

1 **Asian wild apples threatened by gene flow from domesticated apples and by**
2 **their pestified pathogen**

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19

20 **Abstract**

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

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

42

44 **Introduction**

45 The domestication of plants corresponds to genetic and phenotypic differentiation between
46 crops and their wild relatives under human selection (Zeder, Emshwiller, Smith & Bradley,
47 2006). However, crops and their wild relatives often remain interfertile, which can lead to
48 introgression when the two taxa remain geographically close or come into secondary contact
49 (Ellstrand et al., 2013). The hybrids resulting from crop-to-wild pollination events may have a
50 low fitness in natural environments (Wang, Viera, Crawford, Chu & Nielsen., 2017),
51 potentially driving to extinction the wild lineages receiving massive gene flow from its
52 relative crop (Todesco et al., 2016; Wolf, Takebayashi & Rieseberg, 2001). In contrast,
53 hybrids can outcompete pure wild individuals, thus threatening the genetic integrity of wild
54 lineages (Feurtey, Cornille, Shykoff, Snirc & Giraud, 2017; Hooftman, Jong, Oostermeijer &
55 Den Nijs, 2007; Hovick, Campbell, Snow & Whitney, 2012), thereby jeopardizing future
56 adaptation to global changes and leading to a loss of valuable genetic resources for breeding
57 programs.

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

67 and wild host persistence (Lemaire et al., 2016; Leroy, Lemaire, Dunemann & Le Cam, 2013;
68 Leroy et al., 2016). Pathogen spillover (*i.e.*, infection by a pathogen on another host than its
69 endemic host) and hybridization between closely related phytopathogenic fungal species
70 following secondary contact have been reported in several systems (Feurtey & Stukenbrock,
71 2018; Gladieux et al., 2011; Stukenbrock, 2016 a, b). Indeed, when fungal populations have
72 diverged in allopatry and have specialized on new hosts and later met in sympatry (*i.e.*,
73 secondary contact), pre-mating barriers are often weak (Giraud, Refrégier, Le Gac, de Vienne
74 & Hood, 2008; Le Gac & Giraud, 2008) and they can hybridize and/or still infect their
75 ancestral host. The resulting spillover and hybridization events can lead to disease emergence
76 and/or increased disease severity (Ioos, Andrieux, Marçais & Frey, 2006; Lin et al., 2007;
77 Newcombe, Stirling, McDonald & Bradshaw, 2000; Stukenbrock et al., 2012). In anther-smut
78 *Microbotryum* fungi for example, two sister castrating pathogens parasitize two sister plant
79 species and co-occur in sympatry as a consequence of a secondary contact following
80 allopatric divergence (Gladieux et al., 2011). One of the two species, *Microbotryum lychnidis-*
81 *dioicae* (DC. ex Liro, Deml & Oberw.), is however better at infecting both hosts, and
82 spillovers occur in nature (de Vienne, Hood & Giraud, 2009; Gladieux et al., 2011). In
83 poplars, hybrid trees were generated and planted as they were resistant to the two rust species
84 parasitizing the two parental tree species. However, a fungal hybrid between the two rust
85 species rapidly emerged that was able to cause disease on the hybrid poplars (Newcombe et
86 al., 2000). Yet, the consequences of crop-to-wild gene flow on the evolution of wild pathogen
87 populations and on the disease of their wild host has been little investigated. Joint analyses of
88 both wild and agricultural hosts and pathogens are required for a comprehensive
89 understanding of the consequences of secondary contacts of crops and their wild relatives, and
90 of their pathogens, as well as for elucidating coevolutionary histories and local adaptation.

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

93 Here, we addressed these questions on apple trees and their apple scab fungal pathogen,
94 *Venturia inaequalis* (Cooke) G. Winter, which represent good models for investigating the
95 consequences of secondary contact between crops and their wild relatives. They indeed have
96 both wild and agricultural differentiated species/populations that have recently been reunified
97 in their center of origin and may thus possibly hybridize (Figure 1). The cultivated apple
98 (*Malus domestica* Borkh.) is the most cultivated fruit tree of temperate areas worldwide (FAO
99 STATS from 2008-2018, as accessed on 04-07-2020). Analysis of phenotypic diversity,
100 genetic markers, archeological data and demographic inference showed that the cultivated
101 apple was initially domesticated from *M. sieversii* Ledeb. M. Roem (Figure 1A), forming
102 quasi-monospecific natural forests of wild apple in the Tian Shan Mountains in Central Asia
103 (Cornille et al., 2012; Harris, Robinson & Juniper, 2002; Vavilov, 1931; Velasco et al., 2010
104), which have been declared a World Heritage Center by UNESCO. The cultivated apple was
105 imported into Europe by the Romans *via* the Caucasian and Mediterranean coasts, crossing
106 the distribution ranges of various wild apple tree species on the way, leading to major
107 secondary contributions to the cultivated apple tree genepool from several crabapples
108 (Cornille et al., 2012; Cornille, Gladieux & Giraud 2013; Nikiforova, Cavalieri, Velasco &
109 Goremykin, 2013). There has been in particular, in Western Europe, an important contribution
110 from the European crabapple, *M. sylvestris* (L.) Mill, to the *M. domestica* genome (Figure
111 1A). Domestication and breeding has led to high differentiation between *M. domestica* and its
112 various wild progenitors (Cornille et al., 2012). However, because apple species generally
113 have weak interspecific barriers, introgression can still occur between wild and cultivated

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

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

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

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

160 plant and its associated pathogen have not been investigated yet. The lack of diagnostic
161 morphological features for apple species makes the use of genetic markers necessary for
162 species and hybrid identification (Cornille, Giraud, Smulders, Roldán-Ruiz & Gladioux,
163 2014). We therefore used microsatellite markers in apple trees and genome-wide single-
164 nucleotide polymorphisms (SNPs) in *V. inaequalis*, together with pathogenicity assays, to
165 assess the occurrence and impact of secondary contact on both fruit trees and their fungal
166 pathogens in their center of diversity. First, we investigated whether the introduction of
167 domesticated apple trees in Central Asia threatened the genetic integrity of *M. sieversii* wild
168 apple trees via introgressions, and whether orchards planted in forests represent particular
169 threats. We then assessed whether the agricultural-type fungal population occurred on wild
170 apple trees and hybridized with the wild-type population. We tested, using demographic
171 modeling and scenarios comparison, the hypothesis that the co-occurrence and hybridization
172 of agricultural-type and wild-type *V. inaequalis* populations resulted from a secondary contact
173 at the time when domesticated trees were introduced in orchards near wild apple forests, after
174 a period of allopatry. We also tested whether some genomic regions in *V. inaequalis* were
175 good predictors of its ability to parasitize the different host populations. Finally, we tested,
176 using experimental inoculations, whether such gene flow increased the threats to the wild
177 apple tree through increase in virulence of the pathogen populations.

178

179 **Material and Methods**

180 **Sampling**

181 Plant and fungal materials were sampled in 2012 at nine sites in Kazakhstan in the Tian Shan
182 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

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

213

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

215 We used the set of single nucleotide polymorphisms (SNPs) previously detected in the
216 genomes of the 15 wild-type and 18 agricultural-type reference strains (Gladieux et al.,
217 2010b; Le Cam et al., 2019). SNPs were filtered in order to avoid transposable elements and
218 AT-rich regions, as well as more than 20% of missing data (Le Cam et al., 2019). After
219 filtering, the dataset contained 1,210,121 SNPs, with no bias of missing data frequencies
220 between wild and agricultural populations. In order to identify a set of SNPs distinguishing
221 agricultural and wild-type *V. inaequalis* strains, we selected 192 SNPs among the 1,210,121
222 available SNPs as those meeting three requirements: *i*) with moderate ($F_{ST} = 0.21$) to high
223 ($F_{ST} = 1$) differentiation between wild-type and agricultural-type reference populations
224 (Figure S1, Table S3), to obtain reasonable statistical power to assign genotypes to wild,
225 agricultural and hybrid types, *ii*) physically distant enough to ensure lack of linkage
226 disequilibrium between them (correlation coefficient between markers within population $r^2 <$
227 0.1), and *iii*) found in predicted genes (Le Cam et al., 2019).

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

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

238

239 **Genotyping using microsatellite markers for the host apple tree**

240 In order to assign trees to wild, domesticated and hybrid types, all apple trees were genotyped
241 using microsatellite markers. Apple tree DNA was extracted from leaves with the NucleoSpin
242 plant DNA extraction kit II® (Macherey & Nagel). Microsatellite PCR amplifications were
243 performed with a Multiplex PCR Kit® (QIAGEN, Inc.). To genotype the 245 trees, we used
244 33 microsatellite markers spread across the 17 chromosomes using 10 different multiplex
245 reactions, as previously described (Cornille et al., 2012). The genotyping was done at the
246 *Gentyane* platform (INRA, Clermont-Ferrand, France). Only the microsatellite markers with
247 less than 30% of missing data were used and only individuals with less than 50% of missing
248 data were kept for the analyses. The final dataset was thus composed of 240 apple tree
249 samples genotyped at 28 microsatellite markers.

250 Since fungi were sampled on the genotyped host trees, we had genotypes for host and
251 pathogen pairs. We obtained genotypes for 249 host-pathogen pairs, several fungal samples
252 originating from the same trees (132 trees with one fungal strain, 49 trees with two strains,
253 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

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

283 For both apple and *V. inaequalis* samples, we also investigated genetic differentiation and
284 species or genetic lineage assignment through principal component analyses (PCAs) using the
285 ade4 R package (Dray & Dufour, 2007) based on the multilocus genotypes (microsatellite
286 markers for trees and SNPs for fungi). We further investigated the existence of population
287 structure within apple tree species and within the previously delimited *V. inaequalis*
288 populations using the software STRUCTURE v.4.3 (Falush, Stephens & Pritchard, 2003)
289 based on 10 runs of 250,000 Monte Carlo Markov Chain (MCMC) iterations after a burn-in of
290 25,000 iterations using the admixture model and assuming a number of clusters ranging from
291 1 to 6. The output of STRUCTURE was processed with Clumpp (Jakobsson & Rosenberg,
292 2007) and we used the method described in Evanno et al. (2005) and implemented in
293 STRUCTURE HARVESTER (Earl & vonHoldt, 2012) to detect the strongest population
294 subdivision level. We tested the significance of correlations between the hybrid degrees
295 inferred from coordinate values of the first PCA axis, the hybrid index and the ancestry
296 probability given by STRUCTURE using a Pearson's product-moment correlation coefficient
297 in R.

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

302 Significativity of global and pairwise F_{ST} was assessed using Fisher's exact tests of allele
303 frequency differences between sampling sites with 10,000 dememorization and batches and
304 10,000 iterations per batch. We also performed Mantel tests of correlation (Mantel, 1967)
305 between pairwise F_{ST} matrices computed for apple and *V. inaequalis* populations on the one
306 hand, and between each pairwise F_{ST} matrix and a matrix of geographical distance between
307 sampling sites on other hand, using the *mantel.test* function of the ape v5.3 R package
308 (Paradis & Schliep, 2019).

309

310 **Genetic footprints of host adaptation in *Venturia inaequalis***

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

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

330

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

332 In order to test for an effect of the agricultural, hybrid or wild-type status of *V. inaequalis*
333 strains on their pathogenicity on *M. domestica* and *M. sieversii*, we performed artificial
334 inoculations using 57 strains from our sampling, corresponding to 20 strains identified as
335 agricultural-type, 17 strains identified as wild-type and 20 hybrid strains (Table S1). We used
336 one variety of *M. domestica* (GALA[®], X4712) and one accession of *M. sieversii* (GMAL
337 3619.b) for the pathogenicity tests. Gala is a variety planted worldwide despite being highly
338 susceptible to apple scab. GMAL 3619.b has been collected from the Tarbagatai mountain
339 range by American apple breeders (Forsline, Aldwinckle, Dickson, Luby & Hokanson, 2003)
340 and then introduced in France. We grafted the plants on the rootstock MM106 to obtain a
341 vigorous growth favorable to disease development and we inoculated *V. inaequalis* strains
342 when the plants were actively growing. Prior to inoculation, grafted plants were transferred to
343 a quarantine-controlled climatic chamber. Because of the Asian origin of the strains, we used
344 a quarantine chamber to prevent the risk of pathogen escape. Due to the lack of space in
345 climatic chambers, we had to perform experiments at two different dates. In the first
346 experiment, we used 10 agricultural-type strains, 10 hybrids and 8 wild-type strains. In the

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

351 Each strain of *V. inaequalis* was grown on a cellophane sheet placed on malt-agar medium at
352 17°C to obtain spores (Caffier et al., 2014). The cellophane sheets were dried and stored at -
353 20°C. A spore suspension was made by collecting spores from these cellophane sheets and
354 diluting them in water to a final concentration of 1.5×10^5 spores mL⁻¹ (Lê Van et al., 2012).
355 For each strain, the spore suspension was inoculated on three Gala trees and three GMAL
356 3619.b trees using a mechanical air pressure sprayer. As trees actively grew during the
357 experiment, we labelled the youngest fully deployed leaf of each tree as the F0 leaf, one or
358 two days prior to inoculation to facilitate later disease scoring. To favor spore germination
359 and fungal infection, the plants were kept in darkness with moisture maintained at 100% and
360 temperature at 17°C for 48 hours after inoculation (Lê Van et al., 2012). Afterward, moisture
361 was reduced to 80% during the day and 90% at night, with 12 hours of light per day. These
362 climatic conditions are highly favourable to scab infection and thus provide a good indication
363 of the strain ability to infect tree genotypes (MacHardy, 1996). For each host genotype, plants
364 were randomized within three blocks to have in each block one host replicate for each strain.
365 Disease severity, *i.e.*, virulence of the fungal strain, was measured when there was no more
366 increase of the disease symptoms: at 19 days post inoculation (dpi) for *M. sieversii* and at 21
367 dpi for *M. domestica*. Disease severity was measured as the percentage of a leaf displaying
368 sporulation, from 0% when there was no disease symptom to 100% when the whole leaf was
369 covered with spores. The F0 leaf being the last leaf deployed one or two days prior to
370 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 $\partial a \hat{\partial} i$ (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 $\hat{d}a\hat{d}i$ (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 $\hat{d}a\hat{d}i$; 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 $G \times s/S$ (Gutenkunst et al., 2009), where G is the

418 size of the genome used, S is the total number of SNPs called in G , and s the actual number of
419 SNPs used for inference. In our analysis, 1,210,121 SNPs were called on a portion of 34,942
420 Mb of the genome, 6,187 SNPs were used, so that L was computed as $34,942,000 \times (6,187 /$
421 $1,210,121) = 178,648$ bp. All other parameters were scaled by N_{ref} . Times in years were
422 computed using a generation time of one year, as *V. inaequalis* undergoes an obligate sexual
423 reproduction once a year. The model parameters estimated and their 95% confidence intervals
424 were obtained with Godambe methods (Coffman, Hsieh, Gravel & Gutenkunst, 2015) from
425 1000 bootstraps across SNPs.

426

427 **Results**

428 **Contrasting distributions of the wild and agricultural host and pathogen populations in** 429 **the Tian Shan Mountains**

430 We explored the genetic makeup of apple trees and their scab pathogens in a wild
431 environment, by sampling apple trees and fungal strains from the same trees, at nine sites in
432 the forests of the Tian Shan Mountains in Kazakhstan. For apple trees, the PCA (Figure 2A)
433 and STRUCTURE analysis (Figures S3A and B) confirmed the existence of two genetically
434 distinct clusters corresponding to the orchard trees on the one hand, assigned to *M. domestica*,
435 and most of the wild forest trees on the other hand, assigned to *M. sieversii*. We did not detect
436 further population subdivision within the wild species (Figures 2A and S3). Both the first axis
437 of the PCA and STRUCTURE analyses revealed footprints of admixture between the two tree
438 species. The P_{dom} hybrid index values were also estimated for the 240 successfully genotyped
439 trees. All three methods gave congruent results (Figs. 2A and S3C). The correlation
440 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).

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

473 In order to further explore the relationship between host and pathogen genotypes, we
474 compared their genetic diversity and differentiation across the sampling sites. Genetic
475 diversities per sampling site ranged from 0.703 (tuk) to 0.802 (kot) for apple trees and from
476 0.244 (tuk) to 0.487 (kot) for *V. inaequalis* (Table S4). Genetic diversities were minimal and
477 maximal in the same sampling sites for both the host and the pathogen, but no significant
478 correlation was observed between the apple and fungus genetic diversity levels across sites
479 (Kendall's tau=0.389, p-value=0.18; Figure S6). Global F_{ST} among sites were 0.259 for apple
480 trees and 0.243 for *V. inaequalis*. Pairwise F_{ST} estimates ranged from 0.000 to 0.207 for apple
481 trees (Table S5) and from 0.000 to 0.721 for *V. inaequalis* (Table S6). For both the host and
482 the pathogen, the orchard (esi_o) was the most differentiated from other sampling sites
483 (Tables S5 and S6). Host and pathogen pairwise differentiation matrices were significantly
484 correlated (Mantel-test Z-score=0.681, p-value=0.011; Figure S7). Yet, we found no
485 significant correlation between pairwise F_{ST} and geographical distances matrices, neither for
486 apple trees (Mantel test Z-score= 33.10, p-value=0.42) nor for *V. inaequalis* (Mantel test Z-
487 score=103.20, p-value=0.51), indicating a lack of isolation by distance.

488

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, $p\text{-value} = 6.10 \times 10^{-5}$).

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 *Pyraecantha* (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**

534 We then investigated experimentally whether the observed association between fungal
535 pathogen types and host tree species resulted from differences in infection ability, by
536 inoculating 57 fungal strains (20 agricultural-type, 17 wild-type and 20 hybrid strains; Table
537 S1) on one genotype of *M. sieversii* and one genotype of *M. domestica*. All agricultural-type
538 strains could cause disease on *M. domestica* (N=20; Figures 5A and 6A), whereas only one
539 wild-type and two hybrid strains could cause disease on *M. domestica*, and even then, only
540 with very low levels of virulence (less than 2% of diseased leaf area, Figure 5A). All
541 agricultural strains (N=20), hybrids (N=20) and wild strains (N=17) could cause disease on
542 *M. sieversii* (Figure 5B). Both agricultural-type and hybrid strains were significantly more
543 virulent (i.e. caused larger and/or more lesions) than wild-type strains on *M. sieversii* (Figures
544 5B and 6B; Wilcoxon tests: $P=1.7 \times 10^{-5}$ and $P=1.2 \times 10^{-4}$, respectively). The *V. inaequalis*
545 hybrids displayed virulence levels on *M. sieversii* that appeared intermediate between the
546 agricultural-type and wild-type populations although the difference between hybrids and the
547 agricultural-type population was not significant (Figure 5B). Similar results were obtained
548 when assignment to species and hybrids were done using the same hybrid index threshold as
549 for apple trees (wild type: $Pagr < 0.2$; hybrids: $0.2 \leq Pagr \leq 0.8$; agricultural: $Pagr > 0.8$; Figure
550 S11). We found no significant correlation between the proportion of agricultural-type ancestry
551 and the degree of virulence in the hybrid fungal strains (Figure S12; Kendall test: $\tau = -0.047$,
552 $P = 0.7702$).

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

558 two groups of strains corresponding to their alleles at the *V_081690_319* locus, we found that
559 the strains carrying the stop codon (TGA) allele were more virulent than those carrying the
560 tyrosine (TGA) allele, both on *M. domestica* and on *M. sieversii* (Wilcoxon tests: $P=1.4 \times 10^{-8}$
561 and $P=1.2 \times 10^{-3}$, respectively; Figure S13). Most of the strains carrying the tyrosine allele
562 could not cause disease at all on *M. domestica*, but a few of them could induce a low level of
563 symptoms (Figure S13A).

564

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

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

574 The models allowing gene flow received better support than the strict isolation model (AIC=
575 2403,587014; LogL= -1197,793507). The model of isolation with migration (IM; AIC=
576 1975,638252; LogL= -981,8191258) did not perform significantly better than the ancient
577 migration model (AM; AIC= 1977,698045; LogL= -981,8490226). The secondary contact
578 model (SC) received the best support (AIC= 1871,313317, LogL= -928,6566583), which
579 indicated that the agricultural-type *V. inaequalis* population was likely absent in Asian wild
580 apple forests before the recent introduction of domesticated apple trees in orchards (Figure
581 7B; Table 1). Using the estimate of θ obtained from the best model (SC, $\theta=143.729$), a

582 mutation rate μ of 2×10^{-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 ($4,570 \pm 324$ 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 virulence 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-

698 length gene. Taken together with the presence of the full-length gene in an outgroup, the *V.*
699 *inaequalis* lineage parasitizing *Pyracantha* (Gladieux et al., 2010a; Le Cam et al., 2002), this
700 suggests the acquisition of a virulence allele by the agricultural *V. inaequalis* population
701 during crop host tracking, through the loss of a protein recognized by the domesticated apple
702 tree selected for resistance to apple scab. Such an increase in the virulence of a pathogen
703 following domestication of its host is compatible with the pestification process (Salehet al.,
704 2014), and is consistent with virulence evolution mechanisms previously found in many crop
705 pathogens (Raffaele & Kamoun, 2012).

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

714 More comprehensive studies on whole *V. inaequalis* genomes will likely detect further SNPs
715 involved in host adaptation, as suggested by our data. Indeed, the SNP allele corresponding to
716 the stop codon (G allele) was not found in any of the 15 hybrids for which genotypes were
717 available, which significantly deviated from neutral expectations, while agricultural-type
718 strains carrying this allele were found on *M. sieversii*, even if it was at low frequency. In
719 addition, a few strains carrying the full-length allele of the putative avirulence gene were able
720 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).

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984 **Data accessibility**

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

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988 **Author Contributions**

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

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1005 **Tables**

1006

1007 **Table 1:** Comparison of four alternative demographic models of divergence between the
 1008 reference wild-type and agricultural-type populations of *Venturia inaequalis* using $\partial a \partial i$ on the
 1009 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	1.09×10^{-9}	-
SC	6	-928.6	1871.313317	SI***, IM***, AM⁺⁺⁺	0.163	0.991	1.355	4.389	0.227	-	0.006

1010

1011 SI, strict isolation model; IM, isolation with migration model; AM, ancient migration model;
 1012 SC, secondary contact model; k , parameter numbers; logL, log-likelihood; AIC, Akaike
 1013 Information Criterion; N_{Wild} , effective sizes of wild-type lineage; N_{Agri} , effective sizes of
 1014 agricultural-type lineage; M_{WA} , migration rates from wild to agricultural-type lineage; M_{AW} ,
 1015 migration rates from agricultural-type to wild-type lineage; T_{S} , time of split; T_{AM} , duration
 1016 time since ancient migration; T_{SC} , duration time since secondary contact.

1017 The best supported model (SC) is indicated in bold. Model comparisons within and between
 1018 classes of models are shown. Nested models were compared using likelihood ratio tests, with
 1019 subscripts indicating significance levels (abbreviated *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, NS
 1020 non-significant). Non-nested models were compared using AIC with relative likelihood of
 1021 each model compared to the best model $L(\text{Mi}|\text{Mbest}) = \exp((\text{AICmin} - \text{AIC}_i)/2)$ (abbreviated
 1022 +++ $L(\text{Mi}|\text{Mbest}) < 0.001$; ++ $L(\text{Mi}|\text{Mbest}) < 0.01$; + $L(\text{Mi}|\text{Mbest}) < 0.05$, $L(\text{Mi}|\text{Mbest}) > 0.05$:
 1023 AIC difference shown).

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1027 **Figure legends**

1028 **Figure 1:** Summary of previous studies and questions addressed in the current study on *Malus*
1029 apple trees (A) and on their fungal pathogen *Venturia inaequalis* (B). **A.** The divergence and
1030 gene flow are shown at the top between the domesticated apple tree *M. domestica*, its initial
1031 progenitor *M. sieversii* from Asia and its secondary contributor the European crabapple tree
1032 *M. sylvestris* (Cornille et al. 2012). The distribution areas of the two wild apple trees are
1033 shown at the bottom, indicating (1) the initial domestication in Asia (Cornille et al. 2012),
1034 migration along the Silk Road (yellow full line) and (2) secondary gene flow between *M.*
1035 *domestica* and *M. sylvestris* in Europe (Cornille et al. 2013; Feurtey et al. 2017), followed by
1036 dispersion of modern cultivars worldwide (dashed yellow arrows). (3) A couple of previous
1037 studies have investigated the consequence of the secondary contact between the domesticated
1038 apple and its Asian progenitor in terms of gene flow from *M. domestica* to *M. sieversii*
1039 (Cornille et al. 2012; 2012; Omasheva et al., 2017), and here we study the consequences of
1040 planting orchards in forest. **B.** The divergence is shown at the top between the fungal
1041 pathogen *V. inaequalis* populations and their distribution area at the bottom, with colored
1042 trees symbolizing apple tree species and colored circles the fungal populations. (1) Gladieux
1043 et al. (2010b) showed the existence of differentiated populations in Europe on *M. sylvestris*
1044 and *M. domestica* (1A), in anthropic Central Asian areas on *M. sieversii* and *M. domestica*
1045 (1B) and in the wild Central Asian forests on *M. sieversii* (1C). (2) Lemaire et al. (2016)
1046 showed that the differentiated European *V. inaequalis* population on cultivated apples
1047 carrying the Rvi6 resistance originated from *M. floribunda*. (3) Leroy et al. (2016)
1048 investigated the existence of gene flow between populations infecting Rvi6 and non-Rvi6
1049 apple trees in Europe. (4) The present study investigates the occurrence of gene flow among
1050 Central Asian pathogen populations, i.e., the population in anthropic Central Asian areas on
1051 *M. sieversii* and *M. domestica* and the population in the wild Central Asian forests on *M.*
1052 *sieversii*.

1053

1054 **Figure 2:** Genetic diversities and ancestry assignments for apple trees and *Venturia*
1055 *inaequalis*. (A) Principal component analysis on genotypes of 240 apple trees (*Malus spp.*) for
1056 28 microsatellite markers, with their ancestry assignment (P_{dom}) to the cultivated apple tree *M.*
1057 *domestica* indicated by color. The distribution of the P_{dom} index is shown in the inset at the top
1058 left. (B) Principal component analysis on genotypes of 255 *V. inaequalis* strains for 181
1059 SNPs, with their ancestry assignment (P_{agr}) to the agricultural-type of *V. inaequalis* indicated
1060 by color. The distribution of the P_{agr} index is shown in the inset at the top left.

1061

1062 **Figure 3:** Distribution of the samples of apple trees and their fungal pathogen *Venturia*
1063 *inaequalis* in nine sites in the Tian Shan Mountains near Almaty (Kazakhstan). The black dot
1064 on the upper left corner map represents the global sampling location. Proportions of wild
1065 (blue), domesticated/agricultural (yellow) and hybrid (red) genotypes are shown for each
1066 sampling site for *Malus* trees (upper row of pie charts) and for their scab fungal pathogen
1067 (lower row of pie charts). The pie charts below the figure correspond to sampling sites in the
1068 natural forest. The pie charts above the figure refer to a sampling location composed of two
1069 sites, an orchard and the natural forest immediately surrounding it. Codes of the sampling

1070 locations: kot at Koturbulac; tag and tar at Talghar; lac, gec and esi (esi_o for the orchard and
1071 esi_f for the surrounding forest) at Esik; tuk and tum at Turgen.

1072

1073 **Figure 4:** The proportion of *Malus domestica* ancestry in the apple tree (y axis) plotted
1074 against the proportion of agricultural-type ancestry in *Venturia inaequalis* strains (x axis). A)
1075 For each of the 249 host-pathogen pairs, two sampling sites are highlighted: the orchard
1076 (esi_o) in orange and the surrounding forest (esi_f) in blue. B) For each of the 189 host-
1077 pathogen pairs for which the genotype at the *V_081690_319* locus was available, the *V.*
1078 *inaequalis* strains carrying the tyrosine (A) allele were colored in grey and the ones carrying
1079 the stop codon (G) allele in red.

1080

1081 **Figure 5:** Results of pathogenicity experiments in controlled conditions consisting in artificial
1082 inoculation of the fungus *Venturia inaequalis* (57 fungal strains; mean of three replicates per
1083 strain, one genotype per apple species) on apple tree leaves (*Malus sieversii* and *M.*
1084 *domestica*). Boxplots of the percentage of scabbed leaf area for wild-type, agricultural-type
1085 and hybrid *V. inaequalis* strains of A) *M. domestica* at 21 days post inoculation (dpi) and B)
1086 *M. sieversii* at 19 dpi. The box represents the lower and the upper quartiles. The thick
1087 horizontal line represents the median. The whiskers represent the largest and lowest observed
1088 values that fall within the distance of 1.5 times the interquartile range. The points represent
1089 the mean values of the percentage of diseased leaf area for each strain across the three
1090 replicates. Different letters indicate significant differences between populations ($P < 0.05$;
1091 Wilcoxon's rank sum tests).

1092

1093 **Figure 6:** Pictures illustrating the percentage of scabbed leaf area for the most virulent wild-
1094 type, agricultural-type and hybrid *Venturia inaequalis* strains on A) *Malus domestica* at 21
1095 days post inoculation (dpi) and B) *M. sieversii* at 19 dpi.

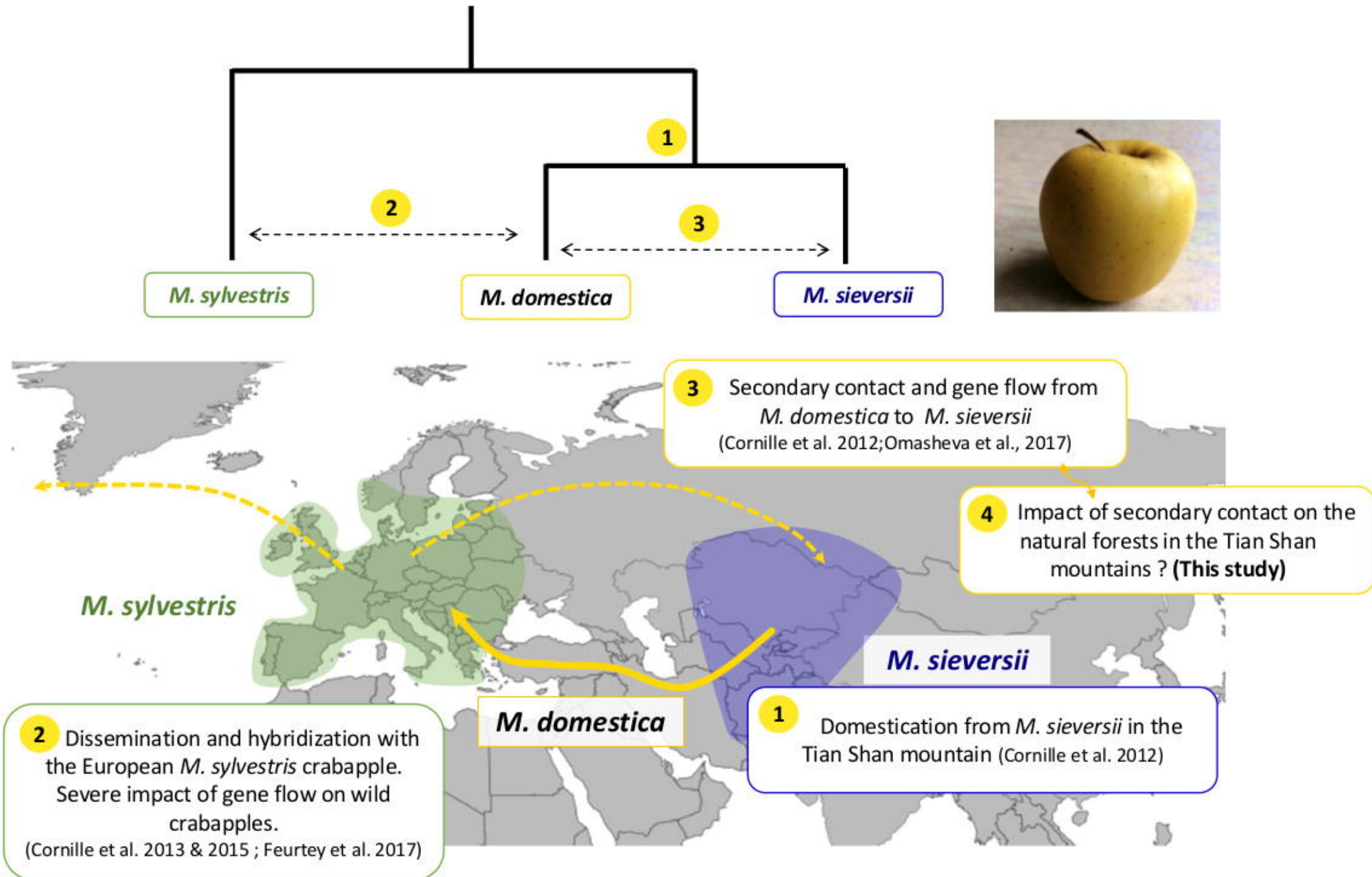
1096

1097 **Figure 7:** Demographic inference on the divergence between wild and agricultural types of
1098 *Venturia inaequalis* in Kazakhstan. **A.** The four contrasted scenarios of divergence compared,
1099 with either strict isolation (SI), isolation with continuous migration (IM), isolation with initial
1100 migration (AM) and secondary contact (SC), with effective populations sizes of the ancestral
1101 population (N_{ref}) and of the two daughter populations (N_w and N_A), the rate of gene flow in
1102 the two directions (M_w and M_A) and the time since the cessation of gene flow (T_{AM}) or the
1103 secondary contact (T_{SC}). **B.** The joint allele-frequency spectrum (AFS) for the wild and
1104 agricultural fungal populations, showing the count of derived allele. Each entry of the joint
1105 AFS is colored by the number of SNPs in it, according to the scale shown. **C.** The secondary
1106 contact (SC) model that obtained the best support and estimates of the parameters.

1107

1108

A

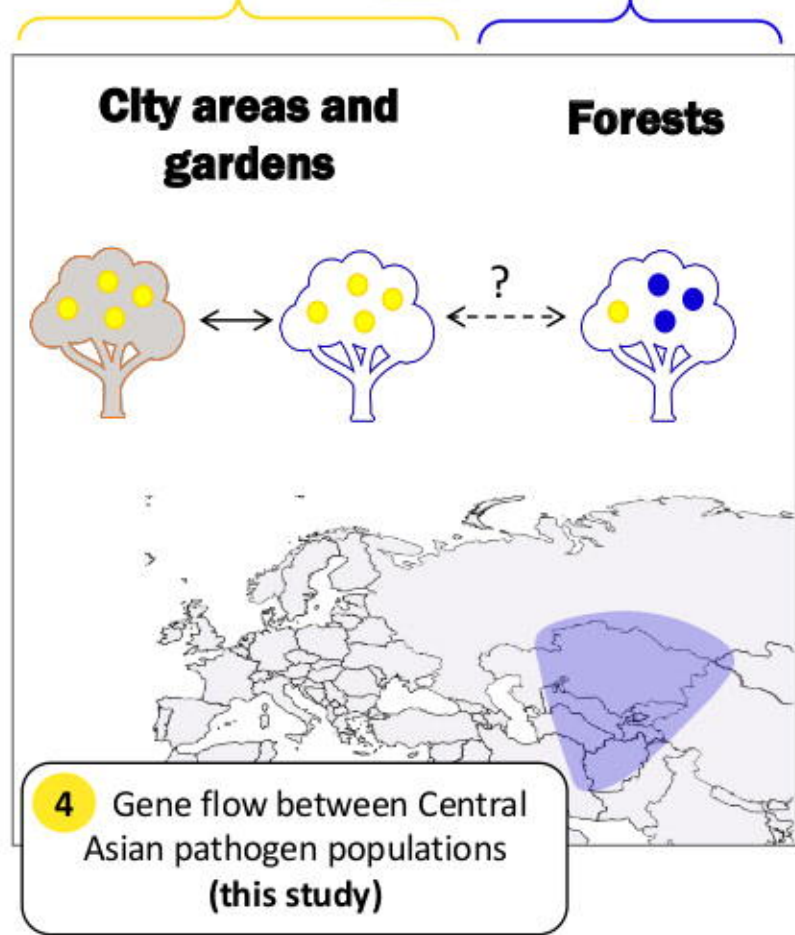
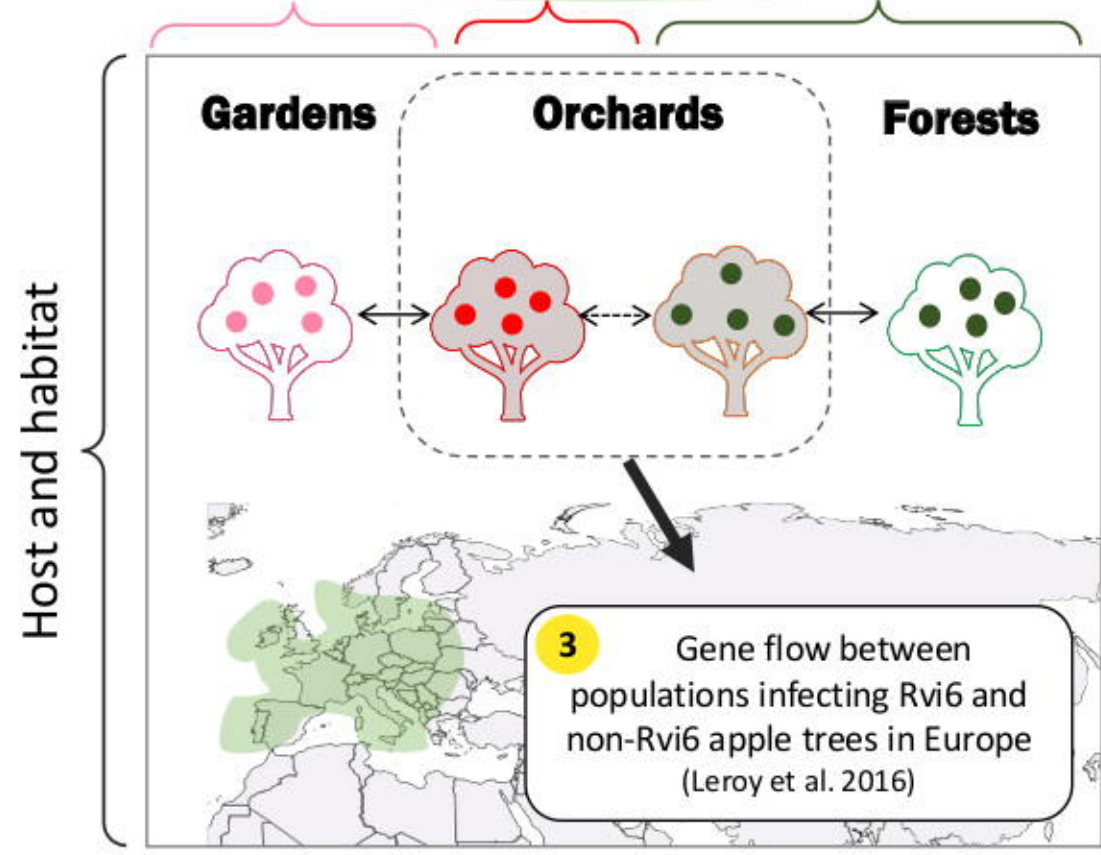
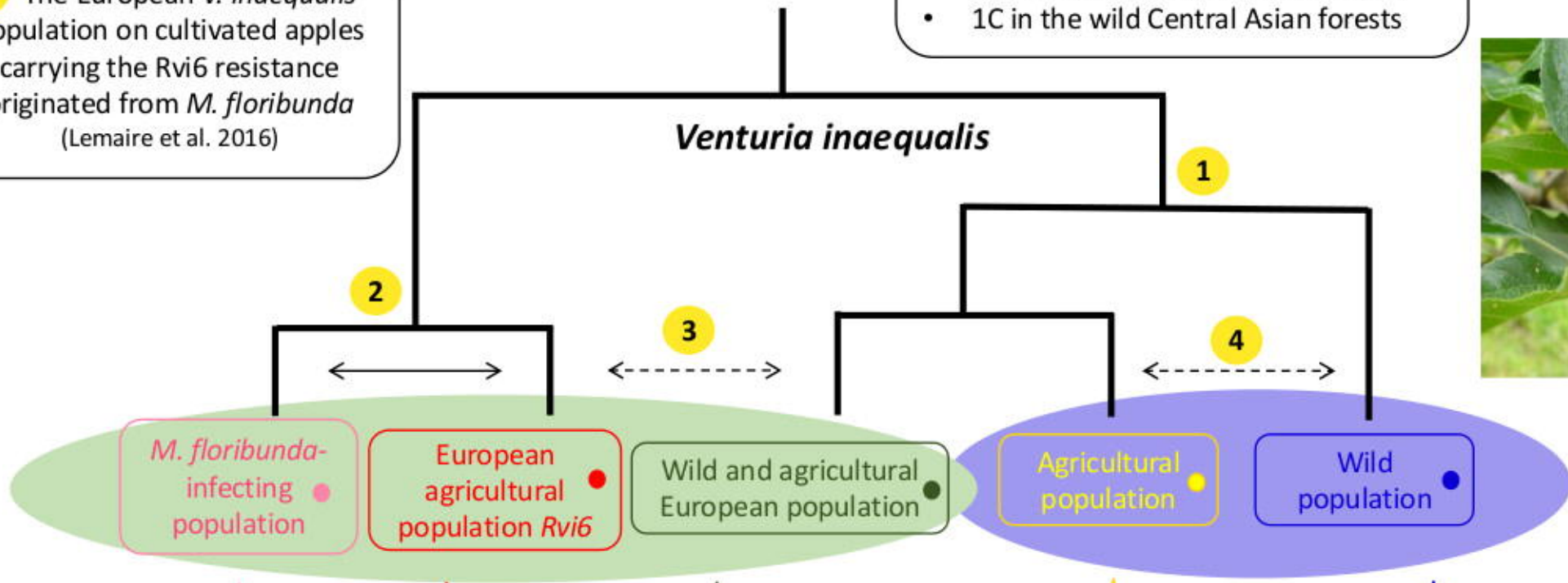


B

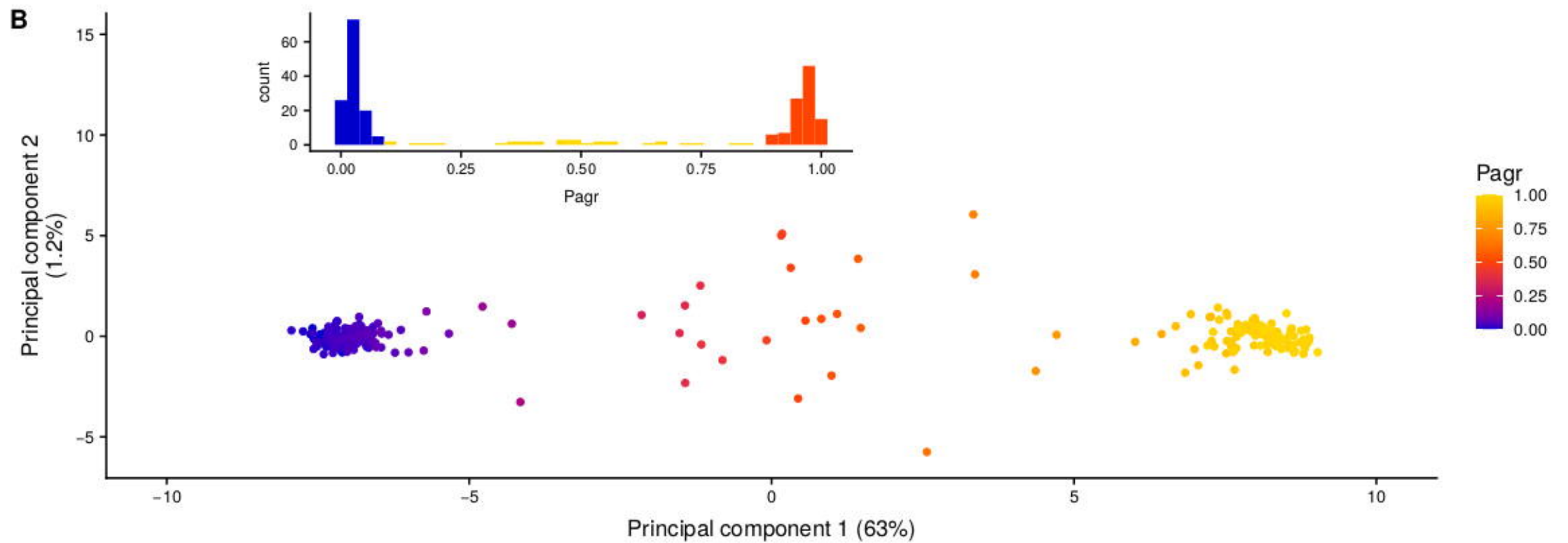
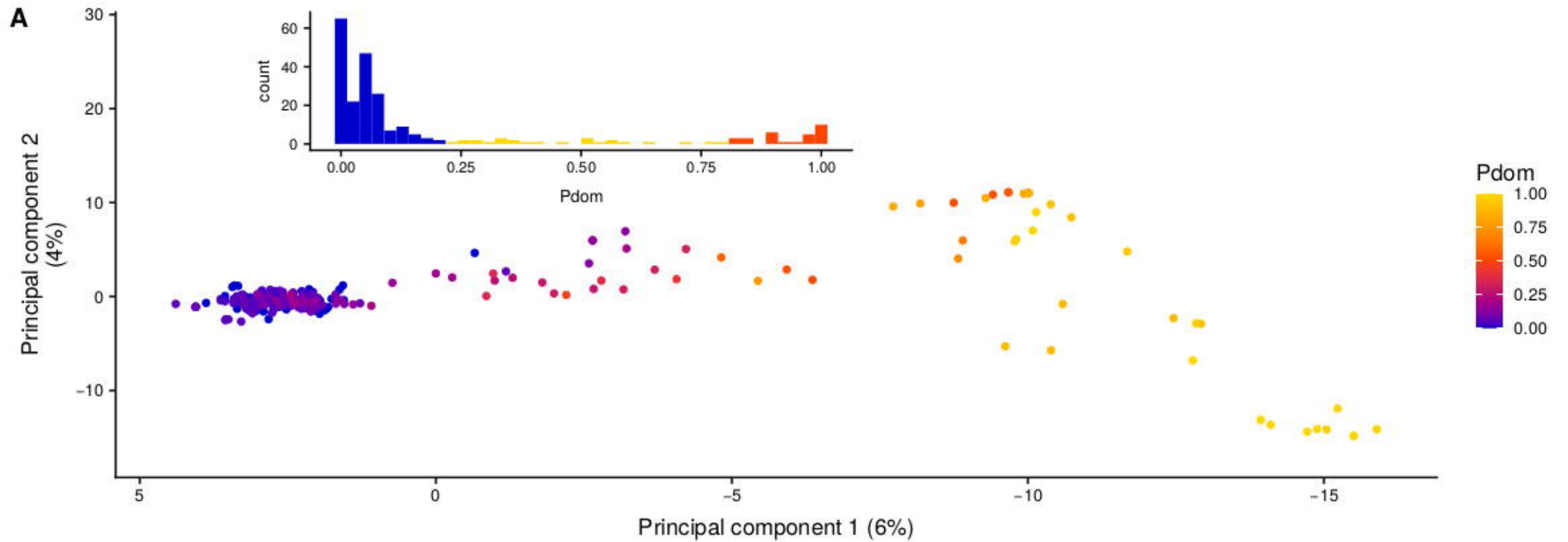
2 The European *V. inaequalis* population on cultivated apples carrying the Rvi6 resistance originated from *M. floribunda* (Lemaire et al. 2016)

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

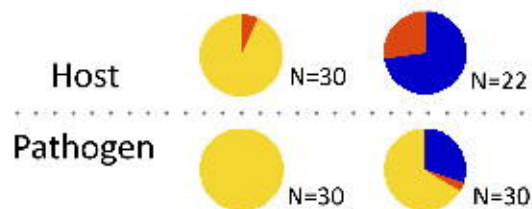


- Legend shapes:**
- *V. inaequalis*
 - 🌳 Wild apple tree
 - 🌳 Domesticated apple tree
- Legend host color:**
- 🌳 *M. domestica* with Rvi6
 - 🌳 *M. domestica* non-Rvi6
 - 🌳 *M. sylvestris*
 - 🌳 *M. sieversii*
 - 🌳 *M. floribunda*





orchard surrounding forest

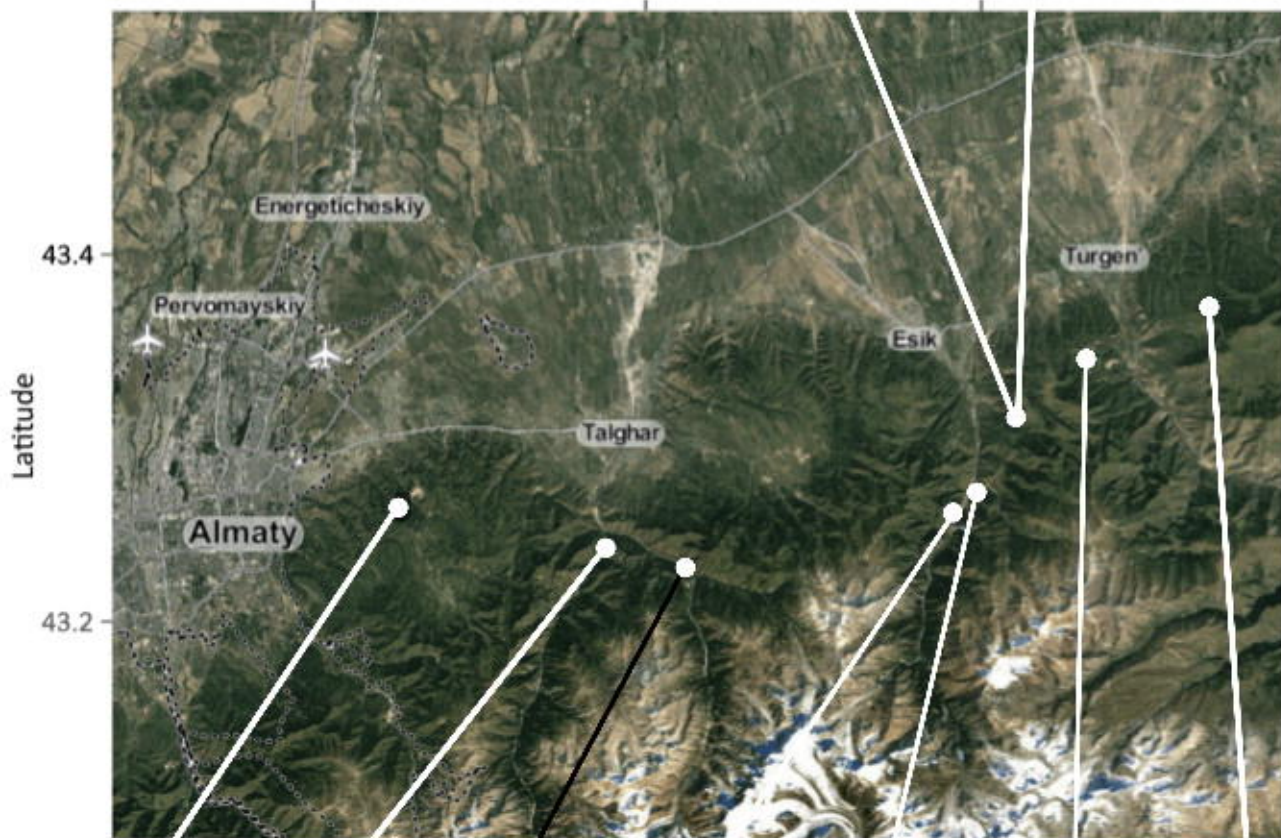


77.00

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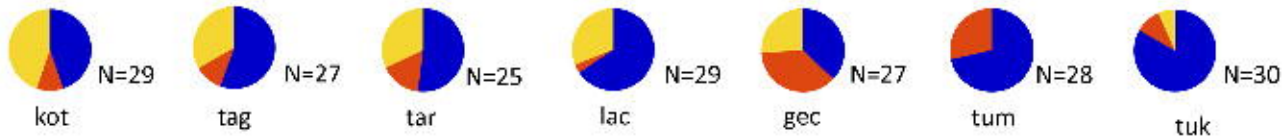
Longitude

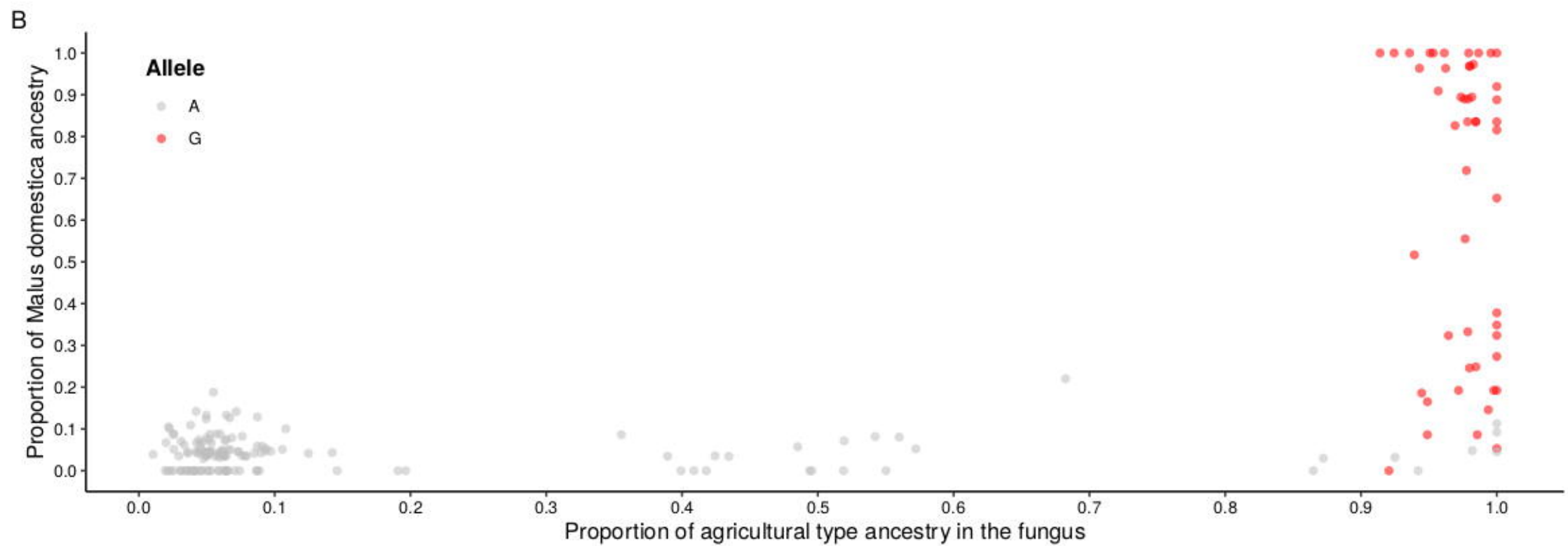
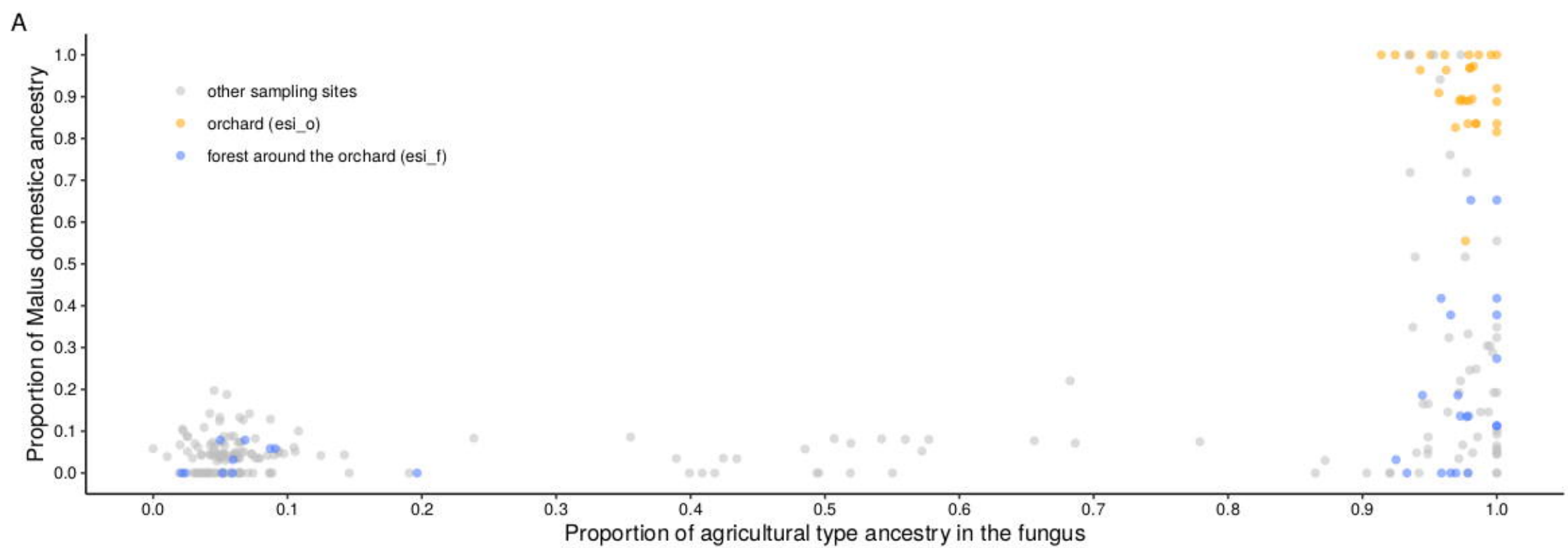


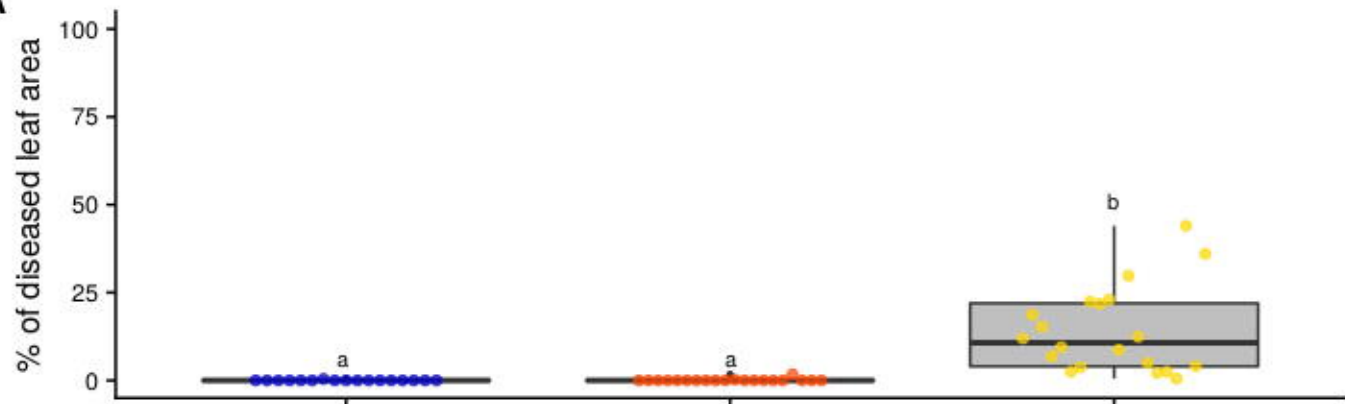
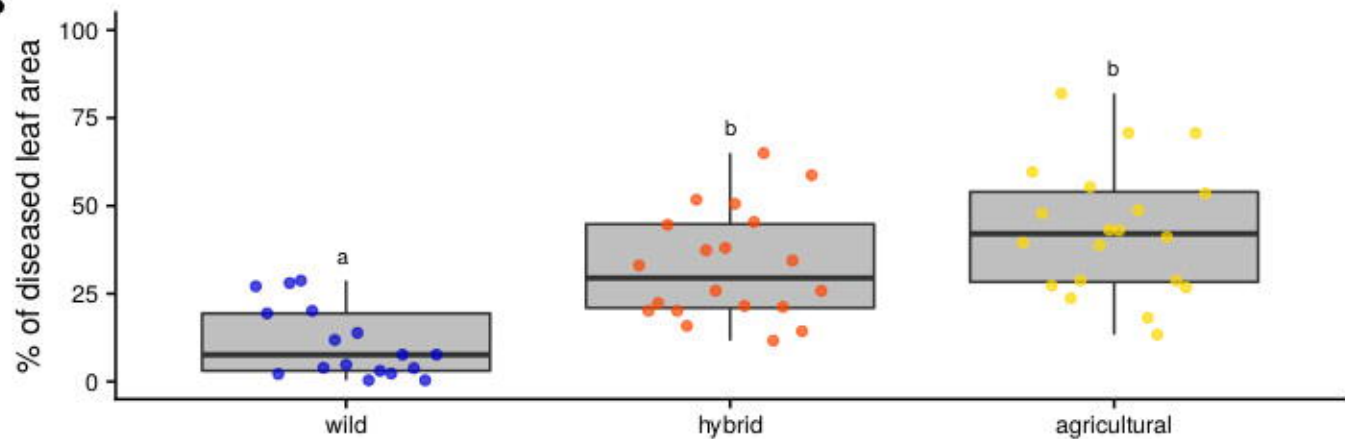
Host



Pathogen





A**B**

A



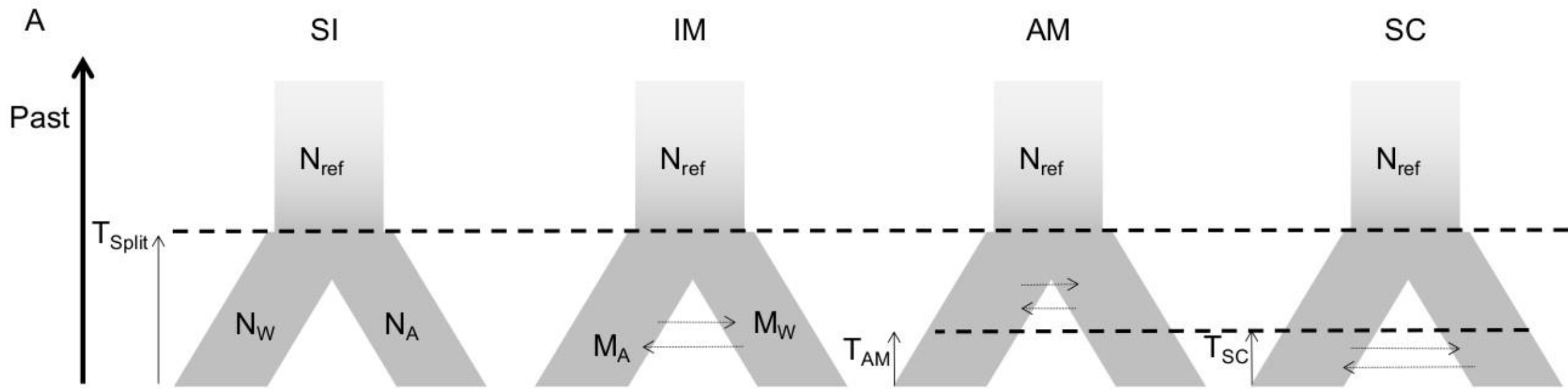
B



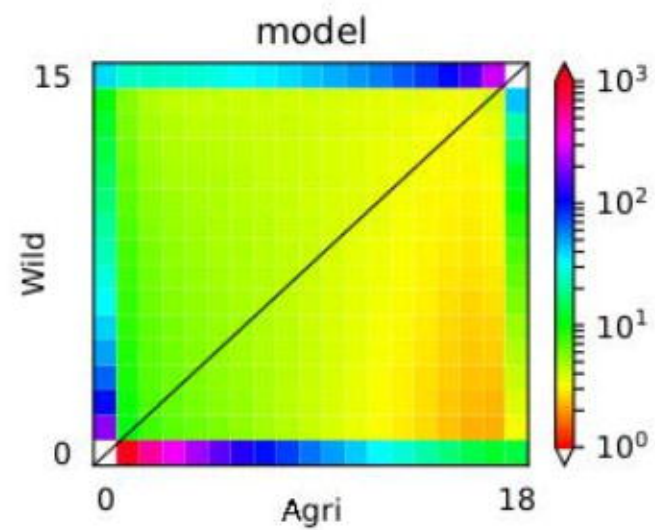
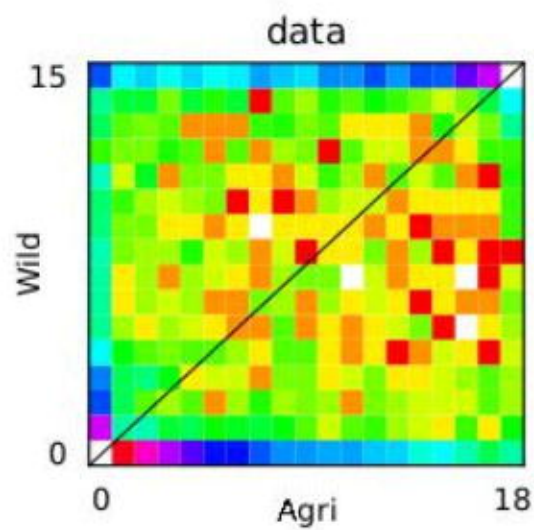
wild

hybrid

agricultural



B



C

