Long-lasting coexistence of multiple asexual lineages alongside their sexual counterparts in a fungal plant pathogen

Ammar Abdalrahem[1], Axelle Andrieux[1], Ronan Becheler[1,2], Sébastien Duplessis[1], Pascal Frey[1], Benoit Marçais[1], Solenn Stoeckel[3], Fabien Halkett[1]

1- Université de Lorraine, INRAE, IAM, F-54000 Nancy, France

2- INRAE, Agrocampus Ouest, Université de Rennes, IGEPP, Le Rheu, France

3- DECOD (Ecosystem Dynamics and Sustainability), INRAE, Institut Agro, IFREMER, Rennes, France

Abstract

Sexual to asexual transition is described as a process whereby asexual lineages emerge within sexual species. This phenomenon gives rise to many questions about the maintenance of sexual reproduction and the evolution of asexuality in these organisms. The poplar rust fungus *Melampsora larici-populina* has a complex life cycle, which is typical of rust fungi (Pucciniales). It alternates between a sexual reproduction phase on larch trees (*Larix* spp.) and rounds of asexual multiplication on poplar trees (*Populus* spp.). This alternation between sexual and asexual reproduction shows some similarities with the cyclical parthenogenesis observed in aphids and daphnia. This study challenges the classic understanding of the *M. larici-populina* life cycle. Our results demonstrate the existence of many distinct lineages that reproduce asexually through the years, skipping the sexual phase. We conducted a comprehensive population genetic analysis, utilizing 21 microsatellite markers and data from over 2000 individuals gathered over an extended period of time from various locations in France. A clustering analysis identified a group of multilocus lineages that displayed all hallmarks of the genetic consequences of asexual reproduction, including highly negative and large variance among loci of the inbreeding coefficient (F_{IS}). This indirect evidence for asexual reproduction was confirmed by the direct observation of these asexual lineages being repeatedly sampled over the years, confirming the findings of variations in *M. larici-populina* life cycle with lineages that bypass the mandatory sexual phase. While sexual lineages are predominant throughout France, asexual lineages are more prevalent in the south of the country, due to possible environmental or ecological factors that allow the

overwintering of asexual forms. The discovery of variations in the life cycle in this species offers insights into the evolution from sexual to asexual reproduction encountered in many pathogen species. It could serve as a model organism for studying the transition from sexual to asexual reproductive mode.

Keywords: sexual-asexual transition | evolution of reproductive mode | population genetics | rust fungi | time samples.

I. Introduction

The evolution of sex remains a fundamental question and intriguing topic in evolutionary biology (Culotta & Hines, 1998; Otto, 2009; Hartfield & Keightley, 2012). Sexual reproduction, which has benefits and costs, is widespread in eukaryotic organisms (Schurko et al., 2009). The key benefit is increased genetic diversity, purging deleterious mutations, and enhancing adaptation to changing environments (Pierre et al. 2022). However, it incurs costs such as time and energy to find mates, combined with the biological cost of meiosis (Roughgarden, 1991; Aanen & Hoekstra, 2007; Hörandl, 2013). Asexual reproduction, on the other hand, produces offspring without a mate, allowing for efficient reproduction, maintenance of lineages adapted to local stable conditions and quick multiplication (Shick et al., 1979; Lodé, 2013). The offspring is genetically identical to the parent barring somatic mutations, which renders asexual lineages more vulnerable to the accumulation of deleterious mutations due to a lack of recombination (Lynch et al., 1993; Peck, 1994; Keightley & Eyre-Walker, 2000), and more prone to loose allele diversity by genetic drift (Reichel et al., 2016). The study of species displaying both sexual and asexual reproductive modes enables pinpointing the pros and cons of each mode of reproduction (Butlin, 2002; Simon et al., 2010; Billiard et al., 2012). In particular, studying these species is key to highlighting cases in which asexual reproduction is favored via the dynamics of transitions from sexual to asexual mode.

The success of asexual reproduction frequently relies on specific environmental conditions. Remarkably, invasive species often shift towards asexual reproduction in their exotic range, while reproducing sexually in their native environment (Song et al., 2011; Platt & Jeschke, 2014; Gladieux et al., 2015; Tilquin & Kokko, 2016). Ant species can exhibit such diverse reproductive strategies, with asexual reproduction frequently being dominant in invasive populations, as observed in *Wasmannia auropunctata* and *Cerapachys biroi* (Foucaud et al., 2006; Kronauer et al., 2012). Plant pathogenic fungi also adopt diverse reproductive strategies, with asexual reproduction being responsible for the most damaging epidemics (Ashu & Xu, 2015; Gladieux et al., 2015; Drenth et al., 2019). Potato late blight is a devastating disease caused by the oomycete pathogen *Phytophthora infestans.* Both sexual and asexual reproduction takes place in the native range, but it is asexual reproduction only that led to the devastating Irish Potato Famine in the 1840s (Gavino et al., 2000; Ristaino, 2002; Fry, 2020). The wheat yellow rust fungus *Puccinia striiformis* f. sp. *tritici* displays a similar pattern, with different reproductive modes depending on

65 the region. The fungus reproduces sexually in China and Pakistan, which is highlighted by its high
66 genotypic diversity, whereas it reproduces asexually in the rest of the world (Mboup et al., 2009;
67 Bahri et al., 2009; Ali et al., 2014). The rice blast fungus *Magnaporthe oryzae* (Syn: *Pyricularia*
68 *oryzae*) reproduces sexually in a limited area of southeast Asia, its native range, whereas epidemics
69 globally are driven by asexual lineages (Saleh et al., 2012).

70 The coexistence of both reproductive modes in the same geographical environment is still poorly
71 documented, despite it offering a unique opportunity to decipher the evolutionary and ecological
72 dynamics of sexually or asexually derived lineages. While such studies are rare on fungal
73 biological models, they have been documented in other taxa such as in aphids (Halkett et al., 2006;
74 Simon et al., 2010), daphnia (Lehto & Haag, 2010) and a cyprinid fish *Carassius gibelio* (Barbuti
75 et al., 2012). The phytopathogenic fungus *Melampsora larici-populina*, causing poplar rust,
76 represents an ideal candidate for elucidating the transition of reproductive systems, as both modes
77 can be found in the same environment. *M. larici-populina* belongs to the taxonomic order of
78 Pucciniales (Steenackers et al., 1996). This species follows a complex life cycle with an obligate
79 sexual reproduction that alternates with rounds of clonal multiplication (Figure S1). Two unrelated
80 host plants are required to complete the life cycle (Ørsted, 1863; Hacquard et al., 2011). Clonal
81 multiplication occurs on poplar trees (*Populus* spp.), while sexual reproduction takes place on
82 larch trees (*Larix* spp.) (Lorrain et al., 2019). In the following, lineages that complete the entire
83 life cycle, including the obligate sexual reproduction phase, are referred to as sexual lineages. The
84 sexual reproduction on larch ensures genetic recombination and obliterates all traces of the
85 previous rounds of clonal multiplication (Xhaard et al., 2011; Rouger et al., 2016). The impact of
86 this fungal disease on larch is insignificant, whereas the many rounds of clonal multiplication that
87 occur on poplar leaves from spring to autumn can be responsible for strong epidemics with large
88 nutrient uptake by the fungus and perturbation of photosynthesis (Jiang et al., 2016). Disease
89 symptoms and damage are greatest on cultivated poplars, and resemble those of cereal rusts caused
90 by several *Puccinia* species. A previous population genetic study (Xhaard et al., 2011) has
91 provided evidence for some variability in reproductive mode, with obligate sexual lineages
92 coexisting with genetically distant lineages that display all hallmarks of long-lasting asexual
93 reproduction. This suggests that some lineages of the poplar rust fungus could survive alone
94 through clonal multiplication on poplar trees, as is the case in wheat rust fungi (Goyeau et al.,

95 2006; Mboup et al., 2009; Bahri et al., 2009; Ali et al., 2014). Yet, this preliminary study did not
96 provide detailed investigations on the ecological and evolutionary dynamics of these asexual
97 lineages, largely because it was based on a single year of sampling.

98 In this study, we provide an in-depth characterization of the reproductive strategies of *M. larici-*
99 *populina*, with an emphasis on the (spatio-temporal) persistence of asexual lineages and their
100 genetic characteristics. Our study also examines the ecological and spatial factors influencing the
101 distribution of sexual and asexual populations, thereby offering insights into the evolution of
102 reproductive modes in rust fungi. This study encompasses the analysis of 2122 individuals sampled
103 across France over 30 years of population surveys that have been genotyped using 21 microsatellite
104 markers.

105 We used a two-fold approach to assess the reproductive mode variation among poplar rust
106 individuals. First, we employed an indirect population genetic approach based on the clustering
107 among lineages and subsequent evaluation of the variation in population genetic indices known to
108 be signatures of asexual reproductive mode, namely: repeated genotypes, linkage disequilibrium,
109 and overall negative F_{IS} value and large variance over loci (Balloux et al., 2003; Halkett, Simon,
110 et al., 2005; Stoeckel & Masson, 2014). Second, we benefited from the time sampling to investigate
111 the resampling through years of the same multilocus lineage (MLL), which would be direct
112 evidence for their asexual survival. Furthermore, the genetic relatedness of all isolates was
113 analyzed to investigate evolutionary relationships among asexual lineages and with their sexual
114 counterparts, which points to multiple events of the emergence of asexual lineages. Finally,
115 examining the coexistence of sexual and asexual lineages, their long-term persistence, and their
116 spatial distribution suggested that environmental and ecological factors potentially play a role in
117 shaping the survival and maintenance of asexual lineages.

118

II. Material and Methods

II.1 Isolate collections

In this study, we examined 2122 individuals of *M. larici-populina* sampled from 61 "départements" across France over 32 years (1992-2024). The dataset includes previously published genotypes from the study by Xhaard et al. (2011) collected from a country-wide survey conducted in France in 2009 (23 locations | 428 samples), and more recent collections with genotype data original to this study. These recent data comprise i) samples from a second country-wide survey conducted in 2011 (60 locations | 1374 samples), ii) individuals from the laboratory collection (1992, 1993, 1994, 1996, 1998, 2008, and 2014 | 34 samples), and genotypes from genetic monitoring of poplar rust epidemics in the Durance River valley (Xhaard et al., 2012; Becheler et al., 2016) that were suspected to belong to the asexual group and thus not included in these previous studies (2004, 2008, and 2020 | 246 samples). All these samples were collected in late summer, at the end of the clonal multiplication phase, therefore before leaf fall and lineage engagement into sexual reproduction (Figure S1). In addition, we benefit from the observation of a distinctive poplar stand in Cavalaire-sur-Mer (south of France) that maintains green leaves until mid-winter, where samples were collected in February 2024 (40 samples).

II.2 Genotyping

The DNA extraction process was guided by the BS96-DNA-plant protocol, which employs the BioSprint 96 DNA plant kit and the BioSprint 96 automated workstation (Qiagen). Samples were genotyped using 21 microsatellite loci [Mlp_91, Mlp_83, Mlp_50, Mlp_71, Mlp_96, Mlp_95, Mlp_55, Mlp_66, Mlp_12, Mlp_68b Mlp_54, Mlp_57, Mlp_58, Mlp_49, Mlp_93, Mlp_82b Mlp_56, Mlp_87, Mlp_97, Mlp_77, Mlp_94 (Xhaard et al. 2009)]. These loci have been extensively utilized in several population genetic studies of this species (Xhaard et al., 2011; Becheler et al., 2017; Persoons et al., 2017; Louet et al., 2023; Saubin et al., 2024). The amplification through PCR with the Multiplex PCR Kit (Qiagen), was performed as described by Xhaard et al. (2011). It was conducted across three multiplex reactions containing eight, five, and eight loci, ranked by amplification size, respectively. The PCR products were then pooled and analyzed using an ABI 3730 Genetic Analyzer (Applied Biosystems) by the company GenoScreen. Fragment sizing utilized a LIZ-1200 size standard, and allele scoring was performed with

148 GENEMAPPER V4.0. Individuals that failed to amplify at more than six loci were excluded from
149 further analysis.

150 **II.3 Population genetic and statistical analyses**

151 First, to handle genetic data of microsatellite markers in the R programming language v.4.4.1, we
152 converted the data format from a data frame to a genuine object using the `df2genind` function
153 from the **adegenet** package v.2.1.10. This function optimizes multilocus genotype data and
154 accommodates missing data if it is found in some individuals or loci. The automated workflow
155 code provides full details on subsequent analyses
156 (https://github.com/ammarabdalrahem/poplar_rust.git). Moreover, specific analyses were
157 executed using the GenAPoPop 1.0 software due to specific methodological requirements.

158 *Identification of repeated genotypes:* The prerequisite to an accurate assessment of reproductive
159 mode is the proper delineation of repeated genotypes derived from clonal multiplication (Halkett
160 et al. 2005, Arnaud-Haond et al. 2007). Individuals with the same combination of alleles (the so-
161 called multilocus genotypes, MLG) were consistently identified through both an ad hoc procedure
162 in Excel during the manual curation of chromatographs and using `mlg.filter` from the **poppr**
163 package v.2.9.3 (Kamvar et al., 2015). Genotypes differing by a few alleles were double checked
164 by returning to the original chromatographs. All remaining differences between genotypes are
165 therefore confirmed and reliable. We computed *Pidu* and *N_Pidu* to test for the discrimination
166 power of our marker set, and to rule out the probability of finding repeated MLG by chance (that
167 is deriving from sexual reproduction) using GenAPoPop 1.0 (Evett & Weir, 1998; Stoeckel et al.,
168 2024).

169 *Delineation of multilocus lineages:* As the accumulation of somatic mutations over time can lead
170 to slight differences among multilocus genotypes within a given asexual lineage (Arnaud-Haond
171 et al., 2007), we subsequently grouped MLG into multilocus lineages (MLL). So as not to apply
172 an arbitrary threshold for MLL delineation, we first computed the distribution of pairwise shared
173 allele distance as advised by Arnaud-Haond et al. (2007) and Bailleul et al. (2016). As intra lineage
174 differentiation can vary across MLLs, we chose to use the UPGMA clustering method, which
175 aggregates MLGs one by one by examining the nearest neighbors until the MLL boundaries are

176 found (Kamvar et al. 2014). The threshold on genetic distance between neighbor genotypes was
177 set using the function `cutoff_predictor` from **poppr**.

178 *Clustering procedure to indirectly identify asexual lineages:* As the asexual reproductive mode
179 freezes genotypes it kept ancestral allelic states. Asexual lineages can thus rapidly diverge from
180 their sexual counterparts, the latter being still influenced by genetic drift (Reichel et al. 2016). In
181 line with this statement, we previously found large genetic differences between sexual and asexual
182 groups of *M. larici-populina* (Xhaard et al. 2011). Examining the genetic apportionment within
183 poplar rust individuals would thus be an effective approach to identifying asexual lineages without
184 any *a priori*. The main pitfall of this method being that the clustering algorithm should not rely on
185 gathering individuals according to Hardy-Weinberg expectations, which is the common
186 assumption of any Structure-like approach (Pritchard et al. 2000). We thus carry out our clustering
187 analysis using a PCA based and discriminant analysis approach based on allelic distribution
188 (Jombart et al., 2010). We used to `find.clusters` function from the **adegenet** v.2.1.10 package
189 to reduce the dimensionality of the dataset. Discriminant analysis of principal components
190 (DAPC) was then used to refine the identification of clusters by maximizing the variance between
191 two clusters while minimizing within cluster variance (Jombart et al., 2010), capturing nonetheless
192 97.86% of genetic variation among individuals by retaining 90 principal components. Individuals
193 are grouped into two clusters using the `dpca` function with K=2. To ensure the reliability of the
194 assignment only individuals with a high membership coefficient (≥0.8) are retained for further
195 analyses of within-cluster genetic characteristics.

196 *Resampling MLLs as direct evidence for asexual reproduction.* Observing the same genotype over
197 several years would indicate that this lineage survived asexually (provided that *Pidu* analysis
198 concludes that we can rule out that sexual reproduction can lead to the same combination of alleles
199 being observed by chance in different individuals). To track the persistence of multilocus lineages
200 (MLLs) over time, we recorded the years each MLL was detected and calculated its frequency
201 across different years. A lineage that persisted for at least two years would be considered asexual.
202 We tested the overlap between the direct and indirect approaches for deciphering of asexual
203 reproductive mode by performing Fisher's exact test (Clarkson et al., 1993) on the contingency
204 table of MLL sorting according to the direct or indirect approach by using `fisher.test` function
205 from the **stats** package. In the following, a given MLL is considered asexual if it meets at least

one of the two criteria: the assignment to an asexual cluster or the inter-annual resampling over a minimum of two years.

Computation of classical population genetic indices: As asexual reproductive mode distorts allele repartition within and among individuals, we compute several classical population genetic indices used to characterize within cluster and within MLL genetic signatures. Observed heterozygosity H_O, unbiased expected heterozygosity H_E (Nei, 1978), genotype richness R, and multilocus linkage disequilibrium corrected for the number of loci $\bar{r}_d$ were calculated using the **poppr** package (basic function). Allelic richness A_R (el Mousadik & Petit, 1996), and F-statistics, including the inbreeding coefficient (F_{IS}) (Weir & Cockerham, 1984) were computed using the **Fstat** package. The clonal evenness was measured by calculating the Pareto $\boldsymbol{\beta}$ index which summarizes the distribution in size of MLLs (Arnaud-Haond et al., 2007), using GenAPoPop 1.0 (Stoeckel et al., 2024).

Spatial analysis of the repartition of sexual and asexual lineages: To illustrate the geographical distribution of sexual and asexual individuals across France, the **maps** package v3.4 was utilized. This enabled us to obtain information not only on spatial distribution, but also on the potential coexistence of the two reproduction modes within the same geographical area. Using the `glmer` function from the **lme4** package, a generalized linear mixed model (GLMM) with a binomial distribution and a logit as a link function was employed to assess the regression coefficients (Bolker et al., 2009). To further test the relationship between the reproductive mode variation and the geographic locations we performed a regression analysis on the count of sexual and asexual lineages per site. The independent variables (fixed effect) were the geographic location represented by latitude and longitude. The effect of the year of sampling was added as a random effect. This analysis was performed on the 2009 and 2011 data originating from the two country-wide surveys, not to emphasize particular locations. To further assess the distribution and relative abundance of asexual MLLs across time and space, we performed Fisher's exact test for fluctuation in MLL abundance across years and locations. Given the large size of the data sets, we employed a Monte Carlo simulation approach with 100,000 replicates to estimate p-values.

Genetic relationships between and among sexual and asexual lineages: To further elucidate the evolutionary trajectories of sexual and asexual individuals, and to address the question of whether

there is an investment of sexual individuals in asexual reproduction and vice versa, a discrete matrix based on relative dissimilarity is calculated by `diss.dist` with the **poppr** package between individuals using microsatellite data to create a neighbor-joining tree (Saitou & Nei, 1987) using `nj` from the **ape** package v5.8.1.

Inference of the rate of clonality: Finally, to estimate the rate of clonality in the *M. larici-populina* populations, we employed the ClonEstiMate Bayesian method. This method analyzes the change of genotype frequencies from the same population over generations (Becheler et al., 2017). We calculated based on two scenarios: 1) merging all individuals within a mixed population (that is any individuals can survive asexually by chance) or 2) considering two distinct sexual and asexual populations, based on the lineage sorting detailed above. Since ClonEstiMate method should be applied on temporal samplings of the same kind of populations, we used the data collected during the country-wide survey surveys carried out in the years 2009 and 2011.

III. Result

III.1 Delineation of multilocus lineages

We identified 1475 multilocus genotypes (MLGs) over the 2121 individuals that have been genotyped. The 21 markers we used provided enough statistical power to ensure unambiguous discrimination between genotypes. The overall probability of obtaining the same combination of alleles by chance under panmictic sexual reproduction (*Pidu*) is approximately 10^{-16}. This implies that we would have to genotype 118 million samples before observing two sexually-produced descendants with identical genotypes (*N_Pidu)*. We thus confidently considered that all the repeated genotypes we found were clones. We then grouped closely related MLGs into multilocus lineages (MLLs) to account for the few somatic mutations that could occur during clonal multiplication. The delineation of MLLs was undertaken using a genetic distance threshold of 4.6, as determined by the UPGMA clustering algorithm. This grouping led to the delineation of 1384 MLLs, so slightly fewer than the number of MLGs, showing that the vast majority of MlGs have no closely related genotype.

III.2 Reproductive mode assessment

The clustering approach indicated that almost all individuals exhibited a high probability of membership in one of two genotypic clusters: 445 individuals were unambiguously assigned to cluster one, while 1676 individuals were assigned to cluster two (Figure S2). Only one isolate had membership coefficients below the threshold and was excluded from further analyses.

A comprehensive population genetic analysis of each cluster (Table 1) was conducted to assess the value of key population genetic indices, thereby revealing a contrast between the two clusters. Cluster 1 exhibits a highly negative F_{IS} value, low genotypic diversity, and high linkage disequilibrium (LD), all of which are indicative of a strong rate of clonal reproduction (Balloux et al., 2003; Halkett et al., 2005; Arnaud-Haond et al., 2007). The high variance of F_{IS} across loci further points to rare events of recombination within a population reproducing essentially asexually over the long term. In contrast, Cluster 2 exhibits slightly positive F_{IS} values and higher genetic diversity, suggesting a higher degree of allelic recombination, a feature of sexual reproduction. The reduced linkage disequilibrium in this cluster further supports this classification.

Consequently, Cluster 1 is labeled as asexual, and Cluster 2 as sexual. Noteworthy, we reported similar levels of observed heterozygosity in both clusters, but a much reduced allelic richness and gene diversity in the asexual Cluster 1, In asexual Cluster 1, the *Pareto* β index was low (~ 0.2), indicating a small number of MLGs consistent with clonality. In sexual Cluster 2, the *Pareto* β was greater than 1, reflecting a more even distribution of genotypes due to genetic recombination.

280 **Table 1.** Characteristics of the two genetic clusters identified in *M. larici-populina* individuals.

	N	*MLG*	*MLL*	*R*	*Pareto β*	A_R	H_O	H_E	F_{IS}	*Var* (F_{IS})	$\bar{r}_d$
Clustering approach											
Cluster 1	445	64	11	0.142	0.226	4.372	0.525	0.360	-0.461	0.085	0.356
Cluster 2	1676	1411	1373	0.842	1.078	9.529	0.506	0.543	0.070	0.004	0.016
Resampling approach											
Multi-years	488	90	19	0.183	0.248	5.364	0.516	0.390	-0.322	0.129	0.337
Single-year	1633	1385	1365	0.848	1.057	9.700	0.508	0.545	0.068	0.004	0.012
Combination of approaches											
Asex	499	96	25	0.191	0.252	5.414	0.516	0.393	-0.315	0.123	0.327
Sex	1622	1379	1359	0.850	1.051	9.749	0.508	0.544	0.068	0.004	0.012

281 *MLG:* number of multilocus genotypes, *MLL:* number of multilocus lineages, *N*: number of individuals, *R*: genotypic diversity, *Pareto* β: evenness among repeated genotypes: A_R:
282 allelic richness, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding coefficient, *Var* (F_{IS}): variance of inbreeding coefficient, and $\bar{r}_d$: multilocus linkage
283 disequilibrium.

A resampling approach revealed two groups: about 488 individuals with lineages spanning more than one year due to asexual reproduction and approximately 1,633 sexual individuals without resampled lineages exceeding two years. The contingency table (Table 2), which compares the clustering and resembling approaches, shows a discrepancy. Among the 54 individuals identified as sexual through cluster analysis, subsequent resampling evidence validated their clonality. In contrast, 11 individuals were clustered as asexual; however, no biological validation was available for these cases.

Table 2. Contingency table showing the relationship between clustering approach and resampling approach for reproductive mode definition in the dataset. Values represent the number of individuals assigned to each category.

	Asex (Resampling approach)	**Sex (Resampling approach)**
Asex (Clustering approach)	434	11
Sex (Clustering approach)	54	1622

A Fisher's exact test was performed to evaluate the correlation between the identification of reproductive modes from clustering analysis and resampling validation. The results obtained a highly statistically significant p-value ($< 2.2 \times 10^{-16}$), thereby strongly rejecting the null hypothesis of no association, with a 95% confidence interval. This finding indicates a robust relationship between the two classification methods. Therefore, a combination of clustering and resampling approaches was used to determine the reproductive mode of each individual. All discrepant individuals between both approaches were identified as asexual. The subsequent analysis involved the calculation of the population genetic indices for both groups. The inference of reproductive mode using two approaches and a combination of both, yield consistent results over all population genetic indices, confirming the robustness of the classification (Table 1). We can nevertheless notice slight differences in the values of population genetic indices among classification methods, with the asexual group determined by the clustering approach revealing a stronger signature of asexual reproduction.

III.3 Spatial distribution of reproductive modes

The spatial distribution of sexual and asexual lineages of *M. larici-populina* in France reveals distinct patterns (Figure 1). Sexual lineages are widespread across most regions, predominating in the northern and central parts of the country. In contrast, asexual lineages are more concentrated in the southern regions.

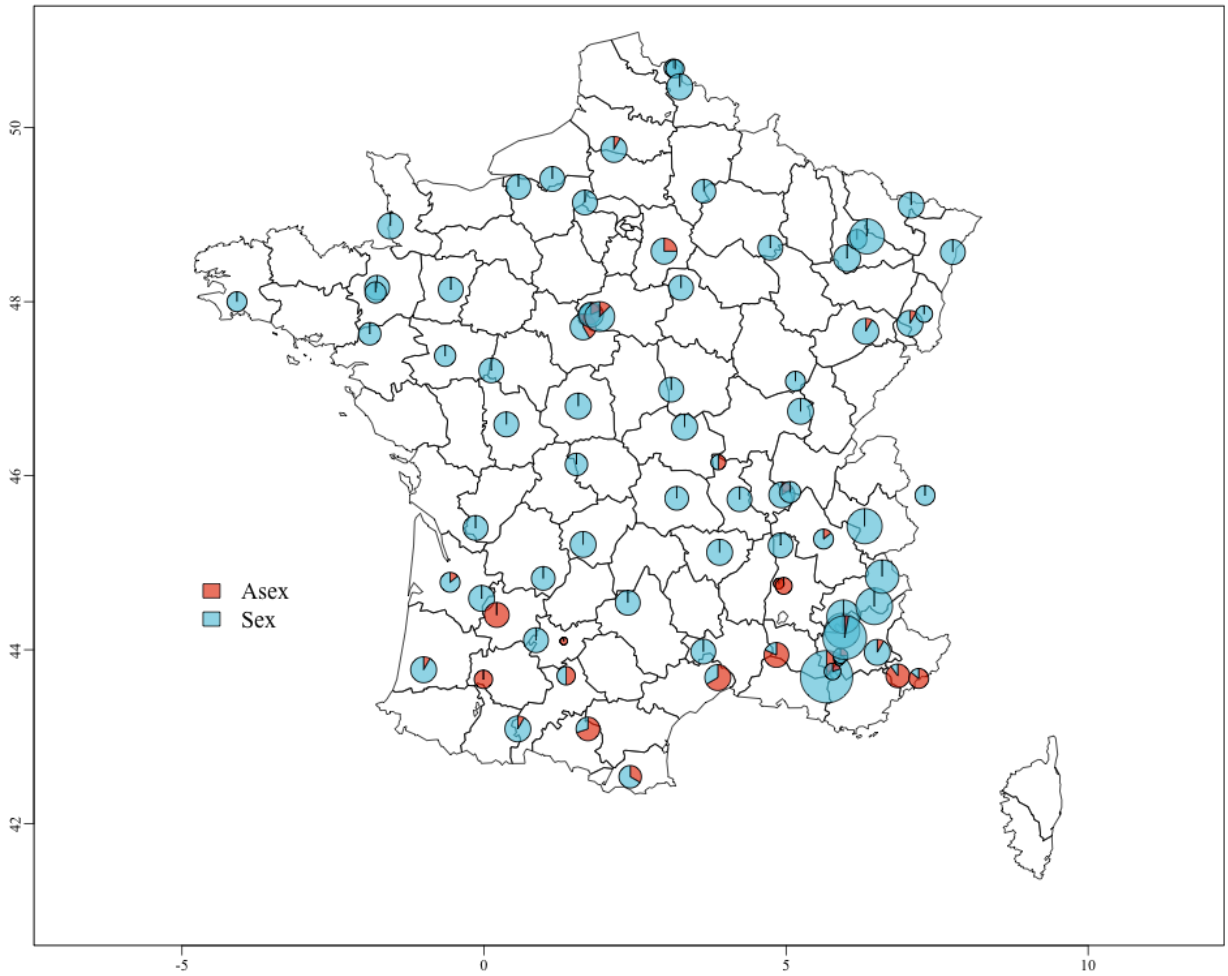

Figure 1

Geographic distribution of sexual and asexual lineages of *Melampsora larici-populina* individuals collected in 2009 and 2011 across France. The pie charts show the proportions of asexual and sexual lineages at each sampling location. The size of each pie chart corresponds to the total number of individuals collected from that site.

To further investigate the factors that influence the prevalence of asexual lineages, GLMM binomial regression analysis was conducted. The analysis (Table 3) revealed a significant effect of both latitude and longitude (Figure S3) on the proportion of sexual and asexual lineages, with positive coefficients indicating that the relative abundance of asexual individuals increases with lower latitude (the closer we get to the south of the country). Additionally, the random effect of years (variance = 18.78, standard deviation = 4.333) indicates that the temporal distribution of individuals is not associated with a specific reproductive mode.

Table 3. Results of the binomial regression model predicting the effect of spatial distribution on the reproductive mode of *M. larici-populina*

Predictor	*Estimate*	*Std. Error*	*z-value*	*p-value*	*Significance*
(Intercept)	-22.99815	2.66959	-8.615	< 2e-16	***
Lat	0.46035	0.04730	9.732	< 2e-16	***
Long	0.09671	0.02912	3.321	0.000896	***

Estimate (regression coefficient), Std. Error (standard error of the estimation), z-value (z-statistic), p-value (statistical significance), and Significance power indicated by asterisks (0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

The ClonEstiMate Bayesian analysis yielded insights into the rate of asexual reproduction in *M. larici-populina*. The 99% credible interval from the posterior probability that was obtained for each group's clonal rates. In the context of a mixed group, where sexual and asexual lineages are merged into one population, the estimated clonal rate was between 0.22 to 0.31, indicating that about 27% of the population reproduces asexually. When the analysis was performed under the assumption of two distinct populations, the clonal rate was between 0.93 to 0.99 for the asexual group and between 0 to 0.21 for the sexual group (Figure 2).

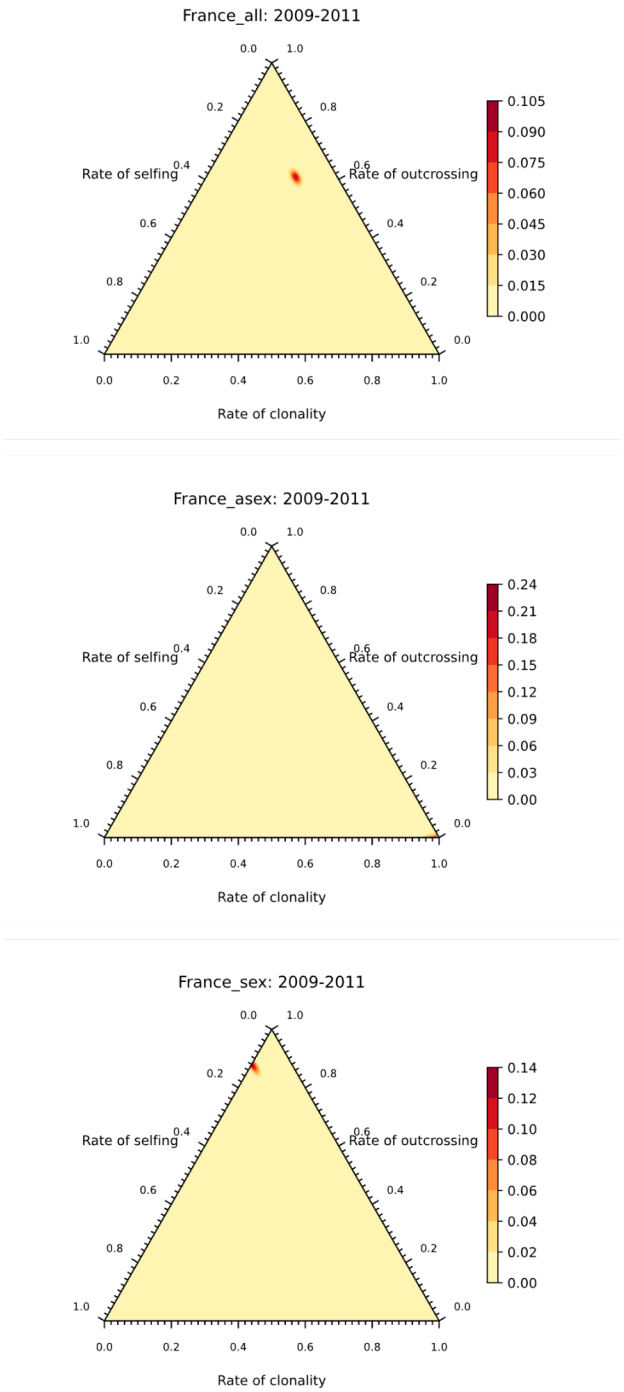

Figure 2

Ternary plot representing the relative proportions of selfing, outcrossing, and clonality rates in three different groups of mixed reproductive, asexual reproductive, and sexual reproductive in France (2009–2011). The color gradient reflects the posterior probability estimates.

III.4 Genetic relationships between sexual and asexual lineages

The dendrogram tree shows different clades for sexual and asexual lineages (Figure 3). This is particularly obvious for six out of the eight most abundant MLLs. However, some asexual lineages also appear as closely related sister branches from the sexual clade. Barring the most abundant MLL9, all of these asexual MLLs that were found closely related to the sexual clade are of small size. These small asexual lineages are thus sporadic, unlike the seven main asexual MLLs that show persistence through time (Figure 3, Table 4). These main asexual MLLs also displayed some reticulation, indicating they consist in different MLGs. In contrast, MLL5 (a main MLL but classified as sexual), consists of individuals sampled only on February 2024 in Cavalaire-sur-Mer that bear exactly the same genotype. This observation underscores the evolutionary relationships and temporal dynamics between reproductive modes within and among M. larici-populina lineages.

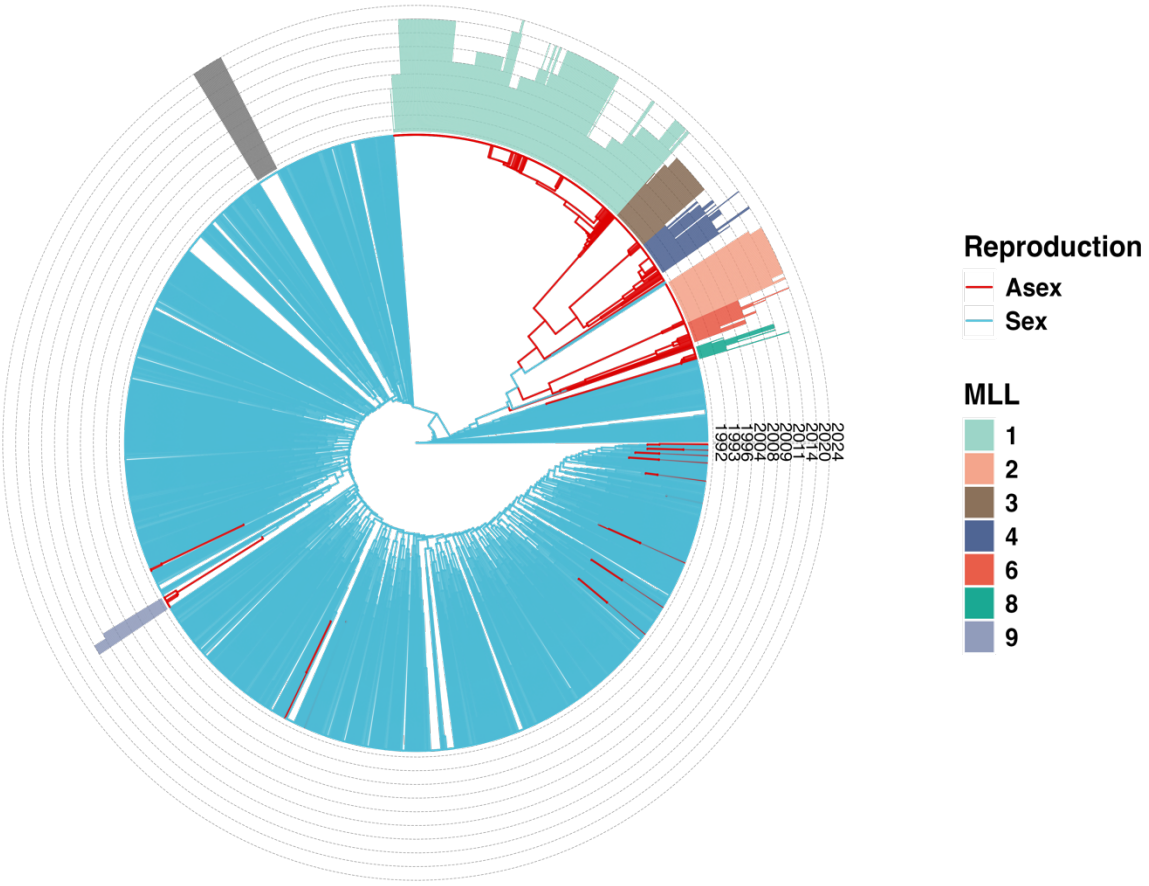

Figure 3

Circular Neighbor-joining (NJ) tree based on microsatellite data (21 markers) illustrating the genetic relationships among *M. larici-populina* individuals. The color of the branches indicates the reproductive mode, with blue representing sexual and red representing asexual modes. The outer rings represent the year of sample collection, with each height corresponding to a year. The nine most abundant multilocus lineages (MLLs) are color-coded.

III.5 Genetic structure and persistence of asexual lineages

A more detailed analysis of the asexual group identified several distinct genetic lineages. Among these, seven lineages were found to be the most abundant. The dominant lineages exhibited varying levels of persistence over the years, as indicated by their range of collection years (Table 4). In particular, MLL1 was consistently detected in almost all collection years and across all regions, highlighting its long-term persistence and widespread occurrence.

Table 4. Characteristics of the seven most abundant asexual multilocus lineages (MLLs) of *M. larici-populina*.

MLL	*MLG*	*N*	*Year Range*	*R*	A_R	H_O	H_E
1	36	280	1992:2024	0.125	1.274	0.519	0.274
2	3	49	2014:2020	0.042	1.290	0.571	0.287
3	4	44	1996:2009	0.070	1.316	0.619	0.312
4	9	38	1996:2020	0.216	1.266	0.499	0.263
6	6	23	1996:2020	0.227	1.196	0.354	0.192
8	5	14	1993:2014	0.308	1.306	0.575	0.296
9	3	14	2009:2011	0.154	1.189	0.350	0.183

MLG: Number of multilocus genotypes, *N*: number of individuals, *R*: Genotypic diversity, Ar: Allelic richness, H_o: Observed heterozygosity, H_s: Expected heterozygosity.

The distribution of these lineages in France varies, with some lineages being more widespread while others are more confined to specific areas (Figure 4).

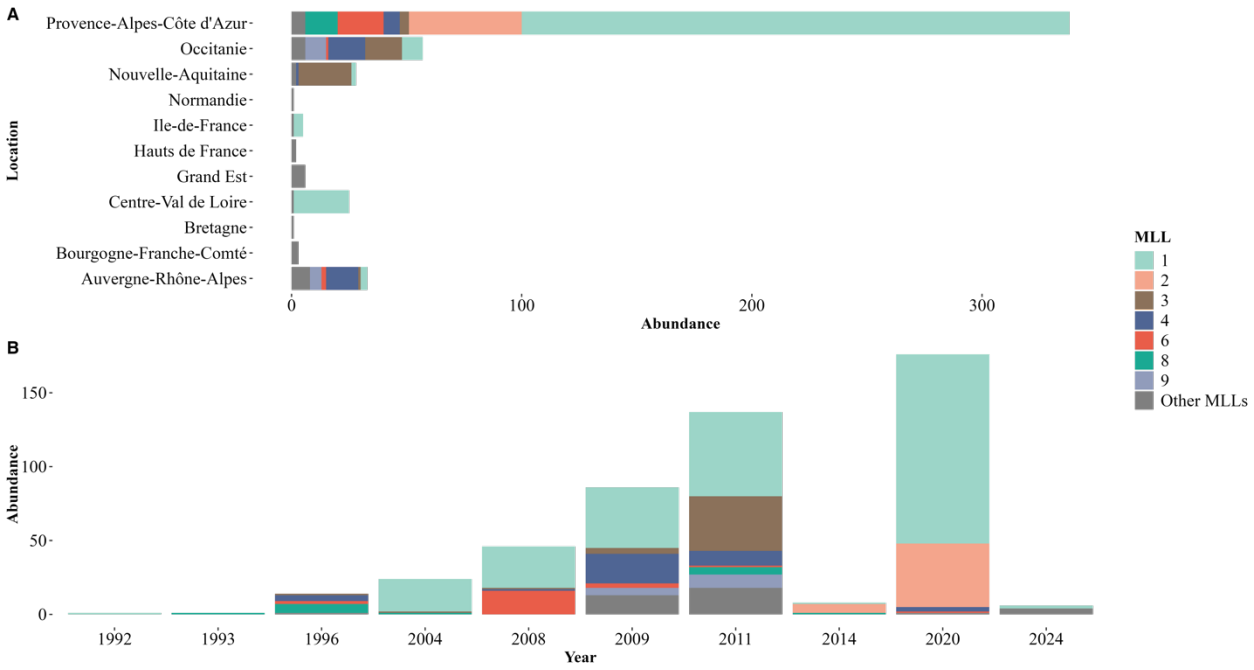

Figure 4

Distribution of asexual lineages over time and across locations. (A) The abundance of asexual MLLs in different French administrative regions. (B) The abundance of asexual MLLs in different years.

Fisher's exact test revealed a highly significant association between MLL distribution and both temporal and spatial factors (P = 1e-05). The analysis revealed that certain MLLs were repeatedly observed in the same geographic regions and over multiple sampling years, suggesting a pattern of persistence. Conversely, other MLLs appear sporadic.

380 # IV. Discussion

381 *Summary of the main findings of this study.* The poplar rust fungus *M. larici-populina* is commonly
382 regarded as an obligate sexual species that needs the presence of both poplars and larches to
383 perform its annual life cycle (Pinon & Frey, 2005; Hacquard et al., 2011). However, a previous
384 population genetic study suggested the existence of a distinct group of individuals that would
385 survive asexually only in poplar hosts (Xhaard et al., 2011). Here 1) we characterized the existence
386 of an asexual group maintaining in the poplar hosts over the years, 2) we found that this asexual
387 group actually consists in specific genetic lineages. We documented that some of these asexual
388 lineages have persisted for nearly three decades despite their coexistence with sexual lineages in
389 some places. These findings challenge the conventional assumptions about the evolutionary
390 dominance of sexual recombination in this species and offer a unique opportunity to investigate
391 the transitions from sexual to asexual reproduction in a plant pathogenic fungus.

392 In the present study, two complementary approaches were employed to reveal and characterize
393 asexual individuals. A clustering approach based on differences in allelic frequency and
394 probability of membership was supplemented by a resampling approach aiming to identify the
395 persistence of lineages over the years. We observed a strong overlap between these indirect and
396 direct approaches, which enables a sound delineation of asexual lineages among *M. larici-populina*
397 individuals. This result further supports the reliability of the clustering approach performed in the
398 initial study of (Xhaard et al., 2011) to identify asexual lineages indirectly. Applying such an
399 individual based clustering approach is all the more relevant that sexual and asexual lineages can
400 be admixed in the same location (Halkett et al., 2006). Consequently, the clustering approach can
401 be regarded as a robust and independent method for detecting cryptic population genetic structures.
402 In addition to the genetic differentiation between sexual and asexual clusters caused by the lack of
403 gene flow, several genetic signatures clearly distinguished asexual lineages thereby reflecting the
404 fundamental differences in evolutionary trajectories between these reproductive modes (Balloux
405 et al., 2003; Halkett, et al., 2005; Reichel et al., 2016). Sexual reproduction ensures the
406 convergence toward Hardy-Weinberg equilibrium (HWE) with recombination among loci that
407 reduces linkage disequilibrium (LD). Asexual reproduction, on the other hand, distorts both allelic
408 and genotypic frequencies mostly because it freezes genotypes (Stoeckel & Masson, 2014;
409 Reichel et al., 2016). As a result, the best way to identify asexual lineages is to apply a multi-

410 criteria method (Kronauer et al., 2012) in which the (diploid) population displays the following
411 hallmarks (Halkett, et al., 2005; Arnaud-Haond et al., 2007): a reduced genotypic diversity
412 combined with a significant excess of heterozygotes (negative F_{IS} values). It should be noted that
413 we also observed a very high variance of F_{IS} across loci, as expected when some recombination
414 events seldom occur (Balloux et al., 2003; Stoeckel & Masson, 2014; Stoeckel et al., 2021). Here,
415 the values found for these indices are consistent with a very high asexuality rate as inferred by
416 ClonEstiMate analysis. The asexual group also displayed significant linkage disequilibrium among
417 loci, which is often reported in asexual and partially asexual species, despite not being consistently
418 expected in neutrally evolving populations (Stoeckel et al., 2021). Linkage disequilibrium was also
419 observed in the sexual group along with a positive F_{IS} value as the result of a Wahlund effect.
420 Indeed, the sexual group gathers different genetic subgroups that have been identified by previous
421 population genetics studies (Xhaard et al., 2011; Persoons et al., 2017) and which are not
422 considered here. Last we observed clone mates in the sexual group too. These can form lineages
423 as abundant as those in the asexual group, with the notable difference that they occur always at a
424 unique sampling site and that, above all, they are never resampled over the years in spite of their
425 local abundance. This local abundance of clone-mate lineages is in accordance with the sampling
426 mostly done in the autumn, at the end of the clonal epidemic phase of *M. larici-populina* life cycle.
427 With this respect, it should be recalled that the mandatory sexual reproduction that takes place on
428 larch resets the population genetic indices in the sexual group each year (Xhaard et al., 2011;
429 Rouger et al., 2016). The contrast in population genetic indices provide a sound framework for
430 distinguishing reproductive modes, supporting the reliable assignment of individuals into either
431 sexual or asexual groups and providing the first insights into their respective evolutionary history
432 (Xhaard et al., 2011).

433 In accordance with the previous study of Xhaard et al. (2011), we found that the asexual group
434 stands was unevenly distributed across the country, and mostly occurred in southern France.
435 Asexual lineages have often been described as distributed at the margins of a species' natural range
436 (Simon et al., 1999; Halkett et al., 2005; Drenth et al., 2019; Pereyra et al., 2023), a phenomenon
437 called the geographic parthenogenesis (Peck et al., 1998). Poplar rust is intimately dependent upon
438 the presence of its two hosts. Although the native range of European Larch (*Larix decidua*) is
439 restricted to the Alps and some other Central-European Mountain ranges, it has been widely

440 planted in many parts of France in mountains and plains since the 18th century. Nevertheless, it is
441 less frequent in the Mediterranean area, which can therefore be considered as a margin for the
442 poplar rust fungus. If we contrast our results with those of other rust fungi, particularly those
443 infecting wheat, we can see a larger spatial segregation between the sexual lineages found only in
444 the disease's center of origin and the asexual lineages that have invaded the crops worldwide.
445 Beyond rusts, this observation is common to many pathogenic species (as for the ascomycete
446 fungus responsible for the rice blast (Saleh et al., 2014), or for the oomycete responsible for the
447 potato late blight (Ristaino, 2002). Interestingly, asexual lineages in these cases are specialized on
448 different cultivars, and therefore on different host genotypes (Goyeau et al., 2007), and the relative
449 abundance of asexual lineages therefore fluctuates over time with the use or disuse of these
450 cultivars (Fontyn et al., 2022). This shows to which extent the asexual evolution of these plant
451 pathogen species is associated with human practices. By contrast, in *M. larici-populina*, asexual
452 lineages are sampled mostly on wild poplar trees and are thus found on different host genotypes.
453 We thus can argue that this rust species enables the incipient stage of sexual to asexual transition
454 to be documented.

455 A puzzling observation is that asexual lineages of *M. larici-populina* coexist with their sexual
456 counterparts in most sampling sites. The absence of larch trees in some areas can be compensated
457 for by the very high dispersal abilities of this species (Barrès et al., 2008), which can be wind
458 dispersed over hundreds of kilometers in a year (Saubin et al., 2024). This can readily explain why
459 sexual and asexual lineages are admixed even if no larch tree exists in the close vicinity of the
460 sampling site. Another non-exclusive hypothesis to account for the long lasting coexistence of
461 sexual and asexual lineages is that of a temporal fluctuation in the environmental conditions that
462 favor the ones over the others. This temporal fluctuation could be responsible for the fluctuations
463 in abundance we observed, with almost half of the asexual lineages sampled in the year 2020 alone.
464 These fluctuations in relative sexual vs. asexual abundances could be linked to peculiar
465 meteorological events that would desynchronize the alternation between poplar and larch during
466 the sexual life cycle and disadvantage sexual lineages that have to complete this complex life cycle.
467 An outstanding example of the effect of a hazardous event on the fate of sexual and asexual
468 lineages was documented in the red alga *Agarophyton chilense* along the Chilean coasts (Becheler
469 et al., 2020). This study monitored wild (mostly sexual) and cultivated (asexually propagated)

470 populations of this red alga before and after a devastating earthquake. Although sexual populations
471 paid a large tribute immediately after the catastrophe, the artificial maintenance of an asexual
472 population caused the algae culture to collapse in the long term, suggesting that each mode of
473 reproduction plays a role in maintaining a dynamic equilibrium.

474 The distribution of asexual lineages of *M. larici-populina* prevailing in southern France points to
475 the first mechanism that can explain their maintenance and diversification. In milder climates the
476 asexual survival could be facilitated by a longer growing period of poplars, creating a so-called
477 green bridge that would enable the rust lineages to jump over the winter from poplar leaves to
478 poplar leaves. According to this hypothesis, the asexual lineages would passively bypass the sexual
479 phase on larch, without the need for any genetic determinism. In support of this hypothesis, it
480 should be noted that (Taris, 1968) experimentally showed that *M. larici-populina* urediniospores
481 could survive for 10 months and still be able to infect poplar leaves. But in these experiments, rust-
482 infected poplar leaves were stored either in a greenhouse, or in a wire cage 2 metres above ground
483 level, which does not mimic real natural overwintering conditions. According to this neutral
484 hypothesis, both the onset and the survival of asexual lineages would be a matter of chance only.
485 In support of this hypothesis, we observed that some asexual lineages branched among the sexual
486 cluster and that these lineages have only been revealed through the resampling over years,
487 indicating they are in very close genetic proximity with the lineages that undergo obligate sexual
488 reproduction each year.

489 The persistence of several asexual lineages over decades, attesting to their evolutionary
490 maintenance and relative success, could also plead in support of some genetic determinism
491 favoring their survival over winter. In support of this hypothesis, the trade-off between
492 overwintering ability and multiplication during the epidemic season has been documented in other
493 fungal species (Hamelin et al., 2016; Suffert et al., 2018), and proved to account for the coexistence
494 and evolutionary maintenance of sexual and asexual lineages (Castel et al., 2014). It is also likely
495 that those long lasting asexual lineages could have lost, at least to some degree, their ability to
496 invest in sexual reproduction, maybe in the benefit of greater asexual survival. Our population
497 genetic analysis, and more especially the high variance of F_{is}, would nonetheless indicate that
498 recombination does not occur among asexual lineages and thus that they do not evolve under strict
499 asexuality but rather as a succession of clones maintaining on poplar leaves over winter, renewed

500 over the years. Furthermore, the rare engagement of asexual lineages into the sexual reproduction
501 can bring additional explanations for the diversity of asexual lineages we observed. In line with
502 the concept of contagious asexuality observed in other organisms with mixed reproductive modes,
503 such as aphids and daphnia (Simon et al., 2002, 2003; Halkett et al., 2006, 2008), asexual lineages
504 that invest in sexual reproduction could be able to produce offspring that inherit their propensity
505 to survive asexually (Halkett et al. 2008). Deciphering further the history and evolutionary success
506 of these asexual lineages would deserve a dedicated experiment to test their performance and
507 investment throughout the life cycle. The complex life cycle of *M. larici-populina* has been
508 mastered in the laboratory (Pernaci et al., 2014), but it remains a challenging experiment that could
509 not be included in the present study.

510 The main original result of our study was to document diversity among asexual lineages in *M.*
511 *larici-populina*, with some lineages persisting over decades and others appearing more
512 sporadically. The fact that sampling campaigns covered the entire French territory twice, and were
513 regularly performed in several locations over more than 30 years, indicate that this pattern could
514 not be attributed to sampling bias. Noteworthy, differences in abundance correlates with
515 differences in the genotypic structure between lineages. We documented that asexual lineages
516 (MLL) can actually consist in different genotypes (MLG), unlike repeated lineages within the
517 sexual group that are the repetition of the same MLG. Interestingly, the most abundant and long
518 lasting asexual lineages were also those displaying the highest genotypic diversity. Here it is
519 important to state that only slight differences distinguish MLG within MLL, in the vast majority
520 up to three loci differ only. Moreover, the changes consist in either heterozygous to homozygous
521 conversion or to increase or decrease in allele length by a one step mutation. This regular pattern
522 of allele changes, found across all MLGs within MLLs, supports the hypothesis that the asexual
523 lineages evolved through single mutation steps. Although mutation rates for the microsatellite
524 markers used in *M. larici-populina* have not yet been estimated, this regular pattern would offer
525 an opportunity to apply a tip calibration approach of molecular clock analysis to estimate the
526 timing of the emergence and persistence of these asexual lineages (Rieux & Balloux, 2016). Yet,
527 the differential abundance and genotypic diversity among asexual lineages reflects heterogeneity
528 within asexual population dynamics and emphasizes the necessity to consider each lineage
529 independently, rather than presuming that the whole asexual population follows the same

530 evolutionary trajectory. The most parsimonious hypothesis is that this diversity has been generated
531 by the repeated emergence of asexual lineages through the evolutionary mechanisms previously
532 discussed (chance of survival or contagious asexuality). Among all asexual lineages, MLL1 stood
533 out. This lineage is the most abundant being repetitively sampled from 1992 and showing an
534 outbreak in 2020. It was detected in all regions where asexual lineages were sampled and forms
535 the only contribution to the unexpected occurrence of asexual lineages northward in the center of
536 France, nearby Paris. The genome of a reference asexual isolate, representative of this MLL, has
537 recently been sequenced, and analysis of its genome structure, polymorphism, and gene content
538 will further document the tale of this dominant asexual lineage.

Data

All analyses were executed using R version 4.2.2 to ensure reproducibility. The relevant scripts and data can be accessed at https://github.com/ammarabdalrahem/poplar_rust.git.

Author Contributions

Sébastien Duplessis, Pascal Frey and Fabien Halkett conceived and designed the study. Axelle Andrieux designed and performed most of the molecular experiments. Ronan Becheler, Ammar Abdalrahem, Axelle Andrieux and Fabien Halkett made the manual curation of genotyping results. Axelle Andrieux, Ronan Becheler and Fabien Halkett did the preliminary analyses of the data. Ammar Abdalrahem performed the final data analyses with help and advice from Benoit Marçais, Ronan Becheler, Solenn Stoeckel and Fabien Halkett. Ammar Abdalrahem and Fabien Halkett prepared the manuscript. All authors revised and approved the manuscript.

Acknowledgments

We thank Jean Pinon for having created the historical poplar rust collection at INRAE Nancy in the 1980s and for having sampled the Cavalaire-sur-Mer population in 2014 and 2024. We warmly thank Kadiatou Schiffer-Forsyth for proofreading an earlier version of the manuscript and **Asmaa Eltahlawy** and her unfailing support. This long-term research program was supported by several grants from the French National Research Agency (ANR-11-BSV7-0007, Clonix project; ANR-12-ADAP-0009, GANDALF project; ANR-13-BSV7-0011, FUNFIT project; ANR-18-CE32-0001, Clonix2D project; ANR-23-CE20-0032, ENDURANCE project). Ronan Becheler was supported by a postdoctoral fellowship from both the Region Lorraine and the French National Research Agency (CLONIX project, ANR-11-BSV7-007). Ammar Abdalrahem was supported by a PhD fellowship from the French Ministry of Education and Research (MESR).

We have used DeepL, Grammarly, and ChatGPT to proofread and suggest synonyms, check grammar and spelling, and debug R scripts. All suggestions made by these tools have been scrutinized and cross-checked by the authors.

Conflicts of Interest

The authors declare no conflicts of interest

Benefit-Sharing Statement

The advantage of this study is that its analysis code is an automated workflow and data are publicly accessible.

References

Aanen, D. K., & Hoekstra, R. F. (2007). Why Sex Is Good: On Fungi and Beyond. In *Sex in Fungi* (pp. 527–534). John Wiley & Sons, Ltd. https://doi.org/10.1128/9781555815837.ch32

Ali, S., Gladieux, P., Rahman, H., Saqib, M. S., Fiaz, M., Ahmad, H., Leconte, M., Gautier, A., Justesen, A. F., Hovmøller, M. S., Enjalbert, J., & de Vallavieille-Pope, C. (2014). Inferring the contribution of sexual reproduction, migration and off-season survival to the temporal maintenance of microbial populations: A case study on the wheat fungal pathogen uccinia striiformis f.sp. tritici. *Molecular Ecology*, *23*(3), 603–617. https://doi.org/10.1111/mec.12629

Arnaud-Haond, S., Duarte, C. M., Alberto, F., & Serrão, E. A. (2007). Standardizing methods to address clonality in population studies. *Molecular Ecology*, *16*(24), 5115–5139. https://doi.org/10.1111/j.1365-294X.2007.03535.x

Ashu, E. E., & Xu, J. (2015). The roles of sexual and asexual reproduction in the origin and dissemination of strains causing fungal infectious disease outbreaks. *Infection, Genetics and Evolution*, *36*, 199–209. https://doi.org/10.1016/j.meegid.2015.09.019

Bahri, B., Leconte, M., Ouffroukh, A., De Vallavieille-Pope, C., & Enjalbert, J. (2009). Geographic limits of a clonal population of wheat yellow rust in the Mediterranean region. *Molecular Ecology*, *18*(20), 4165–4179. https://doi.org/10.1111/j.1365-294X.2009.04267.x

Bailleul, D., Stoeckel, S., & Arnaud-Haond, S. (2016). RClone: A package to identify MultiLocus Clonal Lineages and handle clonal data sets in r. *Methods in Ecology and Evolution*, *7*(8), 966–970. https://doi.org/10.1111/2041-210X.12550

Balloux, F., Lehmann, L., & de Meeûs, T. (2003). The Population Genetics of Clonal and Partially Clonal Diploids. *Genetics*, *164*(4), 1635–1644. https://doi.org/10.1093/genetics/164.4.1635

Barbuti, R., Mautner, S., Carnevale, G., Milazzo, P., Rama, A., & Sturmbauer, C. (2012). Population dynamics with a mixed type of sexual and asexual reproduction in a fluctuating environment. *BMC Evolutionary Biology*, *12*(1), 49. https://doi.org/10.1186/1471-2148-12-49

Barrès, B., Halkett, F., Dutech, C., Andrieux, A., Pinon, J., & Frey, P. (2008). Genetic structure of the poplar rust fungus Melampsora larici-populina: Evidence for isolation by distance in Europe and recent founder effects overseas. *Infection, Genetics and Evolution*, *8*(5), 577–587. https://doi.org/10.1016/j.meegid.2008.04.005

Becheler, R., Guillemin, M.-L., Stoeckel, S., Mauger, S., Saunier, A., Brante, A., Destombe, C., & Valero, M. (2020). After a catastrophe, a little bit of sex is better than nothing: Genetic

consequences of a major earthquake on asexual and sexual populations. *Evolutionary Applications*, *13*(8), 2086–2100. https://doi.org/10.1111/eva.12967

Becheler, R., Masson, J.-P., Arnaud-Haond, S., Halkett, F., Mariette, S., Guillemin, M.-L., Valero, M., Destombe, C., & Stoeckel, S. (2017). ClonEstiMate, a Bayesian method for quantifying rates of clonality of populations genotyped at two-time steps. *Molecular Ecology Resources*, *17*(6), e251–e267. https://doi.org/10.1111/1755-0998.12698

Becheler, R., Xhaard, C., Klein, E. K., Hayden, K. J., Frey, P., De Mita, S., & Halkett, F. (2016). Genetic signatures of a range expansion in natura: When clones play leapfrog. *Ecology and Evolution*, *6*(18), 6625–6632. https://doi.org/10.1002/ece3.2392

Billiard, S., López-Villavicencio, M., Hood, M. E., & Giraud, T. (2012). Sex, outcrossing and mating types: Unsolved questions in fungi and beyond. *Journal of Evolutionary Biology*, *25*(6), 1020–1038. https://doi.org/10.1111/j.1420-9101.2012.02495.x

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Butlin, R. (2002). The costs and benefits of sex: New insights from old asexual lineages. *Nature Reviews Genetics*, *3*(4), 311–317. https://doi.org/10.1038/nrg749

Castel, M., Mailleret, L., Andrivon, D., Ravigné, V., & Hamelin, F. M. (2014). Allee Effects and the Evolution of Polymorphism in Cyclic Parthenogens. *The American Naturalist*, *183*(3), E75–E88. https://doi.org/10.1086/674828

Clarkson, D. B., Fan, Y., & Joe, H. (1993). A remark on algorithm 643: FEXACT: an algorithm for performing Fisher's exact test in r x c contingency tables. *ACM Trans. Math. Softw.*, *19*(4), 484–488. https://doi.org/10.1145/168173.168412

Culotta, E., & Hines, P. (1998). The Evolution of Sex. *Science*, *281*(5385), 1979–1979. https://doi.org/10.1126/science.281.5385.1979

Drenth, A., McTaggart, A. R., & Wingfield, B. D. (2019). Fungal clones win the battle, but recombination wins the war. *IMA Fungus*, *10*(1), 18. https://doi.org/10.1186/s43008-019-0020-8

el Mousadik, A., & Petit, R. J. (1996). Chloroplast DNA phylogeography of the argan tree of Morocco. *Molecular Ecology*, *5*(4), 547–555. https://doi.org/10.1111/j.1365-294x.1996.tb00346.x

Evett, I., & Weir, B. S. (Bruce S.). (1998). *Interpreting DNA evidence: Statistical genetics for forensic scientists*. Sinauer Associates. https://cir.nii.ac.jp/crid/1130282269131400192

Fontyn, C., Zippert, A.-C., Delestre, G., Marcel, T. C., Suffert, F., & Goyeau, H. (2022). Is virulence phenotype evolution driven exclusively by Lr gene deployment in French Puccinia triticina populations? *Plant Pathology*, *71*(7), 1511–1524. https://doi.org/10.1111/ppa.13599

Foucaud, J., Jourdan, H., Breton, J. L., Loiseau, A., Konghouleux, D., & Estoup, A. (2006). RARE SEXUAL REPRODUCTION EVENTS IN THE CLONAL REPRODUCTION SYSTEM OF INTRODUCED POPULATIONS OF THE LITTLE FIRE ANT. *Evolution*, *60*(8), 1646–1657. https://doi.org/10.1111/j.0014-3820.2006.tb00509.x

Fry, W. E. (2020). Phytophthora infestans: The itinerant invader; "late blight": the persistent disease. *Phytoparasitica*, *48*(1), 87–94. https://doi.org/10.1007/s12600-019-00778-3

Gavino, P. D., Smart, C. D., Sandrock, R. W., Miller, J. S., Hamm, P. B., Lee, T. Y., Davis, R. M., & Fry, W. E. (2000). Implications of Sexual Reproduction for Phytophthora infestans in the United States: Generation of an Aggressive Lineage. *Plant Disease*, *84*(7), 731–735. https://doi.org/10.1094/PDIS.2000.84.7.731

Gladieux, P., Feurtey, A., Hood, M. E., Snirc, A., Clavel, J., Dutech, C., Roy, M., & Giraud, T. (2015). The population biology of fungal invasions. *Molecular Ecology*, *24*(9), 1969–1986. https://doi.org/10.1111/mec.13028

Goyeau, H., Halkett, F., Zapater, M.-F., Carlier, J., & Lannou, C. (2007). Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*. *Fungal Genetics and Biology*, *44*(6), 474–483. https://doi.org/10.1016/j.fgb.2007.02.006

Goyeau, H., Park, R., Schaeffer, B., & Lannou, C. (2006). Distribution of Pathotypes with Regard to Host Cultivars in French Wheat Leaf Rust Populations. *Phytopathology®*, *96*(3), 264–273. https://doi.org/10.1094/PHYTO-96-0264

Hacquard, S., Petre, B., Frey, P., Hecker, A., Rouhier, N., & Duplessis, S. (2011). The Poplar-Poplar Rust Interaction: Insights from Genomics and Transcriptomics. *Journal of Pathogens*, *2011*, 716041. https://doi.org/10.4061/2011/716041

Halkett, F., Kindlmann, P., Plantegenest, M., Sunnucks, P., & Simon, J. C. (2006). Temporal differentiation and spatial coexistence of sexual and facultative asexual lineages of an aphid species at mating sites. *Journal of Evolutionary Biology*, *19*(3), 809–815. https://doi.org/10.1111/j.1420-9101.2005.01055.x

Halkett, F., Plantegenest, M., Bonhomme, J., & Simon, J.-C. (2008). Gene flow between sexual and facultatively asexual lineages of an aphid species and the maintenance of reproductive mode variation. *Molecular Ecology*, *17*(12), 2998–3007. https://doi.org/10.1111/j.1365-294X.2008.03798.x

Halkett, F., Plantegenest, M., Prunier-Leterme, N., Mieuzet, L., Delmotte, F., & Simon, J. C. (2005). Admixed sexual and facultatively asexual aphid lineages at mating sites. *Molecular Ecology*, *14*(1), 325–336. https://doi.org/10.1111/j.1365-294X.2004.02358.x

Halkett, F., Simon, J.-C., & Balloux, F. (2005). Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution*, *20*(4), 194–201. https://doi.org/10.1016/j.tree.2005.01.001

Hamelin, F. M., Bisson, A., Desprez-Loustau, M., Fabre, F., & Mailleret, L. (2016). Temporal niche differentiation of parasites sharing the same plant host: Oak powdery mildew as a case study. *Ecosphere*, *7*(11), e01517. https://doi.org/10.1002/ecs2.1517

Hartfield, M., & Keightley, P. D. (2012). Current hypotheses for the evolution of sex and recombination. *Integrative Zoology*, *7*(2), 192–209. https://doi.org/10.1111/j.1749-4877.2012.00284.x

Hörandl, E. (2013). Meiosis and the Paradox of Sex in Nature. In *Meiosis*. IntechOpen. https://doi.org/10.5772/56542

Jiang, Y., Ye, J., Veromann, L.-L., & Niinemets, Ü. (2016). Scaling of photosynthesis and constitutive and induced volatile emissions with severity of leaf infection by rust fungus (Melampsora larici-populina) in Populus balsamifera var. Suaveolens. *Tree Physiology*, *36*(7), 856–872. https://doi.org/10.1093/treephys/tpw035

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. https://doi.org/10.1186/1471-2156-11-94

Kamvar, Z. N., Brooks, J. C., & Grünwald, N. J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, *6*. https://www.frontiersin.org/articles/10.3389/fgene.2015.00208

Keightley, P. D., & Eyre-Walker, A. (2000). Deleterious Mutations and the Evolution of Sex. *Science*, *290*(5490), 331–333. https://doi.org/10.1126/science.290.5490.331

Kronauer, D. J. C., Pierce, N. E., & Keller, L. (2012). Asexual reproduction in introduced and native populations of the ant Cerapachys biroi. *Molecular Ecology*, *21*(21), 5221–5235. https://doi.org/10.1111/mec.12041

Lehto, M. P., & Haag, C. R. (2010). Ecological differentiation between coexisting sexual and asexual strains of Daphnia pulex. *Journal of Animal Ecology*, *79*(6), 1241–1250. https://doi.org/10.1111/j.1365-2656.2010.01726.x

Lodé, T. (2013). Adaptive Significance and Long-Term Survival of Asexual Lineages. *Evolutionary Biology*, *40*(3), 450–460. https://doi.org/10.1007/s11692-012-9219-y

Lorrain, C., Gonçalves dos Santos, K. C., Germain, H., Hecker, A., & Duplessis, S. (2019). Advances in understanding obligate biotrophy in rust fungi. *New Phytologist*, *222*(3), 1190–1206. https://doi.org/10.1111/nph.15641

Louet, C., Saubin, M., Andrieux, A., Persoons, A., Gorse, M., Pétrowski, J., Fabre, B., De Mita, S., Duplessis, S., Frey, P., & Halkett, F. (2023). A point mutation and large deletion at the candidate avirulence locus AvrMlp7 in the poplar rust fungus correlate with poplar RMlp7 resistance breakdown. *Molecular Ecology*, *23*(10), 2472–2483. https://doi.org/10.1111/mec.16294

Lynch, M., Bürger, R., Butcher, D., & Gabriel, W. (1993). The Mutational Meltdown in Asexual Populations. *Journal of Heredity*, *84*(5), 339–344. https://doi.org/10.1093/oxfordjournals.jhered.a111354

Mboup, M., Leconte, M., Gautier, A., Wan, A. M., Chen, W., De Vallavieille-Pope, C., & Enjalbert, J. (2009). Evidence of genetic recombination in wheat yellow rust populations of a Chinese oversummering area. *Fungal Genetics and Biology*, *46*(4), 299–307. https://doi.org/10.1016/j.fgb.2008.12.007

Nei, M. (1978). Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. *Genetics*, *89*(3), 583–590.

Ørsted, A. S. (1863). *Om sygdomme hos planterne, som foraarsages af snyltesvampe, navnlig om rust og brand, og om midlerne til deres forebyggelse: Fire afhandlinger*. JH Schubothes Boghandling.

Otto, S. P. (2009). The Evolutionary Enigma of Sex. *The American Naturalist*, *174*(S1), S1–S14. https://doi.org/10.1086/599084

Peck, J. R. (1994). A Ruby in the Rubbish: Beneficial Mutations, Deleterious Mutations and the Evolution of Sex. *Genetics*, *137*(2), 597–606.

Peck, J. R., Yearsley, J. M., & Waxman, D. (1998). Explaining the geographic distributions of sexual and asexual populations. *Nature*, *391*(6670), 889–892. https://doi.org/10.1038/36099

Pereyra, R. T., Rafajlović, M., De Wit, P., Pinder, M., Kinnby, A., Töpel, M., & Johannesson, K. (2023). Clones on the run: The genomics of a recently expanded partially clonal species. *Molecular Ecology*, *32*(15), 4209–4223. https://doi.org/10.1111/mec.16996

Pernaci, M., De Mita, S., Andrieux, A., Pétrowski, J., Halkett, F., Duplessis, S., & Frey, P. (2014). Genome-wide patterns of segregation and linkage disequilibrium: The construction of a linkage genetic map of the poplar rust fungus Melampsora larici-populina. *Frontiers in Plant Science*, *5*. https://www.frontiersin.org/articles/10.3389/fpls.2014.00454

Persoons, A., Hayden, K. J., Fabre, B., Frey, P., De Mita, S., Tellier, A., & Halkett, F. (2017). The escalatory Red Queen: Population extinction and replacement following arms race dynamics in poplar rust. *Molecular Ecology*, *26*(7), 1902–1918. https://doi.org/10.1111/mec.13980

Pinon, J., & Frey, P. (2005). Interactions between poplar clone and Melampsora populations and their implications for breeding for durable resistance. In *Rust Diseases of Willow and Poplar* (pp. 139–154). https://doi.org/10.1079/9780851999999.0139

Platt, V., & Jeschke, J. M. (2014). Are exotic species red queens? *Ethology Ecology & Evolution*, *26*(2–3), 101–111. https://doi.org/10.1080/03949370.2014.898699

Reichel, K., Masson, J.-P., Malrieu, F., Arnaud-Haond, S., & Stoeckel, S. (2016). Rare sex or out of reach equilibrium? The dynamics of FISin partially clonal organisms. *BMC Genetics*, *17*(1), 76. https://doi.org/10.1186/s12863-016-0388-z

Rieux, A., & Balloux, F. (2016). Inferences from tip-calibrated phylogenies: A review and a practical guide. *Molecular Ecology*, *25*(9), 1911–1924. https://doi.org/10.1111/mec.13586

Ristaino, J. B. (2002). Tracking historic migrations of the Irish potato famine pathogen, Phytophthora infestans. *Microbes and Infection*, *4*(13), 1369–1377. https://doi.org/10.1016/S1286-4579(02)00010-2

Rouger, R., Reichel, K., Malrieu, F., Masson, J. P., & Stoeckel, S. (2016). Effects of complex life cycles on genetic diversity: Cyclical parthenogenesis. *Heredity*, *117*(5), Article 5. https://doi.org/10.1038/hdy.2016.52

Roughgarden, J. (1991). The Evolution of Sex. *The American Naturalist*, *138*(4), 934–953. https://doi.org/10.1086/285261

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Saleh, D., Milazzo, J., Adreit, H., Fournier, E., & Tharreau, D. (2014). South-East Asia is the center of origin, diversity and dispersion of the rice blast fungus, Magnaporthe oryzae. *New Phytologist*, *201*(4), 1440–1456. https://doi.org/10.1111/nph.12627

Saleh, D., Xu, P., Shen, Y., Li, C., Adreit, H., Milazzo, J., Ravigné, V., Bazin, E., Nottéghem, J.-L., Fournier, E., & Tharreau, D. (2012). Sex at the origin: An Asian population of the rice blast fungus Magnaporthe oryzae reproduces sexually. *Molecular Ecology*, *21*(6), 1330–1344. https://doi.org/10.1111/j.1365-294X.2012.05469.x

Saubin, M., Coville, J., Xhaard, C., Frey, P., Soubeyrand, S., Halkett, F., & Fabre, F. (2024). A mechanistic-statistical approach to infer dispersal and demography from invasion dynamics, applied to a plant pathogen. *Peer Community Journal*, *4*. https://doi.org/10.24072/pcjournal.356

Schurko, A., Neiman, M., & Logsdon, J. (2009). Signs of sex: What we know and how we know it. *Trends in Ecology & Evolution*, *24*, 208–217. https://doi.org/10.1016/j.tree.2008.11.010

Shick, J. M., HOFFMANN, R. J., & LAMB, A. N. (1979). Asexual Reproduction, Population Structure, and Genotype-Environment Interactions in Sea Anemones. *American Zoologist*, *19*(3), 699–713. https://doi.org/10.1093/icb/19.3.699

Simon, J.-C., Baumann, S., Sunnucks, P., Hebert, P. D. N., Pierre, J.-S., Le Gallic, J.-F., & Dedryver, C.-A. (1999). Reproductive mode and population genetic structure of the cereal aphid Sitobion avenae studied using phenotypic and microsatellite markers. *Molecular Ecology*, *8*(4), 531–545. https://doi.org/10.1046/j.1365-294x.1999.00583.x

Simon, J.-C., Delmotte, F., Rispe, C., & Crease, T. (2003). Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals: ROUTES TO PARTHENOGENESIS IN ANIMALS. *Biological Journal of the Linnean Society*, *79*(1), 151–163. https://doi.org/10.1046/j.1095-8312.2003.00175.x

Simon, J.-C., Rispe, C., & Sunnucks, P. (2002). Ecology and evolution of sex in aphids. *Trends in Ecology & Evolution*, *17*(1), 34–39. https://doi.org/10.1016/S0169-5347(01)02331-X

Simon, J.-C., Stoeckel, S., & Tagu, D. (2010). Evolutionary and functional insights into reproductive strategies of aphids. *Comptes Rendus. Biologies*, *333*(6–7), 488–496. https://doi.org/10.1016/j.crvi.2010.03.003

Song, Y., Scheu, S., & Drossel, B. (2011). Geographic parthenogenesis in a consumer-resource model for sexual reproduction. *Journal of Theoretical Biology*, *273*(1), 55–62. https://doi.org/10.1016/j.jtbi.2010.12.020

Stoeckel, S., Becheler, R., Bocharova, E., & Barloy, D. (2024). GenAPoPop 1.0: A user-friendly software to analyse genetic diversity and structure from partially clonal and selfed autopolyploid organisms. *Molecular Ecology Resources*, *24*(1), e13886. https://doi.org/10.1111/1755-0998.13886

Stoeckel, S., & Masson, J.-P. (2014). The Exact Distributions of FIS under Partial Asexuality in Small Finite Populations with Mutation. *PLOS ONE*, *9*(1), e85228. https://doi.org/10.1371/journal.pone.0085228

Stoeckel, S., Porro, B., & Arnaud-Haond, S. (2021). The discernible and hidden effects of clonality on the genotypic and genetic states of populations: Improving our estimation of clonal rates. *Molecular Ecology Resources*, *21*(4), 1068–1084. https://doi.org/10.1111/1755-0998.13316

Suffert, F., Goyeau, H., Sache, I., Carpentier, F., Gélisse, S., Morais, D., & Delestre, G. (2018). Epidemiological trade-off between intra- and interannual scales in the evolution of aggressiveness in a local plant pathogen population. *Evolutionary Applications*, *11*(5), 768–780. https://doi.org/10.1111/eva.12588

Taris, B. (1968). Contribution à l'étude des rouilles des Populus observées en France. *Annales Des Épiphyties*, *19*, 5–54.

Tilquin, A., & Kokko, H. (2016). What does the geography of parthenogenesis teach us about sex? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1706), 20150538. https://doi.org/10.1098/rstb.2015.0538

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.2307/2408641

Xhaard, C., Barrès, B., Andrieux, A., Bousset, L., Halkett, F., & Frey, P. (2012). Disentangling the genetic origins of a plant pathogen during disease spread using an original molecular epidemiology approach. *Molecular Ecology*, *21*(10), 2383–2398. https://doi.org/10.1111/j.1365-294X.2012.05556.x

Xhaard, C., Fabre, B., Andrieux, A., Gladieux, P., Barrès, B., Frey, P., & Halkett, F. (2011). The genetic structure of the plant pathogenic fungus Melampsora larici-populina on its wild host is extensively impacted by host domestication. *Molecular Ecology*, *20*(13), 2739–2755. https://doi.org/10.1111/j.1365-294X.2011.05138.x