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1 **Temporal population structure of rubber tree powdery mildew pathogen**

2 **Erysiphe quercicola in Hainan, China**

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## ABSTRACT

1  
2 Rubber tree powdery mildew, caused by the obligate pathogen *Erysiphe quercicola*, is  
3 a major threat to rubber plantations worldwide. To investigate the temporal changes in  
4 the population structure of *E. quercicola*, diseased samples were collected at three  
5 disease epidemic stages (overwinter, early epidemic, and late epidemic) from three  
6 counties in Hainan, China. Population structure was assessed using 16 polymorphic  
7 microsatellite markers. Both permutational multivariate analysis of variance  
8 (PERMANOVA) and analysis of molecular variance (AMOVA) indicated that there  
9 were significant differences among the population structure of *E. quercicola* at  
10 different epidemic stages. Furthermore, the late epidemic populations had higher  
11 genetic diversity than the other two stages. Discriminant analysis of principal  
12 components (DAPC) and STRUCTURE analysis showed that *E. quercicola* samples  
13 grouped into four clusters. One of the clusters only included samples from the late  
14 epidemic stage, while the other three clusters included samples from all three sample  
15 stages. Further analysis confirmed that there were significant ( $P = 0.001$ ) genetic  
16 differences among these four clusters. As the teleomorph stage of *E. quercicola* has  
17 not yet been reported, these findings suggest that the epidemic of rubber tree powdery  
18 mildew is not only established by local inoculum sources, but also may be by  
19 inoculum drifting from other plantations or through host jumps from wild hosts.

## 1 **Introduction**

2 Rubber tree (*Hevea brasiliensis*), native to the rain forests of the tropical region of the  
3 Great Amazonian basin is the primary source of natural rubber, which is a critical raw  
4 material to industries. During the late 1970s, rubber tree cultivation expanded to many  
5 sub-tropical environments including South China, where the plantations experience  
6 stresses such as low temperature, dry periods, typhoons and high altitude (Priyadarshan  
7 et al. 2005). Rubber trees have distinct phenology for annual leaf shedding (wintering)  
8 and leaf flushing (refoliation) in the sub-optimal cultivating habitats (Zhai et al.  
9 2020). The changes of rubber tree phenology and environmental conditions provide  
10 favorable conditions for the occurrence of leaf diseases especially for rubber powdery  
11 mildew, which can reduce latex yields up to 45% (Liyanage et al., 2016). Currently,  
12 rubber powdery mildew control still relies on fungicides because of the absence of  
13 resistant popular commercial clones (Liyanage et al. 2018).

14 The pathogen causing rubber tree powdery mildew was first described as *Oidium*  
15 *heveae*. Recent studies with molecular marker and SEM technology considered that *O.*  
16 *heveae* is the asexual morph of *Erysiphe quercicola* on rubber tree (Liyanage et al.,  
17 2017; Wu et al., 2019). Although the teleomorph of *E. quercicola* has not been reported  
18 on rubber tree, heterothallism was reported in *E. quercicola* from oaks (Gross et al.  
19 2021), reinforcing the opinion of Kirschner & Liu (2014) that the teleomorph is found  
20 only on species of *Quercus* and *Fagaceae*. As a biotrophic pathogen that only infects  
21 tender tissues of rubber leaves, buds, and inflorescences, the disease mainly occurs in  
22 spring when the trees re-foliate (Zhai et al. 2020). The pathogen survives the winter

1 defoliation season of rubber trees on tender leaves of volunteer seedlings in rubber  
2 plantations, on regrowth of trees that were damaged by strong winds e.g. during  
3 typhoons, as well as on seedlings in relatively protected rubber tree propagation  
4 nurseries. Additionally, wild hosts like *Euphorbia hirta*, *Jatropha curcas*, *Alchornea*  
5 *davidii* and *Urema lobata* were considered as potential overwintering hosts of rubber  
6 powdery mildew (Lu et al. 1982; Yu et al. 1996; Liyanage et al., 2016). These infected  
7 leaves serve as sources of primary inoculum to infect the newly produced leaves in  
8 spring in the main rubber tree plantations that otherwise lack tender leaves during the  
9 defoliation season.

10 Conidia of powdery mildew species can be wind-dispersed over long distances  
11 (Brown and Hovmøller, 2002). For example, conidia of *Golovinomyces cichoracearum*  
12 and *Blumeria graminis* were reported to be dispersed 200 km in California and nearly  
13 700 km from the UK to Denmark, respectively (Schnathorst 1959; Hermansen et al.  
14 1978). Therefore, it could be possible for a rubber tree plantation to become infected in  
15 spring from overwintering inoculum within the plantation or from inoculum dispersed  
16 from other plantations. The relative importance or contribution of these two inoculum  
17 sources is currently unknown.

18 Population genetic analyses using molecular markers can be used to infer the origin,  
19 migration and evolution of plant pathogens (Grünwald et al., 2017). For rubber tree  
20 powdery mildew, a few studies based on the ITS and 28S rDNA sequences were  
21 reported to study the population genetics of *E. quercicola*, which indicated high genetic  
22 diversity (Limkaisang et al. 2005; Liyanage et al. 2017). More recently, sixteen

1 polymorphic microsatellite markers for *E. quercicola* were developed (Han et al. 2022)  
2 and used samples from different fields or regions collected at specific phenological  
3 stages.

4 Studying the temporal changes in population structure can provide insight on the  
5 biology and migration of pathogens (Xhaard et al. 2012). Hainan is one of the main  
6 rubber tree plantation areas in China comprising approximately 47% (Liu et al 2022)  
7 and powdery mildew is the most serious leaf disease in the region. We hypothesized  
8 that in a rubber tree plantations, both off season overwintering survival on tender leaves  
9 and migrations from external sources contribute to the temporal maintenance of the  
10 pathogen. Thus, in this study, rubber tree powdery mildew samples were collected at  
11 different disease epidemic stages to (i) evaluate the temporal changes of population  
12 structure of *E. quercicola* in Hainan, China; (ii) infer the relative contributions of off-  
13 season survival and migration to the temporal maintenance of pathogen populations.

## 14 **MATERIALS AND METHODS**

### 15 **Sample collection and DNA preparation**

16 Tender rubber tree leaves with powdery mildew symptoms were collected in 2022 from  
17 three plantations from three counties (Ledong, Qiongzong and Danzhou) of Hainan  
18 province at three disease epidemic stages including overwintering stage (January 9,  
19 January 21 and January 23 for Ledong, Qiongzong and Danzhou, respectively), early  
20 epidemic stage (February 13, March 5 and March 6 for Ledong, Qiongzong and  
21 Danzhou, respectively) and late epidemic stage (March 16, April 10 and April 12 for  
22 Ledong, Qiongzong and Danzhou, respectively). The distance between sampled

1 plantations in Ledong and Danzhou was 98 km, while the distances between sample  
2 sites in Ledong and Qiongzong and Qiongzong and Danzhou were 85 and 47 km,  
3 respectively. The cultivar of the rubber tree sampled was CATAS7-33-97, which is a  
4 major commercial rubber tree variety in Hainan. The samples from Danzhou were  
5 collected from volunteer seedlings, while samples from Ledong and Qiongzong were  
6 collected from seedlings in rubber nurseries. For each seedling, one to two leaves were  
7 sampled.

8 A single powdery mildew circular leaf lesion (colony) on leaves without visual  
9 symptoms of any other diseases was cut with a 1 cm diameter cork borer. Only one  
10 lesion was cut per leaf and was considered as a single sample, which is common practice  
11 for obligate pathogens (Khan et al. 2019; Wallace et al. 2020; Han et al. 2022). As it  
12 was reported that 25 to 30 individuals per population is enough to estimate allele  
13 frequencies accurately using microsatellites (Hale et al. 2012), about 25 samples were  
14 used for each plantation at each sample stage to avoid sample bias and a total of 223  
15 samples were used in this study. Genomic DNA was extracted from each sample using  
16 the Tiangen Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to  
17 the manufacturer's instructions (Han et al. 2022). Extracted DNA was stored at -20°C  
18 for further use.

### 19 **Mating type determination**

20 *Erysiphe quercicola* is heterothallic and mating type can be assayed indirectly by  
21 detection of the *MATI-1* and *MATI-2* idiomorphs (Gross et al. 2021). PCR assays were  
22 conducted as described by Gross et al. (2021) to determine whether samples were

1 *MAT1-1* or *MAT1-2*.

## 2 **Microsatellite genotyping**

3 DNA extracted from single rubber tree powdery mildew circular leaf lesions were used  
4 to genotype *E. quercicola* using sixteen pairs of validated microsatellite primers (Han  
5 et al. 2022).. PCR amplification was performed in a Veriti™ Dx 384-well Thermal  
6 Cycler (Applied Biosystem, Carlsbad, CA, USA) following the protocol described by  
7 Han et al. (2022). Each reaction (10 µl) contained 5 µL 2×TaqPCR MasterMix  
8 (GeneTech, Guangdong, China), 1 µl of template DNA, 0.5 µl of each forward and  
9 reverse primers (10 µM), and 3 µl of double-distilled H<sub>2</sub>O.

10 A touchdown PCR assay was used with the following conditions: initial denaturation  
11 at 95°C for 5 min; 10 cycles of 95°C for 30 s, 62°C for 30 s with a 1°C decrease per  
12 cycle, and 72°C for 30 s; a further 25 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C  
13 for 30 s; and a final extension at 72°C for 20 min. Fragment analyses were conducted  
14 using an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA) with  
15 the GeneScan™500 LIZ (Thermo Fisher Scientific) used for the standard dye size.  
16 Allele sizes were analyzed using GeneMarker software version 3.0.0 (SoftGenetics,  
17 State College, PA)

## 18 **Data analysis**

19 The statistics including the number of alleles, Simpson's diversity index, Nei's gene  
20 diversity and evenness were computed for the 16 microsatellite markers using the *poppr*  
21 package (Kamvar et al. 2014) in R 4.1.0. To assess whether the number of microsatellite  
22 loci was sufficient to discriminate all the different individuals, a genotype accumulation

1 curve was generated using the package *poppr*.

2 For each sample, the multilocus genotype (MLG) was identified based on the allele data  
3 over the 16 microsatellite loci. The MLGs shared between populations collected in  
4 different disease epidemic stages and counties were determined. If an MLG was shared  
5 between different populations, then the MLG was inferred to have migrated (Hovmøller  
6 et al. 2008). Genetic diversity indices including the number of MLGs, the number of  
7 expected MLG at the smallest sample size based on rarefaction (eMLG), Stoddart and  
8 Taylor's index of MLG diversity (G), Simpson's index of genotypic diversity ( $\lambda$ ),  
9 genotypic evenness (E.5) and Nei's gene diversity were computed. Furthermore, the  
10 allelic richness ( $R_a$ ) and private allele richness ( $R_p$ ) were estimated using the rarefaction  
11 approach implemented in the ADZE 1.0 to avoid the sampling size effects (Szpiech et  
12 al., 2008).

13 Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001),  
14 based on a pairwise Bruvo's genetic distance matrix between samples computed in  
15 *poppr*, was used to investigate the statistical differences in *E. quercicola* structure  
16 between sample epidemic stages and counties. 999 permutations were used as  
17 implemented in the *vegan* package (Dixon 2003). Only clone-corrected data were used  
18 to avoid potential biases due to clonality (Balloux et al. 2003). Analysis of molecular  
19 variance (AMOVA) was also used to test the null hypothesis of no temporal or spatial  
20 differentiation with *poppr*. For AMOVA, the total genetic variation was partitioned at  
21 two levels: among stages or counties and among individuals within stages or counties.  
22 Statistical significance was conducted with 999 permutations.

1 Discriminant analysis of principal components (DAPC), a multivariate, model-free  
2 approach (Jombart et al. 2010), was used to study the genetic structure of *E. quercicola*.  
3 DAPC was carried out in the *adegenet* package (Jombart 2008). The *k*-means were used  
4 to identify the optimal number of clusters with lowest Bayesian Information Criterion  
5 (BIC). A Bayesian model implemented in STRUCTURE 2.3.4 was also used to suggest  
6 the number of genetic clusters (Pritchard et al. 2000). In total, 10 replicated runs were  
7 made, each of  $K = 1$  to 10 with 100,000 iterations in Monte Carlo Markov Chain  
8 replications and a burn-in period of 10,000. The optimal number of clusters ( $K$ ) was  
9 suggested based on the estimate of the posterior probability of the data for a given  $K$  ( $\text{LnP}(D)$ )  
10 and the rate of change in the log probability ( $\Delta K$ ) (Evanno et al. 2005).

## 11 **RESULT**

### 12 **Population genetic diversity**

13 MAT1-1 idiomorph was founded in all 223 tested samples used in this study. All  
14 amplified samples exhibited a single peak (i.e., allele) near the expected size for each  
15 microsatellite marker as expected since *E. quercicola* is a haploid. A total of 100  
16 polymorphic alleles were produced using 16 microsatellite markers. The number of  
17 alleles ranged from 3 (OH-SSR38) to 14 (OH-SSR28) (Table 1). The values of  
18 Simpson's diversity index were between 0.06 and 0.69, and evenness varied from 0.36  
19 to 0.63. The genotype rarefaction curve showed a linear increase initially as the number  
20 of the loci increased, before reaching a plateau, indicating that the 16 loci used were  
21 sufficient to distinguish the genotypes among the samples in this study (Fig. S1).

22 Among the 223 samples, a total of 149 MLGs were identified. There were 17 MLGs  
23 that were repeated within 70 samples. Eleven MLGs were repeated at different sample

1 stages within the same county, of which eight were repeated between the overwinter  
2 stage and the other two stages (Figure 1). Furthermore, the MLG108 was repeated at  
3 all three stages in Qiongzong and Danzhou. Ten MLGs were repeated between  
4 different counties.

5 The late epidemic populations had higher genotypic diversity (Simpson's indices),  
6 genotypic evenness, and Nei's gene diversity compared with overwinter and early  
7 populations in all three counties (Table 2). Also,  $R_a$  and  $R_p$ , which were used to avoid  
8 sampling size effects, were higher in late epidemic populations (5.33 and 1.48 for  $R_a$   
9 and  $R_p$ , respectively) than overwinter (3.72 and 0.25 for  $R_a$  and  $R_p$ , respectively) and  
10 early (4.06 and 0.27 for  $R_a$  and  $R_p$ , respectively) populations. The early populations had  
11 similar diversity to the overwinter populations in Ledong and Qiongzong. However,  
12 the early population from Danzhou had higher diversity than overwinter population.

### 13 **Population genetic structure**

14 Although stage, county and their interaction jointly only accounted for 7.75% of the  
15 total genetic variation based on PERMANOVA analysis using clone-corrected data  
16 (Table 3). Sampling stage had significant effects on the population structure of *E.*  
17 *quercicola* from Hainan ( $P = 0.002$ ), while county was not significant ( $P = 0.16$ ).  
18 AMOVA analysis also confirmed the stage-based population subdivision ( $P = 0.006$ )  
19 and a lack of population subdivision in terms of county ( $P = 0.31$ ) (Table 4).

20 The *k*-means method suggested eleven genetic clusters were the optimal number of  
21 clusters with the lowest BIC value (Fig. S2A). However, the DAPC analysis with *E.*  
22 *quercicola* samples with  $K=11$  indicated that these eleven groups were grouped into

1 four distinct clusters (Fig. S2B). Furthermore, the BIC value changed slightly from K  
2 = 11 to K = 4. Thus, four clusters were selected for the final DAPC analysis. Cluster 1,  
3 cluster 2 and cluster 3 had *E. quercicola* samples collected from all the three sampled  
4 stages in three counties. However, cluster 4 only had samples collected from late  
5 epidemic stage in three counties (Fig. 2). AMOVA analysis indicated that there was  
6 significant difference among these four clusters ( $P = 0.001$ ) as 19.47% of the total  
7 genetic variance was among the clusters (Table 4). Based on  $\ln P(D)$  and delta  $K$  values,  
8 STRUCTURE analysis also suggested four clusters within the 223 samples (Fig. 3A  
9 and 3B). STRUCTURE bar plots from  $K=2$  to  $K = 6$  are shown in the supplemental  
10 material and the clusters were not grouped with sample stages and counties (Fig. S3).  
11 Additionally, *E. quercicola* populations showed admixture patterns based on the  
12 STRUCTURE bar plots, i.e.,  $K = 4$  (Fig. 3C).

### 13 **DISCUSSION**

14 Understanding the mode of survival of plant pathogens is an important domain of  
15 microbial ecology research. The temporal changes of population structure of *E.*  
16 *quercicola* were investigated in samples collected at three epidemic stages from three  
17 plantations in Hainan, China. A total of 149 MLGs were observed among 223 *E.*  
18 *quercicola* samples based on 16 published microsatellite markers, which indicated a  
19 high genetic diversity for the pathogen. Also, high genetic diversity was reported using  
20 microsatellite markers on other powdery mildew pathogens like *Podosphaera*  
21 *leucotricha* (Gañán-Betancur et al. 2021) and *B. graminis f. sp. tritici* (Cowger et al.  
22 2016). However, a previous study also reported 119 MLGs were detected among the

1 138 *E. quercicola* samples, collected at the late epidemic stage, from Hainan (Han et  
2 al. 2022), which showed a higher frequency of unique MLG compared with this study.  
3 Additionally, we also found a high frequency of unique MLG (70 MLGs from 75  
4 samples) for samples collected at late epidemic stage in this study. However, only 47  
5 and 48 MLGs were observed in 75 and 73 samples collected at overwinter and early  
6 stage, respectively.

7 Four clusters were defined based on DAPC and there were significant genetic  
8 differences among them. Distinct genetic groups were reported on other powdery  
9 mildew fungi like *E. necator* (Délye et al. 1999; Gur et al. 2021). These may indicate  
10 the founder effect on the *E. quercicola* population in Hainan similarly to that found in  
11 the *E. necator* population in Israel, which was from East America and from Asia (Gur  
12 et al. 2021). The origin of *E. quercicola* is still unknown. Although the rubber tree  
13 originated from the Amazon region, rubber tree powdery mildew was first recorded in  
14 Java in 1918, which suggests that the disease may emerge independently in Southeast  
15 Asia. Conversely, it was suggested that the Brazilian isolates may be ancestral to the  
16 Southeast Asian isolates based on ITS and 28S rDNA sequences (Limkaisang et al.  
17 2005). However, only five rubber powdery mildew specimens were used in the former  
18 study. Therefore, more specimens from different countries from the Amazon and  
19 Southeast Asia should be collected to infer the origin of the pathogen in the future.

20 We cannot exclude the effects of wild hosts on the genetic differentiation of *E.*  
21 *quercicola*. For example, powdery mildew populations from muscadine grapes, *Vitis*  
22 *rotundifolia*, were genetically distinct from populations from other *Vitis* host species

1 (Brewer and Milgroom 2010). Also *Puccinia striiformis* isolates from grasses had a  
2 higher genetic variation than those from cereal crops (Cheng et al., 2016). Although  
3 most powdery mildew pathogens are strictly host specific, *E. quercicola* from rubber  
4 tree can infect other plants like *Euphorbia hirta*, *Jatropha curcas*, *Alchornea davidii*  
5 and *Urema lobata* (Yu et al., 1996; Liyanage et al., 2016). *E. hirta*, *J. curcas* and *A.*  
6 *davidii* are plant species often found under rubber trees in Hainan (Wang et al. 2012).  
7 Further research is needed to study the genetic diversity of *E. quercicola* from these  
8 wild hosts and their effect on the genetic differentiation of the pathogen.

9 Three of the four clusters defined by DAPC included samples from all three stages and  
10 three counties. Eight MLGs were repeated between the winter stage and the other two  
11 stages within the same plantation, and one MLG was repeated at all three stages in two  
12 plantations. All these factors suggest that the pathogen can overwinter in volunteer  
13 seedlings in rubber plantations and seedlings in rubber nurseries to provide inoculum  
14 for the disease to occur on new leaves in spring (Lu et al. 1982; Yu et al. 1996; Liyanage  
15 et al., 2016). The main factors that affect powdery mildew overwintering is the  
16 existence of tender tissues. Young leaves can be produced on a succession of different  
17 seedlings over a prolonged period during winter in Hainan.

18 Interestingly, one of the four clusters defined based on DAPC only included samples  
19 from the late epidemic stage in all three counties. This suggests that this cluster may  
20 not originate from the local overwintered inoculum. Furthermore, the genetic diversity  
21 of the late epidemic stage population was higher than the other two stages, which  
22 indicated that new genotypes may enter the plantations and became established in the

1 late epidemic stage. A similar result was also reported on other obligate, wind-dispersed  
2 plant pathogens like *Pseudoperonospora cubensis* (Naegele et al. 2016) and *P.*  
3 *striiformis* f. sp. *tritici* (Wang et al. 2021).

4 Furthermore, no significant genetic difference was identified among the three counties  
5 based on PERMANOVA and AMOVA analyses, while 9 MLGs were repeated between  
6 counties, indicating a reasonably well mixed population within those three counties and  
7 migration of the pathogen among these counties. *E. quercicola* populations showed  
8 admixture patterns, indicating dispersal of the pathogen between populations, which  
9 agrees with previous reports that airborne conidia of powdery mildews can disperse  
10 over long distances e.g. (Brown and Hovmøller 2002), and for conidia of  
11 *Golovinomyces cichoracearum* which dispersed 200 km (Schnathorst 1959) and for *B.*  
12 *graminis* conidia, which dispersed nearly 700 km (Hermansen et al. 1978). Although  
13 long-distance dispersal does not occur commonly for apple powdery mildew *P.*  
14 *leucotricha* in the United States, inoculum dispersal up to 100 km has likely occurred  
15 in Washington (Gañán-Betancur et al. 2021). The distances between the sampled  
16 plantations were all below 100 km in this study.

17 Sexual reproduction is also thought to contribute to the temporal maintenance of some  
18 other powdery mildew pathogens such as *E. necator* (Pearson and Gadoury 1987), *P.*  
19 *clandestine* (Grove and Boal, 1991), *P. aphanis* (Jin et al. 2013), *B. graminis* f. sp. *tritici*  
20 (Jankovics et al. 2015) and *P. macularis* (Weldon et al. 2021). Some recent studies  
21 considered that *E. quercicola* was the pathogen of rubber tree powdery mildew  
22 (Liyanage et al., 2017; Wu et al., 2019). However, chasmothecia production is rare in

1 tropical and subtropical regions and the teleomorph of *E. quercicola* has not been  
2 observed on rubber tree. Only the *MATI-1* idiomorph was identified in this study and  
3 we cannot exclude whether the *MATI-2* idiomorph exists on wild hosts. For example,  
4 all *P. macularis* isolates from the western U.S. were mating type *MATI-1* from  
5 cultivated hop plants, while both mating type idiomorphs were detected from wild  
6 plants (Gent et al. 2020).

7 *Erysiphe quercicola* seems to have a broad host range of species including tropical fruit  
8 trees or woody plants such as oak, mango, rambutan and cashew (Desprez-Loustau et  
9 al. 2017). Wu et al. (2019) suggested to subdivide *E. quercicola* into subspecies based  
10 on the host range as different *formae specialis* depending on the host may occur for this  
11 species. For example, *Erysiphe quercicola* from oak and mango could infect each other  
12 (Desprez-Loustau et al. 2017) while *E. quercicola* from rubber tree and mango failed  
13 to cross-infect each other (Yu et al. 1996). Whether *E. quercicola* genotypes from other  
14 host species like oak, rambutan and cashew can infect rubber trees requires further  
15 research. Therefore, we infer from the increase in genetic diversity in the late-epidemic  
16 stage that the epidemic of rubber tree powdery mildew is not only established by local  
17 inoculum sources, but also from genotypes migrated from other plantations and  
18 potentially from other hosts. Furthermore, the potential for host jumps from sources of  
19 the pathogen on rubber tree onto other hosts (e.g. *E. hirta*, *J. curcas* and *A. davidii*) also  
20 requires further research.

21

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3

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1 **Table 1** Information about 16 published microsatellite markers used in this study

| Locus    | No. of alleles | Simpson's diversity index | Evenness |
|----------|----------------|---------------------------|----------|
| OH-SSR10 | 6              | 0.43                      | 0.54     |
| OH-SSR11 | 5              | 0.15                      | 0.41     |
| OH-SSR15 | 5              | 0.48                      | 0.62     |
| OH-SSR17 | 4              | 0.16                      | 0.45     |
| OH-SSR26 | 7              | 0.19                      | 0.39     |
| OH-SSR28 | 14             | 0.69                      | 0.62     |
| OH-SSR34 | 5              | 0.37                      | 0.52     |
| OH-SSR36 | 5              | 0.19                      | 0.40     |
| OH-SSR38 | 3              | 0.06                      | 0.38     |
| OH-SSR46 | 5              | 0.37                      | 0.63     |
| OH-SSR47 | 9              | 0.22                      | 0.36     |
| OH-SSR48 | 7              | 0.32                      | 0.46     |
| OH-SSR49 | 6              | 0.24                      | 0.44     |
| OH-SSR50 | 7              | 0.36                      | 0.47     |
| OH-SSR51 | 8              | 0.60                      | 0.63     |
| OH-SSR63 | 4              | 0.07                      | 0.36     |

2

1 **Table 2** Genotypic and genetic diversities of *Erysiphe quercicola* from rubber tree  
 2 based on 16 published microsatellite markers

| County    | Sample stage   | N   | MLG | eMLG  | H    | G     | $\lambda$ | E.5  | Hexp | $R_a$ | $R_p$ |
|-----------|----------------|-----|-----|-------|------|-------|-----------|------|------|-------|-------|
| Ledong    | Overwiner      | 24  | 18  | 16.9  | 2.81 | 15.17 | 0.93      | 0.91 | 0.24 | 2.31  | 0.06  |
|           | Early epidemic | 22  | 12  | 12    | 2.3  | 8.35  | 0.88      | 0.82 | 0.26 | 2.25  | 0.08  |
|           | Late epidemic  | 25  | 25  | 22    | 3.22 | 25    | 0.96      | 1    | 0.35 | 3.46  | 0.52  |
| Danzhou   | Overwiner      | 25  | 15  | 13.55 | 2.37 | 7.02  | 0.86      | 0.62 | 0.27 | 2.53  | 0.07  |
|           | Early epidemic | 27  | 24  | 20.03 | 3.14 | 22.09 | 0.96      | 0.95 | 0.24 | 2.88  | 0.18  |
|           | Late epidemic  | 25  | 25  | 22    | 3.22 | 25    | 0.96      | 1    | 0.47 | 3.64  | 0.37  |
| Qiongzong | Overwiner      | 26  | 18  | 15.81 | 2.72 | 12.07 | 0.92      | 0.79 | 0.2  | 2.15  | 0.13  |
|           | Early epidemic | 24  | 16  | 14.92 | 2.57 | 10.29 | 0.9       | 0.77 | 0.25 | 2.61  | 0.02  |
|           | Late epidemic  | 25  | 22  | 19.69 | 3.05 | 20.16 | 0.95      | 0.95 | 0.31 | 3.18  | 0.25  |
| Overall   | Overwiner      | 75  | 47  | 46.1  | 3.62 | 28    | 0.96      | 0.74 | 0.25 | 3.72  | 0.25  |
|           | Early          | 73  | 48  | 48    | 3.69 | 31.9  | 0.97      | 0.8  | 0.26 | 4.06  | 0.27  |
|           | Late           | 75  | 70  | 68.2  | 4.21 | 63.2  | 0.98      | 0.94 | 0.38 | 5.33  | 1.48  |
|           | Total          | 223 | 149 |       |      |       |           |      |      |       |       |

3 <sup>a</sup> N, number of samples; MLG, number of multilocus genotypes; eMLG, number of expected MLG  
 4 based on rarefaction correction; H, the Shannon-Wiener Index of MLG diversity; G, Stoddart and  
 5 Taylor's index of MLG diversity;  $\lambda$ , Simpson's index of genotypic diversity; E.5, genotypic  
 6 evenness, which is a measure of the distribution of genotypes within a sample; Hexp, Nei's gene  
 7 diversity;  $R_a$ , allelic richness computed using rarefaction,  $R_p$ , private allelic richness computed using  
 8 rarefaction.

1 **Table 3** PERMANOVA of the effects of the stage, county and their interactions for  
2 clone corrected data on the genetic structure of *Erysiphe quercicola* from rubber tree in  
3 Hainan, China<sup>a</sup>

| Source       | df  | SS   | F    | Variation (%) | P     |
|--------------|-----|------|------|---------------|-------|
| Stage        | 2   | 0.09 | 2.31 | 2.57          | 0.002 |
| County       | 2   | 0.06 | 1.39 | 1.55          | 0.16  |
| Stage×County | 4   | 0.13 | 0.03 | 1.63          | 0.02  |
| Residuals    | 166 | 3.39 |      | 92.25         |       |
| Total        | 174 | 3.68 |      | 100           |       |

4 <sup>a</sup> df = Degrees of freedom; SS = Sum of squares; F = F value; P = Probability > F.

1 **Table 4** Analysis of molecular variance (AMOVA) for the clone-corrected data of  
 2 *Erysiphe quercicola* populations from rubber tree based on sample stages or counties  
 3 in Hainan, China

| Source                         | df <sup>a</sup> | Sum of squares | Mean of squares | Variance | Variation % | P <sup>b</sup> |
|--------------------------------|-----------------|----------------|-----------------|----------|-------------|----------------|
| Stage                          |                 |                |                 |          |             |                |
| Among stage                    | 2               | 18.04          | 9.02            | 0.07     | 1.22        | 0.006          |
| Between samples within stage   | 162             | 877.23         | 5.42            | 5.42     | 98.78       |                |
| Total                          | 164             | 895.27         | 5.46            | 5.48     |             |                |
| County                         |                 |                |                 |          |             |                |
| Among county                   | 2               | 12.05          | 6.03            | 0.01     | 0.19        | 0.31           |
| Between samples within county  | 158             | 863.85         | 5.47            | 5.46     | 99.81       |                |
| Total                          | 160             | 875.90         | 5.47            | 5.47     |             |                |
| Cluster <sup>c</sup>           |                 |                |                 |          |             |                |
| Among cluster                  | 3               | 129.85         | 43.28           | 1.20     | 19.47       | 0.001          |
| Between samples within cluster | 145             | 716.86         | 4.94            | 4.94     | 84.64       |                |
| Total                          | 148             | 846.71         | 5.72            | 6.14     |             |                |

4 <sup>a</sup> Degrees of freedom.

5 <sup>b</sup> Probability of variation percentage being significantly greater than zero.

6 <sup>c</sup> Three clusters defined based on DAPC.

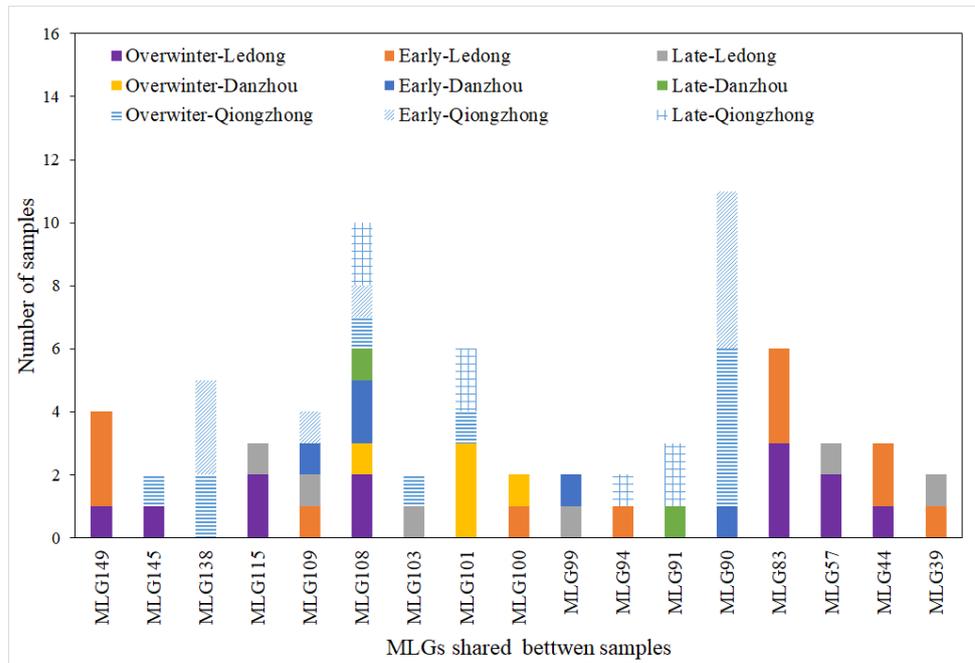
1 **Figure legends**

2

3 **Figure 1** Distribution of multilocus genotypes (MLGs) shared between *Erysiphe*

4 *quercicola* samples of rubber tree collected at different disease epidemic stages from

5 three counties in Hainan.



6

7 **Figure 2** Discriminant analysis of principal components (DAPC) of the *Erysiphe*

8 *quercicola* samples of rubber tree collected at different disease epidemic stages from

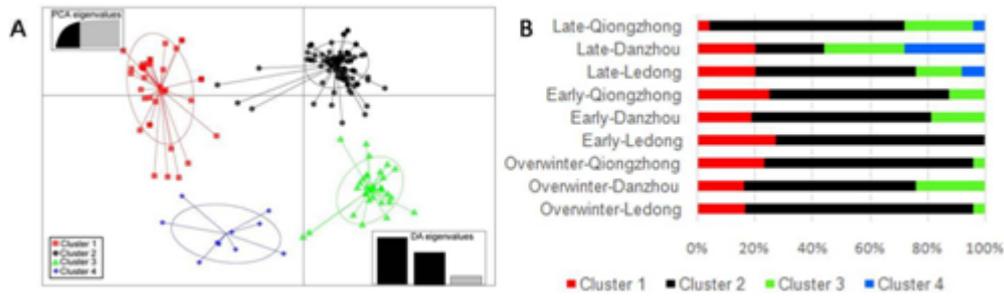
9 three counties in Hainan. A, scatterplot of four clusters based on DAPC. The inset

10 'DA eigenvalues' shows the discriminant analysis eigenvalues retained in the

11 analysis, and the inset 'PCA eigenvalues' shows the number of principal components

12 retained in the analysis and the resulting cumulative variance. B, the *E. quercicola*

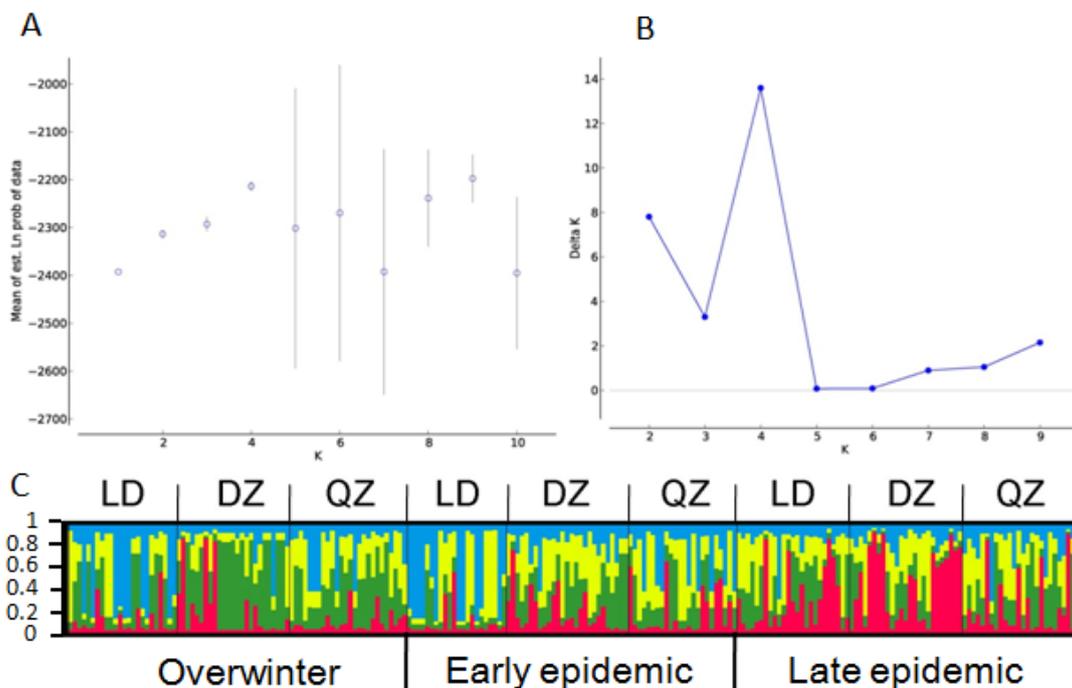
13 sample composition of each cluster.



1

2

3 **Figure 3** STRUCTURE results for the *Erysiphe quercicola* samples of rubber tree  
 4 collected at different disease epidemic stages from three counties in Hainan. A, mean  
 5 estimate of the posterior probability of the data for a given  $K$  ( $\ln P(D)$ ) ( $\pm$  SD). B,  
 6 deltaK curve showing evidence for four genetic clusters. C, different colors represent  
 7 different genetic clusters ( $K=4$ ) with each vertical line representing a sample. LD, DZ  
 8 and QZ represent Ledong, Danzhou, and Qiongzong, respectively.



9

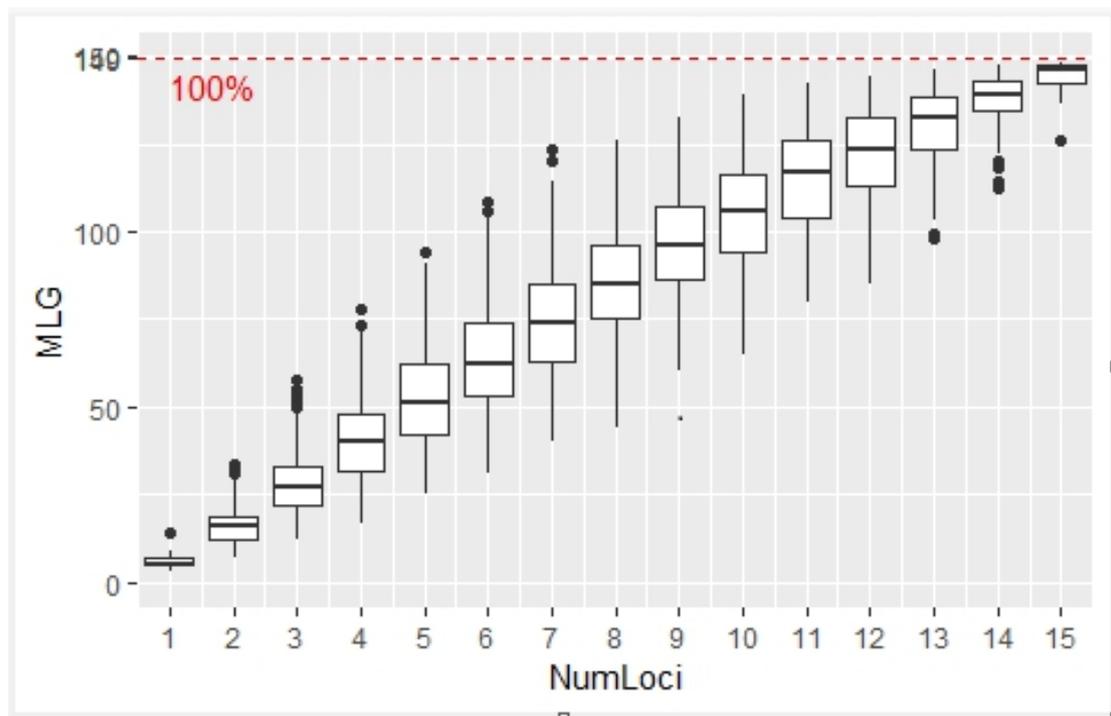
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1 **Supporting information legend**

2

3 **Figure S1** The genotype rarefaction curve for 16 SSR loci used to identify multilocus  
4 genotypes of *Erysiphe quercicola* samples of rubber tree from collected at different  
5 disease epidemic stages from three counties in Hainan.

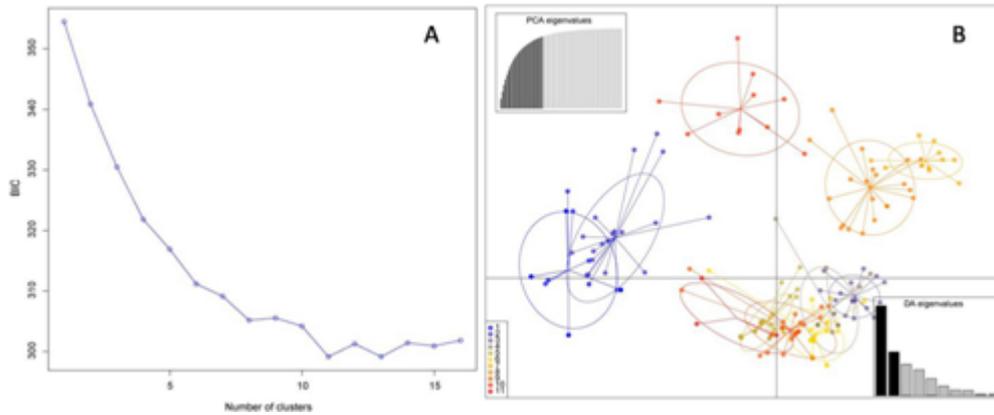


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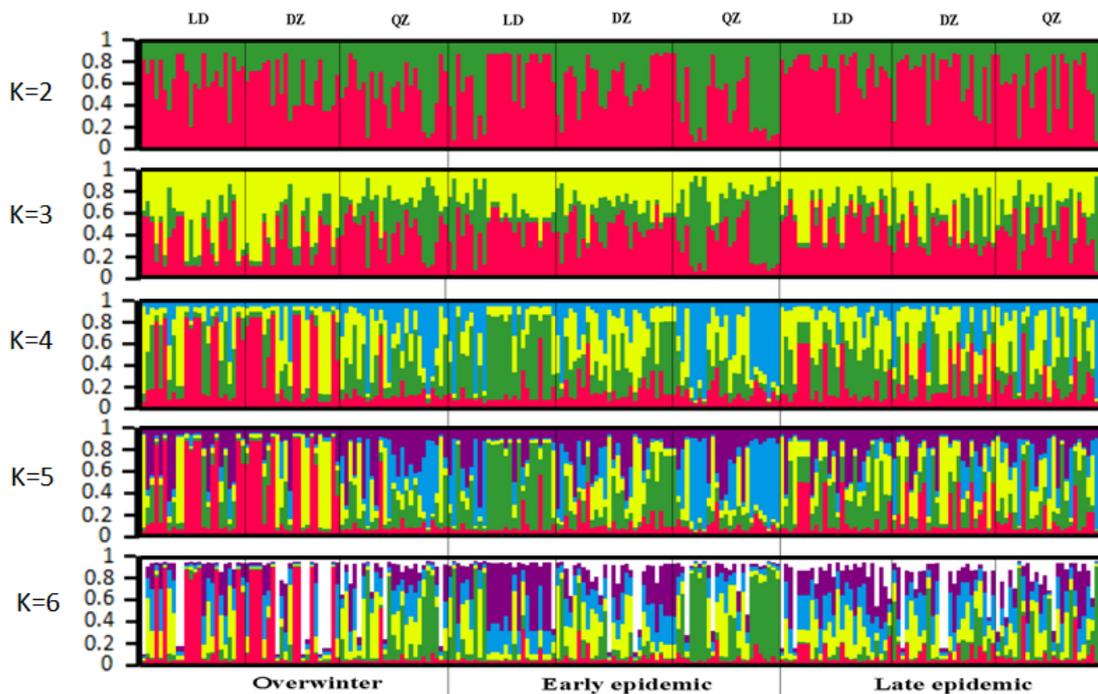
7

8 **Figure S2** Discriminant analysis of principal components (DAPC) of *Erysiphe*  
9 *quercicola* samples of rubber tree based on the lowest associated likelihood (BIC) of  
10 the *K*-means method. A. Bayesian information criterion (BIC) values versus the number  
11 of proposed clusters for the population genetic structure of the *E. quercicola* samples  
12 of rubber tree collected at different disease epidemic stages from three counties in  
13 Hainan. B. Scatterplot for the eleven clusters based on DAPC analysis. . The inset 'DA  
14 eigenvalues' shows the discriminant analysis eigenvalues retained in the analysis, and

1 the inset 'PCA eigenvalues' shows the number of principal components retained in the  
2 analysis and the resulting cumulative variance.



6 **Figure S3** STRUCTURE bar plots of estimated population structure (from K=2 to K=6)  
7 for *Erysiphe quercicola* samples of rubber tree collected at different disease epidemic  
8 stages from three counties in Hainan.



1

2 **Table S1.** Size data for 16 different microsatellite loci from 223 *Erysiphe quercicola*  
3 samples of rubber tree collected at different disease epidemic stages from three counties  
4 in Hainan.

5