) maximum number of consecutive dry days with <1 mm of precipitation (“dry days”); (6) precipitation sum (mm; “precipitation”); and (7) mean of daily average cloud cover (oktas; “cloud cover”). These climate parameters are tabulated in Tables S1–S7.

### 2.2 mtDNA haplotypes

For our comparison between climate parameters and infection prevalence of specific host haplotypes, we used the data set on *B. terrestris* mtDNA haplotypes by Johnson et al. (2011) and Manlik et al. (2017). These studies previously presented haplotype networks of a 420-bp fragment of mitochondrial DNA sequence, which contains the 5-end of the coding region of COI, as well as a non-coding intergenic sequence (IGS) between COI and COII (Crozier et al., 1989; Crozier & Crozier, 1993) for the Neunforn *B. terrestris* population. Johnson et al. (2011) and Manlik et al. (2017) characterized a combined total of 27 mtDNA haplotypes in 876 bumblebees. In this study, we focus on the two most abundant haplotypes, termed A and B, which were found on average in 89% of all spring queens across all the 11 seasons (2000 to 2010; Manlik et al., 2017).

### 2.3 N. bombi infection prevalence of specific host haplotypes

For our analysis, we made use of the data set by Manlik et al. (2017), which presented overall *N. bombi* prevalence (based on genetic screening) and infection of specific haplotypes in spring queens between 2000 and 2010. From this data set, we analyzed the proportion of infected queens, possessing either haplotype A, or B in each of the 11 years. We also analyzed infection prevalence of queens with a given haplotype (i.e. the proportion of queens that possess haplotype A or B being infected with *N. bombi*). Host haplotype-specific *N. bombi* infection prevalence and overall infection prevalence (regardless of host haplotype) for each year are available in table S4 of Manlik et al. (2017). The relative abundance of each haplotype in the population can be found in table S2 of Manlik et al. (2017). Further details on sampling, genotyping of queens, and parasite diagnostics can be found in the “Supplementary Methods” section of the Supplement.

### 2.4 Statistical analyses

A common challenge in studying climate change biology is pseudoreplication, which includes testing for treatment effects of replicates that are not statistically independent (Wernberg et al., 2012). Relating infections from different years to the conditions in each of those years, as we do in this study, would not present pseudo-replication, unless there is a strong dependency across years. To check for this issue we used pairwise tests of successive years to test for autocorrelations of each of the climatic parameters between successive years. For instance, we used Pearson correlation to test for correlations
between average temperature of year 1 (2000) versus year 2 (2001), years 2 (2001) versus year 3, and until year 10 (2009) versus year 11 (2010). Likewise, we tested for autocorrelations of overall infection prevalence, infection prevalence of haplotype A and B between successive years.

To assess whether *N. bombi* infection prevalence of specific mtDNA host haplotype is associated with climate we performed principal component regression (PCR) and generalized linear model (GLM) analyses. Principal component analysis (PCA) combines highly correlated variables into a set of uncorrelated “principal components” (PCs). PCR takes advantage of this by fitting a linear regression model on k PCs, which eliminates multicollinearity in the data by removing PCs associated with small eigenvalues (Park, 1981). PCR tests were performed in GraphPad Prism version 9.4.0 (www.graphpad.com) to test whether *N. bombi* infection is associated with each of the following climate parameters: (1) max. Temp.; (2) avg. temp.; (3) min. Temp.; (4) dry days; (5) humidity; (6) precipitation; and (7) cloud cover. In the first step, PCs were derived from the original seven climate parameters, and the proportion of variance in the data explained by each of the PCs was recorded. For this, variables were transformed to give each variable a mean of 0 and standard deviation of 1, that is, scaling the variables, so that each variable is weighted equally when calculating the PCs: Xstandardized = (Xraw – $\bar{X}$) / sx (where $\bar{X}$ is the mean and sx is the standard deviation of the variable values). PCs were determined based on parallel analysis, which includes simulations to infer the number of PCs to include by determining the point at which the PCs are indistinguishable from those generated by simulated noise (Franklin et al., 1995). Specifically, PCs were only selected, for cases in which the eigenvalue from the input data was larger than the upper percentile from the simulated data (1000 replicates). We obtained the proportional contribution of each of the original climate variables to the calculation of the two PCs that best explained the variance in the data, that is, PC1 and PC2. In the second step, we performed PCR on the dimension reduced PC scores, with *N. bombi* infection data as the dependent variable. This included the following steps: (1) extracting the appropriate number of PCs, based on the parallel analysis; (2) performing multiple linear regression with the selected PC scores as predictors; and (3) changing the parameter coefficient estimates, initially calculated using the PC scores, back to the scale of original variables.

We followed this PCR procedure to check for correlations between climate variables and overall infection prevalence, that is, the proportion of infected queens, irrespective of host genotypes. In our exploratory analysis, we tested for relationships between each of the seven climate variables and overall infection prevalence for all time periods for which we collected climate data, before and after emergence from hibernation, that is, (a) March, (b) February, (c) August to February (hibernation period, before emergence), and (d) August to March (hibernation period and emergence).

For the subsequent PCR analyses, focusing on queens with specific host haplotypes, we only included time periods (i.e. the time period after emergence) that gave significant results in the exploratory analyses on overall infection prevalence. To investigate the relationship between climatic factors and infection of specific host haplotypes by PCR we used two approaches, with two different sets of dependent variables. “Approach 1”: Infection prevalence of specific host haplotypes, that is, the proportion of queens with A and B that were infected with *N. bombi* were set as dependent variables. This approach aimed to investigate whether infection prevalence of each host haplotype (A and B) is correlated with a change in each of the climatic variables. “Approach 2”: The proportion of *N. bombi* infected queens that have either haplotype A or B served as dependent variables: With Approach 2, we aimed to investigate which of the two host haplotypes, A or B, is more likely infected, given that they are infected in the first place. To rule out that one haplotype might be more likely infected because it is more common in the population in any given year, we also tested for correlations between the proportions of a given haplotype observed in the population per year versus infection prevalence of that haplotype at that time. We, thus, also tested for correlations between the proportion of infected individuals that had haplotype A or B versus the observed proportion of haplotypes (A or B, respectively) in the population for each of the 11 years. For both approaches, infection data were transformed as described above. In additional analyses, we excluded the year 2003, when testing for correlations between temperature and infection prevalence for the time period March. We did this because 2003 featured an unusual heatwave throughout Europe (Ciais et al., 2005), including Switzerland (see Tables S1–S3), and we, thus, treated the temperature data for that year as outliers for this additional set of analyses. Proportional data were transformed using arcsine-square-root transformation.

Additionally, we used GLM to test for associations between parasite infection prevalence and infection prevalence of specific host haplotypes and climatic factors. For this analysis, we only included the data set for March, that is, the time period for which the PCR analysis showed significant correlations between climatic variables and infection prevalence (see Table 1). In temperate regions March also coincides with *B. terrestris* queens emerging from hibernation, foraging, and dispersing to search for nest sites (Makinson et al., 2019). To perform GLM analyses we used the lme4 package (Bates et al., 2022) in R v4.0.3 (R Core Development Team, 2018). When analyzing the data, we also took the following considerations into account: We included maximum temperature as the only temperature variable in the GLM model, excluding minimum and average temperature for the following reasons: (1) in particular maximum and average temperatures are highly correlated (Figures S3 and S4); (2) among the three temperature variables, maximum temperature had the largest proportional contribution to the calculation of principal component 1 (PC1; Figure S2); (3) the PCR analysis suggests that the difference between haplotypes might be driven by extremes in temperature (Table 1); (4) minimum temperature showed a positive autocorrelation over years (Table S8). In addition to maximum
temperature, the other climatic variables, dry days, humidity, precipitation, and cloud cover, which were not as highly correlated as maximum and average temperature, were included in the analyses. To improve variance homogeneity, these climatic variables were transformed to zero skewness and expressed on a 0–1 scale prior to the analyses. To investigate the effect of the above-mentioned climatic variables on overall infection prevalence (irrespective of host haplotype), as well as infection prevalence of specific host haplotypes, GLMs were fitted. The models were built based on a forward selection strategy and the final model was selected based on the lowest Akaike information criterion (AIC). In a second analysis, in addition to the climatic variables, infection of host haplotypes A and B were also included in the analyses as explanatory variables for overall infection prevalence. The GLM analyses were performed assuming a Poisson distribution for count data variables (i.e. the number of individuals being infected or not).

### RESULTS

#### 3.1 Association between climate and infection prevalence

We did not observe any auto-correlation for overall infection prevalence or infection prevalence of specific host haplotypes over years (Table S8). Likewise, there was no auto-correlation for the climatic variables over years, except for minimum temperature, which showed a positive autocorrelation (see Table S8). We did not include minimum temperature in the GLM analysis, for other reasons explained above, and thus ignored the effect of year in subsequent analyses for all other climatic variables.

We are reporting here the PCA results for the climate variables recorded for March, which was the only time period that gave significant results in the PCR analysis—see next paragraph below. For March, the PCA calculated a total of seven PCs, two of which

| TABLE 1 | March: Principal component regression (PCR) results for (a) climate variables (March) versus overall infection prevalence (regardless of host haplotype); (b) climate variables versus proportion of spring queens infected with Nosema bombi that possess haplotype A or B (“Approach 1”); climate variable versus infection prevalence of queens with haplotype A and B (“Approach 2”). Relationships are shown for time periods (2000–2010) during March. Climate variables are defined as: “Max temp.” = mean of daily maximum temperatures (°C); “Avg temp.” = mean of daily average temperature (°C); ”Min temp.” = mean of daily minimum temperature (°C); “Dry days” = maximum number of consecutive dry days with <1 mm of precipitation; “Humidity” = mean of daily average relative humidity (%); (6) “Precip.” = precipitation sum (mm); ”Cloud cov.” = mean of daily average cloud cover (oktas). The least square estimates (“Estimate”) and ANOVA F-values (F) are tabulated. Significant values based on PCR: Sig. *p < .05; Sig. **p < .01; ns = nonsignificant (p > .05). Rows that are shaded in green represent positive correlations; blue-shaded rows represent negative correlations. Additional details of this analysis, including the 95% confidence interval, as well as principal component regression analyses for other time periods are tabulated in Tables S10-S13.

| Parameter estimates | Overall infection prevalence | Approach 1: Infection prevalence A and B | Approach 2: Proportion infected (A or B) |
|----------------------|-----------------------------|----------------------------------------|----------------------------------------|
|                      | Estimate | p          | Sig. | Estimate | p          | Sig. | Estimate | p          | Sig. | Estimate | p          | Sig. |
| Max temp.            | 0.02989  | .0012      | **   | 0.03449  | .0145      | ns   | -0.006896 | .0082      | **   | -0.04453 | .0139      | **   |
| Avg temp.            | 0.03473  | .0067      | **   | 0.02449  | .1495      | ns   | -0.00722  | .0145      | **   | -0.0005  | .0966      | ns   |
| Min temp.            | 0.02449  | .0067      | **   | 0.007022 | .0145      | **   | -0.00005  | .0966      | ns   |
| Humidity             | -0.006896| .0082      | **   | -0.00722 | .0145      | **   | -0.00005  | .0966      | ns   |
| Dry days             | -0.0005  | .0966      | ns   | -0.04453 | .0139      | **   | -0.00005  | .0966      | ns   |
| Precip.              | -0.00005 | .0966      | ns   | -0.04453 | .0139      | **   | -0.00005  | .0966      | ns   |
| Cloud cov.           | -0.00477 | .017       | *    | -0.035   | .0205      | *    |

For March, the PCA calculated a total of seven PCs, two of which
(PC1 and PC2) were selected by the parallel analysis, which were later used in the PCR analysis. PC1 and PC2 explained 54.2% and 31.5% of the variance in the data, respectively, and cumulatively 85.8% of the variance (see Figure S1). Minimum temperature had by far the lowest contribution to the calculation of PC1. The proportional contributions of the climate variables to the calculation of PC1, from highest to lowest, were cloud cover (19.8%), max. Temp. (19.4%), humidity (18.2%), precipitation (18.2%), avg. temp. (13.8%), dry days (12.3%) and min. Temp. (2.1%; see Figure S2). The variable loadings of the PCA showed positive correlations between PC1 and the climate variables precipitation, humidity, and cloud cover. PC1 was negatively correlated with the temperature variables and dry days (Table S9; Figure S3). In contrast, PC2 positively correlated with the temperature variables, precipitation, humidity, and cloud cover but negatively correlated with dry days (Table S9; Figure S3).

The PCR analysis showed that *N. bombi* infection prevalence is strongly associated with climate in March, the time period after emergence from hibernation. For this time period, overall infection prevalence (irrespective of host haplotypes) was positively correlated with average and maximum temperature (but not minimum temperature), positively correlated with the consecutive number of dry days, and negatively correlated with humidity and cloud cover (Table 1a; Table S10). In other words, with increasing temperature and drier conditions, more queens were infected. We did not find any association between climate and overall infection prevalence for any other time periods that we considered, that is, February (Table S11), August–February (Table S12), and August–March (Table S13). This suggests that the influence of climate on *N. bombi* infection is only exerted during the time period during or just after emergence from hibernation. Our GLM analysis (see below) thus focused only on the time period of March.

### 3.2 Association between climate and infection of specific mtDNA host haplotypes

Both the PCR and the GLM analyses showed that the effect of climate on infection prevalence during March, the time of emergence is dependent on the host genotypes. The PCR results of Approach 1, for which we set infection prevalence of specific host haplotypes as the dependent variable, showed a negative relationship between the consecutive number of dry days and infection prevalence of both host haplotypes (Table 1b; Table S14). Likewise, humidity and cloud cover were each negatively correlated with infection prevalence of queens with haplotypes A and B (Table 1b; Table S14). However, the PCR analysis showed a divergent response of the two host haplotypes, A and B to temperature. The PCR results for Approach 1 show that temperature is positively correlated only with infection prevalence of haplotype A—not B (Table 1b; Table S14). Specifically, maximum and minimum temperature are highly correlated with infection prevalence of haplotype A (Table 1b; Table S14). This divergent response of the two haplotypes was also apparent from the results of Approach 2, in which the proportion of infected queens that have haplotype A or B served as dependent variables: March temperatures were positively correlated with the proportion of infected queens that had haplotype B, but negatively correlated with the proportions of queens that possess haplotype A (Table 1b; Figure 1; Table S15). This pattern was also true for average, maximum and minimum temperatures (Table 1b; Table S15). The proportion of infected queens that had haplotype A or B was not correlated with the proportions of the respective haplotypes in the population observed for each year (haplotype A: Pearson $r = .2687$; $p = .4244$; haplotype B: Pearson $r = .2938$; $p = .3805$). Thus, the observed correlations between climatic variables and infection prevalence of specific host haplotypes, are independent of the relative abundances of host haplotypes in the population.

The GLM analysis revealed that for March, during the time period after the queens emerge from hibernation, overall parasite infection prevalence was significantly correlated with infection of the specific host haplotypes A and B (Table 2). This is association is irrespective of the relative abundance of the haplotypes in the population given that the proportion of infected haplotypes was not correlated with the proportions of the respective haplotypes in the population (see Section 3.2 above). When we included haplotype A and B in the analyses as explanatory variables, infection prevalence was best explained by the haplotypes, and not the climatic factors (Table 2). The analysis that excluded the haplotypes as explanatory variables showed that *N. bombi* infection prevalence is best explained by the interactions of maximum temperature × dry days × humidity (Table 2; Figure 2a).

In the next step, we performed GLM analyses to investigate the association between climatic factors and infection prevalence of

![Figure 1](image-url)
specific host haplotypes (A and B). For host haplotype A, we found that wet versus dry conditions, in particular the interaction of cloud cover × humidity × precipitation best explained infection prevalence of this haplotype (Table 2; Figure 2b). Infection prevalence of haplotype A was negatively correlated with humidity and precipitation (Table 2; Figure 2b). In contrast, no relationship was found between infection prevalence of haplotype B and any of the moisture parameters (humidity, dry days, precipitation or cloud cover). Instead, infection prevalence of haplotype B, was positively correlated with maximum temperature during March (Table 2; Figure 2b). Overall, these results indicate that N. bombi infection prevalence is associated with interacting climatic variables (temperature, humidity, and consecutive number of dry days) and that warm versus cool (for haplotype B) and wet versus dry conditions (for haplotype A) determine the likelihood of infection of specific host haplotypes. Queens with haplotype A are more susceptible to infection in dry conditions, while those with haplotype B are more susceptible in hotter conditions (Table 2; Figure 2b). Correlations between all variables for March, based on the GLM analysis are shown in Figure S4. The main results of the GLM analysis are schematically presented in Figure 2.

### 4 | DISCUSSION

#### 4.1 | Association between climate and infection prevalence

Climate change impacts individuals and populations but also interspecific interactions, including host–parasite interactions. The complexity of these interactions makes it difficult to assess how host–parasite systems respond to climate change, given that phenotypes of both hosts and parasites can be strongly affected by temperature and other climatic variables (Harvell et al., 2002; Kirk et al., 2018; Raffel et al., 2013). Our decade-long study...
showed a strong association between climate and *N. bombi* infection of bumblebees (*B. terrestris*) in central Europe. The results suggest that climate drives *N. bombi* infections of bumblebee queens during the time of emergence from hibernation, and that the impact of climate depends on the mtDNA COI haplotypes of the host. Overall infection prevalence, irrespective of the host's genotype, was positively correlated with March temperature and dry days but negatively correlated with humidity and cloud cover (Table 1a). When not considering the host haplotypes in the GLM model, the interaction of temperature, humidity, and precipitation best explained *N. bombi* infection prevalence (Table 2; Figure 2). Hot, dry conditions appear to favor *N. bombi* infection, whereas infection is less likely in cooler, wetter conditions. Similarly, a positive correlation between temperature and microsporidian parasite infection has been shown for water fleas (*Daphnia magna*). Kirk et al. (2018) exposed *Daphnia* to the microsporidian parasite *Ordospora colligate* at nine different temperatures, and showed that the rate of infection was greater at higher temperatures.

A study on *B. impatiens* in North America by McNeil et al. (2020) showed a positive correlation between precipitation and *N. bombi* infection. As in the case of our study on the Swiss *B. terrestris* population, the time period that mattered was the spring season. Other studies on honeybees (*Apis mellifera*) showed that *Nosema* infection prevalence is seasonal, with a peak in spring when queens emerge from hibernation (Cisder et al., 2010). Notably, the study by McNeil et al. (2020) assessed parasite load of workers on a spatial scale, while we analyzed parasite infection prevalence of spring queens on a temporal scale. McNeil et al. (2020) investigated the effect of other environmental variables, and the only climatic factor that was considered in that study was precipitation. In addition, two studies on honeybee workers show a strong, but negative, association between temperature and *Nosema* infection. Chen et al. (2012) detected a negative correlation between temperature and *N. ceranae* parasite load in commercial apiaries in Taiwan. Likewise, a study on honeybees in Switzerland showed higher *Nosema* spp. intensities (based on spore counts) during cooler, ambient apiary temperatures over a period of 18 months (Retschnig et al., 2017). Notably, unlike our studies on bumblebee queens, these two studies were conducted on honeybee workers. None of these studies differentiated between different host genotypes or considered any other genetic aspects.

### 4.2 Host-genotype-dependent impact of climate on *N. bombi* infection

Our study also indicates that the impact of climate on *N. bombi* infection is dependent on the mtDNA COI haplotypes of the host. For the GLM model that incorporated the haplotypes as variables, the two host haplotypes (A and B) were the best predictors for *N. bombi* infection under variable climatic conditions (Table 2). The PCR (Approaches 1 and 2) indicates that with increasing temperature, haplotype-B queens were proportionally more likely to be infected compared to queens with haplotype A (Figure 1). These temperature correlations also hold up when we excluded the...
temperatures recorded for 2003, which featured extreme heat throughout Europe (Ciais et al., 2005; Table S18). Notably, these correlations were independent of the haplotype frequencies in the population over time. The strong associations between climate and host genotype-specific *N. bombi* infection were further supported by the GLM analysis, which showed that the influence of several interacting climatic variables on infection depends on the two COI haplotypes. The interaction of humidity, cloud cover and precipitation best explain infection of haplotype A, while infection of haplotype B is best explained by maximum temperature (Table 2: Figure 2b). Notably, those three climatic variables, are positively correlated with one another (Figure S4; also see loads for PC1 and PC2 in Figure S3), and together reflect moisture. The PCR analysis suggests that it may not be the amount of precipitation per se that matters (Table 1), but overall moisture, that is, wet versus dry conditions that affect the likelihood of infection of haplotype-A queens.

In contrast, temperature appears to influence infection of queens with haplotype B. Based on the PCR analysis, average and maximum temperature variables were also the primary factors for the divergent response to *N. bombi* infection between the two host haplotypes (Table 1). Together these results suggest that climate appears to drive *N. bombi* infection, but the host’s response varies according to its genotype. Queens with haplotype A appear more resilient to *N. bombi* infection under wetter conditions. Conversely, for hosts with haplotype B a cooler climate appears to confer greater resistance to infection, while such conditions render host haplotype A relatively more susceptible to infection. Thus, in this case, the two genotypes have different plasticities, in line with Bradshaw’s (1965) early characterization of phenotypic plasticity, in which he explicitly stated that different genotypes may show different phenotypic responses across environments.

### 4.3 Differences between haplotypes offer possible explanations for results

This differential host-genotype-dependent resilience to *N. bombi* infection in a changing climate may result in different immunological and fitness traits that are expressed by the two COI haplotypes (summarized in Table 3). Previous work by Johnson et al. (2011) lends some indirect support to this hypothesis. Their findings suggest that queens possessing haplotype A or B differ in resilience to parasitism because they confer different defense mechanisms against infections: When challenged with lipopolysaccharides (LPS), antibacterial activity of the haemolymph in the bumblebees was significantly greater in haplotype A than in B (Johnson et al., 2011). Furthermore, in comparison to queens with haplotype B, queens harboring haplotype A showed significantly stronger activity of phenoloxidase (Johnson et al., 2011), an enzyme that is considered a key mediator of immune function in insects (Cerenius & Söderhäll, 2004). Perhaps this greater response to LPS and stronger activity of phenoloxidase in haplotype A queens is more pronounced under warmer, or wetter conditions. It is feasible that during warmer temperatures the haplotype-A queens show a comparatively stronger immune response than haplotype-B queens, which may render haplotype-A queens more resilient to *N. bombi* infection during warmer temperatures. At this point, we remark that, in diapausing insects such as bumblebees, the exhaustion of the energy reserves is an important factor to determine the chances of surviving the hibernation period and the acceleration towards early emergence in spring. Parasite-infected and, therefore, energetically stressed, queens should be particularly sensitive to having an efficient metabolism.

As temperatures are rising globally, our results suggest that over time *N. bombi* infection prevalence may increase. Moreover,

| Parameters | Haplotype A | Haplotype B | Study |
|------------|-------------|-------------|-------|
| **Molecular differences** | DNA: Non-synonymous SNP within COI (Position: 164)<sup>a</sup> | Nucleotide: Guanine | Johnson et al. (2011); Manlik et al. (2017) |
| | Nucleotide: Adenine | Amino acid: Isoleucine | |
| | Peptide (COI position: 51)<sup>b</sup> | Amino acid: Valine | |
| **Immunological differences** | Response to challenge with lipopolysaccharides (LPS) | Greater antibacterial activity of haemolymph | Johnson et al. (2011) |
| | Greater Phenoloxidase activity | Lower antibacterial activity of haemolymph | |
| | Greater | Lower | |
| **Nosema bombi** infection prevalence correlates | A-Queens less likely infected when overall infection prevalence is high | B-Queens more likely infected when overall infection prevalence is high | Manlik et al. (2017) |
| **Climatic conditions conferring greater resistance to *N. bombi*** | Warmer, wetter conditions (Figures 1 and 2; Table 2) | Cooler, drier conditions (Figures 1 and 2; Table 2) | This study |

<sup>a</sup>The 449-bp sequence of the haplotypes entails COI, as well as intergenic region between COI and COII. The non-synonymous SNP (single nucleotide polymorphism) is within the COI region of the sequence; NCBI GenBank DNA accession numbers: JF715202.1 (haplotype A), JF715203.1 (haplotype B).

<sup>b</sup>NCBI GenBank accession numbers for the COI peptides encoded by the haplotypes: AEO44562.1 (haplotype A), AEO44563.1 (haplotype B).
with ongoing global warming the results of this study suggest that more individuals with haplotype B and fewer with haplotype A will be infected by N. bombi. This would confer a fitness advantage of haplotype A, and one might expect that haplotype A would become more prevalent in bumblebee populations. This would suggest a shift in haplotype frequencies and a possible fixation of haplotype A in the future. Notably, for the Swiss and one other European bumblebee population, the rate of non-synonymous substitutions (dN) for these haplotypes, exceeded the rate of synonymous substitutions (dS), which is indicative of directional selection (Manlik et al., 2017). It is unclear whether the differential fitness of the two haplotypes will lead to major trans-generational shifts in the haplotype and phenotype frequencies in the populations, or even a genetic assimilation to the changed climatic conditions. However, the observation of temporal fluctuations of haplotype frequencies so far suggests no such trend of a persistent shift: In the Swiss population, and in another B. terrestris population on Gotland, haplotype B remained the most prevalent haplotype (85% on average) between 2000 and 2010 (Manlik et al., 2017). A persistent fluctuation in haplotype frequencies may of course be facilitated by migration.

5  |  CONCLUSION

Changes in temperature and other climatic variables can influence parasite infection and parasite–host interactions, particularly for parasites with ectothermic hosts. Our long-term observations on N. bombi infection of wild buff-tailed bumblebees (B. terrestris) show indeed that climatic conditions influence infection, but the impact of climate is dependent on mitochondrial DNA haplotypes of the host. The two host haplotypes studied here, called A and B, displayed different plastic responses under variable climatic conditions. Queens that possess haplotype A appear more resilient to infection under warmer, wetter conditions. In contrast, host haplotype B confers relatively greater resilience to N. bombi infection in a cooler, drier climate. Given that Nosema infection has been identified as a potential driver of observed population declines of bumblebees, these findings have potentially important implications for the conservation of these economically highly valuable pollinators. The observed plastic response of the two host haplotypes may facilitate the resilience to parasitism and thus persistence in a changing climate. More broadly, our results may also indicate that mtDNA COI could play a pertinent role in climate change adaptations of insect pollinators.

AUTHOR CONTRIBUTIONS

All authors contributed to the design and conceptualization of the study, as well as the writing and revisions of the manuscript. Among other things, the lab of Experimental Ecology (Schmid-Hempel) collected the samples. Oliver Manlik collected and compiled data on climatic variables, infection prevalence and mtDNA haplotypes, performed the PCA, as well as the PCA regression analyses, and drafted the initial manuscript. Sunil Mundra performed the GLM analyses, tabulated the results and provided interpretation of these analyses.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All climatic data that were used for this study are tabulated in Tables S1–S7 (see Supplementary Material). These climate variables for the time periods of this study were collated from the European Climate Assessment & Dataset (ECA&D) project (http://www.ecad.eu; Klein Tank et al., 2002) for the Zurich, Fluntern Station, and can be accessed via the EU-FP6 project UERRA data set: http://www.uerra.eu. The data on host haplotype-specific N. bombi infection prevalence, as well as overall infection versus low levels of climatic conditions table S4 of Manlik et al. (2017): https://doi.org/10.1016/j.meegid.2017.11.019. Data on the relative abundance of each haplotype for each year can be found in Table S2 of the same study. These supplementary tables (Manlik et al., 2017) can also be requested from the corresponding author. Raw data on the number of infected and uninfected queens, as well as collated climate data are also available in Dryad (https://doi.org/10.5061/dryad.s7h44j19). The code for the GLM model is available from S. Mundra (sunilmundra@uaeu.ac.ae) upon request.

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