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Research

Niche differentiation within a cryptic pathogen complex: climatic drivers and hyperparasitism at multiple spatial scales

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Pathogens are embedded in multi-trophic food webs, which often include co-occurring cryptic species within the same pathogen complex. Nonetheless, we still lack an understanding of what dimensions of the ecological niche might allow these cryptic species to coexist. We explored the role of climate, host characteristics (tree autumn phenology) and attack by the fungal hyperparasite *Ampelomyces* (a group of fungi attacking plant pathogens) in defining the niches of three powdery mildew species (*Erysiphe alphitoides*, *E. hypophylla* and *E. quercicola*) within a cryptic pathogen complex on the pedunculate oak *Quercus robur* at the continental (Europe), national (Sweden and France) and landscape scales (a 5 km^2 island in southwestern Finland). Previous studies have shown that climate separated the niches of three powdery mildew species (*E. alphitoides*, *E. hypophylla* and *E. quercicola*) in Europe and two species (*E. alphitoides* and *E. quercicola*) in France. In our study, we did not detect a significant relationship between temperature or precipitation and the distribution of *E. alphitoides* and *E. hypophylla* present in Sweden, while at the landscape scale, temperature, but not relative humidity, negatively affected disease incidence of *E. alphitoides* in an exceptionally warm year. Tree variation in autumn phenology did not influence disease incidence of powdery mildew species, and hyperparasite presence did not differ among powdery mildew species at the continental, national and landscape scale. Climate did not affect the distribution of the hyperparasite at the continental scale and at the national scale in Sweden. However, climate affected the hyperparasite distribution in France, with a negative relationship between non-growing season temperature and presence of the hyperparasite. Overall, our findings, in combination with earlier evidence, suggest that climatic factors are more important than species interactions in defining the niches of cryptic species within a pathogen complex on oak.

Keywords: climate variation, cryptic pathogen species, hyperparasite, niche differentiation, spatial distribution

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Introduction

It has frequently been realized, sometimes after several decades of study, that a single pathogen consisted of a complex of cryptic pathogen species (Taylor et al. 2000). For example, cryptic pathogen complexes have recently been found on plants (Hyde et al. 2010), fish (Xavier et al. 2015), amphibians (Müller et al. 2018), domestic animals (Puig et al. 2016) and humans (Brown et al. 2013). Unravelling cryptic pathogen complexes has contributed to insights into the geographical distribution of species, disease ecology and epidemiology (Fitt et al. 2006, Leavitt et al. 2012, Martel et al. 2013). One question has remained central in the study of cryptic pathogen species: If species are similar enough to be hard or impossible to distinguish, why do they not outcompete each other – or, in other words, what allows them to co-exist? (Gause 1934, Kneitel 2008). One possibility is that the species have evolved separate ecological niches as a response to competition. However, it is unclear which dimensions of the ecological niche are most important. As pathogens are frequently embedded within complex multi-trophic food webs, the ecological niche of cryptic species can differ due to different interactions with hosts and natural enemies (the Eltonian niche), but also due to different responses to climatic conditions (the Grinnellian niche) (de Araújo et al. 2014, Schmeller et al. 2014, Zhao et al. 2019, Mayr et al. 2021). Unravelling the relevant niches of cryptic species, and thereby the drivers of their distribution is crucial, not least for forecasting the distribution and spread of existing and emerging diseases in a changing climate (Beukema et al. 2018).

While climate is well-known to shape the distribution of pathogens (Harvell et al. 2002, Garrett et al. 2006), few studies have investigated whether cryptic species differ in their climatic niches (but see Redondo et al. 2018, Byrne et al. 2019). Cryptic species within the same pathogen complex may respond to the same environmental drivers, as predicted based on phylogenetically conserved traits (Chenuil et al. 2019). However, they may also have evolved separate niches as a result of competition, as predicted by theory on character displacement and competitive exclusion (Hardin 1960, Armstrong and McGehee 1980). Furthermore, niche differentiation of cryptic species has mostly been studied at single spatial scales, and only a handful of studies have investigated the climatic drivers and distribution of cryptic species within pathogen complexes at multiple spatial scales (but see Desprez-Loustau et al. 2018).

While pathogens are commonly attacked by antagonists, like fungal hyperparasites (i.e. fungal parasites whose hosts are other parasites), few studies have focused on top-down control of pathogens in natural systems (Swinton and Gilligan 1999, Tollenaere et al. 2014). This is a clear knowledge gap, since hyperparasites can reduce pathogen population growth, reproduction and overwintering success (Kiss et al. 2004, Tollenaere et al. 2014, Sun et al. 2019, Zewdie et al. 2021). Cryptic species may differ in hyperparasitism rates, for example due to differences in susceptibility to the hyperparasite, and this may allow for the co-existence of fungal pathogens (Fodor 2011, van Hoesel et al. 2020). Importantly, climate may not only affect the distribution of cryptic pathogen species directly, but also indirectly by shaping the distribution of their hyperparasite (Álvarez-Loayza et al. 2011, Wisz et al. 2013). However, only few studies have focused on the climatic drivers of hyperparasite distribution and the role of hyperparasitism in defining niches of cryptic species (Kiss 1998, Grishkan et al. 2003, Parratt and Laine 2016).

We aimed to identify the climatic and trophic factors that shape the coexistence of cryptic species (*Erysiphe* spp.) in a pathogen complex on the pedunculate oak *Quercus robur* at multiple spatial scales. In other words, we targeted both the Grinnellian and Eltonian dimensions of these species' niches (Grinnell 1917, Elton 1927, Gravel et al. 2019). Given variation in the data available at each of the three spatial scales studied, we asked conceptually similar but slightly different questions at each level: 1) At the continental scale, it was previously demonstrated that the cryptic powdery mildew species showed climatic niche differentiation, leading to differences in their geographical distributions (Desprez-Loustau et al. 2018). As a natural extension, we here investigated the niche of a higher trophic level: how temperature and precipitation affect the distribution of the hyperparasite, and whether hyperparasitism differed among cryptic mildew species. 2) At the national scale, we first examined the influence of temperature, precipitation and host characteristics (i.e. oak autumn phenology; Ekholm et al. 2019) on the distribution of the cryptic powdery mildew species in Sweden. As for autumn phenology, we hypothesized that early leaf senescence might reduce the successful production of overwintering structures of powdery mildew species, thus reducing disease levels and providing a strong structuring force. We then examined the impact of the same climatic variables on the hyperparasite in Sweden and France, and whether hyperparasitism differed among the cryptic powdery mildew species. 3) At the landscape scale, we assessed the influence of temperature, relative humidity and oak autumn phenology on the spatiotemporal dynamics of the powdery mildew species at the tree level across four years, as well as the impact of climatic factors on the hyperparasite (see the detailed study predictions in the Supporting information).

Material and methods

Study system

In Europe, the pedunculate oak (*Q. robur*) is commonly attacked by three closely related species of powdery mildew: *Erysiphe alphitoides*, *E. hypophylla* and *E. quercicola* (Mougou et al. 2008, Mougou-Hamdane et al. 2010). The three species cannot be reliably distinguished in the field, and microscopic differences are very minor (Takamatsu et al. 2007). The species *E. alphitoides* and *E. quercicola* are most commonly found on the upper leaf surface, whereas *E. hypophylla* is largely restricted to the lower leaf surface (Takamatsu et al. 2007, Desprez-Loustau et al. 2018).

During the growing season, powdery mildew reproduces asexually and spores are mainly wind-dispersed. At the end of the growing season, *E. alphitoides* and *E. hypophylla* produce sexual structures (chasmothecia), with the help of which they overwinter in the leaf litter or tree bark. *E. quercicola*, on the other hand, is only known to overwinter as mycelium within oak buds (Takamatsu et al. 2007, Marçais et al. 2017). The survival of chasmothecia and spore release in spring depend on climatic conditions during the non-growing season (Marçais et al. 2009, Tack and Laine 2014).

Powdery mildew species, including *Erysiphe* spp., are frequently attacked by fungal hyperparasites in the genus *Ampelomyces* (Tollenaere et al. 2014, Parratt and Laine 2016). When parasitized by *Ampelomyces*, the asexual and sexual sporulation of powdery mildew species is reduced or completely halted (Falk et al. 1995, Legler et al. 2016). Molecular studies revealed that *Ampelomyces* is a genetically diverse group of intracellular fungal hyperparasites, with four major clades (putative species) (Kiss 1998, Park et al. 2010, Kiss et al. 2011, Németh et al. 2021). Hyperparasites can be identified by a change in colour of the powdery mildew colony from white to brown, and microscopically based on the presence of asexual fruiting bodies (pycnidia), but molecular assessment is probably the most accurate mode of detection.

Study design

Continental scale

To assess how variation in climate shapes the distribution of the hyperparasite, we took advantage of a previous distributed sampling campaign of cryptic mildew species on oak trees throughout Europe (Desprez-Loustau et al. 2018). Oak leaves were collected from each of 411 trees (with 318 samples from *Q. robur* and the others from *Q. cerris* [n = 13], *Q. petraea* [n = 27], *Q. ithaburensis* [n = 2], *Q. vulcanica* [n = 40] and *Q. pyrenaica* [n = 11]) in eight European countries in 2014 and 2015 (Fig. 1; Desprez-Loustau et al. 2018). One leaf sample per tree was randomly selected from the subset of leaves for the molecular identification of *Erysiphe* spp. The same samples were used in this study to detect the presence of *Ampelomyces*. To relate the hyperparasite distribution to climatic drivers at the continental scale, we calculated ecologically relevant bioclimatic variables by averaging monthly temperatures and precipitation for the growing and non-growing season over a 30-year period (Supporting information).

National scale

To assess how variation in climate, oak phenology and the hyperparasite shapes the distribution of cryptic powdery mildew species in Sweden, leaves were collected throughout the range of oak. Given that the two native species of oaks found in Sweden, *Q. petraea* and *Q. robur*, hybridize extensively and that there is no clear morphological or genetic distinction between the two species, we treated them as the compound taxon *Q. robur* sensu lato (Aas 1993). The leaves were collected by schoolchildren as part of a citizen science project in autumn 2016 (Ekholm et al. 2019). For each tree, up to twenty randomly selected leaves were collected, with a total of 948 leaves from 92 trees. For each leaf, the percentage of leaf surface covered by powdery mildew was visually scored on

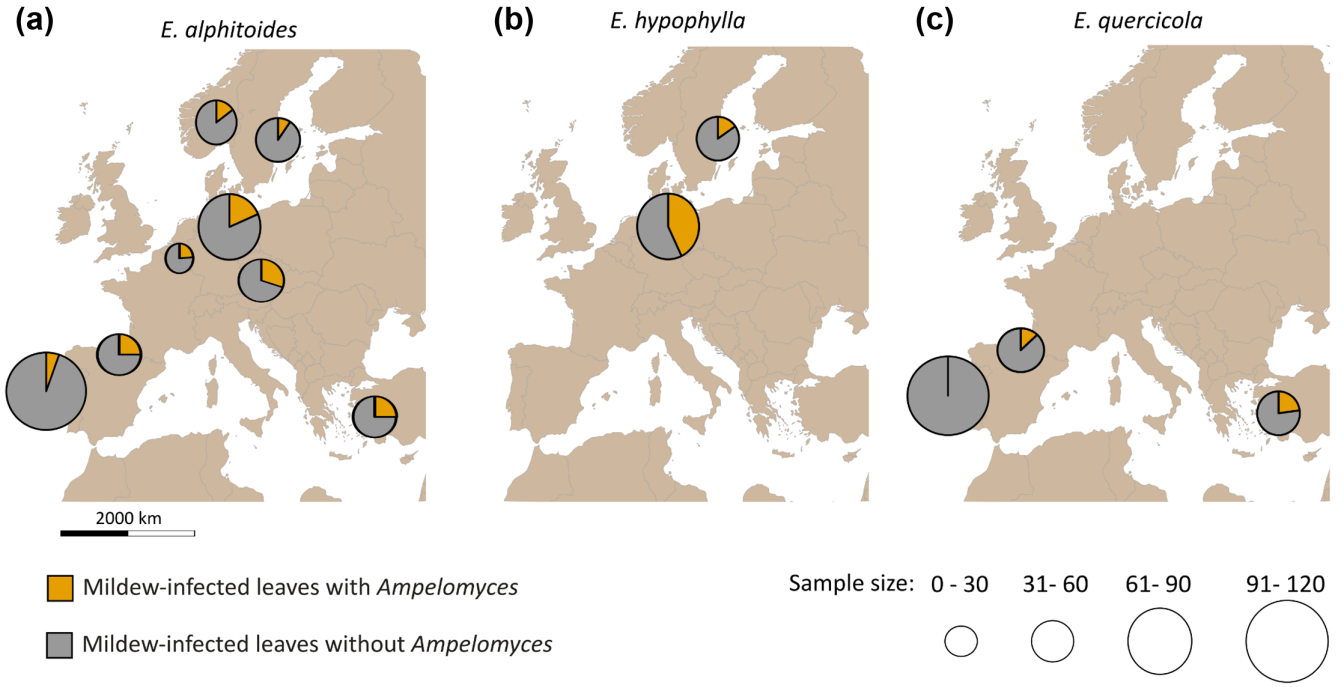


Figure 1. Spatial distribution of *Ampelomyces* spp. on oak powdery mildew (*Erysiphe* spp.) in Europe, shown separately for (a) *E. alphitoides*, (b) *E. hypophylla* and (c) *E. quercicola*. Pie graphs show the fraction of powdery mildew infected leaves with *Ampelomyces* spp. Circle sizes represent number of trees sampled per country (i.e. Austria, Belgium, Germany, Norway, Portugal, Spain, Sweden and Turkey).

the upper and lower leaf surfaces by the same person (MF). We then used four discs from each leaf (pooled into a single leaf-level sample) to molecularly identify the presence of each powdery mildew species and the hyperparasite.

To examine the relationship between climate and the distribution of cryptic powdery mildew species and their hyperparasite, we first focused on the long-term average climatic conditions. For this, we extracted temperature and precipitation data for each sampling location for the period between 1970 and 2000. We then extracted climatic data for the period 2015–2016 to understand if powdery mildew disease incidence (i.e. the proportion of infected leaves at the tree-level; Madden et al. 2017) was affected by climatic conditions in the current year. To identify the relationship between tree autumn phenology and the distribution of powdery mildew species in 2016, we used a tree-level index of autumn phenology (i.e. the proportion of brown leaves at the tree level; Ekholm et al. 2019). For more details on the methodology of the Swedish survey and scoring of oak autumn phenology, see Ekholm et al. (2019).

To investigate the relationship between climate and hyperparasite distribution in France, we used data from a previous multi-year survey of cryptic powdery mildew species on oak saplings in France (Marçais et al. 2017). For molecular identification of *Erysiphe* spp., one leaf sample was collected from each of 400 seedlings (*Q. robur* and *Q. petraea*) in autumn 2012 and 2013, in 32 oak stands across France (Marçais et al. 2017). The same leaf samples were used in this study to detect *Ampelomyces*.

Landscape scale

To investigate the relationship between landscape-scale variation in climate and oak phenology on cryptic powdery mildew species and their hyperparasite, we focused on the 5 km^2 island of Wattkast in southwestern Finland (Tack et al. 2010). The island consists of a patchwork of forests, fields and pastures, and oak trees are patchily distributed across the island. Previous studies on *Q. robur* showed that only two species, *E. alphitoides* and *E. hypophylla*, were present in this landscape (Desprez-Loustau 2018). Since *E. alphitoides* is the only species that occurs on the upper leaf surface, we estimated its disease severity (proportion of leaf area affected by the disease; Madden et al. 2017) visually by recording the infection percentage on the upper leaf side for up to 350 leaves on each of 100 trees from 2015 to 2018. The presence of *E. hypophylla* was assessed molecularly on 13 trees in 2011 (n = 3 or 4 leaves per tree). Presence of the hyperparasite was molecularly analysed on 189 leaves (from 65 trees) collected in 2011.

To characterize variation in microclimate at the landscape scale, we placed EL-USB-2 Lascar dataloggers (Lascar Electronics, Wiltshire, UK) on 20 oak trees. We focused on ecologically relevant bioclimatic variables by averaging temperature and relative humidity for the growing and non-growing season, separately for each year as well as averaged across the four-year period. To estimate tree-level variation in autumn phenology, we scored leaf discoloration (i.e. the proportion of the leaf that is brown) on ten leaves per tree in autumn 2018.

Molecular identification of the cryptic powdery mildew species and their hyperparasite

Identification of the *Erysiphe* species was done based on polymorphisms in the ITS sequences of the ribosomal DNA, using ITS1-Fungi, a universal forward primer for fungi (Gardes and Bruns 1993) and o-micro-rev reverse primer (Desprez-Loustau et al. 2017, 2018). Sequencing of the amplicons was done with Sanger technology (Genewiz, England). Identification of the hyperparasite was done by amplifying and sequencing a part of the ITS region, from the same DNA extracts used for *Erysiphe* spp. identification. For PCR we used a slightly modified version of the method developed by Tollenaere et al. (2014) (Supporting information). *Ampelomyces* detection was based on the presence of the target sequence of the expected size (approximately 150 base pairs) on agarose gel. In addition, amplicons were sequenced by Sanger technology (Genewiz, England) to genetically characterize *Ampelomyces* strains (Supporting information).

Statistical analysis

For the analyses, we used the framework of generalized linear mixed models. All analyses were conducted in R ver. 3.6.0 (<www.r-project.org>). We fitted models using the functions *glm* and *glmer* in the packages *MASS* and *lme4* (Venables and Ripley 2013, Bates et al. 2015), inspected model fit using diagnostic tools in the package *DHARMa* (Hartig 2020) and tested for significance using the function *Anova* in the *car* package (Fox and Weisberg 2019). Continuous variables were scaled to mean zero and unit variance (Schielzeth 2010). We inspected multicollinearity between model variables by calculating variance inflation factors with the function *corvif* (Zuur et al. 2009). Variance inflation factors were lower than the recommended cut-off value of 5 in all final models, indicating that multicollinearity was not interfering too strongly with model inference (Zuur et al. 2009). We used backward model selection to identify the minimal adequate models, where variables were dropped one by one until all p-values were below 0.05 (Crawley 2007). For details on data used, responses examined and model structures, we refer to Supporting information.

To investigate whether the distribution of the hyperparasite was related to long-term average climatic conditions at the continental scale, we modelled the tree-level presence of *Ampelomyces* as a function of temperature and precipitation during the growing (May–September) and non-growing season (November–March), temperature seasonality and precipitation seasonality (the standard deviation of monthly temperature and precipitation averages) over a 30-year period. To identify whether hyperparasitism differed among powdery mildew species, we included the identity of the powdery mildew species as a fixed factor in the model. To account for differences among oak species in the presence of

the hyperparasite, we included oak species identity as a random effect in the model. We further added Location ID as a random effect to account for non-independence of trees from the same location.

At the national scale, we first examined whether climate, oak autumn phenology and hyperparasite presence explained the distribution of powdery mildew species in Sweden. We took a two-step approach, assuming that the geographical distribution of pathogens is driven by long-term climatic averages, whereas disease incidence of pathogens (conditional on presence at the tree level) is mostly affected by climatic conditions during the current year (as determining infection build-up over the season). First, we modelled the presence of each powdery mildew species at the tree level as a function of the 30-year average temperature and precipitation during the growing and non-growing seasons, temperature seasonality and precipitation seasonality. Second, we modelled disease incidence of each powdery mildew pathogen as a function of temperature and precipitation during the growing and non-growing season, temperature seasonality and precipitation seasonality for the year preceding sample collection. To test whether autumn phenology and *Ampelomyces* explained any additional variation in disease incidence (i.e. beyond the effect of climate on phenology), we tested the significance of autumn phenology and *Ampelomyces* presence when adding each to the minimal adequate model. To investigate whether there was a negative or positive association between the occurrences of the two powdery mildew species, we used a Chi-square goodness of fit test.

To assess the relationship between climate and the distribution of the hyperparasite in Sweden, we modelled the presence of *Ampelomyces* at the tree level as a function of the 30-year average temperature and precipitation during the growing and non-growing season, temperature seasonality and precipitation seasonality. We tested whether *Ampelomyces* presence at the leaf level was linked to climate and two aspects of powdery mildew infection – species identity and disease severity. To do this, we first modelled the presence of *Ampelomyces* at the leaf level as a function of temperature and precipitation during the growing (May–September 2016) and non-growing season (November 2015–March 2016), temperature and precipitation seasonality in 2016 and powdery mildew species identity. In a separate model, we modelled leaf-level presence of *Ampelomyces* as a function of the temperature and precipitation during the growing (May–September 2016) and non-growing season (November 2015–March 2016), temperature and precipitation seasonality in 2016 and percentage of the leaf covered by powdery mildew on the upper and lower leaf surface. In both models, we included Tree ID as a random effect. To investigate whether there was a relationship between *Ampelomyces* strain identity and powdery mildew species we used a chi-square goodness of fit test. We note that in the models at the national scale in Sweden, we tested both whether there was a relationship between hyperparasite presence and powdery mildew disease severity, and whether powdery mildew incidence was related to the presence of the hyperparasite. This two-pronged approach was chosen due to the lack of theoretical and empirical insights into the expected direction of causality.

In France, we tested whether hyperparasite distribution was related to long-term average climatic conditions. We modelled tree-level presence of *Ampelomyces* as a function of the 30-year average temperature and precipitation during the growing and non-growing season, and temperature and precipitation seasonality. Furthermore, we tested whether hyperparasite presence differed between cryptic powdery mildew species, by adding the identity of the powdery mildew species as a fixed effect to the model. We included oak stand ID as a random effect.

At the landscape scale, we first investigated the relationship between tree-level variation in microclimate and autumn phenology and the disease incidence of *E. alphitoides*. For this, we first modelled disease incidence as a function of temperature and relative humidity during the growing and non-growing season, year of data collection and the interaction between year and climatic factors. As the interaction between growing and non-growing season temperature and year was significant, we fitted year-specific models for 2015, 2016, 2017 and 2018. We adjusted p-values to account for multiple comparisons using the Benjamini and Hochberg (BH) method. To identify the relationship between climate and *E. hypophylla* distribution, for which we had data from 2011, we modelled *E. hypophylla* presence as a function of the four-year average temperature and relative humidity during the growing and non-growing season. We tested whether tree autumn phenology explained additional variation in *E. alphitoides* disease incidence in 2018 by adding it to the minimal adequate climatic model.

Results

Ampelomyces was found in all countries surveyed in Europe, with an overall infection frequency of 15% (61/411) of mildew-infected leaves (Fig. 1), with almost all sequences being highly similar to the sequence of the AQ strain (AF035783) (see section Genetic diversity of Ampelomyces species in the Supporting information). We found no significant relationship between climatic predictors and *Ampelomyces* distribution at the continental scale (Fig. 1; Supporting information). *Ampelomyces* presence did not differ among the three powdery mildew species (*E. alphitoides*, *E. hypophylla* and *E. quercicola*) in Europe (Fig. 1; Supporting information).

Within the range of oak in Sweden, *E. alphitoides* and *E. hypophylla* were found on 75% and 91% of the 92 trees, respectively, whereas we did not detect *E. quercicola* (Fig. 2a–b). *Erysiphe alphitoides* was detected on 103 out of 666 leaves, *E. hypophylla* on 376 out of 666 leaves and the mixture of two species (*E. alphitoides* and *E. hypophylla*) on 187 out of 666 mildew-infected leaves (Fig. 2a–c). The two species co-occurred on 28% of the leaves (Fig. 2c), which matches the null expectation based on independent species distributions (i.e. no negative or positive association between the two species; chi-square goodness of fit test: $\chi^2_1 = 0.5$, $p = 0.48$).

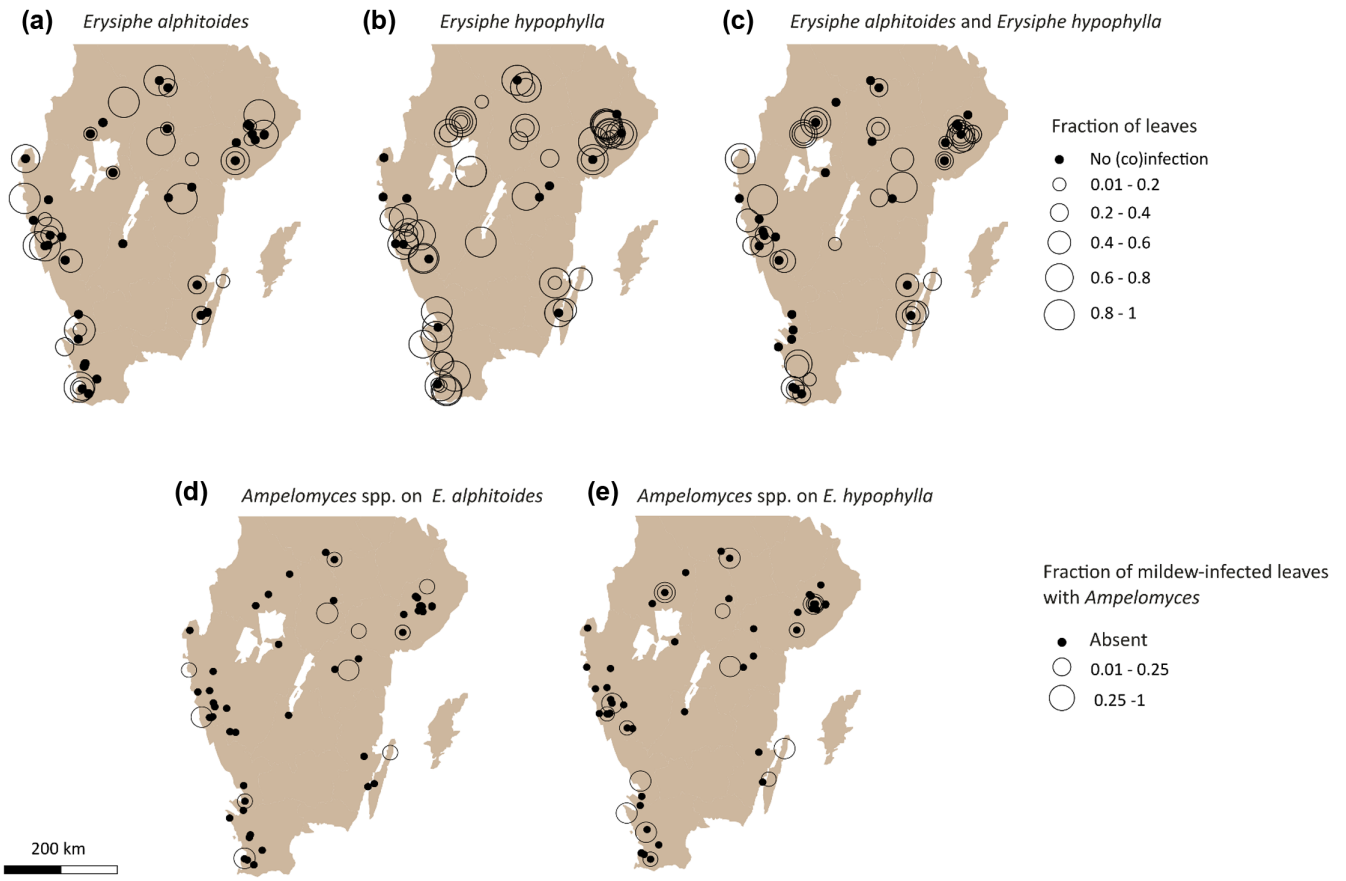


Figure 2. Spatial distribution and co-occurrence of two cryptic powdery mildew species (*Erysiphe alphitoides* and *E. hypophylla*) and *Ampelomyces* spp. across the range of oak in Sweden (n = 92 trees). Panels a–c show the fraction of leaves on each tree infected by (a) *E. alphitoides*, (b) *E. hypophylla* and (c) both *E. alphitoides* and *E. hypophylla*. Panels d and e show the fraction of mildew-infected leaves that are also infected with *Ampelomyces* spp. for (d) *E. alphitoides* and (e) *E. hypophylla*. In panels a–c, circle size scales with the fraction of leaves infected by a single (a and b) or two (c) *Erisyphe* species per tree. In panels d and e, circle size scales with the fraction of mildew-infected leaves showing *Ampelomyces* spp. The small black circles indicate the absence of powdery mildew species from a tree (panels a–c) or the absence of *Ampelomyces* from all mildewed leaves on a tree (panels d and e). Note that the locations of some trees are partially overlapping on the map.

We found no significant relationship between temperature and precipitation and the presence and disease incidence of *E. alphitoides* and *E. hypophylla* (Supporting information). Likewise, tree autumn phenology and hyperparasite presence did not explain powdery mildew disease incidence (Supporting information). In Sweden, *Ampelomyces* was detected on 37% (34 out of 92) of the trees and 15% (102 out of 666) of mildew-infected leaves (Fig. 2d–e). The majority of amplicons yielded a sequence with high homology to AF035783 (i.e. clade 1), but sequences belonging to clades 2, 3 and 4 were also found (Supporting information). We found no significant relationship between hyperparasite presence and temperature or precipitation (Fig. 2d–e and Supporting information). Hyperparasite presence did not differ among the cryptic powdery mildew species and was unaffected by powdery mildew disease severity on the lower or upper leaf surface (Fig. 2d–e and Supporting information). We found no positive or negative association between *Ampelomyces* genetic clade and powdery mildew species identity (chi-square goodness of fit test: $\chi^2_2 = 3.2$, $p = 0.19$).

Within the oak range in France, *Ampelomyces* was found on 12% (48 out of 400) of surveyed seedlings (Fig. 3), with 100% of sequences corresponding to AF035783. There was a significant negative relationship between temperature during the non-growing season and *Ampelomyces* presence (Supporting information). Hyperparasitism did not differ between the two powdery mildew species detected in France, *E. alphitoides* and *E. quercicola* (Supporting information).

At the landscape level, we found a negative relationship between temperature during the non-growing and growing season and disease incidence of *E. alphitoides* in 2016 and 2018, respectively (Supporting information; Fig. 4). In 2015 and 2017, we did not detect an effect of climate variables on *E. alphitoides* disease incidence (Supporting information). We did not detect any significant association between temperature and relative humidity during the growing and non-growing season and the presence of *E. hypophylla* on trees (Supporting information). The timing of leaf senescence did not explain *E. alphitoides* disease incidence in 2018 (Supporting information). *Ampelomyces* was rare within the

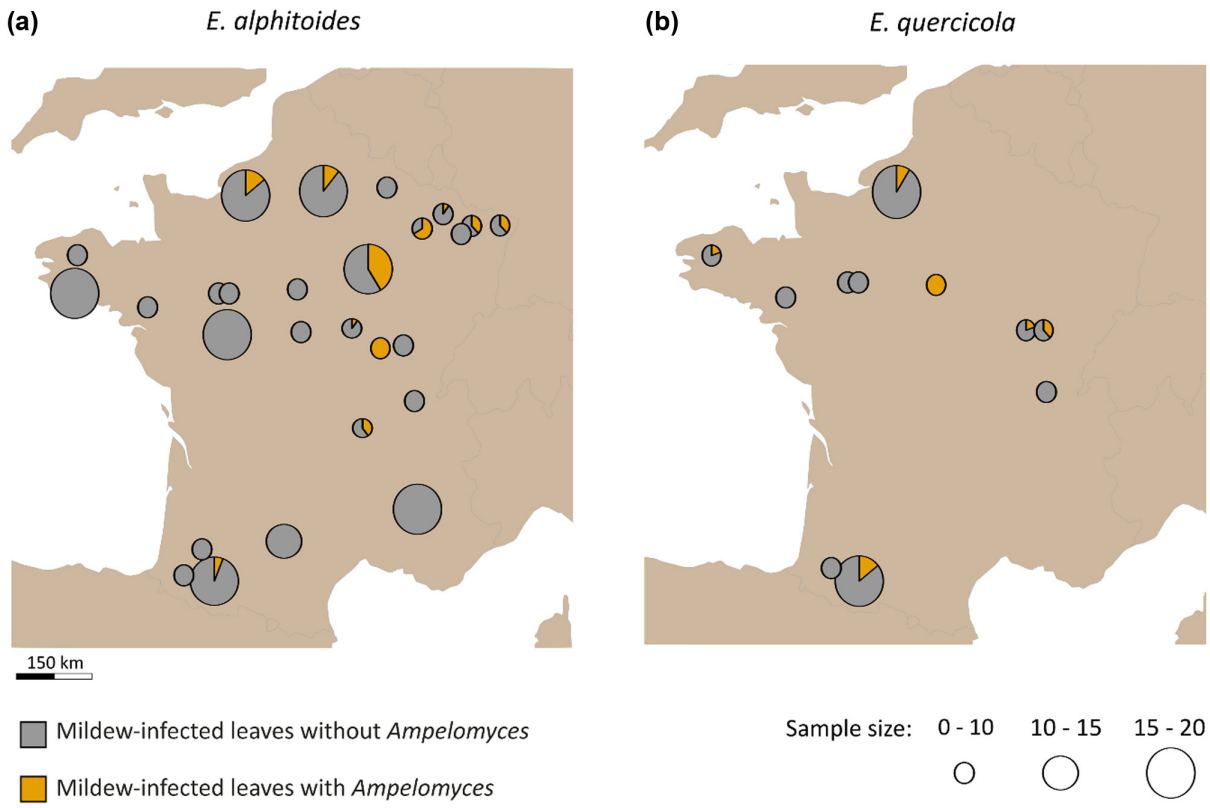


Figure 3. Spatial distribution of *Ampelomyces* spp. on oak powdery mildew (*Erysiphe* spp.) in France, shown separately for (a) *E. alphitoides* and (c) *E. quercicola*. Pie graphs show the fraction of powdery mildew infected leaves with *Ampelomyces* spp. Circle size represent number of seedlings sampled per oak stand across France.

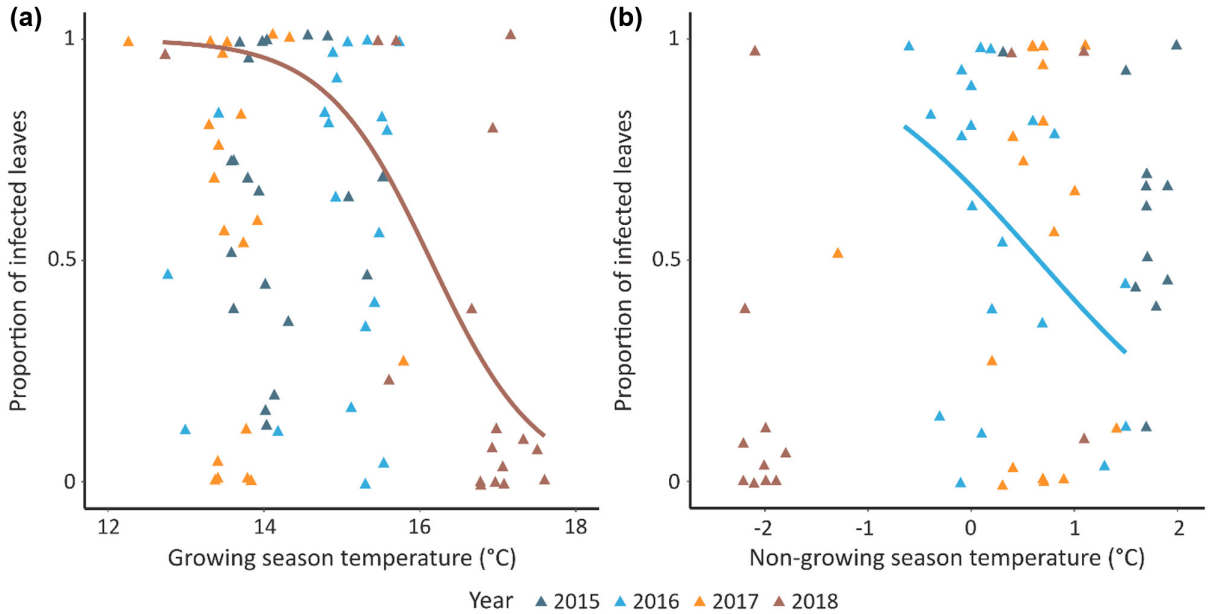


Figure 4. Impact of temperature on *E. alphitoides* disease incidence at the landscape scale in 2015, 2016, 2017 and 2018. Panel a and b show the effect of growing season and non-growing season temperature on *E. alphitoides* disease incidence, respectively. Coloured triangles represent data from individual trees in 2015 (n = 20 trees), 2016 (n = 20), 2017 (n = 20) and 2018 (n = 17). For significant relationships, a trend line is fitted to the data using function *stat_smooth* in *ggplot2* assuming a binomial distribution.

landscape (present on only 3 out of 189 mildew-infected leaves), precluding further analyses.

Discussion

We investigated whether niche differentiation of cryptic species within a pathogen complex on the pedunculate oak might contribute to their co-existence. For this, we focused on species responses to climatic factors, host characteristics (autumn phenology) and hyperparasitism at multiple spatial scales, including the European continent, France, Sweden and a 5 km^2 island in southwestern Finland. In previous studies, cryptic powdery mildew species were shown to differ in their response to climate in Europe (Desprez-Loustau et al. 2018) and France (Marçais et al. 2017). In this study, we demonstrate that in Sweden *E. alphitoides* and *E. hypophylla* were both unaffected by climate, whereas disease incidence of *E. alphitoides*, but not *E. hypophylla*, was negatively affected by temperature in two out of four years at the landscape scale. Tree autumn phenology did not affect cryptic powdery mildew species at the national and landscape scale. Moreover, hyperparasite presence and genetic clade did not differ among cryptic powdery mildew species at the continental and national scales. Overall, these findings and earlier evidence suggest that the climatic dimension is more important than the species interaction dimension for niche differentiation of cryptic powdery mildew species.

Differentiation of the Grinnellian niche of cryptic powdery mildew species

At the continental scale, it was previously shown by Desprez-Loustau et al. (2018) that temperature limited the northern distribution of *E. quercicola* and the southern distribution of *E. hypophylla*, but did not affect the distribution of *E. alphitoides*. In contrast, in our study, we found no significant relationship between temperature or precipitation and the presence and disease incidence of *E. alphitoides* and *E. hypophylla* in Sweden. Furthermore, we found that at the landscape scale, there was a significant negative relationship between temperature and disease incidence of *E. alphitoides*, but not *E. hypophylla*. The negative association between *E. alphitoides* and growing season temperature was only apparent in 2018, when the summer was exceptionally warm and dry in Finland (Hari et al. 2020). Our findings suggest that while there is no significant association between the continental and national macroclimate and the distribution of *E. alphitoides*, this powdery mildew species can be strongly affected by the local microclimate, at least in some years. Such a pattern raises an interesting question: if *E. alphitoides* is not limited by warm temperatures at the southern part of its range, why would it fare worse in a warm year at the northernmost (and coldest) part of its range? We think this might be explained by either adaptation of the pathogen to the prevailing local conditions (Laine 2008, Tack and Laine 2014) or a negative response to plant stress (Velásquez et al. 2018).

Few other studies have focused on the response of cryptic pathogen species to climate, and these have mostly been limited to large spatial scales (Horner et al. 2017, Byrne et al. 2019). For example, Rouxel et al. (2014) demonstrated that three cryptic species (from the clades *riparia*, *quinquefolia* and *vulpina*) within the cryptic pathogen complex *Plasmopara viticola* were limited in their geographical distributions by climate and host distribution, whereas the other two cryptic species (from the clades *vinifera* and *aestivalis*) were widely distributed across North America. In our study, we addressed the niche differentiation of cryptic species at multiple spatial scales. Yet, as the specific methodology and timing differed among scales, we should refrain from direct comparisons of processes across spatial scales. Still, the response of cryptic taxa to climate or species interactions may differ with the spatial scale examined – and whether this is the case should ideally be resolved by future research applying a uniform, nested and hierarchical design.

Differentiation of the Eltonian niche of cryptic powdery mildew species

Interactions with both lower and higher trophic levels can have a profound impact on species niches (Fodor 2011, Arribas et al. 2018). We found that variation in tree autumn phenology did not affect either of the powdery mildew species, indicating that the timing of leaf senescence is not important for the successful production and overwintering of chasmothecia. The absence of an effect of autumn leaf phenology on these parasitic fungi differs from that reported for insect parasites: Ekholm et al. (2019) found that the presence and abundance of several insect gallers and leaf miners on oak were explained by oak autumn phenology. While we lack comparable studies on the relationship between autumn phenology and disease levels, we speculate that the relatively weak response of fungal pathogens to variation in host autumn phenology as compared to that of insect herbivores might be a general pattern in nature, and hope that future studies will set out to validate or refute this hypothesis.

As for the host, we found no evidence that the natural enemy played a role in niche differentiation of the cryptic powdery mildew species: there was no imprint of the hyperparasite on the distribution of the cryptic powdery mildew species, and hyperparasite presence and identity did not depend on powdery mildew species. We lack comparable data on the relationship between hyperparasitism and cryptic species, but some studies have looked at the degree of specialization of *Ampelomyces* on powdery mildew strains (Kiss et al. 2004, Sucharzewska et al. 2011, Tollenaere et al. 2014). These studies have reached different conclusions. Cross-inoculation experiments have shown that *Ampelomyces* collected from a single powdery mildew species can infect other mildew species, indicating that *Ampelomyces* largely lacks host specificity (Kiss et al. 2004, 2011, Pintye et al. 2012, 2015). In contrast, Parratt et al. (2017) found local adaptation of *Ampelomyces* to individual strains of the powdery mildew species *Podosphaera plantaginis*. In agreement with previous studies, our results of

no imprint of hyperparasite identity on powdery mildew species can be explained by low host specificity of *Ampelomyces* at the clade level.

Distribution of the hyperparasite

Ampelomyces was present at all scales investigated, with an average infection frequency of 11%, even though it was very rare in the landscape in southwestern Finland. Interestingly, this finding is in line with Parratt et al. (2017), who demonstrated that the hyperparasite was reasonably common at the metapopulation-level, but was rare when zooming in to the level of individual plants. The previous visual assessments by Kiss (1998; 3 out of 14 leaves) and Topalidou and Shaw (2016; 70 out of 250 leaves) also confirm our findings, suggesting that *Ampelomyces* is a common hyperparasite of oak powdery mildew. Our findings also match the historic records, as the first infection by *Ampelomyces* (synonym *Cicinnobolus cesatii*) was already reported a few years after the discovery of oak powdery mildew in Europe (Vuillemin 1910, Kiss et al. 2004). *Ampelomyces* identified in our study were assigned to four different genetic clades, although the AQ10 type (i.e. clade 1) was the most prevalent (Kiss 1998, Park et al. 2010, Kiss et al. 2011).

We found no significant relationship between climatic factors and the distribution of the hyperparasite at the continental scale or within Sweden, while in France we detected a significant negative relationship between non-growing season temperature and *Ampelomyces* distribution. One plausible explanation might be that hyperparasite presence is related to the distribution of its host (e.g. *E. alphitoides*), which is most abundant in northeastern France, in areas with cold winters. In a similar vein, we found no significant association between powdery mildew species identity and hyperparasite presence at continental and national scales. Interestingly, these results conform to the Eltonian noise hypothesis, which predicts that biotic interactions might play a relatively minor role compared to that of abiotic forces in governing species distributions at large spatial scales (Soberón and Nakamura 2009, de Araújo et al. 2014, Álvarez-Mendizábal et al. 2021, Ashraf et al. 2021, Fecchio et al. 2021). As an alternative explanation, the weak and inconsistent effect of climate variables and powdery mildew species identity on the hyperparasite might be explained by the presence of multiple strains of *Ampelomyces* within each of the clades. Future research might extend the current study upwards in the food chain and use molecular tools to unravel niche differentiation along the climatic and species interaction axes within the cryptic hyperparasite complex.

Conclusions

While cryptic pathogen complexes are common, it remains a conundrum how cryptic species within such complexes can co-exist. Have they evolved different niches? Here, we extended previous work to show the role of climate in differentiating the niches of cryptic powdery mildew species and that climate may influence the distribution of the hyperparasite. In contrast, the powdery mildew species studied were all similarly unaffected by autumn phenology and hyperparasitism, suggesting that these factors did not contribute to niche differentiation among the species. As only few comparable studies exist that examined niche differentiation within a cryptic pathogen complex at multiple spatial scales, it is hard to draw conclusions about the generality of our findings. Clearly, both the Grinnellian and Eltonian niche are highly complex, and other drivers not investigated in our study may be important for cryptic powdery mildew species. Importantly, given that the response of pathogens to abiotic and biotic drivers might differ depending on the spatial scale studied, there is a need for studies using nested, hierarchical designs to explore the drivers and niches of cryptic pathogen species and hyperparasites across spatial scales. The deeper understanding of the multi-scale distribution and climatic drivers of cryptic species within the same pathogen complex is essential for making predictions about the spread of plant diseases under climate warming.

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.02v6wwq4p> (Faticov et al. 2021).

Supporting information

Any supporting information associated with this article is available from the online version.

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