Development of SSR markers linked to QTL reducing leaf hair density and grapevine downy mildew resistance in Vitis vinifera

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Abstract

Dense leaf hairs of grapevines have been known to act as a preexisting defense structure for preventing the incidence of grapevine downy mildew, because the pathogen, *Plasmopara viticola*, needs water to invade grapevines, and water is repelled by a hydrophobic surface due to dense leaf hairs. In the present study, we performed regression analyses of downy mildew resistance with leaf hair density using hybrids of *Vitis labrusca* origin and confirmed the effect of leaf hairs. Reducing the repelling effect of leaf hairs by detergent application cancelled the effect of leaf hair, which confirmed the hypothesis. Thereafter, based on QTL analyses in the population of *V. vinifera* 'Muscat of Alexandria' × the interspecific hybrid 'Campbell Early', we identified a major locus in linkage group (LG) 5 of 'Muscat of Alexandria' controlling leaf hair density. This locus was previously reported as a small effect QTL for downy mildew resistance, however we found that the locus had high LOD scores explaining 71.9%–78.5% of the phenotypic variance of leaf hairs. Moreover, this locus was detected as a QTL for downy mildew resistance. The effect of this locus was confirmed in two other hybrid populations. Finally, we could successfully identify three traditional *V. vinifera* table grapes 'Muscat of Alexandria,' 'Katta Kurgan,' and 'Parkent' as the origin of the allele linked to hairlessness by defining the SSR haplotypes. The use of this locus for marker-assisted selections would

be a promising strategy for producing grapevines with resistance by preexisting defense structure.

Keywords DNA marker ·Grapevine downy mildew · Leaf hairs ·preexisting defense structure · *Vitis vinifera* · *Vitis labrusca*

49 **Introducion**

50

51 Grapevine is a major crop cultivated for wine, fresh fruit, juice, and raisins. *Vitis vinifera* is the most

52 commercially important species, but it is susceptible to many diseases, such as grapevine downy mildew

53 and powdery mildew (Cahoon 1998; Di Gaspero et al. 2012; Reisch et al. 2012; Eibach and Töpfer 2014).

54 Modern grape breeding was prompted by the arrival of North American diseases and insects in Europe

55 (Reisch et al. 2012). Hedrick (1908) stated 'the comparative resistance of the American species to the

56 phylloxera, the mildews, and black-rot has been due to natural selection in the contest that has been waged

57 for untold ages between host and parasite.' Many hybrids between *V. vinifera* and American wild species

58 have been produced to develop disease-resistant grapevines (Reisch and Pratt 1996; Eibach and Töpfer

59 2014). So-called French hybrids have been developed in France by hybridizing *V. vinifera* and American

60 wild species to establish grapevines resistant to phylloxera (*Daktulosphaira vitifoliae*), but resistance to

61 grapevine powdery mildew (*Erysiphe necator*) and downy mildew (DM; *Plasmopara viticola*) are also

62 important reasons for producing hybrids (Cahoon 1998; Di Gaspero et al. 2012; Eibach and Töpfer 2014).

63 Interspecific hybrids with *V. labrusca* parentage are frequently used in the USA, Japan, and some other

64 countries for making juice and as table grapes (Yamada and Sato 2016), and are sometimes collectively
65 referred to as *V. labruscana* Bailey (Bailey and Bailey 1930).

66 Even today, DM is a devastating grapevine disease. Twenty-four resistance loci have been identified
67 (Julius Kühn-Institut 2018). The resistance genes are named *Rpv* (*Resistance to Plasmopara viticola*), and
68 include *Rpv1* (Merdinoglu et al. 2003; Feechan et al. 2013) and *Rpv3* (Fischer et al. 2004; Welter et al.
69 2007; Bellin et al. 2009). *Rpv1* has been introgressed from *Muscadinia* (*Vitis*) *rotundifolia* after several
70 pseudo-backcrosses with different *V. vinifera* cultivars (Pauquet et al. 2001); this gene encodes a TIR-NB-
71 LRR protein (Feechan et al. 2013). *Rpv3* is frequently found in disease-resistant hybrids (Di Gaspero et al.
72 2012); unfortunately, the breakdown of resistance conferred by *Rpv3* has already been reported (Peressotti
73 et al. 2010; Delmotte et al. 2014). Pyramiding of these resistance genes is the main strategy for producing
74 new resistant cultivars (Eibach et al. 2007).

75 In contrast to the above genes, which provide induced resistance, leaf hairs have long been known to
76 act as a physical barrier against DM because *P. viticola* needs water to invade grapevines (Lafon and Bulit
77 1981) and leaf hairs repel water. (Staudt and Kassemeyer 1995; Kortekamp et al. 1999). Leaf hairs are also
78 called trichomes, and Ma et al. (2016) categorized non-glandular trichomes in grapevines into ribbon and

79 simple forms. Ribbon trichomes are the main structures that repel DM infection; hereafter, we use the term
80 'leaf hairs' to refer to ribbon trichomes. Leaf hairs are usually denser on shoot tips and expanding leaves
81 than on fully expanded leaves. In a broader perspective, Agrios (2005) stated that 'a thick mat of hairs on a
82 plant surface may also exert a similar water-repelling effect and may reduce infection.' Such preexisting
83 defense structure would be race non-specific and durable. Staudt and Kassemeyer (1995) mentioned that
84 resistant wild *Vitis* accessions have dense prostrate hairs on the lower leaf surface and hair removal renders
85 several accessions susceptible, although they did not show detailed data. Thick hydrophobic leaf hairs repel
86 water effectively, although not completely. Kortekamp et al. (1999) showed that dense pubescence on the
87 abaxial side of the leaves enhances resistance to DM. By observing *V. vinifera*, hybrids, and wild species,
88 including *V. cinerea*, *V. davidii*, *V. girdiana*, *V. doaniana*, *V. labrusca,* and *M. rotundifolia,* they showed that
89 less force was needed to remove water droplets from the leaves of hairy species than from those of non-
90 hairy species.

91 In this study, regression analyses of downy mildew resistance with leaf hair density using a hybrid
92 population and grapevine germplasms revealed an association between them. Detergent application to the
93 underside leaf hairs of progenies of a population and grapevine germplasms, including 'Pinot Noir' and its

94 bud sport ‘Pinot Meunier,’ confirmed the previously reported hypothesis that leaf hairs reduce DM infection

95 because of their repelling effect. By studying bi-parental populations and grapevine germplasms, we found

96 that downy mildew resistance and underside leaf hair density were co-localized in a QTL on LG5 in hybrid

97 populations of *Vitis labrusca* origin. We also found that *V. vinifera* table grapes harbor a major QTL whose

98 hairless allele drastically reduces the density of leaf hairs.

99

100 **Materials and methods**

101

102 Plant materials

103

104 Grapevines (*Vitis* spp.) were grown in vineyards at the Grape and Persimmon Research Station, NARO,

105 Japan. The mapping population Pop AC (95 individuals) was obtained by crossing *V. vinifera* ‘Muscat of

106 Alexandria’ (‘Heptalilo (synonym: Axina de tres bias)’ × ‘Muscat à Petits Grains Blancs (synonym:

107 ‘Muscat blanc à petits grains’, ‘Moscato Bianco’; Fig 1a)’; Cipriani et al. 2010; Vitis International Variety

108 Catalogue <http://www.vivc.de/>) and the interspecific hybrid ‘Campbell Early’ (‘Moore Early’ ×

109 (‘Belvidere’ × ‘Muscat Hamburg’); Vitis International Variety Catalogue <http://www.vivc.de/>; Fig 1a)

110 from 2007 to 2010. The mapping population Pop 693 (94 individuals) was obtained by crossing the

111 interspecific hybrids 626-84 and Iku 82 in 2002, and was maintained as previously described (Ban et al.

112 2014). The population Pop 777 (89 individuals) was obtained by crossing the interspecific hybrids 700-39

113 and ‘Shine Muscat’ (Akitsu 21 (an interspecific hybrid) × ‘Hakunan’) in 2012. Individuals of all three

114 populations were grafted onto Kober 5BB rootstocks before planting. Among vines listed in Supplementary

115 Table 1, the hybrids were *V. labruscana* (originated from *V. labrusca*) or its descendants, except for ‘Koshu’

116 and ‘Chancellor.’ ‘Koshu’ was previously regarded as *V. vinifera*; however, it was recently shown to be a

117 hybrid between *V. vinifera* and a Chinese wild species (Goto-Yamamoto et al. 2015). ‘Chancellor’ is a DM-

118 resistant French hybrid and was included because it has the DM resistance gene *Rpv3* (Di Gaspero et al.

119 2012).

120

121 Isolation and maintenance of *P. viticola*

122

123 *P. viticola* sporangia were isolated from naturally infected ‘Rizamat’ (‘Katta Kurgan’ × ‘Parkent’; Vitis

International Variety Catalogue <http://www.vivc.de/>) leaves in our vineyard on 5 August 2009. 'Rizamat' is a *V. vinifera* cultivar and was shown to be highly susceptible to DM (Kono et al. 2015a). Young but not shiny 'Rizamat' leaves were surface-sterilized with 1/20 diluted (v/v; available chlorine final 0.25%) sodium hypochlorite solution (Wako, Osaka, Japan) for 2 min, and washed with tap water three times and deionized water three times. Washed leaves were kept in a plastic container with wet paper towel (Kim Towel; Nippon Paper Crecia, Tokyo, Japan) with the abaxial surface upwards. Sporangia on 'Rizamat' leaves or stored sporangia at −80 °C were suspended in sterile water, and the suspension (1×10^4/ml) was inoculated on the abaxial side of the washed leaves. Inoculated leaves were maintained under a 12 h light : 12 h dark photoperiod at 20 °C, and the suspension on the leaves was wiped at one day after inoculation. We inoculated sporangia weekly to obtain sporangia in good condition. Sporangia were collected in a 50ml plastic tubes and stored at −80 °C when 'Rizamat' leaves were not available.

Evaluation of downy mildew resistance of Pop AC in fungicide-free vineyards

Pop AC was grown in a vineyard. Grafted vines (one vine per genotype) were planted on 6 March 2014 at

50 cm between vines with four rows under plastic (6 m × 16 m) to prevent diseases spread by rain in fungicide-free conditions. Three vines of each parent (‘Muscat of Alexandria’ and ‘Campbell Early’) were planted randomly within Pop AC. Two or three short canes with two to three buds were left when the plants were pruned, and ~10 g of fertilizer (N, 8%; P, 9%; K, 8%) and 100 g of cow manure were applied per vine in winter. Emerging shoots were tied upward to the plastic-coated steel pipes (16 mm in diameter × 2.4 m in height) during growth. A suspension of 5×10^4 sporangia/ml was prepared from the maintained *P. viticola*, and was inoculated mainly on the abaxial side of expanding leaves on 4 June 2014, 12 May 2015, and 10 May 2016 with a hand sprayer (DIA Spray; Furupura, Tokyo Japan). In total, 2L of the suspension was inoculated to all the vines in the vineyard (~17.2 ml of inoculum per vine). When inoculated, shoots were growing vigorously, but did not reach to the top of the steel pipes. Water was sprayed daily above the vines from sprinklers installed under the plastic cover to ensure uniform infection. Symptoms on fully expanded leaves were evaluated on 17 July 2014, 18 June 2015, and 9 June 2016 when susceptible genotypes were severely infected. Almost all fully expanded leaves on each vine were evaluated and scored as shown in Supplementary Table 2, and DM resistance was evaluated using the (1) incidence, defined as the ratio of leaves with sporangia (the number of leaves with score 3 or more / total number of leaves examined); and

(2) severity, defined as the overall degree of symptoms calculated as 100 × Σ(score × number of leaves of that score) / (6 (the highest score) × total number of leaves). Fungicides were not applied until DM symptoms were evaluated. After the evaluation, fungicides and pesticides were applied to control DM and powdery mildew. However, a few individuals were lost owing to severe DM infection annually.

Evaluation of downy mildew resistance of 31 grapevine germplasms in fungicide-free vineyards

Vines listed in Supplementary Table 1 were grafted onto Kober 5BB rootstocks and were planted under plastic on 8 March 2013 at 50 cm between vines with two or four vine replicates as shown in Supplementary Table 1 in another plastic-covered vineyard (6 m × 12 m). Three Japanese wild grapevines were included, which were possibly resistant. A suspension of DM sporangia (5×10^4/ml) was inoculated mainly on the abaxial side of expanding leaves on 8 June 2013 and 20 May 2014 (15–20 ml per vine). Water was sprayed daily above the vines from sprinklers installed under the plastic cover to ensure uniform infection. Symptoms on expanded leaves were evaluated as described above on 11 July 2013 and 25 June 2014.

168 Resistance was evaluated as described for Pop AC.

169

170 Evaluation of downy mildew resistance by leaf disc assay

171

172 DM resistance of Pop AC, Pop 693, and Pop 777 were evaluated by a leaf disc assay. A leaf disc assay,

173 which provides a good estimate of the number of sporangia, was performed as previously described with a

174 slight modification (Kono et al. 2015b). Four expanding leaves (no longer translucent and shiny) were

175 sampled; two discs (1.5 cm diameter) were punched from each leaf with a cork borer and were placed on

176 1% agar. A sporangial suspension in water (50 μl of 5×10^4/ml) was inoculated on each disc. At 6 days

177 post-inoculation (dpi), symptoms on each disc were visually rated on the scale depicted in Supplementary

178 Figure 1, and the mean score was calculated.

179

180 Evaluation of leaf hair density

181

182 Fully expanded 5th to 8th leaves from the basal end of shoots of Pop AC, Pop 693, Pop777, vines listed in
183 Supplementary Table 1 and 3 were randomly sampled from the first shoots to elongate from dormant canes;
184 leaves on secondary shoots were not sampled. Images of the abaxial side of leaves were taken using a digital
185 microscope (VH-Z20, Keyence, Osaka, Japan) at 100× magnification. The hair density of each leaf was
186 evaluated on a visual scale (Supplementary Fig. 2), and the mean score was calculated. The scale was
187 modified from Descriptor 6.1.35 (Descriptors for Grapevine; IPGRI 1997). The images in the descriptor of
188 the *V. vinifera* cultivars 'Grenache,' 'Müller-Thurgau,' and 'Clairette' and *V. labrusca* were used to set the
189 scales.

190 Leaf hair density was also estimated by imaging analysis in ImageJ v. 1.50b (Schneider et al. 2012)
191 with the commercially available 'LP_Mouzi' plugin (LPixel Inc. Tokyo, Japan). For each image, the largest
192 possible area in focus was selected as the region of interest (ROI) using the Rectangular Selection tool.
193 Then, the area of leaf hairs within the ROI was determined manually by setting a selection threshold, and
194 the area was measured in pixels with the 'LP_Mouzi' plugin. Hair density was calculated as the ratio of
195 'leaf hair area in pixels' / 'total ROI area in pixels.' The hair density of Pop AC was evaluated with three
196 leaf replicates in 2015 and six in 2016. Hair density of other populations and germplasms was evaluated in

197 one year with three- or four-leaf replicates per genotype.

198

199 Application of detergent to leaves

200

201 Two developing leaves were sampled from each of the *V. vinifera* 'Muscat of Alexandria,' hybrids

202 ('Campbell Early,' 'Kyoho'), wild *Vitis* species (*V. coignetiae* Pulliat *ex* Planch. 'Y0', *V. thunbergii* Siebold

203 et Zucc.), and eight Pop AC individuals (17, 21–24, 26, 44, and 55) on 2 July 2015. Leaves were surface-

204 sterilized with 1/20 diluted (v/v; available chlorine final 0.25%) sodium hypochlorite solution (Wako,

205 Osaka, Japan) for 2 min, and washed with tap water three times and deionized water three times. Eight discs

206 (1.5 cm diameter) were punched from each leaf with a cork borer, and four discs were placed on 1% agar.

207 The other four discs were collected in a 15 ml tube, treated with 3 ml of 0.01% (w/v) Nonidet P-40 for 2

208 min, and washed with tap water three times and with deionized water three times. These discs were placed

209 on 1% agar. A sporangial suspension in water (50 μl of 5×10^4/ml) was inoculated on each leaf disc as

210 described in Kono et al. (2015b), and the discs were maintained under a 12 h light: 12 h dark photoperiod

211 at 20 °C. At 6 dpi, images of the abaxial side of discs were taken using a digital microscope as described

212 above, and sporangia were counted with a disposable hemocytometer. The same experiment was repeated

213 on 9 July 2015. Because water remained within the layer of leaf hairs at 6 dpi, leaf hair density of some

214 discs was not estimated by imaging analysis.

215 Ten developing leaves of *V. vinifera* 'Pinot Noir' (less hairy) and 'Pinot Meunier' (more hairy) were

216 sampled on 2 May 2016. These two cultivars were shown to be bud-sports of each other (Hocquigny et al.

217 2004). Each leaf was halved longitudinally. One half (mock) was surface-sterilized with 1/20 diluted (v/v;

218 available chlorine final 0.25%) sodium hypochlorite solution (Wako, Osaka, Japan) for 2 min and washed

219 with tap water three times and deionized water three times. The other half was surface-sterilized with 1/20

220 diluted (v/v) sodium hypochlorite solution for 2 min, washed with tap water three times, treated with 0.01%

221 (w/v) Nonidet P-40 for 2 min, and washed with tap water three times and with deionized water three times.

222 Four discs (1.5 cm diameter) were punched from each leaf half with a cork borer and were placed on 1%

223 agar. A sporangial suspension in water (50 μl of 5×10^4/ml) was inoculated on each leaf disc, and the discs

224 were maintained under a 12 h light: 12 h dark photoperiod at 20 °C. At 6 dpi, sporangia were counted with

225 a disposable hemocytometer.

226

227 Statistical analyses

228

229 R v. 3.3.3 software (R Core Team, 2017) was used for all statistical analyses and graphical presentation of

230 results except for mapping and QTL analysis. For the variance components analysis (Snedecor and Cochran

231 1989; Crawley 2015) of leaf hair density, the proportion of leaf hair area determined by imaging analysis

232 was analyzed by one-way ANOVA with genotype as a factor. Data on the hair density estimated by imaging

233 analysis were transformed using the arcsine transformation (arcsin(sqrt(x))) before ANOVA to improve

234 homogeneity of variances.

235 To determine the relationship between leaf hair density as an explanatory variable and DM resistance

236 as a response variable, we used linear models. Before linear regression, DM incidence data were arcsine-

237 transformed and sporangial counts were log10-transformed. To avoid zero counts for the log transformation,

238 we added half of the detection limit of the disposable hemocytometer to all data before transformation as

239 proposed by Yamamura (1999).

240 We used the non-parametric Wilcoxon rank-sum test for comparing two medians using the 'wilcox.test'
241 function of R. We used the Wilcoxon rank-sum test for all multiple comparisons using the
242 'pairwise.wilcox.test' function of R with 'p.adj = 'holm' to control the family-wise error rate by the Holm
243 method. The Shapiro-Wilk test was performed to test the normality of the distributions of DM resistance
244 and leaf hair density.
245
246 Genotyping with simple sequence repeat (SSR) markers
247
248 Genotyping with SSR markers was performed as described in Ban et al. (2014) with some modifications.
249 GoTaq (Promega, Madison, USA) was used for PCR amplification. Genomic DNA was extracted from
250 leaves with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) using a protocol modified by Yang et al.
251 (2016), in which polyvinylpyrrolidone (PVP)-40 (2% w/v) was added to the AP1 buffer. To reduce the
252 genotyping cost, we designed sequence-specific forward primers with the M13 (−21) universal sequence
253 (TGTAAAACGACGGCCAGT) at the 5′ end, and added a FAM-labeled M13 (−21) primer to label PCR
254 products as described in Schuelke (2000). The reverse tail sequence (GTTTCTT) was appended to the 5′

255 end of the reverse primers for easier allele scoring. Maps were constructed with published SSR markers,

256 and SSR markers developed by Fechter et al. (2014) were used for the terminal regions of linkage groups

257 (LGs). To obtain a dense genetic map in the regions of interest and to fill gaps in genetic maps constructed

258 by using published SSR markers, we used the Tandem Repeat Finder program (v. 4.04) (Benson 1999) to

259 identify SSRs in the genome sequences of *V. vinifera* downloaded from the Grape Genome Browser (12X)

260 (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/; Jaillon et al. 2007), and developed new SSR

261 markers as summarized in Supplementary Table 4.

262

263 Map construction and QTL analysis

264

265 The parental linkage maps and the consensus linkage map were constructed using the double pseudo-

266 testcross strategy (Grattapaglia and Sederoff 1994) with JoinMap v. 4.1 software (van Ooijen 2006). SSR

267 marker genotyping results were sorted into LGs using reference genome information, and each LG map

268 was constructed separately. Map distances were calculated by the 'Regression mapping' algorithm (Stam

269 1993) using the Kosambi mapping function (Kosambi 1944) with 95 individuals. Marker numbers used for

mapping are summarized in Supplementary Table 5. We found a large area of marker distortions in this population, which were probably caused by lethal genes; to maximize genome coverage, we included distorted markers when they were integrated with other markers at LOD (logarithm of the odds) 2.0 or more by JoinMap 4.1. However, markers in LG18 of 'Campbell Early' were separated into two groups at LOD 2.0; hence, two separate maps, LG18a and LG18b, were constructed. Parental and consensus linkage maps of Pop 693 were reconstructed in JoinMap v. 4.1 after marker additions in LG5.

QTL analysis was carried out using both parental and consensus maps in MapQTL v. 6 software (van Ooijen 2009). QTLs were identified by interval mapping and the non-parametric Kruskal–Wallis rank-sum test. The LOD threshold corresponding to the genome-wide significance level of 0.05 was determined using 1,000 permutations. The number of individuals used for QTL detection for each trait is shown in Table 1. MapChart v. 2.3 software (Voorrips 2002) was used for the graphical presentation of genetic maps and QTL positions.

Results

285 Stability of evaluation of hair density on the abaxial side of leaves

286

287 First, we determined the stability of the evaluation of underside leaf hair density using a hybrid population,

288 Pop AC. The parent 'Muscat of Alexandria' had a few procumbent hairs on the abaxial side, and the

289 epidermis was almost fully exposed, whereas the other parent, 'Campbell Early,' had very dense hairs (Fig.

290 1a).The density of underside leaf hairs of the hybrid population Pop AC was highly dependent on genotype;

291 hence, we analyzed the effect of genotype by ANOVA (Supplementary Table 6). The variance component

292 of genotype was estimated to high, contributing 91% of total variance. The correlation between the mean

293 hair density in 83 individuals of Pop AC in 2015 with that in 2016 was high at $r = 0.91$.

294

295 Regression analysis of underside leaf hair density and DM resistance in Pop AC

296

297 To test the relationship between underside leaf hair density and DM resistance, we evaluated the

298 relationship for Pop AC (Fig. 1b; Supplementary Fig. 3a). Leaf hair density was evaluated by imaging

299 analysis (Fig. 1b) and a visual rating (Supplementary Fig. 3a) for two years. DM resistance was evaluated

300 by incidence and severity (see Materials and Methods) for three years. Linear regression analysis between
301 the 2-year mean of leaf hair density and 3-year mean of DM resistance showed that all the slopes were
302 significant ($P < 0.001$) and leaf hair density had an effect on DM resistance as evaluated by incidence (r^2 =
303 0.64, imaging analysis; $r^2 = 0.67$, visual rating) and severity ($r^2 = 0.53$, imaging analysis; $r^2 = 0.56$, visual
304 rating), suggesting that leaf hairs have a significan and substantial effect on DM resistance, although they
305 did not completely prevent infection.
306
307 Regression analysis of underside leaf hairs and DM resistance using 31 grapevine germplasms
308
309 To confirm this relationship in a wider range of genotypes, we evaluated leaf hair density and DM resistance
310 of 31 grapevine germplasms, which included cultivars, selections, and wild grapevines (Supplementary
311 Table 1). We found high variation in hair density among hybrids (Fig. 1c; Supplementary Fig. 3b). *Vitis*
312 *vinifera* 'Rizamat' had no hairs and 'Muscat Hamburg' had a few hairs, whereas 'Campbell Early' and the
313 American hybrid 'Concord' had very dense hairs such that their epidermis was not visible under the
314 microscope (Supplementary Fig. 4). The hairs on these latter two cultivars were very long and heavily

315 entangled with each other to form a felt-like surface. The relationship between hair density and mean DM

316 resistance in this 2-year evaluation (Supplementary Fig. 5) is shown in Figure 1b and Supplementary Fig.

317 3b in black. Linear regression analysis between leaf hair density and DM resistance showed that all the

318 slopes were significant ($P < 0.001$) and leaf hair density had an effect on DM resistance as evaluated by

319 incidence ($r^2 = 0.45$, imaging analysis; $r^2 = 0.39$, visual rating) and severity ($r^2 = 0.53$, imaging analysis; r^2

320 = 0.51, visual rating). 'Chancellor' and *V. flexuosa* had almost no leaf hairs (Supplementary Fig. 4) but were

321 highly resistant to DM. Two Japanese wild *Vitis* species were also resistant and had dense leaf hairs

322 (Supplementary Fig. 4). By excluding grapevines that were not used as parents in the table grape breeding

323 program in Japan (open circles in Fig. 1c and Supplementary Fig. 3b), we analyzed only table grapes and

324 their parents (Fig. 1c and Supplementary Fig.3b in red). Linear regression analysis between leaf hair density

325 and DM resistance for this group showed that all the slopes were significant ($P < 0.001$) and leaf hair density

326 had a greater effect on DM resistance as evaluated by incidence ($r^2 = 0.75$, imaging analysis; $r^2 = 0.74$

327 visual rating) and severity ($r^2 = 0.72$, imaging analysis; $r^2 = 0.75$, visual rating).

328

329 Regression analysis of underside leaf hairs and previously reported DM resistance by Terai and Yano (1977)

330

331 Similar regression analysis was applied to the DM resistance data by Terai and Yano (1977) to test the

332 relationship between the hair density and DM resistance of hybrid grapes, including those of *V. labrusca*

333 origin. The 27 cultivars used in their study (Supplementary Table 3) were kept in our repository, and we

334 evaluated the underside leaf hair density. Linear regression analysis between underside leaf hair density

335 and DM resistance (Fig. 1d; Supplementary Fig. 3c) showed that all the slopes were significant ($P < 0.001$)

336 and underside leaf hair density had an effect on DM resistance as evaluated by incidence ($r^2 = 0.78$, imaging

337 analysis; $r^2 = 0.70$, visual rating) and severity ($r^2 = 0.38$, imaging analysis; $r^2 = 0.59$, visual rating). Effects

338 on the incidence were stronger than those on severity, and severity varied among grapevines with a few

339 hairs. However, none of the densely haired grapevines were highly susceptible. All the above regression

340 analyses suggest an association between leaf hair density and resistance to DM.

341

342 Detergent application to abolish the hydrophobic effect of leaf hairs

343

344 To test whether the effect of leaf hairs was derived from their water-repelling effect, we applied the non-

345 ionic detergent Nonidet P-40 to leaves to abolish the water-repelling effect and tested the DM resistance.

346 First, we prepared leaf discs from developing leaves of 13 grapevines, including wild *Vitis*, and then

347 inoculated a DM sporangia suspension onto discs pre-treated with or without Nonidet P-40 (Fig 2a). Linear

348 regression analysis between underside leaf hair density and sporangia number per leaf disc without Nonidet

349 P-40 treatment showed that all the slopes were significant ($P < 0.001$) and underside leaf hair density had

350 an effect on sporangia number on discs ($r^2 = 0.71$, visual rating; $r^2 = 0.64$, imaging analysis), whereas there

351 was no relationship between leaf hairs and infection when Nonidet P-40 was applied since no slopes were

352 significant ($P > 0.05$; Fig. 2b). Sporangia were produced even on hairy genotypes, such as 'Campbell Early',

353 *V. thunbergii,* and *V. coignetiae* treated with the detergent.

354 Next, we performed a similar experiment using *V. vinifera* 'Pinot Noir' and 'Pinot Meunier,' which are

355 traditional cultivars and were shown to be bud-sports of each other (Hocquigny et al. 2004). Even though

356 the two cultivars are genetically almost identical, 'Pinot Meunier' has far denser leaf hairs (Fig 2c). If DM

357 resistance of the two cultivars are different and the difference is canceled by detergent application, it would

358 provide additional evidence that leaf hairs provide DM resistance. The results showed that the two cultivars

359 differed significantly in the number of sporangia on discs when the detergent was not applied (Fig. 2d),

360 whereas detergent application abolished the difference. Because the detergent broadened the area of
361 infection after inoculations even on 'Pinot Noir,' detergent application significantly increased the number
362 of sporangia.
363
364 Segregation of DM resistance and underside leaf hair density in Pop AC
365
366 To determine the loci controlling leaf hair density and DM resistance in Pop AC, we analyzed the DM
367 resistance of Pop AC and hair density. The segregation of leaf hair density in Pop AC was clearly bimodal
368 between the parents (Supplementary Fig. 6), and the normality of the distribution was rejected by the
369 Shapiro-Wilk test ($P < 0.001$). No individual had hairs as dense as 'Campbell Early. 'Muscat of Alexandria'
370 was always susceptible and 'Campbell Early' was resistant whether DM incidence or severity was evaluated.
371 The distribution of resistance as evaluated by the leaf disc assay was bimodal and the normality was rejected
372 ($P < 0.001$). However, the normality of the distribution of resistance by incidence and severity was not
373 rejected ($P = 0.08$, incidence; $P = 0.50$, severity), suggesting the continuous distribution (Supplementary
374 Fig. 6).

375

376 QTL analysis of Pop AC for leaf hair traits and DM resistance

377

378 We constructed genetic maps of each parent and the consensus map (Supplementary Fig. 7 and

379 Supplementary Table 5) with 338 markers and performed QTL analysis. We found a QTL located at the top

380 of LG5 of 'Muscat of Alexandria,' which explained 37.7%–49.3% (incidence), 34.5%–43.2% (severity),

381 and 49.2%–76.3% (leaf disc assay) of phenotypic variation in DM resistance (Table 1 and Fig. 3a). The

382 QTL was detected in all 3 years with almost the same effect (Table 1). Because the QTL was detected in

383 'Muscat of Alexandria,' which is susceptible to DM, it confers DM susceptibility. A distinct minor QTL

384 affecting severity was detected in LG18 of 'Campbell Early,' but only in 2014. A QTL on LG7 was detected

385 in 2014 and 2015 by leaf disc assay; this QTL explained approximately 15% of phenotypic variation. In a

386 non-parametric Kruskal–Wallis test, the statistic K* values of all three QTLs were highly significant.

387 QTL analysis for leaf hair traits identified the same locus as that for DM resistance (with Nifts5-50363

388 as the nearest marker). This QTL had a very high LOD score (71.9%–78.5% of phenotypic variance

389 explained; Table 1). Because of its large effect on leaf hair density, we designated the locus *Leaf Hairs 1*

390 (*LH1*). A minor QTL explaining 14.9%–22.8% of phenotypic variance was detected in LG7 of 'Campbell

391 Early.' Both loci were detected in 2015 and 2016. Both QTLs were supported by the Kruskal–Wallis test

392 (Table 1).

393 To analyze the effect of alleles of the *LH1* locus, we compared the mean values of the genotypes of

394 the Nifts5-50363 marker (Fig. 3c). 'Campbell Early' is homozygous not only for Nifts5-50363 (188/188

395 bp; Table 2) but also for several closely linked markers in this region, and 'Muscat of Alexandria' is

396 heterozygous (212/225 bp). Progeny were genotyped as either 188/212 or 188/225. Plants harboring the

397 212-bp allele had fewer leaf hairs, whereas those with the 225-bp allele had significantly denser hairs. By

398 leaf disc assay, the 188/212 genotype was more susceptible to DM than the 188/225 genotype, and the

399 188/212 genotype showed higher incidence and severity of DM under fungicide-free conditions. The mean

400 incidence and severity of DM for the 188/212 genotype were 1.24× and 1.27×, respectively, that of those

401 of the 188/225 genotype.

402

403 Validation of the QTLs in two other populations

404

405 To validate the QTLs, we evaluated DM resistance (leaf disc assay) and leaf hair traits in two other hybrid

406 populations, Pop 693 and Pop 777. A genetic map had already been constructed for Pop 693 (Ban et al.

407 2014); we performed QTL analysis using the map and added two newly developed markers (Nifts5-50304

408 and 50363) near the *LH1 l*ocus. The same QTL was detected at the top of LG5 of both parents (626-84 and

409 Iku 82) and explained 15.4% and 17.0% (leaf disc assay), respectively, and 22.0% and 33.7% (leaf hairs),

410 respectively, of phenotypic variation (Table 1). Because the genotypes of the parents at Nifts5-50363 were

411 heterozygous (188/201; Table 2), the progeny genotypes were 188/188, 188/201, or 201/201. The combined

412 effect of the locus was detected in the consensus map (Fig. 3b; Table 1), and the explained phenotypic

413 variance was as high as 54.4% (leaf disc assay) and 88.4% (leaf hairs). The effect of the QTL at the locus

414 (Nifts5-50363) in Pop 693 is shown in Fig. 3d. Progeny harboring the 201-bp allele had significantly fewer

415 leaf hairs than the 188/188 plants. Surprisingly, the 201/201 genotype resulted in almost no hairs and

416 therefore the leaf hair scores of the 188/201 and 201/201 genotypes were significantly different by the

417 pairwise non-parametric Wilcoxon rank-sum test (P = 0.003). The 188/188 genotype leaf discs had

418 significantly fewer sporangia than the other genotypes, but there was no significant difference between the

419 188/201 and 201/201 genotypes.

420 No genetic map was available for Pop 777, and therefore we analyzed the effect of Nifts5-50363 by
421 using only the leaf hair score and leaf disc assay (Fig. 3e). Because the parents are heterozygous with three
422 alleles (700-39, 188/212; 'Shine Muscat,' 188/225; Table 2), there are four possible progeny genotypes
423 (188/188, 188/212, 188/225, 212/225). Plants harboring the 212-bp allele had significantly fewer leaf hairs
424 than the 188/188 and 188/225 genotypes. Accordingly, the 188/188 and 188/225 genotype leaf discs had
425 significantly fewer sporangia than the genotypes carrying the 212-bp allele (Fig. 3e).
426
427 *Vitis vinifera* origin of the hairless allele
428
429 Because the size of the hairless allele of Nifts5-50363 in Pop 693 differed from that in the other populations,
430 their origins might be different. We traced them back to the original cultivars by genotyping the ancestors
431 of the three populations with the closely linked SSR markers Nifts5-50304, 50363, and 50437 near *LH1*
432 (Table 2; Supplementary Fig. 8). A SSR haplotype, 206/201/235, was derived from *V. vinifera* 'Katta
433 Kurgan.' Another haplotype, 206/212/235, was found in *V. vinifera* 'Muscat of Alexandria' and 'Parkent.'
434 We could not genotype *V. vinifera* 'Heptakilo,' a parent of 'Muscat of Alexandria.' However, it might have

435 the latter haplotype because the other parent, *V. vinifera* 'Muscat à Petits Grains Blancs,' does not have the

436 haplotype. 'Muscat of Alexandria' and 'Parkent' have the same haplotype, whereas the haplotype found in

437 Pop AC came from 'Muscat of Alexandria' and that found in Pop 777 came from 'Parkent' (Supplementary

438 Fig. 7). In accordance with the genotype, all three *V. vinifera*—'Katta Kurgan,' 'Muscat of Alexandria,' and

439 'Parkent'—had a few or no hairs (Fig. 1a; Supplementary Fig. 4).

440

441 **Discussion**

442

443 We have quantitatively shown that underside leaf hairs are associated with resistance to DM by regression

444 analyses of Pop AC and other germplasms, and detergent application to the underside of the leaf supported

445 the hypothesis that the underside leaf hairs function to impart preexisting structural defense to DM. This

446 hypothesis was proposed previously by Kortekamp and Zyprian (1999), and Divilov et al. (2018) also found

447 that leaf hairs had an effect on disease resistance in their F1 family. In this study, we also confirmed that

448 the effect of leaf hairs could be completely canceled by detergent application using our progenies, as well

449 as 'Pinot Noir' and its bud sport 'Pinot Meunier.' 'Pinot Meunier' could be regarded as a hairy mutant of

450 'Pinot Noir,' and we confirmed that leaf hair could inhibit DM infection, and the inhibition was canceled
451 by detergent application. This is additional new evidence that leaf hairs function to create preexisting
452 structural defense.

453 This resistance is not qualitative or true resistance, and disease progression cannot be completely
454 prevented, but DM incidence and severity in less haired individuals in Pop AC were significantly higher
455 (1.24× and 1.27×, respectively) than those of haired individuals. Leaf hairs provide typical 'quantitative
456 disease resistance,' and the *LH1* locus is an example of 'quantitative resistance loci,' which confer
457 quantitative disease resistance (Poland et al. 2009). This locus was a major QTL for DM resistance in this
458 population because all the values for phenotypic variance explained (Table 1) exceeded the threshold (20%–
459 25%) defined by St. Clair (2010).

460 We observed significant differences in leaf hair density among cultivars, selections, and species. Leaf
461 hair density on fully expanded leaves was evaluated stably as shown by the variance component analysis
462 of Pop AC. Accordingly, hairs on the lower leaf surface are often important in identification of *Vitaceae*
463 species (Gerrath et al. 2015). These hairs are described as 'prostrate hairs between main veins on lower side
464 of blade' (Characteristic 28) in the DUS (Distinctness, Uniformity and Stability) guidelines of UPOV

(International Union for the Protection of New Varieties of Plants); this is also the Descriptor 6.1.35 for Grapevine in IPGRI (1997), in which *V. labrusca* is indicated as a grapevine with the densest hairs.

Gerrath et al. (2015) mentioned that *V. labrusca* plants have dense brownish or whitish prostrate hairs covering the lower leaf surface; the hairs are so dense that they obscure the lower leaf surface except for veins. This species was an important parent of American hybrids. The exact genetic mechanisms that increase leaf hair density are not clear. In the Japanese grapevine breeding program, American hybrids, especially 'Steuben' and 'Campbell Early,' are important parents. One of their ancestors is 'Concord,' which is a hybrid between 'Catawba' and *V. labrusca*; 'Catawba' is a hybrid between *V. vinifera* 'Sémillon' and *V. labrusca* (Huber et al. 2016; Vitis International Variety Catalogue <http://www.vivc.de/>), suggesting a strong *V. labrusca* background. Cadle-Davidson et al. (2008) compared their data with several previous studies and concluded that 'Concord' remains the only consistently resistant or moderately resistant cultivar among 89 cultivars. This suggests that 'Concord' has a broad-spectrum, durable foliar resistance, which is rarely found in cultivated *Vitis*. The present study suggests that thick leaf hairs might be a mechanical cause of the durable resistance of 'Concord,' and that this trait originated from a wild species, *V. labrusca*, during long-term interaction between this species and downy mildew in North America.

480 Kortekamp and Zyprian (1999) suggested that *V. labrusca* and *V. cinerea* might have defense
481 mechanisms different from leaf hairs. The presence of such mechanisms was implied in our regression
482 analyses as variation from the regression lines. Because r^2 explains the variance derived from leaf hair
483 density, $1 - r^2$ explains the unexplained variances, including those caused by other resistance mechanisms
484 and experimental error. As $1 - r^2$ was 0.33 to 0.47 (Fig. 1b; Supplementary Fig. 3a), considerable variance
485 may result because of other resistance mechanisms, although their effect was smaller than that of leaf hairs
486 in this population. However, we found that 'Chancellor' and *V. flexuosa* had almost no hairs but were highly
487 resistant to DM (Supplementary Fig. 5). 'Chancellor' has the *Rpv3* gene (Di Gaspero et al. 2012), but the
488 resistance genes in *V. flexuosa* are unknown. These are examples of germplasm with high resistance to DM
489 unrelated to leaf hairs.

490 In Pop 777, no significant difference in leaf hair density was detected between the 188/188 and
491 188/225 genotypes (Fig. 3e), suggesting that QTL linked to the 225-bp allele has the same effect as the 188-
492 bp allele, and both are recessive to the hairless 212-bp allele. Throughout the analysis of the locus, the
493 hairless 201- and 212-bp alleles at Nifts5-50363 were dominant to other alleles and significantly reduced
494 the density of underside leaf hairs. We used SSRs closely linked to the *LH1* locus (Nifts5-50304, 50363,

and 50437) to define the hairless haplotypes, as Di Gaspero et al. (2012) analyzed for the *Rpv3* locus. We found that the haplotype 206/201/235 originated from 'Katta Kurgan' and 206/212/235 originated from 'Muscat of Alexandria' and 'Parkent' (Table 2; Supplementary Fig. 8). 'Muscat of Alexandria' and its parent 'Heptakilo' (a probable source of the hairless allele; Cipriani et al. 2010) originated in Greece, whereas 'Katta Kurgan' and 'Parkent' originated in Uzbekistan (Vitis International Variety Catalogue <http://www.vivc.de/>). These *V. vinifera* cultivars are important parents in the Japanese breeding program (Yamada and Sato 2016), hence the hairless traits might have been reducing the resistance of resultant populations to DM. More thorough investigation of *V. vinifera* genotypes is necessary, but our data suggest the presence of at least two distinct hairless alleles that originated from southeastern Europe and central Asia. Because wine grape populations are not included in this study, we do not know whether the hairless alleles found in this study prevail in *V. vinifera* wine grapes. The isolation of gene(s) responsible for the hairless trait and the explanation of the evolutionary benefit of this trait for the table grape *V. vinifera* are the main challenges.

A DM resistance locus with small effects, designated as *Rpv11,* was previously identified in LG5 (Fischer et al. 2004; Schwander et al. 2012). The QTL was detected based on the leaf disc assay, and the 1-

510 LOD confidence interval was 14.6-22.8 cM on the consensus map of Gf.Ga-52-42 (‘Bacchus’ × ‘Villard

511 blanc’) × ‘Solaris’ (‘Merzling’ × Geisenheim 6493), which corresponds to the region between the VMC3B9

512 and VRZAG79 markers (Schwander et al. 2012). Almost the same region was found by Bellin et al. (2009)

513 by the observation of mesophyll invasion of DM, and the confidence interval (1-LOD) was 10.6-39.2 cM

514 on the ‘Chardonnay’ map. The confidence interval (1.5-LOD) of *LH1* defined by leaf hair density was on

515 the top of LG5 (0-2.3 cM of the ‘Muscat of Alexandria’ map; Table 1), and it does not overlap with the

516 region between VMC3B9 and VRZAG79. Hence, *Rpv11* and *LH1* could be regarded as different loci.

517 Recently, QTL for leaf hair density was identified on LG5 from a hybrid grapevine ‘Horizon’ (Divilov et

518 al. 2017; Divilov et al. 2018). The percentage of variance explained by the sporulation trait on the leaf disc

519 assay was 11.27%, and the 95% credible interval is 0.844-5.511 Mbp (Divilov et al. 2018). The interval

520 includes *LH1* because the locus is located around Nifts5-50363 (0.877 Mbp). However, Divilov et al. (2018)

521 performed another analysis, the multiple phenotype Bayesian network analysis, and found the physical

522 locations of QTL on LG7, 8, and 15, but not LG5, for the leaf trichome (Lt) phenotype. Thus, they did not

523 designate QTL on LG5. In this study, however, we successfully located the QTL on LG5 by analyzing

524 hybrid populations originating from *V. labrusca* and identified the origin of the hairless alleles in traditional

525 *V. vinifera* cultivars, and hence designated the locus as *LH1*. Our developed SSR markers enabled us to

526 detect the hairless genotypes at the *LH1* locus.

527 We also found a small effect QTL in LG7 on the 'Campbell Early' map, and its confidence interval

528 (1.5-LOD) was 25.8-41.7cM (the largest interval by visual rating, 2015), and the nearest marker was Nifts7-

529 59300 (9.903Mbp). *Rpv7* (Bellin et al. 2009), *Rpv9* (Moreira et al. 2011), and *Rpv21* (Divilov et al. 2018)

530 are located near the QTL. Another QTL found in LG18 of the 'Campbell Early' map was close to *Rpv3*

531 (Fischer et al. 2004; Welter et al. 2007; Bellin et al. 2009), however, it was detected only in one year (2014).

532 Owing to the large confidence intervals of these two QTLs on LG7 and 18, further work is necessary to

533 determine which *Rpv* genes are equivalent to the QTLs on LG7 and 18, and whether they are the same

534 genes or not.

535 Durability of resistance is always the concern when resistance genes are used for breeding, and

536 resistance is sometimes effective for just a few years (Parlevliet 2002). This is consistent with the emergence

537 of aggressive *P. viticola* isolates that overcame the resistance of 'Bianca' (Peressotti et al. 2010) and 'Regent'

538 (Delmotte et al. 2014). Delmotte et al. (2014) stated that the erosion of 'Regent' resistance may have

539 occurred in less than 5 years and at least three times independently. Both cultivars harbor the *Rpv3* gene

(Di Gaspero et al. 2012). Gene pyramiding (Eibach et al. 2007) and the use of quantitative resistance with a different mechanism would be promising strategies for producing cultivars with durable resistance. As Kortekamp and Zyprian (1999) suggested, breeding 'hairy' grapevines could be an alternative to the use of R-gene-mediated resistance. In this respect, the *LH1* on LG5 would be an important locus and a promising DNA marker that could be effectively used in grapevine breeding for discarding hairless genotypes.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank Takeshi Hayashi (NARO, Tsukuba, Japan) for support in Pop AC mapping and manuscript

revision, Ryosuke Mochioka (Kagawa University, Kagawa, Japan) and Hino Motosugi (Kyoto Prefectural University, Kyoto, Japan) for providing Japanese wild *Vitis* species, Natsumaro Kutsuna (LPixel Inc. Tokyo, Japan) for support in imaging analysis, Technical Support Center Operations Unit 1 in Akitsu for their technical support in vineyards. We are grateful to Mirai Nakahara, Miho Kohata, Tamami Nakasumi, and Sumie Kurokawa (NARO, Hiroshima, Japan) for technical assistance, and to Takao Ito (NARO, Hiroshima, Japan) for critical reading of the manuscript. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, HOR-2006).

Author's contributions

A. K. wrote the manuscript. A. K., Y. B., K. S., A. A., N. O., and A. S. designed the experiments. A. A. contributed to the experiments using 'Pinot Meunier.' A. K. and A. S. contributed to statistical data analyses. A. K. performed the experiments. A. K., Y. B., N. M., and S. S. genotyped Pop AC. Y. B. and N. M. developed populations. H. F. extracted SSRs from the grapevine reference genome.

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712

713 **Figure captions**

714

715 **Fig. 1** (a) Leaf hairs on the abaxial side of fully expanded leaves of 'Muscat of Alexandria' (left) and

716 'Campbell Early' (right). Bars, 250 μm. (b) Regression of mean incidence and severity of DM (2014–2016)

717 in Pop AC on leaf hair density. The latter was evaluated as the proportion of leaf hair area by imaging

718 analysis. The linear regression equations are y = 1.45^{***}- 0.73^{***}x (incidence) and y = 69.9^{***}- 33.6^{***}x

719 (severity). $^{***}P < 0.001$. (c) Regression of mean incidence and severity of DM (2013, 2014) in 31 grapevines.

720 Black, all data. The linear regression equations are y = 1.05^{***}- 0.82^{***}x (incidence) and y = 55.1^{***}- 33.7^{***}x

721 (severity). $^{***}P < 0.001$.; Red, excluding 'Chancellor' and 'Koshu,' *V. coignetiae*, *V. thunbergii,* and *V.*

722 *flexuosa* (○), which are not used in our table grape breeding. The linear regression equations are y =

723 1.16^{***}- 0.93^{***}x (incidence) and y = 58.1^{***}- 36.2^{***}x (severity). $^{***}P < 0.001$. Leaf hair density was

724 evaluated by imaging analysis. (d) Regression of mean incidence and severity of DM in 27 cultivars and

725 selections evaluated by Terai and Yano (1977). The linear regression equations are y = 1.45^{***}- 1.31^{***}x

726 (incidence) and y = 44.8^{***}- 62.5^{***}x (severity). $^{***}P < 0.001$. Leaf hair density was evaluated by imaging

727 analysis. $^{***}P < 0.001$

728

729 **Fig. 2** (a) Inoculated leaf discs at 1 dpi. Note that inoculum on a detergent (Nonidet P-40)-treated disc of

730 'Campbell Early' was absorbed within the layer of leaf hairs, whereas that on the untreated disc was clearly

731 repelled by thick hairs. (b) Regression of number of sporangia on leaf discs treated with or without detergent

on leaf hair density. Each data point is the mean number of sporangia on the four discs derived from one halved leaf. The linear regression equations are $y = 5.31^{***} - 0.56^{***}x$ (leaf hair score/without detergent), $y = 3.97^{***} - 2.22^{***}x$ (proportion of leaf hair area/without detergent), $y = 4.30^{***} - 0.00^{ns}x$ (leaf hair score/with detergent), and $y = 4.47^{***} - 0.22^{ns}x$ (proportion of leaf hair area/with detergent). $^{***}P < 0.001$; ns, not significant ($P > 0.05$). (c) Underside hairs on leaf discs prepared from young developing leaves of 'Pinot Noir' and 'Pinot Meunier.' Bars, 250 μm. (d) Number of sporangia on discs prepared from 10 leaves of 'Pinot Noir' and 'Pinot Meunier' treated with or without Nonidet P-40. Bars labeled with the same letter are not significantly different (pairwise Wilcoxon rank-sum test; $\alpha = 0.01$). Error bars denote SE

Fig. 3 (a) QTL-LOD profiles of leaf hair density and downy mildew resistance traits in Pop AC. QTLs for mean incidence and severity under fungicide-free conditions (2014–2016) and mean leaf hair density (the proportion of leaf hair area by imaging analysis; 2015, 2016) on the 'Muscat of Alexandria' map are shown. The proportion of leaf hair area were arcsine-transformed. (b) QTL-LOD profiles of leaf hair density and downy mildew resistance traits in Pop 693. QTLs for mean scores of leaf disc assay (2016) and mean leaf

746 hair density (mean scores by visual rating) on the consensus map are shown. Colored boxes in (a) and (b)

747 represent 1.5-LOD confidence interval. (c) Effect of the Nifts5-50363 locus on downy mildew resistance

748 and leaf hair trait in Pop AC. Mean score of leaf disc assay (2013–2015), mean incidence and severity under

749 fungicide-free conditions (2014–2016), and leaf hair density (2015, 2016) were compared between different

750 genotypes at the locus. (d, e) Effect of the Nifts5-50363 locus on downy mildew resistance and leaf hair

751 trait in (d) Pop 693 and (e) Pop 777. Mean scores of leaf disc assay (2015, Pop 777; 2016; Pop 693) and

752 leaf hair density by visual rating (2015) were compared between different genotypes at the locus. (c–e) Bars

753 labeled with the same letter are not significantly different (pairwise Wilcoxon rank-sum test; $\alpha = 0.01$).

754 Error bars denote SE

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