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Research article

Climatic variability, spatial heterogeneity and the presence of multiple hosts drive the population structure of the pathogen *Phytophthora ramorum* and the epidemiology of Sudden Oak Death

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We implement a population genetics approach to clarify the role that temporal and environmental variability, spatially distinct locations and different hosts may have in the epidemiology of a plant disease and in the microevolution of its causative pathogen. In California and southern Oregon (USA), the introduction of the invasive pathogen *Phytophthora ramorum*, causal agent of the widespread disease Sudden Oak Death (SOD), has resulted in extensive mortality of various oaks *Quercus* sp. and of tanoak *Notholithocarpus densiflorus*. Although the disease can infect over a hundred hosts, California bay laurel *Umbellularia californica* is the most competent transmissive host but is not lethally affected by the disease. Using population genetics data, we identify the relationship among *P. ramorum* populations in bay laurels, oaks and tanoaks to clarify the contribution of each host on the epidemiology of SOD and on the microevolution of its causal agent and to explore differences in population structure across sites and years. We conclude that bay laurel is the primary source for infections of both tanoak and oak, and that tanoak contributes minimally to oak infection but can infect bay laurel, creating a secondary pathogen amplification process. Overall, pathogen diversity is associated with rainfall and presence of bay laurels, which sustain the largest populations of the pathogen. Additionally, we clarify that while bay laurels are a common source of inoculum, oaks and tanoaks act as sinks that maintain host-specific pathogen genotypes not observed in bay laurel populations. Finally, we conclude that different sites support a dominance of different pathogen genotypes. Some genotypes were widespread, while others were limited to a subset of the plots. Sites with higher bay laurel densities sustained a higher genotypic diversity of the pathogen. This work provides novel insight into the ecology and evolutionary trajectories of SOD epidemics in natural ecosystems.

Keywords: competence, disease transmission, host-mediated evolution, landscape heterogeneity, multi-host disease, population structure

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Introduction

Multihost pathosystems have been extensively studied to better our understanding of many human, animal and plant diseases (Barrett et al. 2009, Johnson and Thieltges 2010, Ostfeld and Keesing 2012). Differences in the epidemiological role of different hosts have been shown to modify not only the course of animal and human diseases (Civitello et al. 2015), but also the structure and composition of host populations (Johnson et al. 2008) and the evolutionary trajectories of pathogens themselves (Levin 1996). The similarities between animal and plant pathosystems are many, however, when studying plant diseases, a landscape-scale component needs to be additionally integrated into the study of multihost plant diseases, given that plants are sessile (Plantagenest et al. 2007). Many studies have investigated the various roles that each of multiple hosts may have on disease progression (Simpson et al. 2012, Johnson et al. 2013, Rosenthal et al. 2021b), on pathogen persistence in the absence of primary hosts (LoGiudice et al. 2003) and on pathogen evolution (Betts et al. 2016), especially when hosts have clearly different competence. Here, we present a study focused on the multihost plant disease Sudden Oak Death (SOD) in California, with each of the three hosts studied being characterized by strikingly different competence and morbidity and by a variable distribution across the landscape.

SOD caused by the nonnative pathogen *Phytophthora ramorum* (Stramenopila, Peronosporales) (Werres et al. 2001, Rizzo et al. 2002) is an introduced forest disease (Rizzo and Garbelotto 2003) that has recently become endemic in coastal northern California and southwestern Oregon (Garbelotto et al. 2020). The pathogen was introduced to California in the 1980s or 1990s via infected nursery stock (Croucher et al. 2013). To date, only four clonal lineages have been detected worldwide (Ivors et al. 2006, Van Poucke et al. 2012) outside the region in Asia where it is genetically diverse and possibly native (Jung et al. 2021). However, only two lineages, named NA1and EU1, have been detected in forest populations in California (Ivors et al. 2006, Garbelotto et al. 2021), while three lineages (NA1, NA2 and EU1) are currently present in Oregon forests (Grünwald et al. 2016, LeBoldus et al. 2022, Peterson et al. 2022).

Phytophthora ramorum causes high levels of mortality in California forest settings, mostly of coast live oak *Quercus agrifolia* and of tanoak *Notholithocarpus densiflorus* Bole infections of oaks and tanoaks cause lesions that destroy the cambium and the outer vascular tissue and eventually kill the hosts by girdling them, however these infections themselves are generally not transmissive (Garbelotto and Hayden 2012). Conversely, the primary infectious hosts and most commonly infected trees in California forests are California bay laurels *Umbellularia californica* and tanoaks (Garbelotto et al. 2003, Davidson et al. 2005). Bay laurels and tanoaks, in addition to over 100 other (minor) species, serve as foliar hosts for the pathogen (Anonymous, USDA APHIS 2008), meaning that production of infectious sporangia occurs on the leaves of these hosts. Foliar and petiole infections cause localized tissue necrosis and branch die back in some hosts, but the plants themselves are not killed by the pathogen, if not when infections occur multiple times in the course of multiple years on the same plant (Garbelotto and Hayden 2012). Bay laurels and tanoaks are not equally competent. A few studies (Garbelotto et al. 2017, Kozanitas et al. 2022) have indicated that there is a strong relationship between disease prevalence on bay laurels and oak infection, while tanoak may not support the levels of sporulation observed on bay laurel (Garbelotto et al. 2017, Rosenthal et al. 2021a). Nonetheless, infected tanoak leaves are able to infect themselves and their neighbors, causing both bole cankers and petiole lesions (Davidson et al. 2005, Cobb et al. 2012, Garbelotto et al. 2017).

Sporulation on foliar hosts is triggered by environmental cues and increases markedly in late spring, when conditions are both warm and wet (Davidson et al. 2005, Dodd et al. 2008, Hüberli et al. 2011). Several studies have shown a strong positive correlation between rainfall amounts, disease incidence and sporulation levels (Kozanitas et al. 2017, 2022, Lione et al. 2017, Garbelotto et al. 2020). Furthermore, Garbelotto et al. (2017) and Kozanitas et al. (2022) have shown that new oak infections only occur in years with extremely high rainfall levels. Oak infection, in fact, requires very high levels of inoculum (Garbelotto et al. 2017) and is thus mediated by the presence of high precipitation levels and by the presence of favorable climatic conditions, resulting in high sporulation by the pathogen. For these reasons, studies focusing on understanding the epidemiology of SOD should be multi-year and include both dry and wet years.

Not only do transmission rates vary by year and host species, but studies at the landscape level have identified great variability in disease incidence among sites and even in infection status among trees in the same site. Sites characterized by high bay laurel density, lower bay laurel basal area and with an easterly aspect are possible hotspots that include multiple superspreader trees and refugial trees, i.e. trees where the pathogen survives during prolonged unfavorable climatic conditions, such as drought or extreme heat (Kozanitas et al. 2022). However, to date, no study has looked at the landscape-level spatial distribution of individual pathogen genotypes in California forests or has determined which sites may harbor the greatest genetic diversity of the pathogen, an aspect that is of great importance for the continued adaptation process of an exotic organism (Sakai et al. 2001, Pérez et al. 2006).

Based on population genetics studies in California mixed oak woodlands dominated by bay laurels (Eyre et al. 2013, Eyre and Garbelotto 2015), we do know that the epidemiology of SOD is driven by pathogen populations on bay laurel leaves, where, during favorable climatic conditions, a few pathogen genotypes produce the infectious inoculum that is responsible for most plant infections (Eyre et al. 2013) and for the inoculation of soil and water (Eyre and Garbelotto 2015). Soil and water generally act as dead-end-sinks in which selection pressure is laxed and where genotypes that are not detectable on leaves may be detected (Eyre et al. 2013, Eyre and Garbelotto 2015). This differential niche-driven

selection pressure results in the survival of different genotypes in the three different substrates, with soil and water populations experiencing an annual turnover of genotypes, while the most abundant genotypes on bay laurel persist in time. A similar process occurs in dead-end hosts such as oaks and tanoak boles, where the chemical environment of the wood and bark triggers a series of chromosomal variations resulting in the duplication, deletion and translocation of chromosomal blocks, with significant changes in genome structure and phenotype (Kasuga et al. 2012, 2016).

In summary, while competent hosts lead to the emergence of new genotypes because they are responsible for the demographic growth of the pathogen, dead-end hosts and substrates allow for the insurgence or survival, respectively, of pathogen genotypes that are normally undetected in the pathogen population on competent hosts. Thus, it is possible that genetic diversity of the pathogen may increase when competent and non-competent hosts are sympatric.

Here, for the first time, we employ a population genetics approach to identify the relationship among *P. ramorum* populations in bay laurels, oaks and tanoaks in multiple sites across a watershed in a four year period, in order to clarify the contribution that host, site and weather may have on the epidemiology of SOD and on the microevolution of its causal agent. First, we ask whether sites that are conducive to disease spread may harbor more diverse pathogen populations, and whether that diversity may be generated on competent bay laurel leaves during wet springs and may be stable through years because of the dominance of fit genotypes. Second, we ask whether bay laurels may be the source of infection for both tanoaks and oaks, whether infection from tanoaks to other hosts may be epidemiologically significant, and whether genetic evidence may be gathered regarding the presumed epidemiological dead-end nature of oaks. Third, we want to investigate whether the presence of multiple hosts in a variable environment may increase the genetic diversity of the pathogen and may be associated with the presence of a number of unique genotypes that, although less widespread than the most infectious ones, may provide the basis for future selection processes.

Material and methods

Field site selection/habitat description

The field portion of this study was conducted within an existing plot network, established in 2008 (Kozanitas et al. 2017, 2022) in the San Francisco Public Utility Commission (SFPUC) watershed in central San Mateo County, California (37°31′10.3″N, 122°22′08.2″W). A total of 15 research plots were monitored repeatedly from 2008 to 2012, during pre-selected times of the year. Plots could be divided in two groups based on the density of bay laurels. Plots 0, 1, 2, 3, 5, 7, 11, 12, 16 were characterized by lower density of bay laurels and lack of tanoaks. Plots 6, 8, 9, 10, 14, 15 were all characterized by higher bay laurel density and by the occasional presence of tanoaks (Supporting information) (Kozanitas et al. 2022). The 9300 ha watershed ranges in elevation between 95 and 1050 m, and plots were located in one of two major drainages, either the Pilarcitos or the Crystal Springs drainage, with the Pilarcitos drainage being on average higher in elevation than the Crystal Springs drainage. Each plot contained three transects, 100 m long and 10 m wide, radiating from a center point. A bay laurel stem or a tanoak stem (if present) was selected and tagged for repeated surveying at 10 m increments along each transect. A stem was defined as any major branch of a tree separated from the main stem below breast height (1.4 m) with a diameter at breast height (DBH) greater than 1 cm. All oak stems along each transect were tagged and monitored once per year for five years. At three plots per drainage (total of six) three additional transects were added. Additionally, circles 30 m in diameter and with an area of 707 m^2 were also added around the six plot centers: all host species within the circles were tagged for a more robust surveying schematic. All plots were located a minimum of 2 km apart to avoid spatial autocorrelation between sites. This distance was based on a priori knowledge of infectious propagule mobility stating airborne inocula typically travel either locally (between 10 and 100 m) in rain events or long distance (up to 1 km) in high wind events (Mascheretti et al. 2008). Rainfall data were retrieved from the CA Department of Water Resources (CA-DWR; RAWS database, Western Regional Climate Center, https://raws.dri.edu) and SFPUC data archives of the Crystal Springs Cottage rain gauge (37°28′08.4″N, 122°19′44.4″W).

Sampling schematic

Three times per year, for three years, all bay laurels and tanoaks along the transects and within the circles surrounding the plot centers were surveyed for visible symptoms of *P. ramorum* infection and were sampled if deemed symptomatic. Surveys were conducted three times per year in order to determine the variation of isolation success and therefore pathogen viability in different seasons. Surveys took place in the late spring when sporulation and transmission is highest including transmission from foliar hosts (bay laurel) to dead end hosts (coast live oak); in the autumn, when pathogen viability is at its lowest after its dormant phase over the hot dry summer; and in the early winter when activity levels are intermediate (Eyre et al. 2013, Johnston et al. 2016, Kozanitas et al. 2022). A bay laurel leaf was considered to be 'symptomatic' if it displayed the dark pixilated spots along the outer margin or lesions at the tip of the leaf, characteristic of infection by *P. ramorum*. A tanoak leaf was deemed symptomatic if necrotic tissue extended up the midrib of the leaf onto the petiole (Garbelotto et al. 2002, Davidson et al. 2003). All coast live oaks within 5 m from a transect were part of the study and were assessed for symptoms once per year, in the Fall, over a four-year period. If bleeding bole cankers were present, the outer layer of the bark was removed and the margin of the infected cambial tissue excised and embedded directly into selective media. In the final year of the study (2012), only

isolates from oaks were genotyped and included in this study. Given that it may take up to a year for symptoms of SOD to manifest themselves in oaks, this additional sampling was performed in order to capture any new infections on oak that may have potentially occurred during the peak transmissive season of the previous year.

Sample processing

All sampled leaves from bay laurel and tanoak were processed within 72 h of collection. Processing involved excising and embedding the advancing margin of a leaf or petiole lesion into the *Phytophthora*-selective media PARP (Erwin and Ribeiro 1996). Plates were then incubated in the dark at 20°C for up to seven days or until mycelial growth was observed and hyphae were subcultured onto clean plates. Oak samples, which were plated in the field, were subcultured onto clean PARP as soon as any mycelial growth was visible. All samples exhibiting mycelial growth were then scored under the microscope as *P. ramorum*-positive or -negative using distinguishable morphological features. Once mycelial isolates were large enough, they were inoculated into 12% pea broth liquid (Eyre et al. 2013) and grown for seven days in six well culture plates at room temperature. Isolates were then transferred into 2 ml screw cap tubes, lyophilized and amalgamated using 5 mm glass beads. DNA was extracted using a NaOH extraction method (Eyre et al. 2013).

Genotyping

Six sets of primers were used on the extracted *P. ramorum* DNA to amplify the following 10 microsatellite loci known to be variable in the NA1 lineage; Ms18 and Ms 64 (Ivors et al. 2006), Ms39a, Ms39b, Ms43b, Ms43b, Ms45 (Prospero et al. 2007) and MsILVO145a, MsILVO145b, MsILVO145c (Vercauteren et al. 2010). PCR reactions were carried out as described by Eyre et al. (2013) and the thermal cycling program for each primer was set by following the varying protocols outlined in Ivors et al. (2006), Prospero et al. (2007), Mascheretti et al. (2008) and Vercauteren et al. (2010). Fragment analysis was performed with a 3730 ABI Sequencer using a LIZ 500 size standard (Applied Biosystems). Fragment sizes were scored using Peakscanner ver. 1.0 (ABI Biosystems) and were then converted to the appropriate number of microsatellite motif repeats for analysis. Each isolate was assigned a multilocus genotype (MLG) isolate using the Poppr package in R ver. 3.4.0 (www.r-project.org) for genetic analysis of populations with clonal reproduction (Kamvar et al. 2014).

Statistical analysis

All statistical analyses, with the exception of the coalescent analysis, were conducted in R ver. 3.4.0 (www.r-project.org). Proportions of MLG abundance per plot were calculated and plotted using the 'viridisLite' package in R. Expected genotypic richness eMLG was calculated using rarefaction and 999 permutations to account for unequal sample sizes and to visualize genotype accumulations curves by host species, using the package 'vegan' in R. Two metroplots were constructed in R, one to visualize the distribution of each unique MLG across sampling year and another to visualize the proportion of MLG abundance per plot as well as number of samples per MLG in each plot.

In order to view potentially existing clusters of MLGs, a discriminant analysis of principal components (DPAC), i.e. a cartesian method that uses linear combinations of alleles to infer the structure of putative populations, was implemented using the R package 'adegenet'. The DAPC is derived from a principal component analysis (PCA), and it differs from PCA in that PCA creates combinations of alleles that describe variation within the analysis, while DAPC creates combinations of alleles that seek to maximize differences among a priori assigned populations. The DAPC was executed by retaining 37 principal components that yielded two discriminant axes.

The following diversity indices were calculated using the 'Poppr' package for R. First, Nei's Gst values were calculated from pairwise comparisons of the three host species, weighted by sample size to accommodate the unbalanced number of samples from each host. Nei's Gst was then calculated by year, regardless of host. In order to study the level of genetic differentiation among plots, the Fst among plots, regardless of host or year, was also calculated. Additionally, the Shannon–Weiner diversity index (H), the Stoddard and Taylor's index (G), the Simpson's index (lambda), evenness (E_5), and Nei's gene diversity (Hexp) were calculated for the three populations of *P. ramorum* isolated from each host and then again using the populations of *P. ramorum* isolated in each year of the study regardless of host. Finally, we identified any private alleles, i.e. alleles that are found only in a single host population, by year.

To test the directional rate of *P. ramorum* migration among tanoak (*Node*), coast live oak (*Quag*) and bay laurel (*Umca*), coalescent analyses were performed using the software MIGRATE-N ver. 3.7.2 (Beerli 2009). First, the length of both flanking regions of each microsatellite was subtracted from the total size of amplicons, and the resulting lengths were transformed into number of repeats, then a Bayesian inference method (Beerli 2006) was used to estimate direction and migration rates between the three host types. It has been shown that most infection and particularly cross-host infection in mixed oak woodlands occurs during the spring (Hüberli et al. 2011, Eyre et al. 2013, Garbelotto et al. 2017), therefore only genotypes isolated from bay laurel in the spring sampling events were used for this analysis. The run was executed excluding a priori the routes (Beerli et al. 2019) from oak to tanoak and from oak to bay laurel, given the dead-end epidemiological status of oaks (Garbelotto and Hayden 2012). The Brownian motion microsatellite evolution model with an assumption of a constant mutation rate for all loci, a burn in of 10 000, and a static heating scheme with four chains were used in the analysis. Genic migration (M) was estimated as the immigration rate *m* divided by the

mutation rate *l*. For Theta and M, prior distribution parameters were assumed to be uniform: ranging between 0 and 0.1 (mean 0.05, Delta 0.01, Bins 200) for Theta, and between 0 and 1000 (mean 500, Delta 100, Bins 200) for M.

Results

A total of 491 bay laurel trees were surveyed nine times for a total of 4419 trees: 3972 trees were found to be symptomatic and were sampled, resulting in 1320 isolates. A total of 952 oaks were surveyed four times, with 251 being symptomatic and sampled, yielding 138 isolates. A total of 45 tanoaks were surveyed nine times, with 214 sampled yielding 71 isolates. Cumulatively, a total of 1529 isolates from all three host types yielded 237 unique MLGs (Supporting information). The breakdown of MLGs per host is as follows: 71 isolates from tanoak were represented by 18 MLGs, 138 isolates from coast live oak were represented by 60 MLGs and 1320 isolates from bay laurel were represented by 205 MLGs. Of these, 169 MLGs were detected on bay laurel only, 28 MLGs on oak only and 4 MLGs on tanoak only. The number of MLGs detected on combinations of hosts is as follows: 22 MLGs on both bay laurel and oak, Four on bay laurel and tanoak, 0 MLGs on both oak and tanoak and 10 MLGs were found on all three hosts (Fig. 1). No comparisons were made between these MLGs and MLGs obtained in other studies, due to allelic differences that can arise when analyses are performed separately and years apart.

Genotypic richness per host species was assessed using rarefaction and determined that the sampling effort of bay laurel populations was sufficient to get a reasonable estimate of unique MLG abundance per host species (Fig. 2). The rarefaction curves for oak and tanoak populations did not indicate sample saturation levels had been met, however at sample size = 71, i.e. a sample size reached for each of the three host species, MLG diversity was markedly lower in tanoak than in the two other hosts. Additionally, MLG diversity was comparable when comparing oaks and bay laurels.

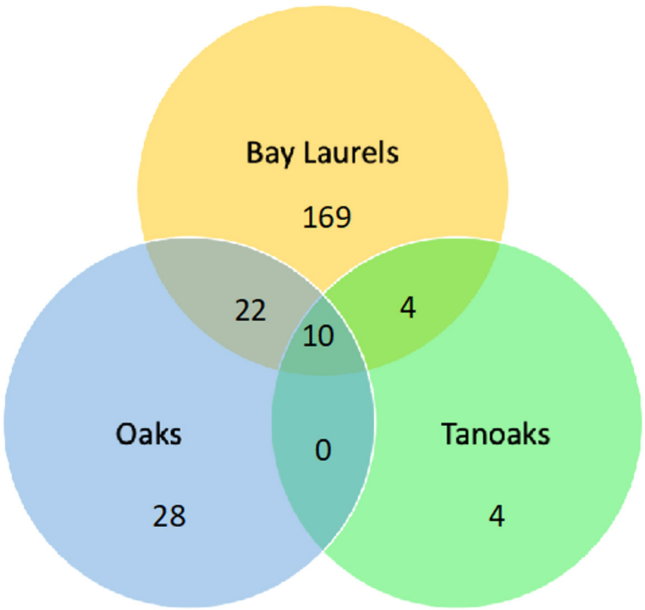

Figure 1. A Venn diagram illustrating the distribution of multilocus genotypes (MLGs) of *Phytophthora ramorum* by host species.

The two metroplots constructed to help visualize the abundance of MLGs both per year and per plot showed the distribution of MLGs spatially and temporally. The first illustrated the pattern through time, and indicated that the most abundant MLGs persist and were detected in each year of the study (Fig. 3). The number of singletons was highest in 2011, the year with the highest isolation success (Table 3b). Three main groups of MLGs emerged when considering abundance through time: those that are both abundant and persistent (e.g. MLG 299), those that are not abundant yet persist (e.g. MLG 220), and those that are neither abundant nor persistent study (e.g. MLG 233) (Fig. 3–4). When considering the abundance of MLGs spatially, at the plot level in this case, it became apparent that different plots had different most abundant MLGs and that different plots were not equal in terms number of MLGs detected (Fig. 4). It is also interesting that MLGs that were widespread, each tended to be most abundant in a different plot (Fig. 4). Plots could be divided in two groups based on the number of MLGs. One group (plots 0, 1, 2, 3, 7, 11, 12, 16) was characterized by lower density of bay laurels, lack of tanoaks and on average contained a smaller number of MLGs, mostly the ones that were most common. The second group (plots 6, 8, 9, 10, 14, 15), characterized by higher bay laurel density and by the presence of tanoaks, contained a much larger number of MLGs. Plot 5 had too few isolates to be included in the analyses. Site pairwise F_{st} values were variable depending on the pair of sites that was compared, however, 98% of pairwise comparisons had a Fst < 0.06, suggesting very limited genetic structure. Only 2% of comparisons had a $F_{st} > 0.06$ suggestive of moderate isolation and of presence of limited genetic structure.

Isolation success from bay laurel varied greatly depending on season and year. The sizes of samples that yielded live cultures were uneven both throughout and across years. Tanoaks and oaks also had unequal sampling sizes as many infected individuals died, and uninfected individuals became symptomatic over the course of the study. Over the three years in which all three host species were sampled (excluding 2012) isolation success was highest in the wettest year (2011, 726 isolates), intermediate in the year with moderate rainfall (2010, 528 isolates) and lowest in the driest year of the study (2009, 240 isolates).

The distribution of the ten most commonly occurring MLGs in each host is shown in Table 1. The most common MLG overall (MLG 213) was also the most common MLG in both bay laurel and oak populations. However, MLG 213 was not the most common in the tanoak population, having been isolated only three times from that host. The most common MLG in tanoak (MLG 167) was the third most common MLG overall, and only the 7th most common in bay laurel. The top two MLGs found in the tanoak population

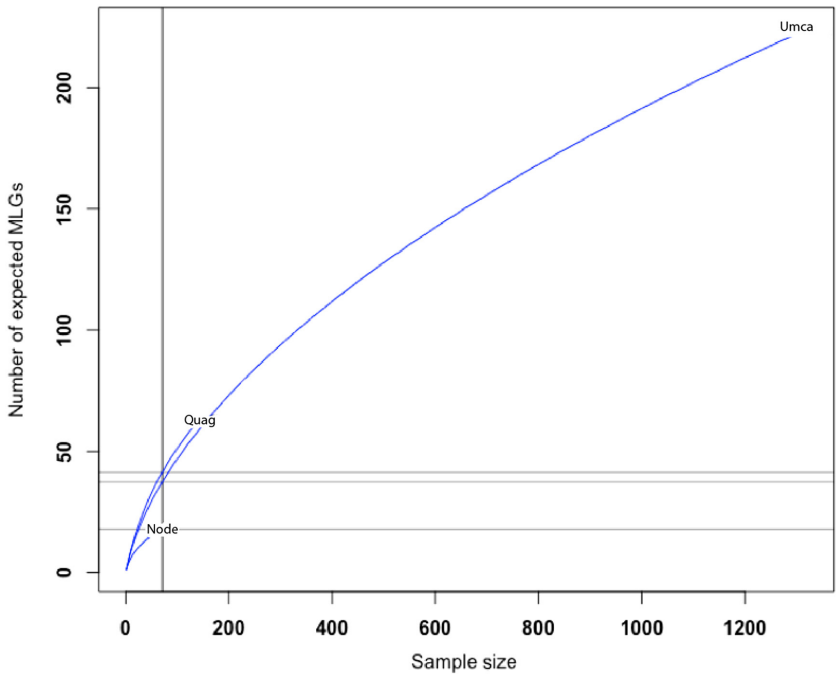

Figure 2. A rarefaction curve to visualize genotypic richness and to determine whether sample size was sufficient in order to get a reasonable estimate of unique multilocus genotype (MLG) abundance per host species. The three host species are tanoak *Notholithocarpus densiflorus* (*Node*), coast live oak *Quercus agrifolia* (*Quag*) and California bay laurel *Umbellularia californica* (*Umca*).

MLG 167 and MLG 236 showed very low representation in the oak population (five and one instances respectively), while the top two MLGs in oak (MLG 213 and MLG 153) were either barely observed or not detected at all in tanoak (three and zero instances respectively). No MLGs were shared exclusively by oaks and tanoaks.

Results from the DAPC showed overlap between bay laurel and oak populations, as well as between bay laurel and tanoak populations, while oak and tanoak populations showed a significant area that did not overlap between the two (Fig. 4). This pattern indicates that these populations are connected to one another, but that the oak and tanoak populations are divergent. The genetic distance (G_{st}) between the three populations isolated from each host species was calculated and the interspecific pairwise measures of differentiation ranged from 0.0012 between coast live oak and bay laurel to 0.02 between coast live oak and tanoak (Table 2a). While these low G_{st} values indicated little to no degree of differentiation among genotypes of *P. ramorum* in various host taxa, the pairwise G_{st} between oak and tanoak at 0.02 was one order of magnitude higher than any other pairwise comparison between hosts. Pairwise comparisons among years were also conducted but showed little to no difference between populations collected in different years (Table 2b). The evenness of genotypes in the studied populations ranged from 0.4 to 0.7 along a scale of 0–1, with one being the most even. The oak population was the most even with a score of 0.7 (Table 3a). While there was not differentiation in the yearly population with regard to genetic distance, there were differences in evenness among years, with values ranging from 0.42 to 0.86 (Table 3b). The highest value of 0.86 in 2012 is only reflective of isolates from the oak population as bay laurel and tanoak isolates were not collected in 2012, indicating another very even population of oak. The values for years 2009–2011 however include all three host populations. The most even population was detected in 2009, the driest year of the study, with an E_5 value of 0.57. The least even population was detected in 2011, the wettest year of the study, with an E_5 value of 0.42. The population from 2010, with reported rainfall levels slightly lower than that of 2012, had an intermediate E_5 value of 0.49 (Table 3). The Stoddard and Taylor index or G value in this situation is reflective of the number of MLGs analyzed taking sample size into account (Table 3). An important pattern emerged, associating rainfall levels, the measure of evenness and the abundance of MLGs detected in a year. With an increase in rainfall, there is an increase in the number of MLGs detected regardless of host, and a decrease in the evenness of the population, as some MLGs become dominant.

A search for private alleles unique to a particular host species found that bay laurel exhibited the highest number of such alleles, although it should be noted that it was also the host taxon with the greatest sample size and is represented by the most MLGs (Table 4a). Oak and tanoak populations had much fewer private alleles than bay laurel. When looking at the number of private alleles in the yearly populations, regardless of host species, the most private alleles were detected in 2011, the wettest year of the study and the year with the highest number of MLGs (Table 4b).

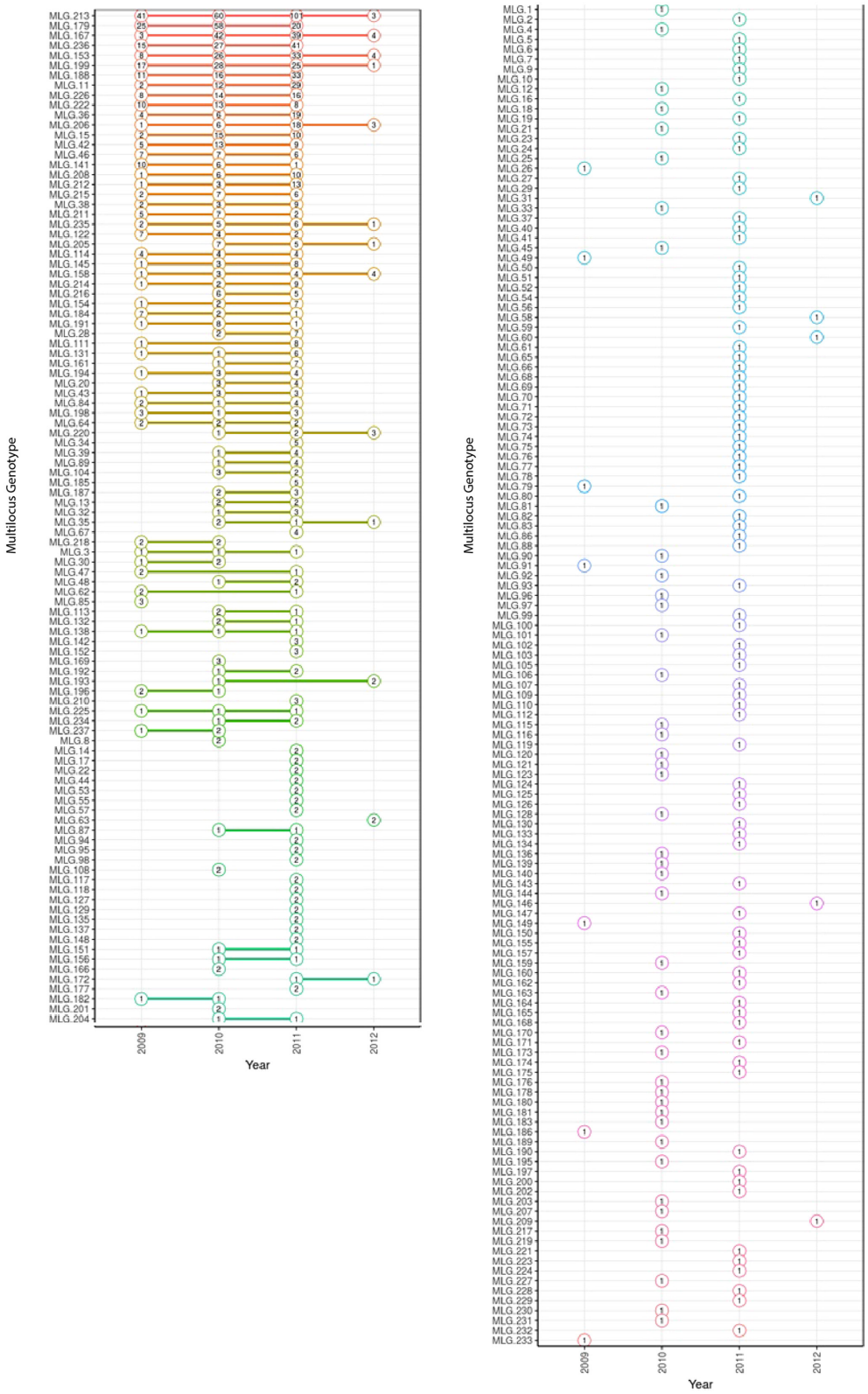

Figure 3. A Metroplot showing the abundance and persistence of each multilocus genotype (MLG) detected per year.

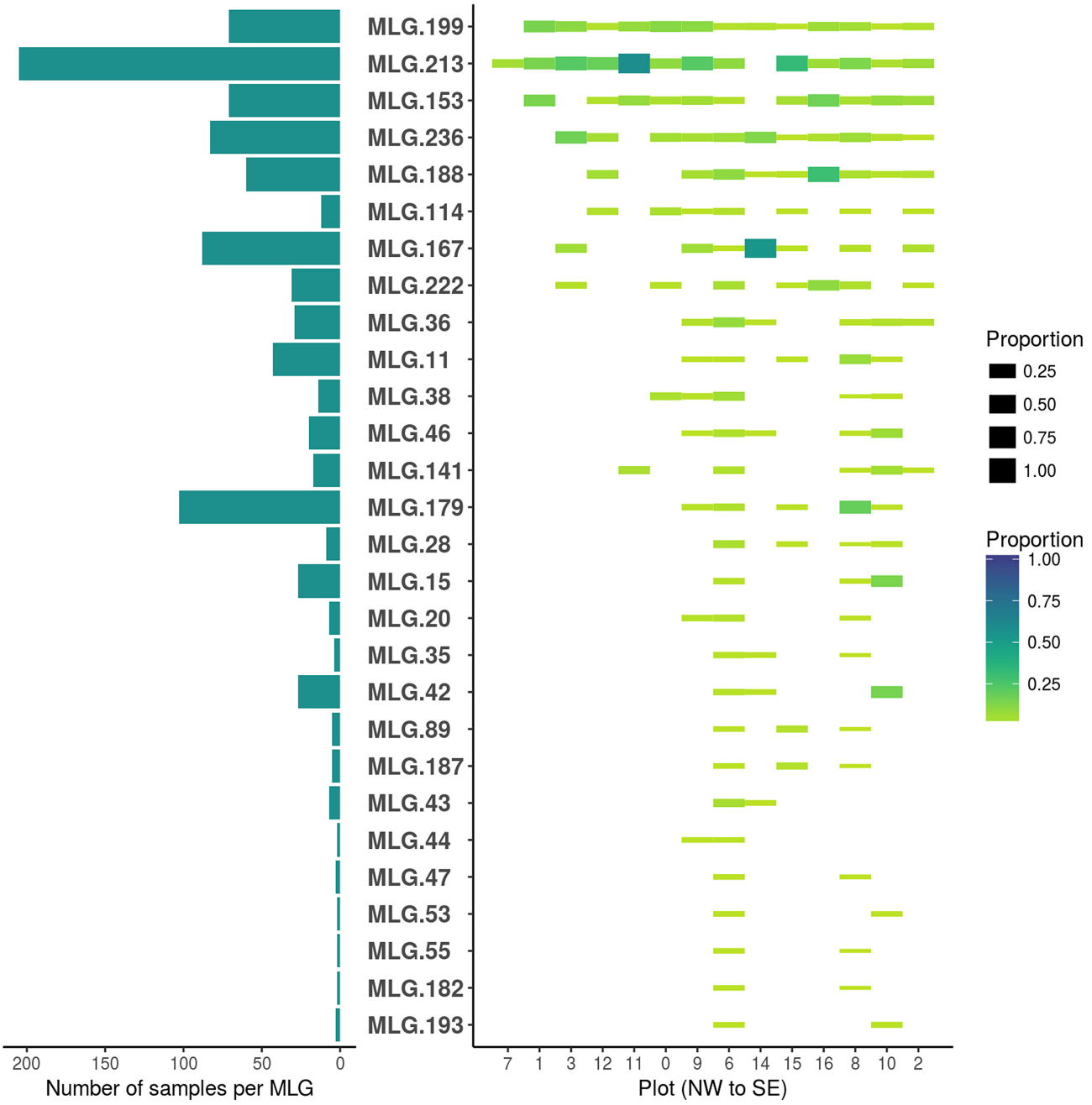

Figure 4. Proportion of multilocus genotypes (MLGs) or MLG abundance per plot and number of samples per MLG. The figure only includes MLG's found in more than one plot. Size of the rectangle indicates proportion of a MLG represented by isolations from each plot. Color of rectangle represents the proportion of isolates from any given plot that is represented by a specific MLG.

Coalescent analyses were performed to test the rate and direction of migration of *P. ramorum* among hosts. Given that oak infection only occurs in the spring and given that oaks are not infectious, only isolates from bay laurel that had been collected during the spring sampling events were included, and any migration from oak was excluded. Migration was highest from bay laurel to both oak and tanoak, migration occurring from tanoak to bay laurel was the lowest detected in this study and migration from tanoak to oak was low to intermediate (Table 5).

Discussion

The SFPUC watershed in San Mateo County was selected to examine the population genetics of *P. ramorum* among three key hosts of SOD in multiple sites and in different seasons and years. In previous publications, it has been demonstrated that soil, water and bay laurel populations of *P. ramorum* in the SFPUC watershed were all interconnected, and that the genetic diversity of all three was largely driven by the bay laurel population, with further differentiation or structure

Table 1. Distribution of the ten most commonly occurring MLG in each of the three host species; tanoak *Notholithocarpus densiflorus* (*Node*), coast live oak *Quercus agrifolia* (*Quag*) and California bay laurel *Umbellularia californica* (*Umca*).

	MLG.213	MLG.179	MLG.167	MLG.236	MLG.153	MLG.199	MLG.188	MLG.11	MLG.226	MLG.222
Node	3	0	29	10	0	4	3	0	4	0
Quag	15	1	5	1	7	5	2	0	5	0
Umca	187	102	54	72	64	62	55	43	29	31

likely to be accentuated by the differential selection processes in each of the three substrates (Eyre et al. 2013, Eyre and Garbelotto 2015). With the exception of a small-scale size by Kasuga et al. (2012), no other study has investigated the effect that multiple hosts with different epidemiological role and from multiple sites may have on the population size, diversity and microevolution of *P. ramorum* in California.

Although several studies exist on the population dynamics of *P. ramorum* in forests, only some have explicitly dealt with the possibility of multiple introductions (Croucher et al. 2013, Harris et al. 2018, Carleson et al. 2021), while other studies (McPherson et al. 2005, Meentemeyer et al. 2008, Haas et al. 2011) often have not considered the likely possibility that exotic *P. ramorum* populations may have been subject to different and multiple founder effects, thus making results hard to interpret (Sherpa et al. 2020). Here, all samples were obtained from three hosts within a single watershed with a relatively long and shared history of infection (Croucher et al. 2013), so that age of infestation should not represent a significant problem. The assumption of a homogeneous genetic background was confirmed by our population genetics analyses. F_{st} values among study plots, in fact, were insignificant for 98% of pairwise comparisons among plots, while only 2% of comparisons had a F_{st} of about 0.06, still a low value.

It has been shown that the spread of SOD in California mixed oak–woodlands is highly dependent on yearly and seasonal weather patterns (Eyre et al. 2013, Meentemeyer et al. 2015, Garbelotto et al. 2017, Kozanitas et al. 2017, 2022). As expected, in this study, pathogen populations increased in size and diversity as rainfall increased. At the same time, the increase in MLGs was mirrored by a decrease in evenness of MLGs (Table 3b). This decrease has been observed in other population genetic studies on *P. ramorum* (Eyre et al. 2013, Eyre and Garbelotto 2015), and is normally explained by the dominance of a few infectious genotypes during outbreak phases driven by favorable weather (Frank et al. 1992). These infectious fit genotypes were 13,5% (n = 32) of all MLGs (n = 237) and were the most common genotypes, independent of bay laurel density, both during favorable and unfavorable weather conditions (Fig. 3). A second group included genotypes that were not abundant but persisted through time (40/237; 16.9% of MLGs), and a third group, finally, included genotypes that were neither abundant nor persistent (165/237; 69.6%MLGs). The MLGs from the first group are bound to be the most important epidemiologically, however, it is possible that persistent MLGs in the second group, only found in sites with high bay laurel density, may also contribute to the adaptation and evolutionary processes of the exotic pathogen, as the pathogen faces changing and possibly novel environmental conditions in different locations. It is interesting to note that our results are different from those of Prospero et al. (2007) in Oregon who identified only a single widespread genotype that was also persistent. However, that study was performed in an infestation that may have been younger than that at the SFPUC, and in a later study in Oregon (Carleson et al. 2021), multiple persistent genotypes were found, with some characterized by lower abundance, as reported in this study.

Due to the long-term survival of epidemiologically relevant MLGs, we found little genetic structure when comparing *P. ramorum* populations from different years within the SFPUC watershed (Table 2b). This last finding was in perfect agreement with the findings of Mascheretti et al. (2008, 2009) and Croucher et al. (2013). It is also intriguing that, despite the rapid evolutionary potential of the pathogen (Kasuga et al. 2012, Yuzon et al. 2020), the most abundant genotypes have remained unchanged in time. We suggest that multihost generalism may actually be slowing down local adaptation (Morley et al. 2017), due to gene exchange among populations

Table 2. Nei's G_{st} values from pairwise comparisons among (a) the three host species, namely tanoak *Notholithocarpus densiflorus* (*Node*), coast live oak *Quercus agrifolia* (*Quag*) and California bay laurel *Umbellularia californica* (*Umca*) and (b) sample year 2009–2012.

(a)

	Umca	*Quag*	*Node*
Umca	0.0000000	0.0011751	0.0044148
Quag	0.0011751	0.0000000	0.0204225
Node	0.0044148	0.0204225	0.0000000

(b)

	2009	2010	2011	2012
2009	0.0000000	0.0010052	0.0019234	0.0040379
2010	0.0010052	0.0000000	0.0011183	0.0017303
2011	0.0019234	0.0011183	0.0000000	0.0013991
2012	0.0040379	0.0017303	0.0013991	0.0000000

Table 3. Diversity tables summarizing the diversity indices for populations of *P. ramorum* isolated from each of the three host species, tanoak *Notholithocarpus densiflorus* (*Node*), coast live oak *Quercus agrifolia* (*Quag*) and California bay laurel *Umbellularia californica* (*Umca*) in (a) and in each year of the study in (b). Where n is the samples size, MLG is the total number of multilocus genotypes in a population, eMLG is the estimated number of MLGs when n = 71, H is the Shannon–Weiner diversity, G is the Stoddard and Taylor's index, lambda is the Simpson's index, E_5 is evenness, and Hexp is Nei's gene diversity.

(a)

Pop	n	MLG	eMLG (SE)	H	G	lambda	E_5	Hexp
Node	71	18	18.00000 (0)	2.169909	4.898931	0.7958738	0.5026026	0.4250025
Quag	138	59	39.75335 (2.6)	3.731567	29.571429	0.9661836	0.7012349	0.4957181
Umca	1320	206	36.70857 (3.4)	4.016354	23.486911	0.9574231	0.4126159	0.4766213
Total	1529	237	37.59686 (3.45)	4.103311	25.144835	0.9602304	0.4055204	0.4780799

(b)

Pop	n	MLG	eMLG	G	lambda	E_5	Hexp
2009	240	55	19.52723	16.02671	0.9376042	0.5681507	0.4643528
2010	528	115	21.69159	21.80385	0.9541365	0.4921291	0.4801571
2011	726	169	23.91651	26.38282	0.9620965	0.4194376	0.4795522
2012	35	18	18.00000	13.17204	0.9240816	0.8599117	0.4788406
Total	1529	237	23.03097	25.14483	0.9602304	0.4055204	0.4780799

from different hosts or substrates limiting the selection of host- or substrate-dependent new genotypes. Because no new highly transmissive hosts have emerged in California besides bay laurel and tanoak, novel host-driven genotypic differentiation, such as that reported for *P. ramorum* populations emerging on the novel larch *Larix* host (Harris et al. 2018), may have been limited until the date of the study.

In spite of lack of genetic structure among sites, our results indicated that the identity and incidence of the most abundant MLGs varied strikingly across the network of study plots (Fig. 4), and that sites with high bay laurel density, dubbed 'hotspots' because of their ability to support higher levels of infection throughout the year (Kozanitas et al. 2022), were also genetically the most diverse. These results thus confirm the presence of variation at the landscape scale of pathogen populations, in spite of a common and recent history. Recently, Yuzon et al. (2020) have determined that site ecology drives evolutionary processes, and that the same genomic variations occur in different sites characterized by similar ecology. Our results indicate that genotypic composition may be similar in geographically distinct but ecologically similar sites, thus facilitating convergent evolutionary processes as those discussed by Yuzon et al. (2020). Additionally, our results indicate that different ecology may allow different genotypes to become dominant, even in the absence of different founder effects. It can be hypothesized that these different site types may start different microevolutionary processes, due to founder effects determined by the presence of different genotypes and of different existing alleles (Prentis et al. 2008). The importance of ecology in selection of MLGs, and possibly of intraspecific competition among genotypes is further reinforced by the fact that each widespread MLG is most abundant in a different plot.

We conclude that niche-driven selection is in action here in two ways. First, high bay laurel density and favorable environmental conditions lead to larger populations, which in turn results in a larger number of genotypes in a clonally reproducing microorganism (Croucher et al. 2013). This result agrees with those of studies reporting that bay laurel abundance increases disease transmission (Haas et al. 2011, Rosenthal et al. 2021b). Second, and for those plots in which tanoaks are present, the most abundant genotypes isolated from tanoaks were different from those that were most abundant in bay laurels and oaks, suggesting that, while there may be no strict host specificity, some genotypes may be preferentially associated with tanoaks. Hence, the presence of tanoaks is also bound to increase the overall genotypic diversity of the pathogen, or, at a minimum, to skew

Table 4. Private alleles occurring in populations of *P. ramorum* separated into groups by (a) host species Umca = *Umbellularia californica* (bay laurel), Quag = *Quercus agrifolia* (coast live oak), Node = *Notholithocarpus densiflorus* (tanoak) and (b) by sample year.

(a)

Host	ms18	ms39b	ms43a	ms43b	ms64	ms145a	ms145b	ms145c
Umca	11	12	18	12	6	5	4	3
Quag	0	0	0	8	0	0	0	0
Node	0	0	2	0	0	0	0	0

(b)

Year	ms18	ms39b	ms43a	ms43b	ms64	ms145a	ms145b	ms145c
2009	1	0	0	0	0	1	0	0
2010	0	4	2	8	2	3	3	8
2011	10	0	18	12	2	0	1	0
2012	0	0	0	0	0	0	0	0

Table 5. Results of coalescent analysis from Migrate-N, using the mean data of all loci from bay laurel isolates collected during spring sampling events. The populations are as follows 1: tanoak (*Node*), 2: coast live oak (*Quag*), 3: bay laurel (*Umca*).

		Theta					Migration rate				
Locus	Pop	2.5%	Mode	97.5%	Median	Mean	Direction	2.5%	Mode	97.5%	Median	Mean
All	1 (*Node*)	0.01400	0.01775	0.02600	0.01975	0.02066	3−>1	860.000	927.000	1000.000	932.500	926.051
All	2 (*Quag*)	0.04400	0.04725	0.05900	0.04825	0.04019	1−>2	40.0000	72.5000	100.000	427.500	306.705
All	3 (*Umca*)	0.09600	0.09875	0.10000	0.09875	0.09821	3−>2	695.000	817.500	955.000	832.500	827.234
							1−>3	45.0000	67.5000	90.0000	72.5000	69.6530

the abundance of specific genotypes. It is also possible that if tanoaks were to grow in microsites where bay laurels do not, due to the reported different ecology of the two species (Garbelotto et al. 2017), mixed tanoak–bay stand may carry a higher inoculum load. The same would not be true if tanoaks were to simply replace bay laurels.

We further investigated the presence of differences in populations of *P. ramorum* among its three main hosts in California mixed oak woodlands, taking into consideration the epidemiological role that each host may have. Based on the knowledge that, in California, sporulation on bay laurel exceeds levels observed on other hosts (Garbelotto et al. 2003, Davidson et al. 2005), and based on the strong association repeatedly identified between bay laurel density and infection rates on oaks and tanoaks (Cobb et al. 2010, 2012, Kozanitas et al. 2022), our main hypothesis was that the California bay laurel population of *P. ramorum* would represent a major source of inoculum for both oaks and tanoaks. Oaks, instead, due to their lack of competence, would represent a sink, maybe even a dead-end decoy, while tanoaks would be a secondary source and also a sink.

Our results convincingly show the primary role that bay laurels play in oak infection for the following reasons: the most abundant MLG in bay laurel was also the prominent MLG in oaks, proving for the first time direct contagion between sympatric bay laurel and oaks; private allele richness was much higher in bay laurels than in oaks further corroborating the role of bay laurels as a source population; 50% of MLGs found in oak were also found in bay laurel; G_{st} between bay laurel and oak populations was the lowest recorded in the study, and, finally, the number of bay laurel to oaks migrants, estimated through coalescent analysis, was close to 1000, thus it can be regarded to be extremely high. The number of migrants from bay to oak was, in fact, significantly higher than that determined for bay-to-bay infection between adjacent or interconnected forest sites in California (Mascheretti et al. 2009).

Based on other studies (Garbelotto et al. 2017, Kozanitas et al. 2022), it has been deduced that oak infection occurs only in exceptionally wet years. This selection process would limit the number of viable genotypes in oaks because only those that meet the epidemiological requirements necessary for infection of this host would be reisolated from it. If this were the case, we would expect a reduction of genotypic diversity in oaks compared to that in bay laurels. However, this pattern was not observed in this study. Alternatively, given that oaks are not transmissive, the oak environment may allow for the survival of genotypes that, by lacking a transmission-related trait, are outcompeted in bay laurels. In support of the second scenario, oaks had the highest expected pathogen genotypic diversity among the three hosts. This is in spite of their reported general lack of competence, which implies no new genotypes are generated on oaks. Likewise, the low number of private alleles in oaks confirms that little genetic variability is created on oaks and confirms their lack of competence. Further support of oaks as a dead-end host is given by the high values of the evenness

index in oak populations reported to have reached 0.85 in the 2012 oak sampling. High evenness is normally associated with pathogen populations that are not in outbreak phase or by populations in which individuals are not directly competing for a resource, as shown for plants (Rajaniemi 2011). Given that, occasionally, low intensity sporulation occurs on oaks (Rosenthal et al. 2021a), the pathogen diversity associated with oaks may have evolutionary consequences even if generally it does not have epidemiological outcomes.

Although migration was intermediate in scale from tanoak to oak populations, this result may be spurious since oaks and tanoaks are infected by the same bay laurel source. In support of this interpretation, our analysis determined that no MLGs were exclusively shared between oaks and tanoaks, while about 50% of MLGs in tanoaks were shared with bay laurels, not unlike what was found for oaks. Furthermore, the top ten MLGs detected in oaks and tanoaks were all also found in bay laurels, while private allelic diversity was low in both oaks and tanoaks, suggesting once again a bay laurel source for these MLGs isolated from the two other hosts. Even if we recognize G_{st} values were overall low in the study, it is striking that the G_{st} between pathogen populations from oak and those from tanoaks was one order of magnitude higher than the G_{st} of other pairwise host combinations. This result further suggests that oak and tanoak pathogen populations are genetically isolated, with no direct migration between them. Migration of the pathogen instead was estimated to be very high from bay laurel to tanoak populations, giving credibility to other studies reporting a strong association between bay laurel density and tanoak infection (Cobb et al. 2012, Kozanitas et al. 2022), and further suggesting that the connection between oak and tanoak may be mediated by the bay laurel population that is the source of infection for both. Conversely, the number of migrants from tanoaks to bay laurel was rather low, indicating that tanoak does not play a major epidemiological role in mixed oak woodlands with a significant component of bay laurels present. Nonetheless, infections of bay laurels by tanoaks are in addition to infections resulting from bay laurel-to-bay laurel autoinfection. Based on the difference in migration rates initiated by bay laurels with those initiated by tanoaks, we estimate that bay laurels may amplify tanoak inoculum by one order of magnitude.

Finally, we tested whether having three hosts, each with a different epidemiological role and morbidity, may affect the overall genetic diversity of the pathogen. The DAPC analysis, clearly showed that oak and tanoak populations both overlap with the bay laurel population, but are clearly divergent from one another (Fig. 5). This genetic difference between

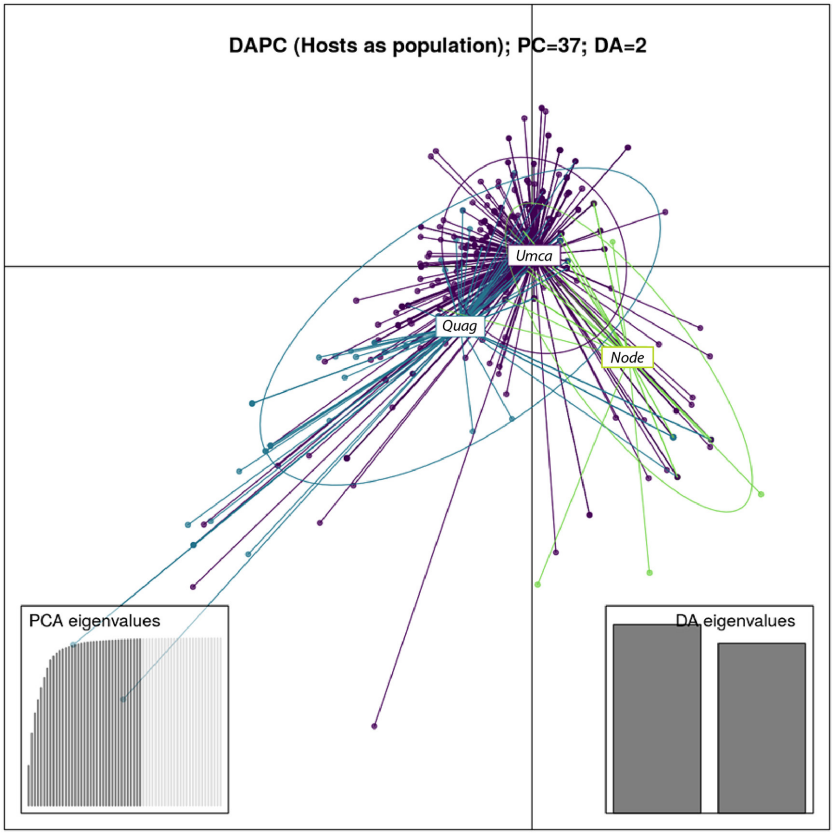

Figure 5. Results from a discriminant analysis of principal components (DAPC) as implemented in adegenet, showing any overlap that exists between the populations of *Phytophthora ramorum* isolated from the three different host species. The DAPC was executed by retaining 37 principal components that yielded two discriminant axes. The three host species are tanoak *Notholithocarpus densiflorus* (*Node*), coast live oak *Quercus agrifolia* (*Quag*) and California bay laurel *Umbellularia californica* (*Umca*).

two populations that supposedly originated from the same bay laurel source may be in part explained by the different selection pressure experienced by MLGs of the pathogen in the two hosts, combined with the lack of direct migration between the two. A similar process has been suggested to explain the genetic differences among populations from bay laurel and soil or water (Eyre et al. 2013, Eyre and Garbelotto 2015). Additionally, the fact that the most common MLG in tanoaks is not the most common MLG in bay laurels, suggests a niche-driven selection, with some genotypes being preferentially associated with tanoaks.

Overall, our data suggest that having three hosts, each with a different epidemiological role, is generating more diversity in pathogen populations than having one or two hosts. Unless hosts are epidemiologically identical, it is possible that, as more hosts are added to the system, the greater will be the genetic variability in the pathogen. It should be noted that when host-driven adaptation exists, rapid evolution of host-adapted populations can occur (Bacigalupe et al. 2019). Finally, by showing the prevalence of different genotypes in various hosts and sites, we also show, for the first time in California, that landscape level variations and host species complexity are not only able to drive disease dynamics at the epidemiological level, but at the genetic level as well. Note that the effects of host diversity on evolutionary processes may be different than those on disease progression: in the latter case, in fact, host diversity has been reported to decrease disease transmission (Haas et al. 2011). We conclude that scenarios in which bay abundance is paired with the presence of tanoaks may have a higher adaptation and evolutionary potential for *P. ramorum*, compared to that in dense but pure bay stands. In conclusion, our work provides novel insights into multihost epidemiological and evolutionary dynamics of a forest pathogen.

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Author contributions

Melina Kozanitas: Data curation (equal); Formal analysis (equal); Investigation (equal); Writing – original draft (equal). **Brian J. Knaus:** Formal analysis (equal). **Javier F. Tabima:** Formal analysis (equal). **Niklaus J. Grünwald:** Formal analysis (equal); Writing – review and editing (supporting). **Matteo Garbelotto:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.hqbzkh1ps (Kozanitas et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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