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Abstract

Massive gene flow between crops and their wild relatives may threaten the genetic integrity of wild species. Such threats are now well documented, but little is known about indirect consequences involving the spillover of crop pathogens to wild plants or introgression between crop and wild pathogens. To address these questions, we used population genetics approaches, demographic inference and pathogenicity tests on host-pathogen pairs composed of wild or domesticated apple trees of Central Asia and their fungal pathogen, *Venturia inaequalis*, itself showing differentiated agricultural-type and wild-type populations. We confirmed the occurrence of gene flow from cultivated to wild apple trees in Asian forests, threatening the Asian wild apple genetic integrity. SNP markers and demographic modeling revealed the occurrence of a secondary contact followed by hybridization between agricultural-type and wild-type fungal pathogen populations, and the dispersal of the agricultural-type pathogen in wild forests. We detected a SNP predicting the ability of the fungus to parasitize the different host populations, which induced an early stop codon in a gene coding for a small secreted protein in the agricultural-type fungal population, thus representing a putative avirulence gene which function loss would enable to parasitize cultivated apples. Pathogenicity tests in fact revealed the pestification of *V. inaequalis*, with higher virulence of the agricultural-type population on both wild and domesticated trees. Our findings highlight the threat posed by cultivating a crop near its center of origin, with the invasion of a pestified pathogen on wild plants and introgression in the wild-type pathogen.

Keywords: apple, crop-to-wild gene flow, hybridization, *Malus*, secondary contact, avirulence gene, *Venturia*

43

Introduction

The domestication of plants corresponds to genetic and phenotypic differentiation between crops and their wild relatives under human selection (Zeder, Emshwiller, Smith & Bradley, 2006). However, crops and their wild relatives often remain interfertile, which can lead to introgression when the two taxa remain geographically close or come into secondary contact (Ellstrand et al., 2013). The hybrids resulting from crop-to-wild pollination events may have a low fitness in natural environments (Wang, Viera, Crawford, Chu & Nielsen., 2017), potentially driving to extinction the wild lineages receiving massive gene flow from its relative crop (Todesco et al., 2016; Wolf, Takebayashi & Rieseberg, 2001). In contrast, hybrids can outcompete pure wild individuals, thus threatening the genetic integrity of wild lineages (Feurtey, Cornille, Shykoff, Snirc & Giraud, 2017; Hooftman, Jong, Oostermeijer & Den Nijs, 2007; Hovick, Campbell, Snow & Whitney, 2012), thereby jeopardizing future adaptation to global changes and leading to a loss of valuable genetic resources for breeding programs.

A much less widely studied, but just as alarming, consequence of secondary contact between crops and their wild relatives, is the possible gene flow between their respective pathogens, which are themselves often closely related. Such gene flow between differentiated pathogen populations or species parasitizing wild and domesticated hosts can promote the emergence of new diseases or the breakdown of resistance, through the generation of pathogens with an expanded host range (Depotter, Seidl, Wood & Thomma, 2016), higher resistance to antibiotics (Hanage, Fraser, Tang, Connor & Corander, 2009) or enhanced virulence (i.e. degree of damage caused by the pathogen to its host) (Stukenbrock, Christiansen, Hansen, Dutheil & Schierup, 2012), thus potentially representing a major threat to both crop health

and wild host persistence (Lemaire et al., 2016; Leroy, Lemaire, Dunemann & Le Cam, 2013; Leroy et al., 2016). Pathogen spillover (*i.e.*, infection by a pathogen on another host than its endemic host) and hybridization between closely related phytopathogenic fungal species following secondary contact have been reported in several systems (Feurtey & Stukenbrock, 2018; Gladieux et al., 2011; Stukenbrock, 2016 a, b). Indeed, when fungal populations have diverged in allopatry and have specialized on new hosts and later met in sympatry (i.e., secondary contact), pre-mating barriers are often weak (Giraud, Refrégier, Le Gac, de Vienne & Hood, 2008; Le Gac & Giraud, 2008) and they can hybridize and/or still infect their ancestral host. The resulting spillover and hybridization events can lead to disease emergence and/or increased disease severity (Ioos, Andrieux, Marçais & Frey, 2006; Lin et al., 2007; Newcombe, Stirling, McDonald & Bradshaw, 2000; Stukenbrock et al., 2012). In anther-smut *Microbotryum* fungi for example, two sister castrating pathogens parasitize two sister plant species and co-occur in sympatry as a consequence of a secondary contact following allopatric divergence (Gladieux et al., 2011). One of the two species, *Microbotryum lychnidis-dioicae* (DC. ex Liro, Deml & Oberw.), is however better at infecting both hosts, and spillovers occur in nature (de Vienne, Hood & Giraud, 2009; Gladieux et al., 2011). In poplars, hybrid trees were generated and planted as they were resistant to the two rust species parasitizing the two parental tree species. However, a fungal hybrid between the two rust species rapidly emerged that was able to cause disease on the hybrid poplars (Newcombe et al., 2000). Yet, the consequences of crop-to-wild gene flow on the evolution of wild pathogen populations and on the disease of their wild host has been little investigated. Joint analyses of both wild and agricultural hosts and pathogens are required for a comprehensive understanding of the consequences of secondary contacts of crops and their wild relatives, and of their pathogens, as well as for elucidating coevolutionary histories and local adaptation.

Yet, such studies combining both host and pathogen population genetic analyses remain scarce (Croll and Laine 2016).

Here, we addressed these questions on apple trees and their apple scab fungal pathogen, *Venturia inaequalis* (Cooke) G. Winter, which represent good models for investigating the consequences of secondary contact between crops and their wild relatives. They indeed have both wild and agricultural differentiated species/populations that have recently been reunified in their center of origin and may thus possibly hybridize (Figure 1). The cultivated apple (*Malus domestica* Borkh.) is the most cultivated fruit tree of temperate areas worldwide (FAO STATS from 2008-2018, as accessed on 04-07-2020). Analysis of phenotypic diversity, genetic markers, archeological data and demographic inference showed that the cultivated apple was initially domesticated from *M. sieversii* Ledeb. M. Roem (Figure 1A), forming quasi-monospecific natural forests of wild apple in the Tian Shan Mountains in Central Asia (Cornille et al., 2012; Harris, Robinson & Juniper, 2002; Vavilov, 1931; Velasco et al., 2010), which have been declared a World Heritage Center by UNESCO. The cultivated apple was imported into Europe by the Romans *via* the Caucasian and Mediterranean coasts, crossing the distribution ranges of various wild apple tree species on the way, leading to major secondary contributions to the cultivated apple tree genepool from several crabapples (Cornille et al., 2012; Cornille, Gladieux & Giraud 2013; Nikiforova, Cavalieri, Velasco & Goremykin, 2013). There has been in particular, in Western Europe, an important contribution from the European crabapple, *M. sylvestris* (L.) Mill, to the *M. domestica* genome (Figure 1A). Domestication and breeding has led to high differentiation between *M. domestica* and its various wild progenitors (Cornille et al., 2012). However, because apple species generally have weak interspecific barriers, introgression can still occur between wild and cultivated

forms (Cornille et al., 2012; Nikiforova et al., 2013). This situation raises serious concerns regarding possible gene flow and its deleterious consequences for the conservation of important wild genetic resources. Yet, most studies on gene flow between domesticated and wild apples have focused on the European wild apple tree. These studies have shown that extensive crop-to-wild gene flow is threatening the genetic integrity of the European crabapple *M. sylvestris* (Cornille et al., 2015; Feurtey et al. 2017; Figure 1A). In Central Asia, at the end of the 19th century, western European *M. domestica* apple varieties have been planted in orchards close to *M. sieversii* forests, leading to recent secondary contact between *M. domestica* and *M. sieversii*. A couple of studies have suggested the occurrence of gene flow from *M. domestica* to the Asian crabapple *M. sieversii*, although without investigating the contact sites that orchards represent (Cornille et al. 2012; Omasheva et al., 2017). Such gene flow may affect the genetic integrity of *M. sieversii*, which would amplify the current threat on Asian wild-apple forest ecosystems, which are already endangered by forest destruction, *M. sieversii* being included in the International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List) as a vulnerable organism.

Apple domestication has also fostered divergence in the ascomycete fungus *V. inaequalis*, a haploid fungus with an obligatory sexual reproduction event each year. This fungus is responsible for the apple scab disease, producing gray-brown lesions on leaves and fruits, and leading to major economic losses on cultivated apples. *Venturia inaequalis* parasitizes *M. domestica* and several wild apple tree species, such as *M. sieversii*, *M. sylvestris* and *M. floribunda* (Siebold ex Van Houtte), and differentiated populations occur on these different *Malus* species (Figure 1B; Gladieux et al., 2010b). Apple trees and *V. inaequalis* share a common geographical origin in Central Asia (Cornille et al., 2012; Gladieux et al., 2008), *M.*

sieversii being the wild host of origin of the fungus (Gladieux et al., 2010b). In the Central Asian Mountains, in forests where *M. domestica* is absent, a wild-type *V. inaequalis* population that represents a relic of the ancestral population has been found on *M. sieversii* trees (Gladieux et al., 2010b). An agricultural-type *V. inaequalis* population has been found in Central Asia (Gladieux et al., 2010b) in the peri-urban or agricultural environments on *M. domestica* and on *M. sieversii* (Figure 1B). These wild-type and agricultural-type populations of *V. inaequalis* began diverging in Central Asia between 2,000 and 4,000 years ago (Gladieux et al., 2010b), the agricultural-type population having then spread into Europe together with the domesticated apple (Figure 1B; Gladieux et al., 2010b). It has not been studied yet whether the current co-occurrence of the agricultural-type and wild-type *V. inaequalis* populations represents a secondary contact between the two fungal populations, and what are its epidemiological consequences through potential spillover and introgressions (Wang et al., 2017). Indeed, gene flow between wild-type and agricultural-type *V. inaequalis* populations may lead to the emergence of hybrids harboring new epidemiological traits, potentially harmful to both wild and cultivated apple trees, and/or to the dispersal of the agricultural-type population into wild forests. As a matter of fact, previous studies have shown that the *V. inaequalis* population parasitizing *M. floribunda*, and highly differentiated from all the populations on *M. sieversii* and *M. sylvestris*, migrated to resistant varieties of *M. domestica*, which promoted a resistance breakdown and gene flow between pathogen populations (Figure 1B; Lemaire et al. 2016; Leroy et al. 2016).

Despite the importance of wild Asian apple trees as an endangered wild species and as a valuable genetic resource for future breeding programs, the potential impacts of the secondary contact between domesticated and wild apple trees in Central Asia on the fitness of both the

plant and its associated pathogen have not been investigated yet. The lack of diagnostic morphological features for apple species makes the use of genetic markers necessary for species and hybrid identification (Cornille, Giraud, Smulders, Roldán-Ruiz & Gladieux, 2014). We therefore used microsatellite markers in apple trees and genome-wide single-nucleotide polymorphisms (SNPs) in *V. inaequalis*, together with pathogenicity assays, to assess the occurrence and impact of secondary contact on both fruit trees and their fungal pathogens in their center of diversity. First, we investigated whether the introduction of domesticated apple trees in Central Asia threatened the genetic integrity of *M. sieversii* wild apple trees via introgressions, and whether orchards planted in forests represent particular threats. We then assessed whether the agricultural-type fungal population occurred on wild apple trees and hybridized with the wild-type population. We tested, using demographic modeling and scenarios comparison, the hypothesis that the co-occurrence and hybridization of agricultural-type and wild-type *V. inaequalis* populations resulted from a secondary contact at the time when domesticated trees were introduced in orchards near wild apple forests, after a period of allopatry. We also tested whether some genomic regions in *V. inaequalis* were good predictors of its ability to parasitize the different host populations. Finally, we tested, using experimental inoculations, whether such gene flow increased the threats to the wild apple tree through increase in virulence of the pathogen populations.

Material and Methods

Sampling

Plant and fungal materials were sampled in 2012 at nine sites in Kazakhstan in the Tian Shan Mountains, the origin center of the apple tree domestication where the natural forest is

183 dominated by wild apple trees (Cornille et al., 2014). Seven of the nine sampling sites were
184 located in natural apple forests, farther than dozens of kilometers from any orchard. The two
185 remaining sampling sites corresponded to a 20-30 year-old orchard and the wild natural forest
186 immediately surrounding the orchard, respectively. We sampled apple trees in the orchard site
187 and wild apple trees in the surrounding forest. This orchard was situated directly in the middle
188 of the natural forest and constituted a rare situation allowing to investigate the consequences
189 of the secondary contact for both the host and the pathogen. We obtained plant material from
190 245 apple trees, originating from the nine sites. The 245 plant samples corresponded to 185
191 leaves and 60 fruit skin pieces, all harbouring visible scab lesions (Table S1). No
192 morphological differences between *M. domestica* and *M. sieversii* allow to distinguish them
193 reliably in the field. Therefore, all sampled trees were genotyped with 33 microsatellite
194 markers and were assigned to *M. domestica* or *M. sieversii* species or to a hybrid class using
195 as references 50 apple genotypes previously characterized as being non-hybrid *M. domestica*
196 genotypes and 25 apple genotypes previously characterized as being non-hybrid *M. sieversii*
197 genotypes (Cornille et al., 2012).

198 Monospore isolation of the fungus was performed from disease lesions on 205 of these 245
199 trees: 24 to 48 h after spreading a spore suspension obtained from the diseased material on a
200 Petri dish with malt-agar medium, we took a single germinated spore using a needle under a
201 stereomicroscope for culture on a new Petri dish. We performed one monospore isolation per
202 tree for 148 trees and two to four monospore isolations per tree for 57 trees. In total, we
203 obtained 269 strains (Table S1). In addition to the fungal collection sampled for this study, we
204 included in our final dataset 15 *V. inaequalis* strains previously assigned to the pure wild type
205 population and 18 *V. inaequalis* strains previously assigned to the agricultural-type population
206 (Gladieux et al., 2010b). These 33 *V. inaequalis* reference strains whose genomes have been

previously sequenced (Le Cam et al., 2019) had been sampled in Kazakhstan on *M. sieversii,* either from a peri-urban environment near Almaty (43°15000N-76°54000E) or from a natural forest site in the Tian Shan Mountains (43°13807N-77°16783E). Despite being sampled on *M. sieversii*, the 18 strains from the peri-urban environment clustered with strains sampled on domesticated apples in Kazakh orchards, thus belonging to the agricultural type (Gladieux et al., 2010b) (Figure 1B; Table S2).

SNP design and genotyping for the apple scab fungus *Venturia inaequalis*

We used the set of single nucleotide polymorphisms (SNPs) previously detected in the genomes of the 15 wild-type and 18 agricultural-type reference strains (Gladieux et al., 2010b; Le Cam et al., 2019). SNPs were filtered in order to avoid transposable elements and AT-rich regions, as well as more than 20% of missing data (Le Cam et al., 2019). After filtering, the dataset contained 1,210,121 SNPs, with no bias of missing data frequencies between wild and agricultural populations. In order to identify a set of SNPs distinguishing agricultural and wild-type *V. inaequalis* strains, we selected 192 SNPs among the 1,210,121 available SNPs as those meeting three requirements: *i)* with moderate (F_{ST} = 0.21) to high (F_{ST} = 1) differentiation between wild-type and agricultural-type reference populations (Figure S1, Table S3), to obtain reasonable statistical power to assign genotypes to wild, agricultural and hybrid types, *ii)* physically distant enough to ensure lack of linkage disequilibrium between them (correlation coefficient between markers within population $r^2 <$ 0.1), and iii) found in predicted genes (Le Cam et al., 2019).

For each monospore strain, DNA was extracted from 30 to 40 mg of mycelium as previously described (Leroy et al., 2016). The 269 strains were genotyped at the 192 SNP markers (Table

S3) using the *KASPAR* method (KBioscience, competitive allele-specific polymerase chain reaction assay). *KASPAR* genotyping was performed at the *Gentyane* platform (INRA, Clermont-Ferrand, France) using 10 ng of haploid DNA mixed with the KASP genotyping master mix (Catalogue number: KBS-1016-017, LGC Genomics, Hoddesdon, UK) and custom KASP SNP assays (LGC Genomics, Hoddesdon, UK). Only SNPs and individuals with less than 20% missing data were kept for analyses. After filtering for missing data, the dataset was composed of 255 fungal strains genotyped for 181 SNPs. Details about SNP location on the *V. inaequalis* reference genome are given in Table S3.

Genotyping using microsatellite markers for the host apple tree

In order to assign trees to wild, domesticated and hybrid types, all apple trees were genotyped using microsatellite markers. Apple tree DNA was extracted from leaves with the NucleoSpin plant DNA extraction kit II® (Macherey & Nagel). Microsatellite PCR amplifications were performed with a Multiplex PCR Kit® (QIAGEN, Inc.). To genotype the 245 trees, we used 33 microsatellite markers spread across the 17 chromosomes using 10 different multiplex reactions, as previously described (Cornille et al., 2012). The genotyping was done at the *Gentyane* platform (INRA, Clermont-Ferrand, France). Only the microsatellite markers with less than 30% of missing data were used and only individuals with less than 50% of missing data were kept for the analyses. The final dataset was thus composed of 240 apple tree samples genotyped at 28 microsatellite markers.

Since fungi were sampled on the genotyped host trees, we had genotypes for host and pathogen pairs. We obtained genotypes for 249 host-pathogen pairs, several fungal samples originating from the same trees (132 trees with one fungal strain, 49 trees with two strains, five trees with three strains and one tree with four strains).

254

255 **Analyses of diversity, differentiation and hybridization**

256 Reference genotypes of *M. domestica* (N=50) and *M. sieversii* (N=25) (Cornille et al., 2012)
257 were used to estimate the proportion of ancestry from these two species in the 240 genotyped
258 apple trees. We computed a hybrid index (*HI*) implemented in the add-on R-package
259 *Introgress* (Gompert & Buerkle, 2010), an estimate of the proportion of alleles that were
260 inherited from one of the two parental populations, *i.e.* the level of ancestry in one of two
261 populations. In our case, we set pure *M. domestica* trees to *HI*=1 while *M. sieversii* had *HI*=0.
262 This index therefore represents the proportion of ancestry in the *M. domestica* genepool (thus
263 called here P_{dom}). For the pathogen, we used the same package to compute a hybrid index
264 based on the reference genotypes of strains previously identified as belonging to the
265 agricultural-type population (N=18) or to the wild-type (N=15) population (Gladieux et al.,
266 2010b). Here, we set $HI = 1$ for the agricultural type, so that the hybrid index represents the
267 proportion of ancestry in the agricultural-type population (here called P_{agr}) genepools.

268 In most of our analyses, we used the hybrid indices as continuous values, and not discrete
269 classes, to avoid potential biases generated by arbitrary thresholds. Only for the analyses of
270 apple and fungal population or species spatial distribution, of the genetic determinants of
271 adaptation and of pathogenicity tests, we assigned apple and fungal genotypes to discrete
272 classes. Apple tree genotypes with a P_{dom} index below 0.2 and those with a P_{dom} index greater
273 than 0.8 were considered to belong to *M. sieversii* and *M. domestica*, respectively, based on
274 the distribution of the hybrid index (Figure 2A). A fungal strain with a P_{agr} below 0.1 was
275 considered to be a wild-type strain, whereas a strain with a P_{agr} greater than 0.9 was
276 considered to be an agricultural-type strain, based on the distribution of the hybrid index
277 (Figure 2B). The threshold was more stringent for fungi than for trees, as more markers were

available and the power was, therefore, higher. For both pathogens and hosts, genotypes with intermediate hybrid index values were considered to be hybrids. Assigning hybrids to more precise hybrid classes (*ie* F1, F2 or backcrosses) is not possible with the current dataset for haploid organisms such as *V. inaequalis*. When we used discrete classes for analyses, we checked that the choice of the thresholds did not affect the inference.

For both apple and *V. inaequalis* samples, we also investigated genetic differentiation and species or genetic lineage assignment through principal component analyses (PCAs) using the ade4 R package (Dray & Dufour, 2007) based on the multilocus genotypes (microsatellite markers for trees and SNPs for fungi). We further investigated the existence of population structure within apple tree species and within the previously delimited *V. inaequalis* populations using the software STRUCTURE v.4.3 (Falush, Stephens & Pritchard, 2003) based on 10 runs of 250,000 Monte Carlo Markov Chain (MCMC) iterations after a burn-in of 25,000 iterations using the admixture model and assuming a number of clusters ranging from 1 to 6. The output of STRUCTURE was processed with Clumpp (Jakobsson & Rosenberg, 2007) and we used the method described in Evanno et al. (2005) and implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012) to detect the strongest population subdivision level. We tested the significance of correlations between the hybrid degrees inferred from coordinate values of the first PCA axis, the hybrid index and the ancestry probability given by STRUCTURE using a Pearson's product-moment correlation coefficient in R.

For both apple and *V. inaequalis* populations, we estimated classical population genetics statistics, i.e., expected heterozygosity (H_e, Weir & Cockerham, 1984), as well as global and pairwise differentiation F_{ST} (Weir & Cockerham, 1984) between the nine sampling sites. All statistics were computed and tested for significance using GENEPOP v.4.7.5 (Rousset, 2008).

Significativity of global and pairwise F_{ST} was assessed using Fisher's exact tests of allele frequency differences between sampling sites with 10,000 dememorization and batches and 10,000 iterations per batch. We also performed Mantel tests of correlation (Mantel, 1967) between pairwise F_{ST} matrices computed for apple and *V. inaequalis* populations on the one hand, and between each pairwise F_{ST} matrix and a matrix of geographical distance between sampling sites on other hand, using the *mantel.test* function of the ape v5.3 R package (Paradis & Schliep, 2019).

Genetic footprints of host adaptation in *Venturia inaequalis*

In order to detect genetic signatures of adaptation to host species in *V. inaequalis* populations, we performed a discriminant analysis on principal components (DAPC; Jombart, Devillard & Balloux, 2010) implemented in the R package *adegenet v.2.12* (Jombart, 2008). Unlike PCA which focuses on overall genetic variance, this procedure seeks variables, the discriminant functions, that maximize differences between *a priori* delimited groups, while minimizing the variance within these groups. We applied this analysis by defining groups in *V. inaequalis* based on the assignment of their apple tree groups of collection (*M. domestica*, *M. sieversii* and hybrids). This approach also aims at detecting the genetic factors (here SNPs) that contributed the most to this ecologically-based clustering. If one of the 181 SNPs in *V. inaequalis* was closely linked to a genetic variant involved in adaptation to host species, its loading on the first discriminant functions would indeed be greater than that of other SNPs. In addition, we estimated monolocus pairwise F_{ST} between the three host-based fungal clusters, using Genepop v4.7.5 (Rousset, 2008).

To assess what allele was ancestral or derived at the SNP found to predict the host species of collection, we used five available genomes (Le Cam et al., 2019) belonging to the outgroup *V. inaequalis* lineage parasitising Pyracantha (Gladieux, Caffier, Devaux & Le Cam, 2010a; Le Cam, Parisi & Arene, 2002), with the following NCBI accession numbers SAMN07816619, SAMN07816620, SAMN07816621, SAMN07816617, SAMN07816618, corresponding to the strains 186, 1669, 2266, 2269 and 2507 respectively (Le Cam et al., 2019).

Pathogenicity tests of *V. inaequalis* on *M. domestica* and *M. sieversii*

In order to test for an effect of the agricultural, hybrid or wild-type status of *V. inaequalis* strains on their pathogenicity on *M. domestica* and *M. sieversii*, we performed artificial inoculations using 57 strains from our sampling, corresponding to 20 strains identified as agricultural-type, 17 strains identified as wild-type and 20 hybrid strains (Table S1). We used one variety of *M. domestica* (GALA®, X4712) and one accession of *M. sieversii* (GMAL 3619.b) for the pathogenicity tests. Gala is a variety planted worldwide despite being highly susceptible to apple scab. GMAL 3619.b has been collected from the Tarbagatai mountain range by American apple breeders (Forsline, Aldwinckle, Dickson, Luby & Hokanson, 2003) and then introduced in France. We grafted the plants on the rootstock MM106 to obtain a vigorous growth favorable to disease development and we inoculated *V. inaequalis* strains when the plants were actively growing. Prior to inoculation, grafted plants were transferred to a quarantine-controlled climatic chamber. Because of the Asian origin of the strains, we used a quarantine chamber to prevent the risk of pathogen escape. Due to the lack of space in climatic chambers, we had to perform experiments at two different dates. In the first experiment, we used 10 agricultural-type strains, 10 hybrids and 8 wild-type strains. In the

second experiment, we used 10 agricultural-type strains, 10 hybrids and 9 wild-type strains. In addition, for normalizing the two experiments, six strains (two strains of each type) that were used in the first experiment were also included in the second experiment and used as calibration strains in the analysis.

Each strain of *V. inaequalis* was grown on a cellophane sheet placed on malt-agar medium at 17°C to obtain spores (Caffier et al., 2014). The cellophane sheets were dried and stored at -20°C. A spore suspension was made by collecting spores from these cellophane sheets and diluting them in water to a final concentration of $1.5x10^5$ spores mL^{-1} (Lê Van et al., 2012). For each strain, the spore suspension was inoculated on three Gala trees and three GMAL 3619.b trees using a mechanical air pressure sprayer. As trees actively grew during the experiment, we labelled the youngest fully deployed leaf of each tree as the F0 leaf, one or two days prior to inoculation to facilitate later disease scoring. To favor spore germination and fungal infection, the plants were kept in darkness with moisture maintained at 100% and temperature at 17°C for 48 hours after inoculation (Lê Van et al., 2012). Afterward, moisture was reduced to 80% during the day and 90% at night, with 12 hours of light per day. These climatic conditions are highly favourable to scab infection and thus provide a good indication of the strain ability to infect tree genotypes (MacHardy, 1996). For each host genotype, plants were randomized within three blocks to have in each block one host replicate for each strain. Disease severity, *i.e.*, virulence of the fungal strain, was measured when there was no more increase of the disease symptoms: at 19 days post inoculation (dpi) for *M. sieversii* and at 21 dpi for *M. domestica*. Disease severity was measured as the percentage of a leaf displaying sporulation, from 0% when there was no disease symptom to 100% when the whole leaf was covered with spores. The F0 leaf being the last leaf deployed one or two days prior to inoculation, the assessment was done on the F0 leaf and on the first leaf under F0, named F1.

371

372 **Statistical analysis of phenotypic data**

373 The data of the two distinct inoculation experiments were standardized based on the data from
374 the six calibration strains: the disease severity values were corrected to obtain the same mean
375 between the two distinct experiments for the six calibration strains. The virulence of each
376 strain was estimated as the mean percentage of scabbed leaf area across the two leaves F0 and
377 F1 and across the three inoculated plants of a given genotype (either *M. domestica* or *M.*
378 *sieversii*), so that there was one value per fungal strain and per apple tree genotype in the
379 analysis. As the assumptions of normality were not met, we compared the medians of
380 virulence between the three different populations (agricultural-type, wild-type and hybrids)
381 using a Kruskal-Wallis and a *post-hoc* Wilcoxon test at 21 dpi on *M. domestica* and 19 dpi on
382 *M. sieversii* in R (version 3.4.4; R Core Team, 2018), considering the different strains in each
383 population as replicates for the population effect. In addition, a Kendall test was performed to
384 analyse the correlation between virulence and hybrid index P_{agr} for the 20 hybrid strains.

385

386 **Demographic inference for wild and agricultural types of the apple scab fungus *Venturia***
387 ***inaequalis***

388 In order to test demographic scenarios, and in particular the likelihood of divergence history
389 without gene flow followed by a secondary contact in the fungal pathogen, we used the
390 composite-likelihood, diffusion approximation-based approach for demographic inference
391 implemented in ∂a∂i (Gutenkunst, Hernandez, Williamson & Bustamante, 2009). We used the
392 33 reference *V. inaequalis* genomes described above, belonging to 15 wild-type and 18
393 agricultural-type strains. In order to meet the requirement of marker independence, we

394 thinned the 1,210,121 available SNPs for these genomes to keep only one SNP every 5kb (a
395 threshold set based on the LD decay curve, Figure S2) using vcftools (Danecek et al., 2011),
396 which left 6,187 SNPs. Using ∂a∂i (Gutenkunst et al., 2009), we tested a set of four basic
397 models (Tine et al., 2014): strict isolation (SI) in which the two lineages diverge without gene
398 flow, isolation with migration (IM) in which the two lineages diverge with constant gene
399 flow, ancient migration (AM) in which the two lineages diverge with gene flow and stop
400 exchanging genes at a time noted T_{AM}, and secondary contact (SC) in which the two lineages
401 diverge without gene flow and then exchange gene since a secondary contact occurring at T_{SC}.
402 Each of these four models was evaluated and fitted with the observed joint allele frequency
403 spectrum (jAFS) using 20 independent runs. Each run started with perturbed starting
404 parameters, from which a global optimisation was done with a simulated annealing
405 optimisation procedure and was followed by an optimisation phase with a maximum number
406 of 100 iterations (https://popgensealab.wordpress.com/dadi-inference/; Christe et al., 2017;
407 Tine et al., 2014). For nested models, likelihood ratio tests (LRTs) were used to identify the
408 best model. For non-nested models, the relative likelihood of Akaike criterion (AIC) was used
409 instead (Christe et al., 2017).

410 Demographic parameters were estimated for the four models. These parameters included
411 migration rates in the two directions, effective sizes in the two lineages, divergence time and
412 time elapsed since other demographic events (T_{AM} in AM model and T_{SC} in SC model). The
413 ancestral effective size before the split was calculated as $N_{ref} = \theta / (2 \times \mu \times L)$ (Gutenkunst et
414 al., 2009), where θ was estimated by∂a∂i; μ is the mutation rate per nucleotide per generation,
415 estimated to be 2×10^{-8} from an analysis of divergence with the closely related species
416 *Venturia pirina* parasitizing pear trees (Le Cam et al., 2019; data not shown). *L* is the effective
417 genome length analyzed, being computed as G x s/S (Gutenkunst et al., 2009), where *G* is the

size of the genome used, *S* is the total number of SNPs called in *G*, and *s* the actual number of SNPs used for inference. In our analysis, 1,210,121 SNPs were called on a portion of 34,942 Mb of the genome, 6,187 SNPs were used, so that *L* was computed as 34,942,000 x (6,187 / 1,210,121) = 178,648 bp. All other parameters were scaled by N_{ref}. Times in years were computed using a generation time of one year, as *V. inaequalis* undergoes an obligate sexual reproduction once a year. The model parameters estimated and their 95% confidence intervals were obtained with Godambe methods (Coffman, Hsieh, Gravel & Gutenkunst, 2015) from 1000 bootstraps across SNPs.

Results

Contrasting distributions of the wild and agricultural host and pathogen populations in the Tian Shan Mountains

We explored the genetic makeup of apple trees and their scab pathogens in a wild environment, by sampling apple trees and fungal strains from the same trees, at nine sites in the forests of the Tian Shan Mountains in Kazakhstan. For apple trees, the PCA (Figure 2A) and STRUCTURE analysis (Figures S3A and B) confirmed the existence of two genetically distinct clusters corresponding to the orchard trees on the one hand, assigned to *M. domestica*, and most of the wild forest trees on the other hand, assigned to *M. sieversii*. We did not detect further population subdivision within the wild species (Figures 2A and S3). Both the first axis of the PCA and STRUCTURE analyses revealed footprints of admixture between the two tree species. The P_{dom} hybrid index values were also estimated for the 240 successfully genotyped trees. All three methods gave congruent results (Figs. 2A and S3C). The correlation coefficient between P_{dom} and the coordinates of the first PCA axis was high and significant

441 (r=-0.97; p-value < 0.001; Figure S4A). The correlations between the ancestry proportion
442 inferred by STRUCTURE and P_{dom} on the one hand, and between the ancestry proportion
443 inferred by STRUCTURE and the coordinates of the first axis of the PCA on the other hand,
444 were also high and significant (r=0.96, p-value<0.001, and r=-0.97, p-value<0.001,
445 respectively). Based on the P_{dom} hybrid index, we identified 78% pure wild *M. sieversii* trees,
446 ca. 12% *M. domestica* trees and near 10% hybrid trees (Figure 2A; Table S1). The species
447 identified genetically were consistent with our expectations during sampling: only one *M.*
448 *domestica* was identified in the natural forest sites (Figure 3), whereas most of the trees
449 sampled in the orchard belonged to *M. domestica* (28 out of 30 trees sampled in the orchard,
450 with 14 different genotypes). Three of the forest sites contained only *M. sieversii* trees, but the
451 four other forest sites contained both *M. sieversii* and hybrid trees (with hybrid trees
452 representing 11.1 to 27.6%, Figures 3), as did the forest site directly surrounding the orchard
453 (with hybrid trees reaching there 27.3%).

454 For *V. inaequalis*, the PCA (Figure 2B) and STRUCTURE analysis (Figures S5A and B)
455 confirmed the existence of two differentiated populations and the lack of further subdivision,
456 and revealed admixture between the wild and agricultural populations (Figures 2B and S5B).
457 The hybrid index P_{agr}, the ancestry coefficient given by STRUCTURE and the first axis of the
458 PCA, separating individuals according to their degree of ancestry into the two divergent
459 populations (Figure 2B), gave congruent results (Figure S5C). The correlation coefficient
460 between P_{agr} and the first axis of the PCA was high and significant (r=0.99; p-value=0.001)
461 (Figure S4B). The correlations between the ancestry proportion inferred by STRUCTURE
462 and P_{agr} on the one hand, and between the ancestry proportion inferred by STRUCTURE and
463 the coordinates of the first axis of the PCA on the other hand, were also high and significant
464 (r=0.99, p-value<0.001 and r=0.98, p-value<0.001, respectively).

The P_{agr} hybrid index estimated for the 255 *V. inaequalis* strains displayed a U-shaped distribution, with 33 fungal strains (12.9%) having P_{agr} values between 0.1 and 0.9, thus being considered to be hybrids, and with high frequencies of pure agricultural-type (38.5%) and pure wild-type (48.6%) fungal genotypes (Figure 2B; Table S1). All the fungal strains sampled in the orchard carried alleles typical of the agricultural-type (Figure 3), and the fungal strains sampled on the surrounding forest were mostly either agricultural-type or wild-type genotypes. A single hybrid strain was detected in the forest surrounding the orchard, whereas 3.4% to 37% of the fungal strains were hybrids in the other forest sites (Figure 3).

In order to further explore the relationship between host and pathogen genotypes, we compared their genetic diversity and differentiation across the sampling sites. Genetic diversities per sampling site ranged from 0.703 (tuk) to 0.802 (kot) for apple trees and from 0.244 (tuk) to 0.487 (kot) for *V. inaequalis* (Table S4). Genetic diversities were minimal and maximal in the same sampling sites for both the host and the pathogen, but no significant correlation was observed between the apple and fungus genetic diversity levels across sites (Kendall's tau=0.389, p-value=0.18; Figure S6). Global F_{ST} among sites were 0.259 for apple trees and 0.243 for *V. inaequalis*. Pairwise F_{ST} estimates ranged from 0.000 to 0.207 for apple trees (Table S5) and from 0.000 to 0.721 for *V. inaequalis* (Table S6). For both the host and the pathogen, the orchard (esi_o) was the most differentiated from other sampling sites (Tables S5 and S6). Host and pathogen pairwise differentiation matrices were significantly correlated (Mantel-test Z-score=0.681, p-value=0.011; Figure S7). Yet, we found no significant correlation between pairwise F_{ST} and geographical distances matrices, neither for apple trees (Mantel test Z-score= 33.10, p-value=0.42) nor for *V. inaequalis* (Mantel test Z-score=103.20, p-value=0.51), indicating a lack of isolation by distance.

489 **All pathogen types occur on wild apple trees, but wild-type pathogens occur only on**
490 **trees with no domesticated ancestry**

491 We investigated the association between the fungal pathogen types and the *Malus* genotypes.
492 From the 240 trees and 255 fungal strains successfully genotyped, we could obtain 249 host-
493 pathogen pairs, several strains having been isolated from the same host trees. A sharp L-
494 shaped pattern was observed when the hybrid index values of the host trees were plotted
495 against those of their associated fungal genotype (Figure 4A). Hybrids were found among
496 both trees and fungi, but no hybrid pathogens were collected from hybrid trees. Hybrid and
497 wild-type fungi were found only on wild apple trees, whereas agricultural-type fungi were
498 found on all types of apple trees, across the whole range of the hybrid index.

499

500 **Genetic determinants of host adaptation in *Venturia inaequalis***

501 In order to identify SNPs predicting the ability of fungal genotypes to parasitize the host
502 species, we performed a DAPC on the genotypes of the 249 *V. inaequalis* strains, with groups
503 defined *a priori* based on the assignment of their collection tree as *M. domestica*, hybrid or *M.*
504 *sieversii*. The first linear discriminant function (LD1) clearly separated the strains sampled on
505 domesticated apple trees from those sampled on wild and hybrid apple trees (Figure S8). The
506 second linear function separated strains found on hybrid apple trees from the ones sampled on
507 *M. sieversii* (LD2, Figure S8). The analysis of allele contribution (loadings) to the first
508 discriminant function revealed that the SNP *V_08160_319* was strongly involved in the
509 genetic differentiation between strains parasitizing *M. domestica* and those found on hybrid
510 and wild hosts (loading of 0.123, Figure S9), while the other SNPs showed much lower

511 contributions (from 8.723×10^{-8} to 0.024). The estimate of global F_{ST} was accordingly much
512 higher for the V_08160_319 locus (F_{ST} = 0.887) than for other loci (Figure S10).

513 The SNP *V_08160_319* was located in a gene identified as a small secreted protein (SSP)
514 using the protocol described in Le Cam et al. (2019). The two alleles of this SNP were A and
515 G and corresponded to a non-synonymous site. According to the gene allelic sequences in the
516 33 reference genomes, the *V_08160_319* SNP was associated to a second one in the same
517 codon, which thus corresponded to a TGA stop codon versus a TAC codon, coding for a
518 tyrosine, at the 140th codon in the 295 amino-acid long protein. Out of the 189 strains for
519 which genotypes for this SNP were available, the stop codon allele indeed had frequencies of
520 1.00 on *M. domestica* (N=28), 0.92 on hybrid apple trees (N=13) and 0.06 on *M. sieversii*
521 (N=148) (Figure 4B). Considering ancestry of fungal strains, the stop codon allele had a
522 frequency of 0.89 (N=56) in the agricultural-type population and 0 in the wild-type population
523 (N=107) and in hybrids (N=26). The detection of only A (tyrosine) alleles in hybrids
524 significantly deviated from expectations under the hypothesis of neutral segregation at this
525 locus given the allele frequencies in the pure populations (Binomial-test, p-value=6.10×10^{-5}).

526 To assess which allele was ancestral, we used five available genomes (Le Cam et al., 2019)
527 belonging to the outgroup *V. inaequalis* lineage parasitising Pyracantha (Gladieux et al.,
528 2010a; Le Cam et al., 2002). These genomes carried the TAC codon, as did the wild-type *V.*
529 *inaequalis* population on *M. sieversii*. This means that the stop codon truncating the small
530 secreted protein is a derived allele, and has evolved in the agricultural *V. inaequalis*
531 population on *M. domestica*.

532

533 **Agricultural-type and hybrid fungal strains are more virulent than wild-type strains**

We then investigated experimentally whether the observed association between fungal pathogen types and host tree species resulted from differences in infection ability, by inoculating 57 fungal strains (20 agricultural-type, 17 wild-type and 20 hybrid strains; Table S1) on one genotype of *M. sieversii* and one genotype of *M. domestica*. All agricultural-type strains could cause disease on *M. domestica* (N=20; Figures 5A and 6A), whereas only one wild-type and two hybrid strains could cause disease on *M. domestica*, and even then, only with very low levels of virulence (less than 2% of diseased leaf area, Figure 5A). All agricultural strains (N=20), hybrids (N=20) and wild strains (N=17) could cause disease on *M. sieversii* (Figure 5B). Both agricultural-type and hybrid strains were significantly more virulent (i.e. caused larger and/or more lesions) than wild-type strains on *M. sieversii* (Figures 5B and 6B; Wilcoxon tests: $P=1.7 \times 10^{-5}$ and $P=1.2 \times 10^{-4}$, respectively). The *V. inaequalis* hybrids displayed virulence levels on *M. sieversii* that appeared intermediate between the agricultural-type and wild-type populations although the difference between hybrids and the agricultural-type population was not significant (Figure 5B). Similar results were obtained when assignment to species and hybrids were done using the same hybrid index threshold as for apple trees (wild type: $Pagr<0.2$; hybrids: $0.2 \leq Pagr \leq 0.8$; agricultural: $Pagr>0.8$; Figure S11). We found no significant correlation between the proportion of agricultural-type ancestry and the degree of virulence in the hybrid fungal strains (Figure S12; Kendall test: $\tau=-0.047$, $P=0.7702$).

The allele at the outlier locus *V_081690_319* partially explained the virulence on *M. domestica*. Fourty-seven genotypes were available out of 57 strains used in the experiment. Out of the 15 agricultural-type strains with available genotypes, 13 (87%) carried the stop codon (TGA) allele. Conversely, all the 15 hybrid strains with available genotypes (100%), as well as all the 17 wild strains (100%), carried the tyrosine (TAC) allele. When comparing the

two groups of strains corresponding to their alleles at the *V_081690_319* locus, we found that the strains carrying the stop codon (TGA) allele were more virulent than those carrying the tyrosine (TGA) allele, both on *M. domestica* and on *M. sieversii* (Wilcoxon tests: P=1.4x10^{-8} and P=1.2x10^{-3}, respectively; Figure S13). Most of the strains carrying the tyrosine allele could not cause disease at all on *M. domestica*, but a few of them could induce a low level of symptoms (Figure S13A).

Demographic inference on the divergence history of wild-type and agricultural-type *Venturia inaequalis* populations

We performed demographic inferences in order to test if the observed co-occurrence and hybridization of wild and agricultural-type *V. inaequalis* populations in Kazakhstan resulted from divergence with gene flow or originated from a secondary contact, following the introduction of *M. domestica*, about one century ago in a few orchards in forests of wild *M. sieversii*. We compared the likelihoods of four contrasted scenarios of divergence, with either strict isolation, isolation with continuous migration, isolation with initial migration or secondary contact (Figure 7A).

The models allowing gene flow received better support than the strict isolation model (AIC= 2403,587014; LogL= -1197,793507). The model of isolation with migration (IM; AIC= 1975,638252; LogL= -981,8191258) did not perform significantly better than the ancient migration model (AM; AIC= 1977,698045; LogL= -981,8490226). The secondary contact model (SC) received the best support (AIC= 1871,313317, LogL= -928,6566583), which indicated that the agricultural-type *V. inaequalis* population was likely absent in Asian wild apple forests before the recent introduction of domesticated apple trees in orchards (Figure 7B; Table 1). Using the estimate of θ obtained from the best model (SC, θ=143.729), a

582 mutation rate μ of $2x10^{-8}$, we found that the ancestral effective size before split, N_{ref}, was near
583 20,000 individuals (Figure 7C). Parameter estimates for the secondary contact model
584 indicated that the divergence between the wild-type and agricultural-type *V. inaequalis*
585 populations occurred 4,570 years ago (4,570 ± 324 SD) followed by a secondary contact 130
586 years ago (130 ± 9 SD). This scenario and estimates are consistent with a fungal divergence
587 triggered by apple domestication under strict genetic isolation, followed by a recent secondary
588 contact promoted by modern orchards (Figure 7C).
589

590 **Discussion**

591 **Gene flow between apple trees species may threaten the genetic integrity of the Asian**
592 **wild apple**

593 Our data show that *M. sieversii* wild apple trees are affected by gene flow following the
594 introduction of the domesticated apple trees in orchards near the natural apple forests of the
595 Kazakh Tian Shan Mountains. Gene flow from *M. domestica* to *M. sieversii* had previously
596 been suggested (Cornille et al. 2012; Omasheva et al., 2017), and we reveal here the strong
597 impact of the presence of orchards close to wild forests. Currently, gene flow appears to pose
598 a less severe threat to *M. sieversii* than it does to *M. sylvestris* in Europe, where wild
599 European crabapple tree populations display massive introgression from the cultivated apple
600 tree, to the extent that the European wild species is considered as endangered (Cornille et al.,
601 2015; Feurtey et al., 2017). The lower level of introgression in *M. sieversii* wild populations
602 in Central Asia is probably due to more recent and much less extensive secondary contact
603 between wild and cultivated species than in Europe. The cultivated apple tree was introduced
604 into Europe before the 3rd century BC (Cornille et al., 2014), whereas it was not introduced

605 into the area sampled in this study until about 100 years ago, and much less extensively than
606 in Europe. Furthermore, the higher density of *M. sieversii* trees in Kazakh wild forests (Harris
607 et al., 2002; Vavilov, 1931) than of *M. sylvestris* in European wild forests probably also
608 restricts gene flow from cultivated apple trees. Indeed, pollination distances decrease with
609 density around pollinated trees in European wild apple trees, longer pollination distances
610 increasing the risk of interspecific mating with cultivated trees (Feurtey et al. 2017; Reim et
611 al. 2015).

612 While the overall percentage of apple tree hybrids in the wild forest remained low, near 25%
613 of the trees were found to be hybrids in the forest immediately adjacent to a cultivated
614 orchard, as well as in the forest location that was the closest to the peri-urban environment of
615 Almaty. These findings confirm the weak interspecific reproductive barriers reported for the
616 *Malus* genus (Zohary & Hopf, 2000) and raise concerns about the consequences of increasing
617 human-induced changes in this region. As indicated by the IUCN Red List, *M. sieversii* is
618 already threatened by its range shrinking in Central Asia (Eastwood, Lazkov & Newton,
619 2009), with a disappearance of more than 70% of the apple wild forest area over the last 30
620 years due to agricultural expansion and overgrazing. In addition to this range reduction, our
621 results support the view that the genetic integrity of *M. sieversii* may be also threatened. Thus,
622 unless preventive measures are taken to protect Central Asian forests, it may only be a
623 question of time before the genetic integrity of *M. sieversii* deteriorates in Central Asia, as
624 already observed for *M. sylvestris* in Europe (Cornille et al., 2015; Feurtey et al., 2017), as
625 well as for other European native plant species such as poplars (Bleeker, Schmitz & Ristow,
626 2007; Rhymer & Simberloff, 1996; Vanden Broeck, Villar, Van Bockstaele & Van Slycken,
627 2005).

628

629 **Recent invasion of the wild forests by the agricultural *V. inaequalis* population**

630 We confirm in this study the existence of two differentiated *V. inaequalis* populations in
631 Central Asia. The high differentiation level between the agricultural-type and wild-type *V.*
632 *inaequalis* populations raises the question of whether they should be considered as separate
633 species. However, further studies are needed to assess the taxonomic status of the *V.*
634 *inaequalis* lineages, especially as hybrids were found.

635 Most importantly, we show that agricultural-type and wild-type *V. inaequalis* populations
636 came into a recent secondary contact after having diverged in strict isolation during apple tree
637 domestication. Indeed, demographic inferences showed that agricultural-type and wild-type
638 fungal populations initially diverged without gene flow about 4,500 years ago, a time
639 consistent with that of apple tree domestication, and came into secondary contact about one
640 century ago, i.e., when domesticated apple trees were introduced in Central Asia. This
641 secondary contact has led to the recent invasion of the agricultural-type population in the wild
642 *M. sieversii* forest and to the production of hybrids.

643

644 **The agricultural-type *V. inaequalis* population is able to parasitize both wild and**
645 **domesticated host trees**

646 We uncovered contrasting host range patterns among *V. inaequalis* populations: wild-type and
647 hybrid fungal strains were only found on *M. sieversii* trees, whereas agricultural-type fungal
648 strains were found on trees of all types of ancestry. Pathogenicity tests in controlled
649 conditions matched the distribution observed in nature. Indeed, the agricultural-type fungal
650 strains were able to cause disease on *M. domestica* and *M. sieversii* whereas the wild-type and

651 hybrid fungal strains could induce symptoms only on wild host trees. These results, obtained
652 with high spore concentration and in favorable climatic conditions, are conservative and
653 confirm conclusions that were previously obtained with a limited number of *V. inaequalis*
654 strains (Lê Van et al., 2012). Furthermore, we detected agricultural-type *V. inaequalis* strains
655 at eight different sites in the wild forests on numerous different trees, suggesting that the
656 agricultural-type population can parasitize a large range of *M. sieversii* genotypes. In contrast,
657 no wild-type or hybrid fungal strain was detected on any of the *M. domestica* genotypes
658 present in the orchard. This host range pattern likely explains the significant correlation found
659 between apple tree and *V. inaequalis* pairwise F_{ST} matrices, which was not due to isolation by
660 distance patterns in hosts and pathogens. Indeed, it is likely that the *M. domestica* ancestry
661 proportion in each site drives the allele frequency distribution in *V. inaequalis* by selection,
662 because *M. domestica* can only be parasitized by agricultural-type strains, and not by wild-
663 type or hybrid pathogens. This situation contrasts with that reported for poplars, in which the
664 hybrid fungal pathogen *Melampsora x columbiana* can parasitize both pure species and hybrid
665 trees (Newcombe et al., 2000).

666 In addition, the agricultural *V. inaequalis* population and hybrids displayed greater virulence
667 on wild apple trees than its own endemic wild-type population. This is consistent with the
668 process known as “pestification” (Saleh, Milazzo, Adreit, Fournier & Tharreau, 2014), under
669 which selection by humans of more resistant plants unwittingly leads pathogens to accumulate
670 more virulences traits and to cause more severe symptoms. This could add further threats for
671 the wild tree species in its natural habitat. Indeed, the scab disease on apple tree leaves
672 reduces photosynthesis (Spotts & Ferree, 1979), which may affect the growth of the plants.
673 While such increase in virulence may have a limited impact on the growth of adult trees, its
674 effect on the growth of young seedlings could be severe. Furthermore, scab disease can lead

675 to early fruit fall (MacHardy, 1996), which may decrease seed number, thereby affecting host
676 population dynamics. On *M. sieversii*, hybrid strains were more virulent than wild-type fungal
677 strains but not significantly different from agricultural-type fungal strains. Thus, although the
678 hybridization between fungal lineages does not currently appear to increase damages on wild
679 trees compared to the pure agricultural pathogen, it might participate in the invasion of the
680 disease on the wild host. Future experiments are still needed to estimate precisely the disease
681 fitness effect on seedlings and on the number of seeds produced by adult trees in order to
682 ascertain the long-term impact of this pathogen population on the wild tree natural
683 populations. Nevertheless, our results already suggest that the invasion of the wild forests by
684 the agricultural-type *V. inaequalis* population could further threaten natural *M. sieversii*
685 populations.

686

687 **The genomic determinants of pestification: a likely new virulence gene**

688 Our findings suggest the evolution of *V. inaequalis* populations by host tracking of the
689 domesticated apple tree without the loss of its ability to parasitize the wild host. Many
690 resistance genes have been introgressed into *M. domestica* during domestication and modern
691 breeding (Cornille et al., 2012). During its evolutionary tracking of apple trees evolution, the
692 agricultural *V. inaequalis* population has probably accumulated multiple alleles allowing to
693 counteract the crop resistance genes, potentially including the truncated allele of the small
694 secreted protein identified in this study as a putative avirulence gene. Indeed, we found that
695 the host lineage on which *V. inaequalis* strains were sampled was predicted by a SNP in a
696 gene encoding a small secreted protein. In the fungal strains collected on *M. domestica*, the
697 allele corresponded to a stop codon while strains sampled on *M. sieversii* carried the full-

length gene. Taken together with the presence of the full-length gene in an outgroup, the *V. inaequalis* lineage parasitizing Pyracantha (Gladieux et al., 2010a; Le Cam et al., 2002), this suggests the acquisition of a virulence allele by the agricultural *V. inaequalis* population during crop host tracking, through the loss of a protein recognized by the domesticated apple tree selected for resistance to apple scab. Such an increase in the virulence of a pathogen following domestication of its host is compatible with the pestification process (Salehet al., 2014), and is consistent with virulence evolution mechanisms previously found in many crop pathogens (Raffaele & Kamoun, 2012).

The identification of a putative avirulence gene from a small set of 181 SNPs can be explained by the initial choice of these SNPs as the most differentiated between the fungal populations and located within genes. However, we cannot exclude that the *V_081690_319* SNP is not directly involved in host adaptation but is instead linked to a genomic determinant of host adaptation. Future functional experiments are required to confirm that the identified gene is an avirulence gene. On the plant side, the identification of the receptor involved in the recognition of this putative avirulent protein could lead to the discovery of a new resistance gene.

More comprehensive studies on whole *V. inaequalis* genomes will likely detect further SNPs involved in host adaptation, as suggested by our data. Indeed, the SNP allele corresponding to the stop codon (G allele) was not found in any of the 15 hybrids for which genotypes were available, which significantly deviated from neutral expectations, while agricultural-type strains carrying this allele were found on *M. sieversii*, even if it was at low frequency. In addition, a few strains carrying the full-length allele of the putative avirulence gene were able to cause some symptoms on *M. domestica* in artificial inoculations. This suggests that the stop

721 codon allele was not selected against on *M. sieversii* in itself but may be deleterious in
722 association with alleles at other loci in hybrids. These results therefore suggest that the locus
723 *V_081690_319* is involved in epistatic interactions with other genes and that hybridization
724 breaks up beneficial allelic combinations required to parasitize *M. domestica*. Furthermore,
725 the *V. inaequalis* hybrids appeared quantitatively intermediate in virulence on *M. sieversii*,
726 although the virulence difference with the agricultural population was not significant.
727 Intermediate values in hybrids for haploid organisms suggest the implication of multiple
728 genes in virulence.

729

730 **Conclusion**

731 We found that natural *M. sieversii* populations are currently only mildly affected by gene flow
732 from *M. domestica,* which was introduced into the area about a century ago but still remains
733 very rare in the Kazakh mountains. Further disturbances in this area might however lead to
734 much higher levels of crop-to-wild gene flow, as already reported in Europe for the European
735 crabapple (Cornille et al., 2013; 2015; Feurtey et al. 2017). In addition, the agricultural-type
736 *V. inaequalis* population is invading wild forests and is introgressing the wild-type *V.*
737 *inaequalis* population, probably due to the greater virulence of the agricultural-type fungal
738 population acquired during its tracking of apple tree domestication. Furthermore, our study
739 represents one of the very rare joint analyses of host and pathogen populations (Croll &
740 Laine, 2016), despite their importance for understanding the evolutionary mechanisms and
741 histories leading to host specialization and local adaptation. Thanks to the study of host and
742 pathogen pairs, we could identify a putative avirulence gene that may be of great importance

743 on a cultivated crop, and a similar approach may be widely used in future studies on host and
744 pathogen populations.

745

746 **Acknowledgments**

747 The authors thank C. Peix and kazakh forestry agents for their assistance in sampling, M.
748 Cascales for her assistance in SNPs data analyzing, N. Bierne, R. Nielsen and M. Slatkin for
749 helpful discussions. They acknowledge the staff at the GENTYANE genotyping platform
750 (INRA GDEC, Clermont-Ferrand, France) for SNP and microsatellite genotyping. Authors
751 are also grateful to Pascal Heitzler for providing *M. sieversii* budwoods. They are also most
752 grateful to the PHENOTIC core facility (Angers, France) for its technical support. This study
753 was funded by grants from ANR-12-ADAP-0009 (Gandalf project), from INRA-SPE
754 Department (Escapades project), by a Louis D. grant (Institut de France) to TG, a PhD grant
755 from the Région Ile-de-France to AF, and by the program "Objectif Végétal, Research,
756 Education and Innovation in Pays de la Loire", supported by the French Region Pays de la
757 Loire, Angers Loire Métropole and the European Regional Development Fund (Escapes
758 project).

759

760 ## References

761 Bleeker, W., Schmitz, U., & Ristow, M. (2007). Interspecific hybridisation between alien and
762 native plant species in Germany and its consequences for native biodiversity. *Biological*
763 *Conservation*, *137*(2), 248-253. doi: 10.1016/j.biocon.2007.02.004

764 Caffier, V., Lasserre-Zuber, P., Giraud, M., Lascostes, M., Stievenard, R., Lemarquand, A., ...
765 Le Cam, B. (2014). Erosion of quantitative host resistance in the apple × *Venturia inaequalis*
766 pathosystem. *Infection, Genetics and Evolution*, *27*, 481-489. doi:
767 10.1016/j.meegid.2014.02.003

768 Christe, C., Stölting, K. N., Paris, M., Fraïsse, C., Bierne, N., & Lexer, C. (2017). Adaptive
769 evolution and segregating load contribute to the genomic landscape of divergence in two tree
770 species connected by episodic gene flow. *Molecular Ecology*, *26*(1), 59-76. doi:
771 10.1111/mec.13765

772 Coffman, A. J., Hsieh, P. H., Gravel, S., & Gutenkunst, R. N. (2015). Computationally
773 efficient composite likelihood statistics for demographic inference. *Molecular Biology and*
774 *Evolution*, *33*(2), 591-593. doi: 10.1093/molbev/msv255

775 Cornille, A., Feurtey, A., Gélin, U., Ropars, J., Misvanderbrugge, K., Gladieux, P., & Giraud,
776 T. (2015). Anthropogenic and natural drivers of gene flow in a temperate wild fruit tree: a
777 basis for conservation and breeding programs in apples. *Evolutionary Applications*, *8*(4), 373-
778 384. doi: 10.1111/eva.12250

779 Cornille, A., Giraud, T., Smulders, M. J., Roldán-Ruiz, I., & Gladieux, P. (2014). The
780 domestication and evolutionary ecology of apples. *Trends in Genetics*, *30*(2), 57-65. doi:
781 10.1016/j.tig.2013.10.002

782 Cornille, A., Gladieux, P., & Giraud, T. (2013). Crop-to-wild gene flow and spatial genetic
783 structure in the closest wild relatives of the cultivated apple. *Evolutionary Applications*, *6*(5),
784 737-748. doi: 10.1111/eva.12059

785 Cornille, A., Gladieux, P., Smulders, M. J., Roldan-Ruiz, I., Laurens, F., Le Cam, B., ...
786 Gabrielyan, I. (2012). New insight into the history of domesticated apple: secondary
787 contribution of the European wild apple to the genome of cultivated varieties. *PLoS genetics*,
788 *8*(5), e1002703. doi: 10.1371/journal.pgen.1002703

789 Croll, D., Laine, A. L. (2016). What the population genetic structures of host and pathogen
790 tell us about disease evolution. *New Phytologist*, *212*(3):537-539. doi: 10.1111/nph.14203.

791 Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... McVean,
792 G. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156-2158. doi
793 :10.1093/bioinformatics/btr330

794 Depotter, J. R. L., Seidl, M. F., Wood, T. A., Thomma, B. P. H. J. (2016). Interspecific
795 hybridization impacts host range and pathogenicity of filamentous microbes. *Current Opinion*
796 *in Microbiology*, *32*:7–13

797 de Vienne, D. M., Hood, M. E., & Giraud, T. (2009). Phylogenetic determinants of potential
798 host shifts in fungal pathogens. *Journal of Evolutionary Biology*, *22*(12), 2532-2541. doi:
799 10.1111/j.1420-9101.2009.01878.x

800 Dray, S., & Dufour, A. B. (2007). The ade4 package: implementing the duality diagram for
801 ecologists. *Journal of statistical software*, *22*(4), 1-20. doi: 10.18637/jss.v022.i04

802 Earl, D. A. & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program
803 for visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
804 *Genetics Resources*, *4* (2), 359-361. doi: 10.1007/s12686-011-9548-7

805 Eastwood, A., Lazkov, G., & Newton, A. (2009). *The red list of trees of Central Asia*. Fauna
806 and Flora International.

807 Ellstrand, N. C., Meirmans, P., Rong, J., Bartsch, D., Ghosh, A., De Jong, T. J., ... Strasburg,
808 J. L. (2013). Introgression of crop alleles into wild or weedy populations. *Annual Review of*

809 *Ecology, Evolution, and Systematics*, *44*, 325-345. doi: 10.1146/annurev-ecolsys-110512-
810 135840

811 Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals
812 using the software structure: a simulation study. *Molecular Ecology*, 14, 2611-2620.
813 doi:10.1111/j.1365-294X.2005.02553.x

814 Falush, D., Stephens, M. & Pritchard, J. K. (2003). Inference of population structure:
815 Extensions to linked loci and correlated allele frequencies. *Genetics*, 164(8), 1567-1587.

816 Feurtey, A., Cornille, A., Shykoff, J. A., Snirc, A., & Giraud, T. (2017). Crop-to-wild gene
817 flow and its fitness consequences for a wild fruit tree: Towards a comprehensive conservation
818 strategy of the wild apple in Europe. *Evolutionary Applications*, *10*(2), 180-188. doi:
819 10.1111/eva.12441

820 Feurtey, A., & Stukenbrock, E. H. (2018). Interspecific gene exchange as a driver of adaptive
821 evolution in fungi. *Annual Review of Microbiology*, *72*, 377-398. doi: 10.1146/annurev-micro-
822 090817-062753

823 Forsline, P. L., Aldwinckle, H. S., Dickson, E. E., Luby, J. J., & Hokanson, S. C. (2003).
824 Collection, maintenance, characterization, and utilization of wild apples of Central Asia.
825 *Horticultural reviews*, *29*, 1-62.

826 Giraud, T., Refrégier, G., Le Gac, M., de Vienne, D. M., & Hood, M. E. (2008). Speciation in
827 fungi. *Fungal Genetics and Biology*, *45*(6), 791-802. doi: 10.1016/j.fgb.2008.02.001

828 Gladieux, P., Caffier, V., Devaux, M., Le Cam, B. (2010a). Host-specific differentiation
829 among populations of *Venturia inaequalis* causing scab on apple, pyracantha and loquat.
830 *Fungal Genetics and Biology*, *47*(6), 511-521. doi: 10.1016/j.fgb.2009.12.007

831 Gladieux, P., Vercken, E., Fontaine, M. C., Hood, M. E., Jonot, O., Couloux, A., & Giraud, T.
832 (2011). Maintenance of fungal pathogen species that are specialized to different hosts:
833 allopatric divergence and introgression through secondary contact. *Molecular Biology and*
834 *Evolution*, *28*(1), 459-471. doi: 10.1093/molbev/msq235

835 Gladieux, P., Zhang, X. G., Afoufa-Bastien, D., Sanhueza, R. M. V., Sbaghi, M., & Le Cam,
836 B. (2008). On the origin and spread of the scab disease of apple: out of central Asia. *PLoS*
837 *One*, *3*(1), e1455. doi: 10.1371/journal.pone.0001455

838 Gladieux, P., Zhang, X. G., RóLdan-Ruiz, I., Caffier, V., Leroy, T., Devaux, M., ... Le Cam,
839 B. (2010b). Evolution of the population structure of *Venturia inaequalis*, the apple scab
840 fungus, associated with the domestication of its host. *Molecular Ecology*, *19*(4), 658-674. doi
841 :10.1111/j.1365-294X.2009.04498.x

842 Gompert, Z., & Alex Buerkle, C. (2010). INTROGRESS: a software package for mapping
843 components of isolation in hybrids. *Molecular Ecology Resources*, *10*(2), 378-384. doi:
844 10.1111/j.1755-0998.2009.02733.x

845 Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009).
846 Inferring the joint demographic history of multiple populations from multidimensional SNP
847 frequency data. *PLoS Genetics*, *5*(10), e1000695. doi: 10.1371/journal.pgen.1000695

Hanage, W. P., Fraser, C., Tang, J., Connor, T. R., & Corander, J. (2009). Hyper-recombination, diversity, and antibiotic resistance in pneumococcus. *Science*, *324*(5933), 1454-1457. doi: 10.1126/science.1171908

Harris, S. A., Robinson, J. P., & Juniper, B. E. (2002). Genetic clues to the origin of the apple. *Trends in Genetics*, *18*(8), 426-430. doi: 10.1016/S0168-9525(02)02689-6

Harshman, J. M., Evans, K. M., Allen, H., Potts, R., Flamenco, J., Aldwinckle, H. S., … Norelli, J. L. (2017). Fire blight resistance in wild accessions of *Malus sieversii*. *Plant disease*, *101*(10), 1738-1745. doi: 10.1094/PDIS-01-17-0077-RE

Hooftman, D. A. P., Jong, M. J. D., Oostermeijer, J. G. B., & Den Nijs, H. J. C. M. (2007). Modelling the long-term consequences of crop–wild relative hybridization: a case study using four generations of hybrids. *Journal of Applied Ecology*, *44*(5), 1035-1045. doi: 10.1111/j.1365-2664.2007.01341.x

Hovick, S. M., Campbell, L. G., Snow, A. A., & Whitney, K. D. (2012). Hybridization alters early life-history traits and increases plant colonization success in a novel region. *The American Naturalist*, *179*(2), 192-203. doi: 10.1086/663684

Ioos, R., Andrieux, A., Marçais, B., & Frey, P. (2006). Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology*, *43*(7), 511-529. doi: 10.1016/j.fgb.2006.02.006

Jakobsson, M. & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23(14), 1801-1806.

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403-1405. doi: 10.1093/bioinformatics/btn129

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*: 94.

Le Cam, B., Parisi, L. & Arene, L. (2002). Evidence of two *formae speciales* in *Venturia inaequalis*, responsible for apple and Pyracantha scab. *Phytopathology*, *92*, 314-320.

Le Cam, B., Sargent, D., Gouzy, J., Amselem, J., Bellanger, M. N., Bouchez, O., ... Lemaire, C. (2019). Population genome sequencing of the scab fungal species *Venturia inaequalis*, *Venturia pirina*, *Venturia aucupariae* and *Venturia asperata*. *G3: Genes, Genomes, Genetics*, *9*(8), 2405-2414. doi: 10.1534/g3.119.400047

Le Gac, M. L., & Giraud, T. (2008). Existence of a pattern of reproductive character displacement in Homobasidiomycota but not in Ascomycota. *Journal of evolutionary biology*, *21*(3), 761-772. doi: 10.1111/j.1420-9101.2008.01511.x

Lê Van, A., Gladieux, P., Lemaire, C., Cornille, A., Giraud, T., Durel, C. E., ... Le Cam, B. (2012). Evolution of pathogenicity traits in the apple scab fungal pathogen in response to the domestication of its host. *Evolutionary applications*, *5*(7), 694-704. doi: 10.1111/j.1752-4571.2012.00246.x

Lemaire, C., De Gracia, M., Leroy, T., Michalecka, M., Lindhard-Pedersen, H., Guerin, F., ... Le Cam, B. (2016). Emergence of new virulent populations of apple scab from

889 nonagricultural disease reservoirs. *New Phytologist*, *209*(3), 1220-1229. doi:
890 10.1111/nph.13658

891 Leroy, T., Caffier, V., Celton, J. M., Anger, N., Durel, C. E., Lemaire, C., & Le Cam, B.
892 (2016). When virulence originates from nonagricultural hosts: evolutionary and
893 epidemiological consequences of introgressions following secondary contacts in *Venturia*
894 *inaequalis*. *New Phytologist*, *210*(4), 1443-1452. doi: 10.1111/nph.13873

895 Leroy, T., Lemaire, C., Dunemann, F., & Le Cam, B. (2013). The genetic structure of a
896 Venturia inaequalis population in a heterogeneous host population composed of different
897 *Malus* species. *BMC evolutionary biology*, *13*(1), 64. doi: 10.1186/1471-2148-13-64

898 Lin, X., Litvintseva, A. P., Nielsen, K., Patel, S., Floyd, A., Mitchell, T. G., & Heitman, J.
899 (2007). αADα hybrids of *Cryptococcus neoformans*: evidence of same-sex mating in nature
900 and hybrid fitness. *PLoS Genetics*, *3*(10), e186. doi: 10.1371/journal.pgen.0030186

901 Liu, B. H., Cheng, L., Ma, F. W., Liang, D., & Zou, Y. J. (2012). Influence of rootstock on
902 drought response in young 'Gale Gala' apple (*Malus domestica* Borkh.) trees. *Journal of the*
903 *Science of Food and Agriculture*, *92*(12), 2421-2427. doi: 10.1002/jsfa.5647

904 MacHardy, W. E. (1996). *Apple scab: biology, epidemiology, and management*. American
905 Phytopathological Society (APS Press).

906 Mantel, N. (1967). The detection of disease clustering and a generalized regression approach.
907 *Cancer Research 27*, 209-20.

908 Newcombe, G., Stirling, B., McDonald, S., & Bradshaw, H. D. (2000). *Melampsora*×
909 *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. *Mycological Research*,
910 104(3), 261-274. doi: 10.1017/S0953756299001665

911 Nikiforova, S. V., Cavalieri, D., Velasco, R., & Goremykin, V. (2013). Phylogenetic analysis
912 of 47 chloroplast genomes clarifies the contribution of wild species to the domesticated apple
913 maternal line. *Molecular Biology and Evolution*, *30*(8), 1751-1760. doi:
914 10.1093/molbev/mst092

915 Omasheva, M. Y., Flachowsky, H., Ryabushkina, N. A., Pozharskiy, A. S., Galiakparov, N.
916 N., & Hanke, M. V. (2017). To what extent do wild apples in Kazakhstan retain their genetic
917 integrity? *Tree genetics & genomes*, *13*(3), 52. doi: 10.1007/s11295-017-1134-z

918 Paradis, E. & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and
919 evolutionary analyses in R, *Bioinformatics*, 35(3), 526–528.
920 https://doi.org/10.1093/bioinformatics/bty633

921 Raffaele, S., Kamoun, S. (2012). Genome evolution in filamentous plant pathogens: why
922 bigger can be better. *Nature Reviews Microbiology 10***,** 417-430. doi: 10.1038/nrmicro2790

923 R Core Team (2018). R: A language and environment for statistical computing. R Foundation
924 for Statistical Computing, Vienna. URL: http://www.R-project.org/

925 Reim, S., Proft, A., Heinz, S., Lochschmidt, F., Höfer, M., Tröber, U., & Wolf, H. (2017).
926 Pollen movement in a *Malus sylvestris* population and conclusions for conservation measures.
927 *Plant Genetic Resources*, *15*(1), 12-20. doi:10.1017/S1479262115000301

928 Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression.
929 *Annual Review of Ecology and Systematics*, *27*(1), 83-109. doi:
930 10.1146/annurev.ecolsys.27.1.83

931 Rousset, F. (2008). Genepop'007: a complete re□implementation of the genepop software for
932 Windows and Linux. *Molecular Ecology Resources*, *8*, 103-106. doi:10.1111/j.1471-
933 8286.2007.01931.x

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

937 Spotts, R. A., & Ferree, D.C. (1979). Photosynthesis, transpiration, and water potential of
938 apple leaves infected by *Venturia inaequalis*. *Phytopathology*, *69*(7), 717-719. doi:
939 10.1094/Phyto-69-717

940 Stukenbrock, E. H., Christiansen, F. B., Hansen, T. T., Dutheil, J. Y., & Schierup, M. H.
941 (2012). Fusion of two divergent fungal individuals led to the recent emergence of a unique
942 widespread pathogen species. *Proceedings of the National Academy of Sciences*, *109*(27),
943 10954-10959. doi: 10.1073/pnas.1201403109

944 Stukenbrock, E. H. (2016a). Hybridization speeds up the emergence and evolution of a new
945 pathogen species. *Nature genetics*, *48*(2), 113. doi: 10.1038/ng.3494

946 Stukenbrock, E. H. (2016b). The role of hybridization in the evolution and emergence of new
947 fungal plant pathogens. *Phytopathology*, *106*(2), 104-112. doi: 10.1094/PHYTO-08-15-0184-
948 RVW

949 Tine, M., Kuhl, H., Gagnaire, P. A., Louro, B., Desmarais, E., Martins, R. S., ... Dieterich, R.
950 (2014). European sea bass genome and its variation provide insights into adaptation to
951 euryhalinity and speciation. *Nature communications*, *5*, 5770. doi: 10.1038/ncomms6770

952 Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S., ...
953 Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary Applications*, *9*(7), 892-
954 908. doi: 10.1111/eva.12367

955 Vanden Broeck, A. V., Villar, M., Van Bockstaele, E., & Van Slycken, J. (2005). Natural
956 hybridization between cultivated poplars and their wild relatives: evidence and consequences
957 for native poplar populations. *Annals of Forest Science*, *62*(7), 601-613. doi:
958 10.1051/forest:2005072

959 Vavilov NI. (1931). The role of Central Asia in the origin of cultivated plants. *Bulletin of*
960 *Applied Botany Genetics and Plant Breeding* 26(3), 3-44.

961 Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., ... Salvi,
962 S. (2010). The genome of the domesticated apple (*Malus*×*domestica* Borkh.). *Nature*
963 *genetics*, *42*(10), 833. doi: 10.1038/ng.654

964 Wang, H., Vieira, F.G., Crawford, J. E., Chu, C. & Nielsen, R. (2017).Asian wild rice is a
965 hybrid swarm with extensive gene flow and feralization from domesticated rice. *Genome*
966 *Research*, 27(6),1029-1038. doi: 10.1101/gr.204800.116.

Wang, Y., Arenas, C. D., Stoebel, D. M., Flynn, K., Knapp, E., Dillon, M. M., ... Cooper, T. F. (2016). Benefit of transferred mutations is better predicted by the fitness of recipients than by their ecological or genetic relatedness. *Proceedings of the National Academy of Sciences*, *113*(18), 5047-5052. doi: 10.1073/pnas.1524988113

Weir, B. S., & Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution 38*: 1358-70.

Wolf, D. E., Takebayashi, N., & Rieseberg, L. H. (2001). Predicting the risk of extinction through hybridization. *Conservation Biology*, *15*(4), 1039-1053. doi: 10.1046/j.1523-1739.2001.0150041039.x

Zeder, M. A., Emshwiller, E., Smith, B. D., & Bradley, D. G. (2006). Documenting domestication: the intersection of genetics and archaeology. *Trends in Genetics*, *22*(3), 139-155. doi: 10.1016/j.tig.2006.01.007

Zohary, D., & Hopf, M. (2000). *Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley*. Oxford University Press, New York.

Data accessibility

All data will be archived and available on the following link, doi: 10.5281/zenodo.3565732

Author Contributions

AF, EG, TG and CL wrote the manuscript with inputs from BLC, VC, JS and LD. Sampling in Kazakhstan was performed by CL and BLC. Apple microsatellite genotyping was performed by AF. Apple scab SNP design and genotyping was performed by MDG, MS and CL. SNP mapping and calling was performed by LD and JS. EG, MNB, PE and VC managed *in vitro* cultures of *Venturia inaequalis* and pathology assays. AF, EG and CL performed population genetics and statistical analyses. CL, BLC and TG conceived the project and obtained financial support.

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Tables

Table 1: Comparison of four alternative demographic models of divergence between the reference wild-type and agricultural-type populations of *Venturia inaequalis* using ∂a∂i on the SNP dataset.

Model	*k*	logL	AIC	Model Comparison	N_{Wild}	N_{Agri}	M_{WA}	M_{AW}	T_S	T_{AM}	T_{SC}
SI	3	-1197.8	2403.587014	-	0.094	1.360	-	-	0.092	-	-
IM	5	-981.8	1975.638252	SI***	0.139	0.898	1.259	1.131	0.460	-	-
AM	6	-981.8	1977.698045	SI***, IM^{NS}	0.139	0.892	1.270	1.140	0.460	$1.09x10^{-9}$	-
SC	**6**	**-928.6**	**1871.313317**	**SI***, IM***, AM^{+++}**	**0.163**	**0.991**	**1.355**	**4.389**	**0.227**	-	**0.006**

SI, strict isolation model; IM, isolation with migration model; AM, ancient migration model; SC, secondary contact model; *k*, parameter numbers; logL, log-likelihood; AIC, Akaike Information Criterion; N_{Wild}, effective sizes of wild-type lineage; N_{Agri}, effective sizes of agricultural-type lineage; M_{WA}, migration rates from wild to agricultural-type lineage; M_{AW}, migration rates from agricultural-type to wild-type lineage; T_S, time of split; T_{AM}, duration time since ancient migration; T_{SC}, duration time since secondary contact.
The best supported model (SC) is indicated in bold. Model comparisons within and between classes of models are shown. Nested models were compared using likelihood ratio tests, with subscripts indicating significance levels (abbreviated ***P<0.001; **P <0.01; *P<0.05, NS non-significant). Non-nested models were compared using AIC with relative likelihood of each model compared to the best model L(Mi|Mbest)=exp((AICmin-AICi)/2) (abbreviated +++L(Mi|Mbest)<0.001; ++L(Mi|Mbest) <0.01; +L(Mi|Mbest)<0.05, L(Mi|Mbest)> 0.05 : AIC difference shown).

Figure legends

Figure 1: Summary of previous studies and questions addressed in the current study on *Malus* apple trees (A) and on their fungal pathogen *Venturia inaequalis* (B). **A.** The divergence and gene flow are shown at the top between the domesticated apple tree *M. domestica*, its initial progenitor *M. sieversii* from Asia and its secondary contributor the European crabapple tree *M. sylvestri*s (Cornille et al. 2012). The distribution areas of the two wild apple trees are shown at the bottom, indicating (1) the initial domestication in Asia (Cornille et al. 2012), migration along the Silk Road (yellow full line) and (2) secondary gene flow between *M. domestica* and *M. sylvestris* in Europe (Cornille et al. 2013; Feurtey et al. 2017), followed by dispersion of modern cultivars worldwide (dashed yellow arrows). (3) A couple of previous studies have investigated the consequence of the secondary contact between the domesticated apple and its Asian progenitor in terms of gene flow from *M. domestica* to *M. sieversii* (Cornille et al. 2012; 2012; Omasheva et al., 2017), and here we study the consequences of planting orchards in forest. **B.** The divergence is shown at the top between the fungal pathogen *V. inaequalis* populations and their distribution area at the bottom, with colored trees symbolizing apple tree species and colored circles the fungal populations. (1) Gladieux et al. (2010b) showed the existence of differentiated populations in Europe on *M. silvestris* and *M. domestica* (1A), in anthropic Central Asian areas on *M. sieversii* and *M. domestica* (1B) and in the wild Central Asian forests on *M. sieversii* (1C). (2) Lemaire et al. (2016) showed that the differentiated European *V. inaequalis* population on cultivated apples carrying the Rvi6 resistance originated from *M. floribunda*. (3) Leroy et al. (2016) investigated the existence of gene flow between populations infecting Riv6 and non-Riv6 apple trees in Europe. (4) The present study investigates the occurrence of gene flow among Central Asian pathogen populations, i.e., the population in anthropic Central Asian areas on *M. sieversii* and *M. domestica* and the population in the wild Central Asian forests on *M. sieversii* .

Figure 2: Genetic diversities and ancestry assignments for apple trees and *Venturia inaequalis*. (A) Principal component analysis on genotypes of 240 apple trees (*Malus spp*.) for 28 microsatellite markers, with their ancestry assignment (P_{dom}) to the cultivated apple tree *M. domestica* indicated by color. The distribution of the P_{dom} index is shown in the inset at the top left. (B) Principal component analysis on genotypes of 255 *V. inaequalis* strains for 181 SNPs, with their ancestry assignment (P_{agr}) to the agricultural-type of *V. inaequalis* indicated by color. The distribution of the P_{agr} index is shown in the inset at the top left.

Figure 3: Distribution of the samples of apple trees and their fungal pathogen *Venturia inaequalis* in nine sites in the Tian Shan Mountains near Almaty (Kazakhstan). The black dot on the upper left corner map represents the global sampling location. Proportions of wild (blue), domesticated/agricultural (yellow) and hybrid (red) genotypes are shown for each sampling site for *Malus* trees (upper row of pie charts) and for their scab fungal pathogen (lower row of pie charts). The pie charts below the figure correspond to sampling sites in the natural forest. The pie charts above the figure refer to a sampling location composed of two sites, an orchard and the natural forest immediately surrounding it. Codes of the sampling

locations: kot at Koturbulac; tag and tar at Talghar; lac, gec and esi (esi_o for the orchard and esi_f for the surrounding forest) at Esik; tuk and tum at Turgen.

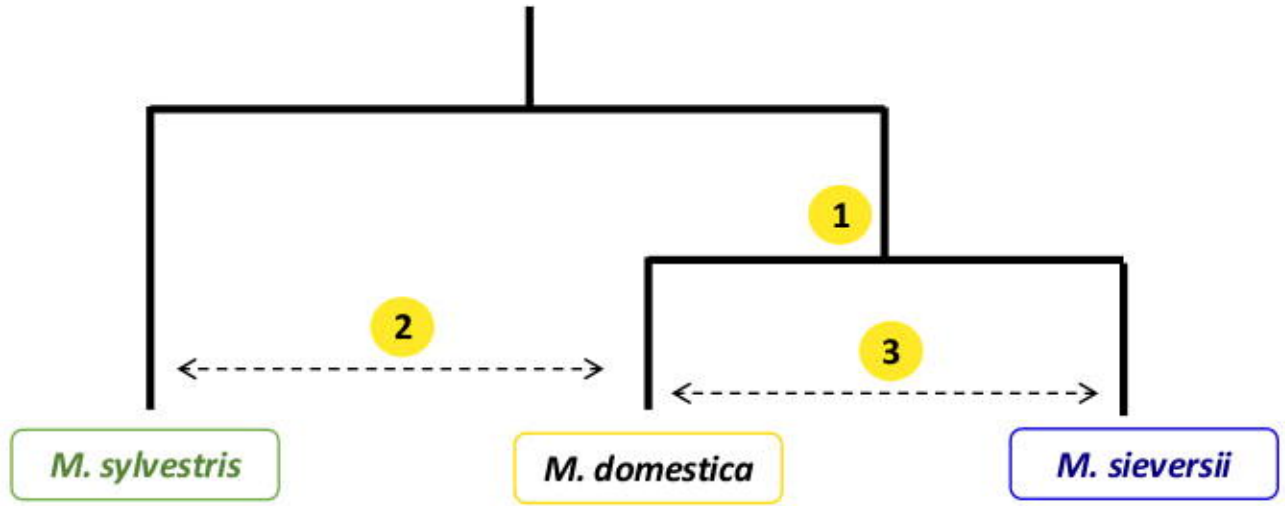
Figure 4: The proportion of *Malus domestica* ancestry in the apple tree (*y* axis) plotted against the proportion of agricultural-type ancestry in *Venturia inaequalis* strains (x axis). A) For each of the 249 host-pathogen pairs, two sampling sites are highlighted: the orchard (esi_o) in orange and the surrounding forest (esi_f) in blue. B) For each of the 189 host-pathogen pairs for which the genotype at the *V_081690_319* locus was available, the *V. inaequalis* strains carrying the tyrosine (A) allele were colored in grey and the ones carrying the stop codon (G) allele in red.

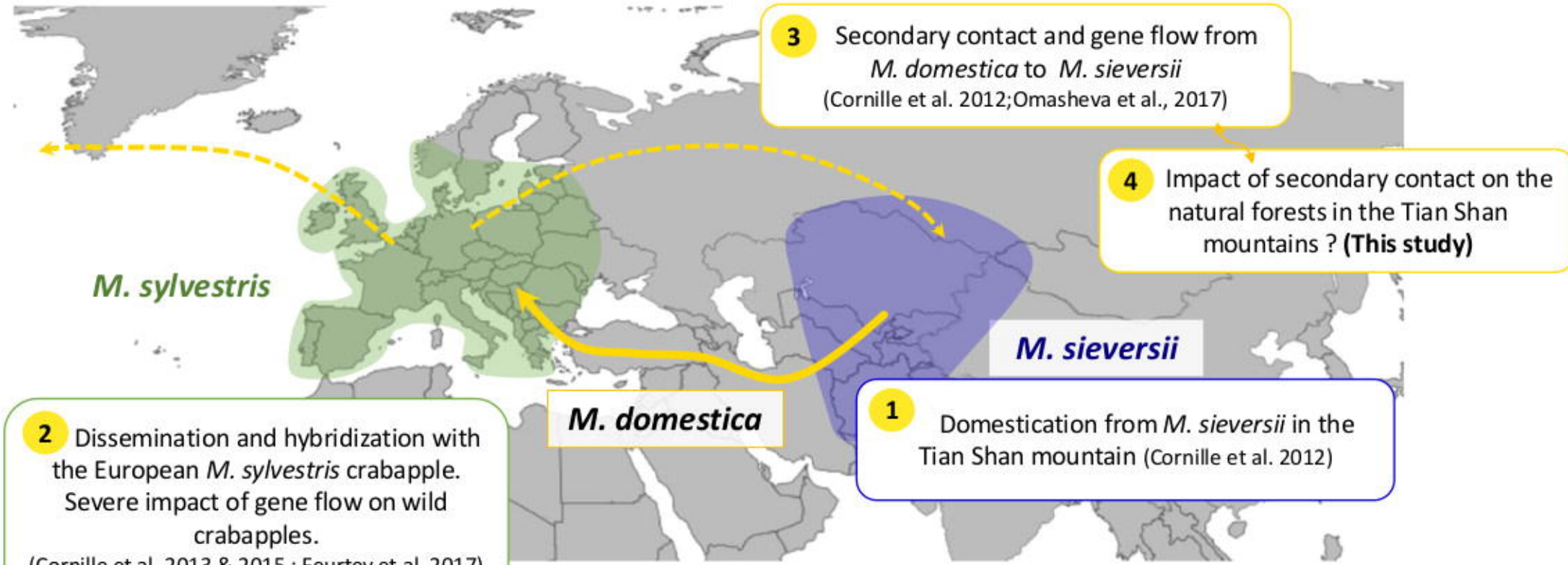
Figure 5: Results of pathogenicity experiments in controlled conditions consisting in artificial inoculation of the fungus *Venturia inaequalis* (57 fungal strains; mean of three replicates per strain, one genotype per apple species) on apple tree leaves (*Malus sieversii* and *M. domestica*). Boxplots of the percentage of scabbed leaf area for wild-type, agricultural-type and hybrid *V. inaequalis* strains of A) *M. domestica* at 21 days post inoculation (dpi) and B) *M. sieversii* at 19 dpi. The box represents the lower and the upper quartiles. The thick horizontal line represents the median. The whiskers represent the largest and lowest observed values that fall within the distance of 1.5 times the interquartile range. The points represent the mean values of the percentage of diseased leaf area for each strain across the three replicates. Different letters indicate significant differences between populations (P<0.05; Wilcoxon's rank sum tests).

Figure 6: Pictures illustrating the percentage of scabbed leaf area for the most virulent wild-type, agricultural-type and hybrid *Venturia inaequalis* strains on A) *Malus domestica* at 21 days post inoculation (dpi) and B) *M. sieversii* at 19 dpi.

Figure 7: Demographic inference on the divergence between wild and agricultural types of *Venturia inaequalis* in Kazakhstan. **A.** The four contrasted scenarios of divergence compared, with either strict isolation (SI), isolation with continuous migration (IM), isolation with initial migration (AM) and secondary contact (SC), with effective populations sizes of the ancestral population (N_{ref}) and of the two daughter populations (N_w and N_A), the rate of gene flow in the two directions (M_w and M_A) and the time since the cessation of gene flow (T_{AM}) or the secondary contact (T_{SC}). **B.** The joint allele-frequency spectrum (AFS) for the wild and agricultural fungal populations, showing the count of derived allele. Each entry of the joint AFS is colored by the number of SNPs in it, according to the scale shown. **C.** The secondary contact (SC) model that obtained the best support and estimates of the parameters.

A


1
2
3
M. sylvestris
M. domestica
M. sieversii


3 Secondary contact and gene flow from *M. domestica* to *M. sieversii* (Cornille et al. 2012;Omasheva et al., 2017)
4 Impact of secondary contact on the natural forests in the Tian Shan mountains ? **(This study)**
M. sylvestris
M. domestica
M. sieversii
2 Dissemination and hybridization with the European *M. sylvestris* crabapple. Severe impact of gene flow on wild crabapples. (Cornille et al. 2013 & 2015 ; Feurtey et al. 2017)
1 Domestication from *M. sieversii* in the Tian Shan mountain (Cornille et al. 2012)

B

1 Identification of 3 *V. inaequalis* populations (Gladieux et al 2010b):
- 1A in Europe
- 1B in anthropic Central Asian areas
- 1C in the wild Central Asian forests

2 The European *V. inaequalis* population on cultivated apples carrying the Rvi6 resistance originated from *M. floribunda* (Lemaire et al. 2016)

Venturia inaequalis

1 2 3 4

M. floribunda-infecting population

European agricultural population *Rvi6*

Wild and agricultural European population

Agricultural population

Wild population

Host and habitat

Gardens **Orchards** **Forests**

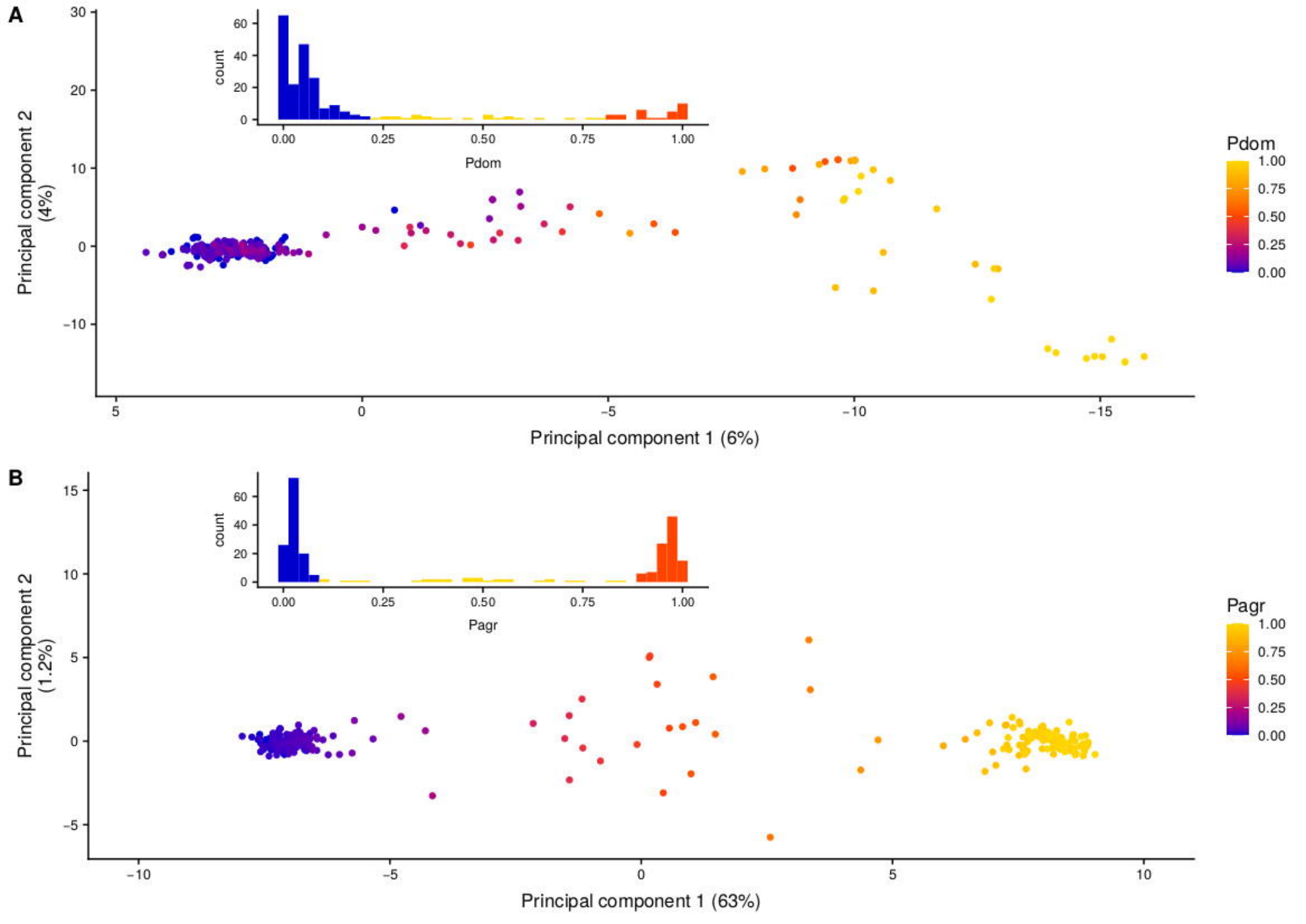
3 Gene flow between populations infecting Rvi6 and non-Rvi6 apple trees in Europe (Leroy et al. 2016)

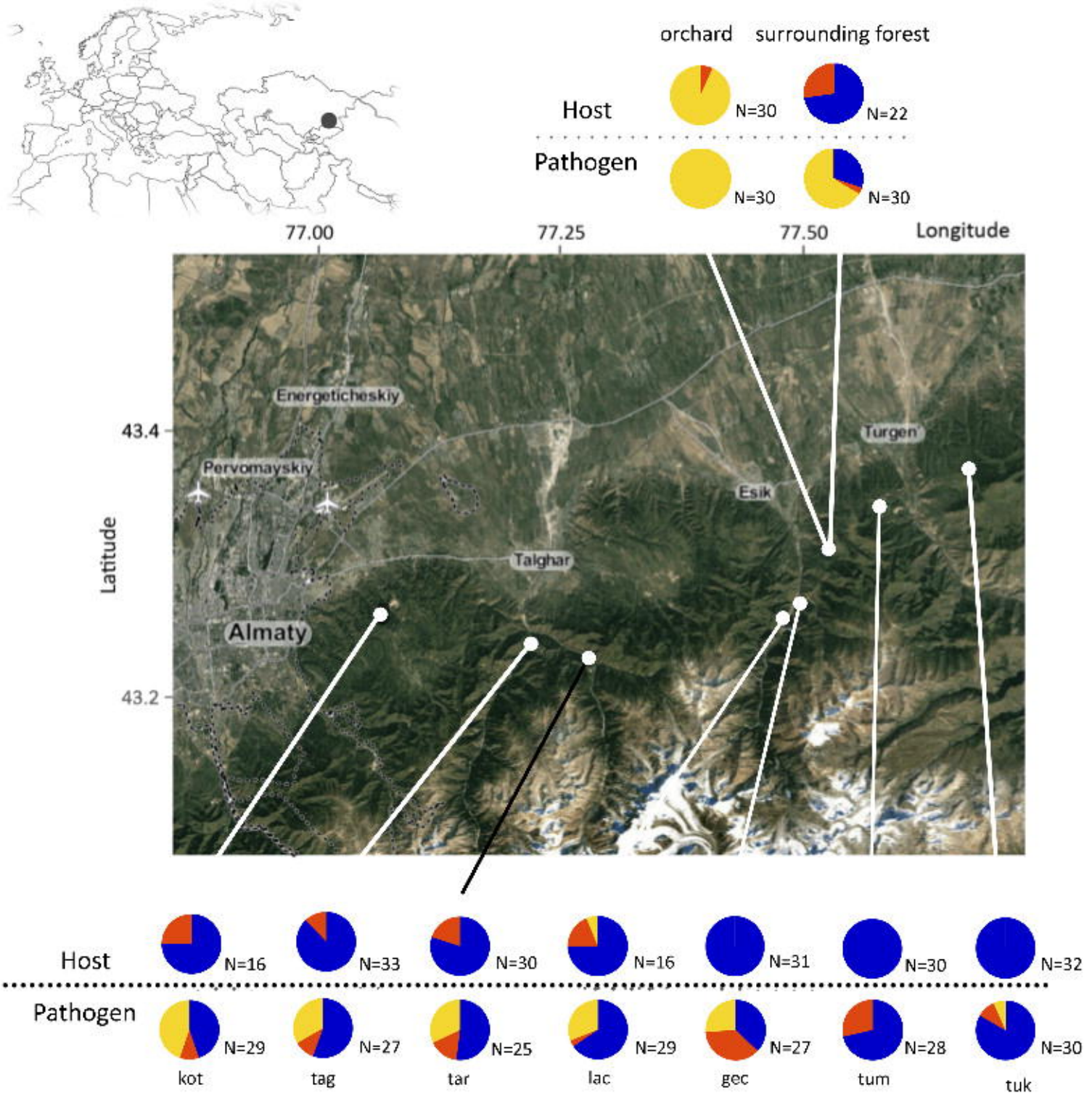
City areas and gardens **Forests**

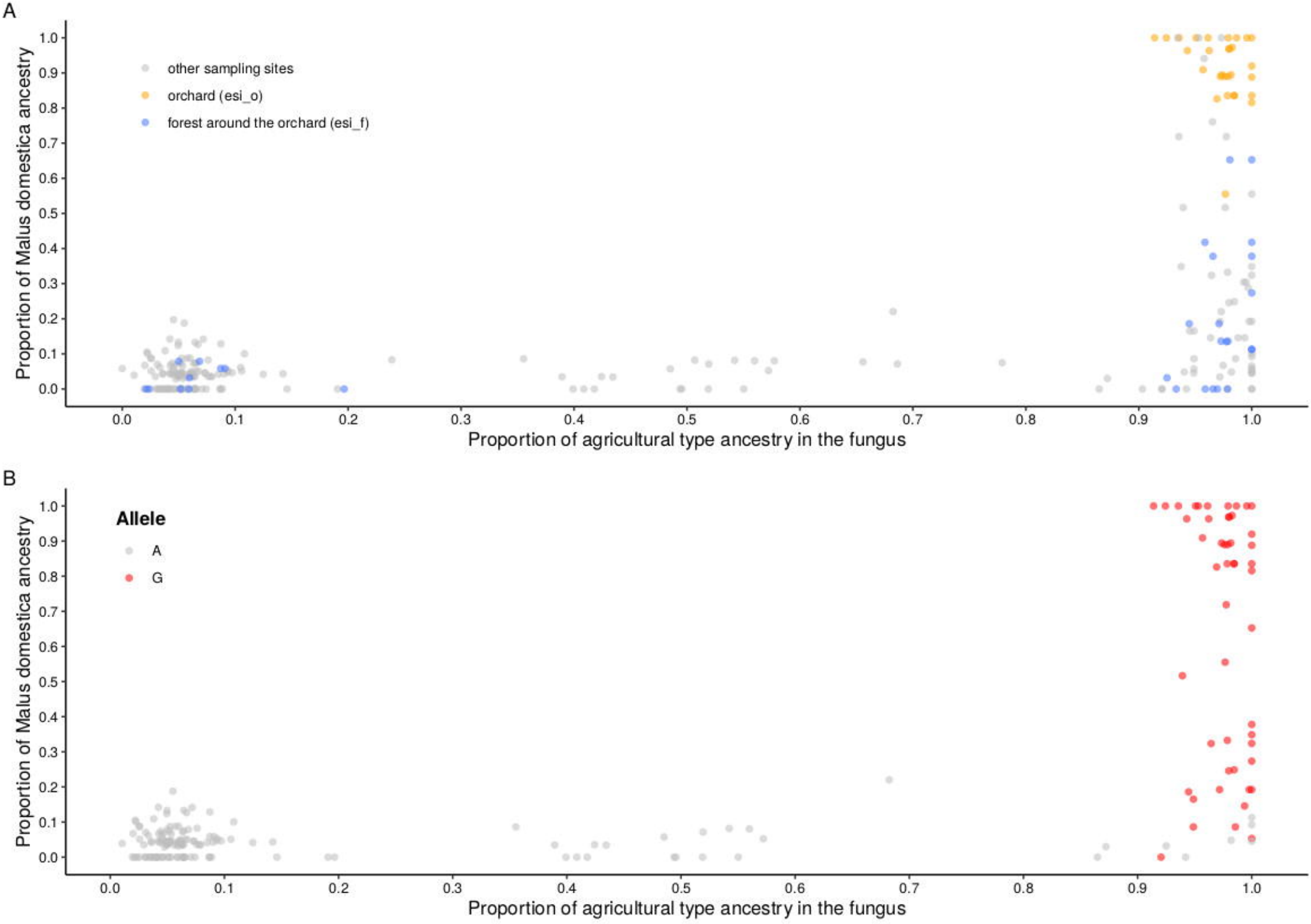
4 Gene flow between Central Asian pathogen populations **(this study)**

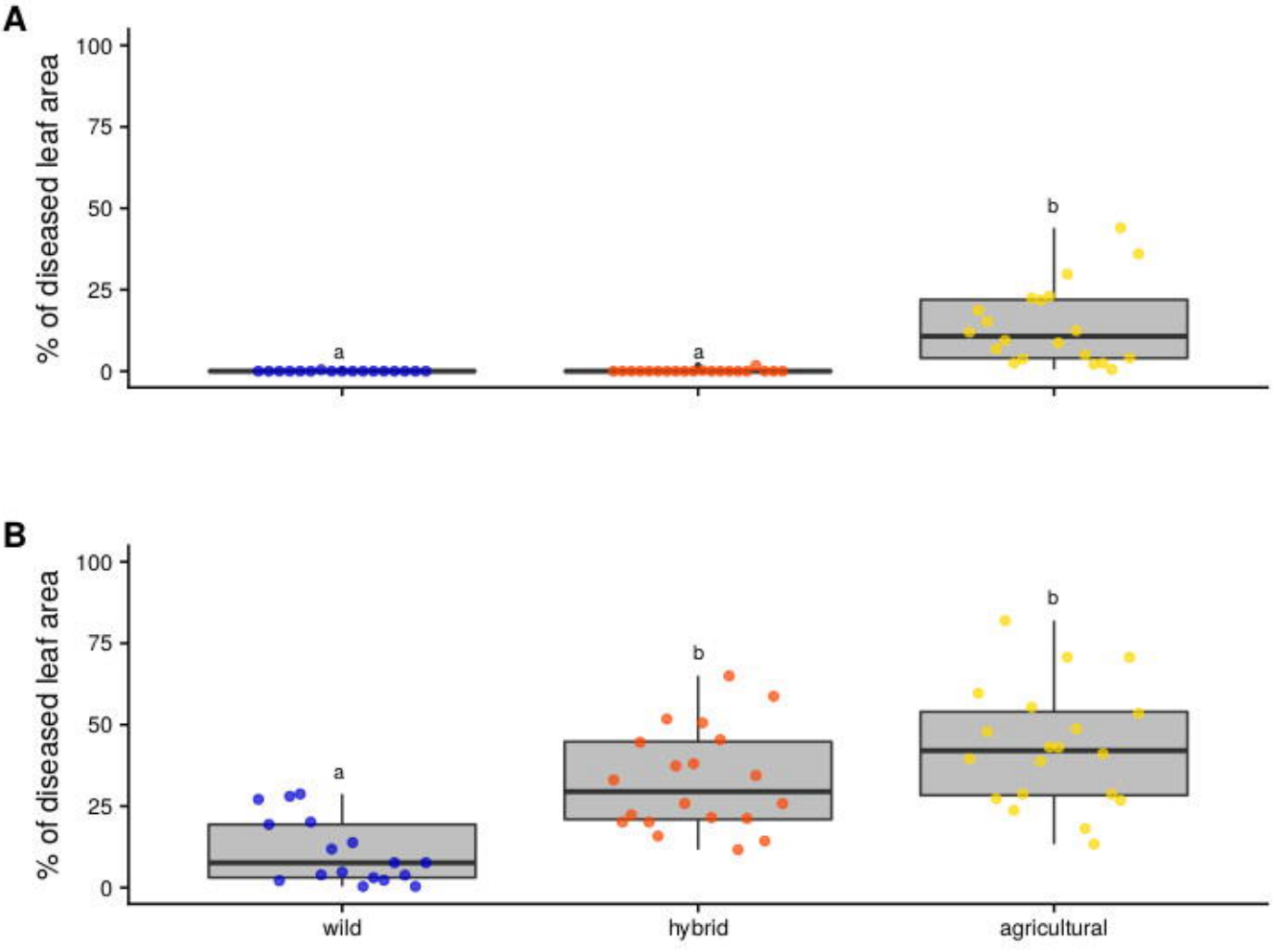
Legend shapes:
- *V. inaequalis*
- Wild apple tree
- Domesticated apple tree

Legend host color:
- *M. domestica with Rvi6*
- *M. domestica non-Rvi6*
- *M. sylvestris*
- *M. sieversii*
- *M. floribunda*

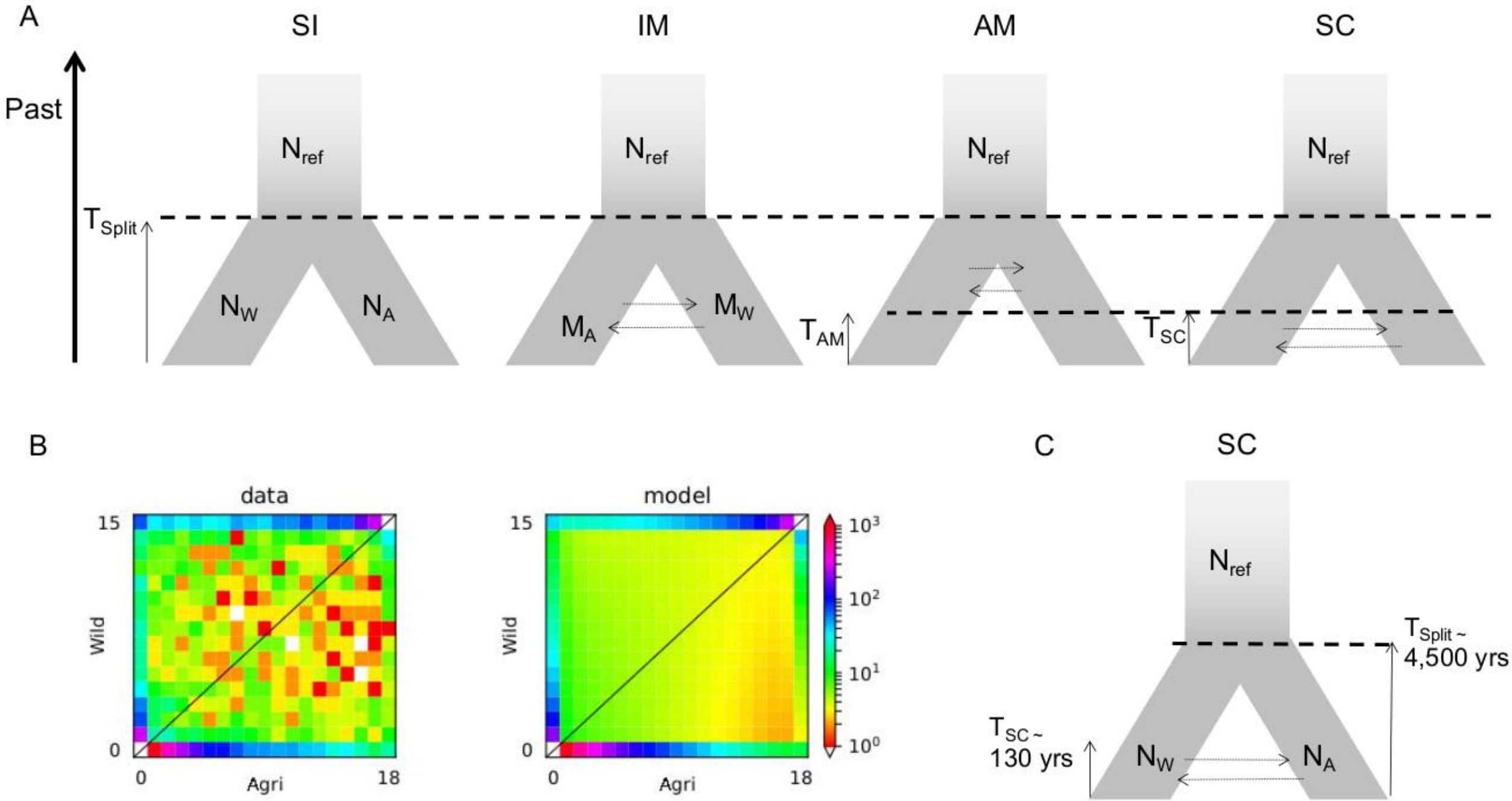

A
Principal component 2 (4%)
Principal component 1 (6%)
count
Pdom
Pdom
1.00
0.75
0.50
0.25
0.00
B
Principal component 2 (1.2%)
Principal component 1 (63%)
count
Pagr
Pagr
1.00
0.75
0.50
0.25
0.00


orchard
surrounding forest
Host
N=30
N=22
Pathogen
N=30
N=30
77.00
77.25
77.50
Longitude
Latitude
43.4
43.2
Energeticheskiy
Pervomayskiy
Esik
Turgen'
Talghar
Almaty
Host
N=16
N=33
N=30
N=16
N=31
N=30
N=32
Pathogen
N=29
N=27
N=25
N=29
N=27
N=28
N=30
kot
tag
tar
lac
gec
tum
tuk


A
other sampling sites
orchard (esi_o)
forest around the orchard (esi_f)
Proportion of Malus domestica ancestry
Proportion of agricultural type ancestry in the fungus
B
Allele
A
G
Proportion of Malus domestica ancestry
Proportion of agricultural type ancestry in the fungus


A
% of diseased leaf area
100
75
50
25
0
a
a
b
B
% of diseased leaf area
100
75
50
25
0
a
b
b
wild
hybrid
agricultural


A
B
wild
hybrid
agricultural


A
SI
IM
AM
SC
Past
N_ref
T_Split
N_W
N_A
M_A
M_W
T_AM
T_SC
B
data
model
15
0
Wild
Agri
18
10^3
10^2
10^1
10^0
C
SC
T_Split ~
4,500 yrs
T_SC ~
130 yrs