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3 **Regional microbial signatures positively correlate with differential wine phenotypes: evidence for**

4 **a microbial aspect to *terroir***

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7 RSS feed title: Evidence for a microbial aspect to *terroir*

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23 ## Abstract

24 Many crops display differential geographic phenotypes and sensorial signatures, encapsulated by the
25 concept of *terroir*. The drivers behind these differences remain elusive, and the potential
26 contribution of microbes has been ignored until recently. Significant genetic differentiation between
27 microbial communities and populations from different geographic locations has been demonstrated,
28 but crucially it has not been shown whether this correlates with differential agricultural phenotypes
29 or not. Using wine as a model system, we utilize the regionally genetically differentiated population
30 of *Saccharomyces cerevisiae* in New Zealand and objectively demonstrate that these populations
31 differentially affect wine phenotype, which is driven by a complex mix of chemicals. These findings
32 reveal the importance of microbial populations for the regional identity of wine, and potentially
33 extend to other important agricultural commodities. Moreover, this suggests that long-term
34 implementation of methods maintaining differential biodiversity may have tangible economic
35 imperatives as well as being desirable in terms of employing agricultural practices that increase
36 responsible environmental stewardship.

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39 Key Words: *Saccharomyces cerevisiae*, *terroir*, wine, agriculture

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44 Many important crops that comprise the same or very similar genotypes display differential
45 geographic phenotypes in terms of the physical and sensorial signatures of their produce: this is
46 generally encapsulated by the concept of *terroir*[1]. Often the chemical descriptors of these
47 differential geographic phenotypes are well documented[2-9]; however, the factors that drive these
48 differences remain elusive[10]. Classically, differential agricultural geographic phenotypes are thought
49 to result from complex interactions between specific crop genotypes and local soils, topography,
50 climate and agricultural practices, and these differential manifestations are commercially important
51 as they add distinctiveness and thus value to products[10]. Microbes play key roles in the production of
52 quality agricultural commodities for reasons ranging from their effect on crop nutrient availability via
53 rhizosphere interactions with roots, through to their role in crop disease pressure: ultimately
54 microbes influence plant and fruit health[11-13]. Additionally microbes transform plant products to
55 economically and socially important commodities such as coffee, chocolate, bread, beer and a range
56 of other fermented beverages including wine[14]. The potential contribution of, and link between,
57 microbes and differential geographic phenotypes, or *terroir*, of agricultural products is assumed to
58 exist, but to date has not been objectively verified[1,15-17].

59

60 Wine has been made by humans since the dawn of civilization and is an important social and
61 economic commodity. It arguably displays the strongest geographic signatures of all agricultural
62 products and thus is a superb model to evaluate the degree to which there might be a microbial
63 aspect to *terroir*. However, even for wine the drivers of *terroir* remain largely untested[10]. Microbes,
64 predominantly fungi, may significantly affect the 'phenotype' of wine firstly by affecting grapevine
65 and fruit health and development, and thus quality[18], and secondly by manipulating wine flavor,
66 aroma and style due to their actions during fermentation[19,20]. During alcoholic fermentation fungi
67 including *Saccharomyces cerevisiae*, the primary yeast involved in wine fermentation, not only
68 convert sugars into ethanol but also produce an array of secondary metabolites, including volatile
69 compounds, that are important to wine aroma and flavor[21,22]. While grape-derived compounds may

70 provide varietal distinctions, at least yeast-derived acids, alcohols, carbonyl compounds, phenols,

71 esters, sulfur compounds and monoterpenoids all significantly contribute to wine quality and

72 aroma[22,23].

73

74 It is well documented that different species of microbes differentially affect vine health and

75 development, and that different species of yeast, and even different genotypes of *S. cerevisiae*,

76 produce different aroma profiles in wine[18,19,24,25]. Only recently has evidence been provided for the

77 regional delineations of both microbial communities, and populations of *S. cerevisiae,* associated

78 with vines and the populations driving the spontaneous ferment of fruit from these vines into

79 wine[1,16,17]. On the face of it, together, these two sets of observations might seem enough to

80 conclude that microbes have an influence on differential agricultural geographic signatures, at least

81 for wine. However, the critical assumption here is that there is a positive correlation between

82 microbial relatedness, and aroma profiles in wine: i.e. that closely related microbes and their

83 communities produce closely related agricultural geographic signatures. This has not been shown,

84 but here we provide the first evidence for such a link.

85

86 To evaluate this idea we focused on the potential for microbes to influence differential geographic

87 wine phenotypes via fermentation. Wine may be made by either attempting to remove the array of

88 microbes that are naturally associated with grapes and then deliberately inoculating with a

89 commercial strain of yeast, or allowing the microbes naturally associated with grapes to conduct the

90 ferment[26]. The former inoculated option reduces the potential for microbes to contribute to *terroir*,

91 during fermentation at least, and has only been available commercially to winemakers since 1965[27].

92 The latter has been employed by humans since the dawn of civilization and is known as spontaneous

93 or wild fermentation, and may comprise at least tens of species and hundreds of strains of *S.*

94 *cerevisiae*[1,28]. Since spontaneously fermented wine comprises a diversity of yeast species and strains

95 of *S. cerevisiae*, metabolic interactions between these different types may also potentially be the key
96 to any microbial signature contributing to *terroir.* Due to the complex and often unpredictable
97 nature of microbial interactions, community effects on the chemical and sensorial properties of wine
98 are hard to experimentally control. As a first step towards understanding the impact microbes have
99 on the regional distinctiveness of wine, we focus on the dominant species driving fermentation: *S.*
100 *cerevisiae*.

101

102 We have recently shown there are genetically differentiated natural sub-populations of *S. cerevisiae*
103 associated with vineyards and spontaneous ferments in major regions in New Zealand (NZ)[17]. Using
104 population genetic analyses, here we select appropriate genetic representatives from these regional
105 *S. cerevisiae* sub-populations and analyze their fermentative effects on a suite of chemicals known to
106 significantly affect the phenotype of wine. We test for the presence of correlations between the
107 genetic relatedness of these natural regional *S. cerevisiae* sub-populations and their resulting wine
108 phenotypes, to conduct the first empirical test for whether there is a microbial aspect to *terroir*.

109

110

111 ## Results

112 Selection of *S. cerevisiae* genotypes

113 Recently Knight and Goddard[17] isolated 3,900 *S. cerevisiae* from native forests, vineyards, and the
114 spontaneous ferments of *Vitis vinifera* var Sauvignon Blanc fruit from six major regions in NZ
115 (Hawke's Bay, Martinborough, Nelson, Wairau Valley, Awatere Valley and Central Otago).
116 Microsatellite genotype profiling of these isolates revealed the presence of 295 different genotypes.
117 Bayesian population structure methods, and statistical analyses of the resulting ancestry profiles[29],
118 showed significantly distinct sub-populations residing in each of these regions[17]. Here we use the

119 genetic ancestry profiles produced from Bayesian analysis, in combination with regional allele
120 frequencies, to select *S. cerevisiae* genotypes from each of these regional sub-populations that span
121 and represent the genetic diversity within each region. Genotypes that belong to the main inferred
122 population correlating with each region, that also maximize the diversity of alleles present in each
123 region, were selected, including at least one genotype that harbored regionally unique alleles.
124 Supplementary Table S1 shows the Bayesian ancestry profiles for the genotypes originally analyzed
125 by Knight and Goddard[17]: the inferred sub-populations common in each regions are noted and the
126 genotypes selected for use in this study are highlighted. The allele frequencies within each regional
127 population are shown in Supplementary Table S2 with the alleles harbored by the selected
128 genotypes in this analysis highlighted. Due to the large diversity of alleles observed in each regional
129 population, and the constraints on the number of ferments we could perform and analyze here,
130 clearly not every allele could be represented. Rather, we included those genotypes harboring the
131 more common alleles in each region: genotypes selected ensured that the average proportion of
132 each population that harbored the represented alleles was no lower than 60% (Supplementary Table
133 S2).

134

135 <u>Ferment Performance</u>

136 All ferments were conducted using the same commercially derived batch of homogenized and
137 sterilized Sauvignon Blanc juice from Marlborough in NZ. Six individual *S. cerevisiae* genotypes from
138 each region, and co-inoculations of all six genotypes representing regional populations, were
139 fermented in triplicate across three separate batches totaling 126 ferments. The extent to which
140 sugars were fermented was analyzed by weight loss[30], and most lost approximately 25 g indicating
141 complete fermentation given the 220 g of sugar in the juice initially. One genotype from the Wairau
142 Valley failed to ferment at all and was removed from all analyses. Eleven single genotype ferments,
143 all in the third batch, displayed significantly less weight loss than the remaining ferments ($F_{1, 108}$ =

144 905.9, $P < 0.0001$), indicating incomplete fermentation which is known to affect the volatile profiles

145 of wines[31]. Also consistent with incomplete fermentation, the final concentration of ethanol in these

146 ferments reduced (Dataset S1). It would also be expected that these ferments would have higher

147 residual sugar but curiously the residual sugar reported for all of these ferments is below 2.5 g/L

148 suggesting the majority of the sugar has been consumed (Dataset S1). To confirm this observation

149 the wines from the third batch were also analyzed for residual sugar using an alternate enzymatic

150 assay (Megazyme D-Fructose/D-Glucose assay kit), which confirmed the low residual sugar levels,

151 reporting concentrations between 0 – 1.1 g/L. This suggests these ferments may not have had as

152 much sugar at the start of fermentation, potentially caused by incomplete mixing of the initial juice

153 before allocation into flasks. We therefore conservatively removed these ferments from all further

154 analyses. Lag phase, the time taken for fermentation to initiate, differed significantly between

155 batches ($F_{2, 89} = 7.73$, $P = 0.0008$), and since each batch contained one replicate of each sample, this

156 was controlled for in subsequent statistical analyses by introducing a "batch" factor.

157

158 <u>Chemical profiles produced by single genotype ferments correlate with region of microbe origin</u>

159 We quantified the concentrations of 39 volatile compounds and wine quality parameters produced

160 in each of the 112 successful ferments using targeted GC-MS and FTIR analyses. First we analyzed

161 the volatile profiles deriving from ferments conducted by single yeast genotypes only. A

162 Permutational Multivariate Analysis of Variance (PERMANOVA) employing a full factorial model with

163 "region" and "batch" as main effects, and where permutations kept replicates of each genotype

164 together, revealed that both factors significantly affected volatile profiles (both $P = 0.001$), but

165 provided no evidence of an interaction between these main effects (Table 1a). The $R^2$ value for the

166 region effect was greatest reporting the geographic origin of the *S. cerevisiae* genotypes explained

167 approximately 10 % of the total variation in the chemical profiles (Table 1a). The lack of significance

168 for the interaction term indicates this result is not confounded by the differences between batches.

169 In addition, we analyzed these differential chemical profiles by accounting for human perception

170 thresholds of compounds. Where available, we used empirically determined odor activity values
171 (OAVs) to standardize the various chemical concentrations in these ferments[32,33]. The results of the
172 subsequent PERMANOVA agreed with the initial analyses and again revealed a highly significant
173 effect of the region of *S. cerevisiae* isolation on these wine phenotypes (Region: $R^2 = 0.127$, $P =$
174 $0.002$; Table 1b). Thus, we can categorically reject the null hypothesis, and move to accept that there
175 is a significant correlation between the region of isolation of *S. cerevisiae* and aroma profiles in wine.

176

177 Regional pairwise PERMANOVA analyses revealed different degrees of distinction between the
178 chemical profiles produced by *S. cerevisiae* genotypes originating from different regions
179 (Supplementary Table S3). *P*-values can be misleading when multiple comparisons are performed[34],
180 and it has been argued that more emphasis should be placed on the magnitude of the effect when
181 dissecting differences[35]: we therefore examined the magnitude of the *F*-statistics from these
182 multiple comparisons as a measure of the strength of evidence for a regional effect (i.e. the higher
183 the *F*-statistic, the stronger the support for a regional effect). The chemical profiles of yeasts
184 originating from Nelson are the most distinct compared to other regions with the mean of the
185 pairwise *F*-statistics involving this region being the highest at 3.20 (Fig. 1; Supplementary Table S3).
186 Nelson's similarity to all regions is low with the exception of the Awatere Valley (Fig. 1). The Awatere
187 and Wairau Valleys are the most similar to other regions (Fig. 1) and report the least distinct
188 chemical profiles compared to other regions with mean *F*-statistics of 1.19 and 1.73 respectively
189 (Supplementary Table S3). Central Otago, Martinborough and Hawke's Bay are intermediate with a
190 mix of both highly similar and more distinct relationships with other regions (Fig. 1; Supplementary
191 Table S3).

192

193 To effectively visualize the differences in chemical profiles, the data were transformed and plotted
194 using Constrained Correspondence Analysis (CCA)[36]. Overall a large overlap is observed between

195 chemical profiles derived from genotypes from different regions (Fig. 2); however, the chemical
196 profiles of Central Otago genotypes cluster in the upper half and those from Nelson mostly toward
197 the lower left quadrant, with the exception of the three replicate samples from one genotype that
198 are located in the upper right quadrant (Fig. 2a). The genotypes from Wairau and Awatere Valleys
199 have the largest ellipses indicating a larger variability in the chemical profiles of these samples (Fig.
200 2b).

201

202 Chemical drivers of regional differentiation in single ferment samples

203 Next we evaluated which components of the volatile profiles might be driving these differences in
204 wine phenotype. Individual ANOVA analyses were performed for each of the chemical properties
205 measured. As explained above, *F*-statistics are reported here in place of *P*-values as they are a more
206 appropriate measure of support for multiple comparisons. We designate *F*-statistics larger than two
207 as having a sizeable effect (i.e. region explains more the twice the variation in the model compared
208 to the residuals), and thus 29 of the 39 compounds vary with respect to the region of origin of the
209 yeast genotype (Supplementary Table S4). $R^2$ values range from zero to 38% of the variation being
210 explained by the *S. cerevisiae* genotype region of isolation, but no one class of chemical compound is
211 exclusively responsible for the regional signal for wine phenotypes (Supplementary Table S4).

212

213 CCA additionally provides vectors indicating the direction and magnitude of influence that each
214 chemical property has on the positioning of the sample aroma profiles within the plot, and
215 potentially provides a mechanism to infer which chemicals differentiate each region. Four
216 compounds (three esters and one fatty acid) have the greatest impact on the distribution of these
217 wine phenotypes generally with vectors of a magnitude larger than 0.25 (Fig. 3a); however these
218 chemical compounds are not necessarily correlated to the differentiation calculated between
219 regions. To focus on and visualize the vectors of the chemical properties most important to the

220 differences in chemical profiles between ferments conducted by yeasts derived from different
221 regions we identified those chemicals that reported $R^2$ values above 0.25, and *F*-statistics above 5 in
222 the individual ANOVA analyses (Fig. 3b and c; Supplementary Table S4). This reveals that
223 concentrations of ethyl isobutyrate and ethyl-2-methyl butanoate, which have apple and sweet fruit
224 sensory descriptors, are on average both greatest in the ferments conducted by the genotypes
225 deriving from Nelson and least in those from Central Otago and Martinborough. In addition,
226 concentrations of ethyl butanoate (sensory descriptors of peach, apple and sweet) are on average
227 greatest in ferments conducted by genotypes derived from Martinborough, and least in ferments
228 conducted by genotypes derived from Nelson (Fig. 3). β-damascenone (sensory descriptors of apple,
229 honey and floral) concentrations are on average greater in the ferments conducted by yeast
230 genotypes derived from the Awatere and Wairau Valleys comprising the larger Marlborough region,
231 and least from the ferments conducted by genotypes deriving from the Hawke's Bay. Together this
232 paints an intuitively sensible picture and reveals that the differential wine phenotype signatures
233 driven by yeasts derived from different regions are not one-dimensional but multi-faceted.

234

235 <u>The genetic basis for differences in chemical profiles</u>

236 While not exclusively genetically determined, the types and concentrations of metabolites produced
237 by *S. cerevisiae* are significantly influenced by yeast genotype[37-39]. It is thus not surprising that a
238 Mantel test evaluating the correlation between *S. cerevisiae* genotype genetic distance (using
239 microsatellite profiles)[17] and volatile chemical profile distance (calculated using Jaccard dissimilarity)
240 reveal they are significantly correlated ($R^2 = 0.189$; $P < 0.0001$). This formally allows us to accept the
241 alternate hypothesis at the core of this study: that there is a significant correlation between the
242 genetic relatedness of natural *S. cerevisiae* sub-populations and their effect on resulting wine
243 phenotypes. Additionally, PERMANOVA analysis using the assignment of genotypes to inferred
244 genetic clusters calculated using InStruct[17] as a factor, as opposed to region of origin, increased the

245 $R^2$ value by 0.051 to 0.151 or 15% ($P$ = 0.007). Some of the genotypes do not have a high proportion
246 of ancestry to any one inferred population, and thus have mixed ancestry to different regions
247 (Dataset S1). If these hybrid genotypes are removed and only those genotypes with a 'clean'
248 geographic signal are analyzed, the PERMANOVA analysis reveals an increase in the $R^2$ for the factor
249 "region" to 0.198 ($P$ = 0.006), double that of the original analysis (Table 1c).

250

251 <u>The effect of regionally co-fermented genotypes and blended wines on volatile profiles</u>

252 There is evidence to show that the presence of other yeasts during fermentation, be they
253 conspecifics or other species, may affect the subsequent volatile profiles of wine compared to the
254 profiles produced when genotypes ferment in isolation[25,40-42]. We moved to evaluate whether
255 interactions between genotypes from each region may affect and potentially alter regional signals
256 for wine phenotypes. We compared the volatile profile of regional co-ferments, produced by
257 inoculating all six genotypes from a region together in equal proportions, to regional blends, created
258 by mixing the final wine produced by single genotypes from each region in equal proportions.
259 PERMANOVA reveals that the type of ferment (co-ferment or blend) has a significant effect on
260 chemical profiles ($R^2$ = 0.061, $P$ = 0.014; Table 1d). Again CCA was used to visualize the differences
261 between the chemical profiles, and while overlap between the blends and co-ferments is evident,
262 the blended ferments show less variability than the co-ferments, and are typically placed in the
263 lower right of the plot (Supplementary Fig. S1a). Individual chemical ANOVA and the resulting CCA
264 plot show the main differences between the co-ferments and blends are driven by ethyl decanoate,
265 ethyl dodecanoate, ethyl octanoate and ethyl acetate (Supplementary Fig. S1b).

266

267 While the co-fermentation of multiple genotypes significantly affects the phenotype of wine
268 compared to blending, it appears to erode signal for wine phenotype regionality, as PERMANOVA
269 analysis reveals no strong regional co-ferment effect on volatile profiles ($R^2$ = 0.346, $P$ = 0.073; Table

270 1e). However, this may be an issue of statistical power – only three replicates of regional co-
271 ferments and blends were implemented compared to the six volatile profiles from each of six
272 genotypes from each region in the initial analysis. It is worth noting that the *P*-value for the effect of
273 region reported by the co-ferments is marginal ($P$ = 0.073), but the value for blends is not ($P$ = 0.196)
274 (Table 1 e and f), and might suggest that blending more greatly erodes any signal for regional wine
275 phenotype than co-fermentation does.
276
277

278 ## Discussion

279 We experimentally tested and quantified the extent to which genetically distinct regional
280 populations of *S. cerevisiae* affect wine phenotype in terms of volatile composition. We show
281 significant positive correlations between the genetic and geographic relatedness of natural *S.*
282 *cerevisiae* sub-populations and their effect on resulting wine phenotypes. As far as we are aware this
283 is the first empirical test for whether there is potential for a microbial aspect to *terroir*. This result
284 aligns with the belief that microbes significantly contribute to the regional identity or *terroir* of wine
285 and may potentially extend to the differential effects of microbes on other important agricultural
286 crops and produce generally.
287

288 The ability of microbes to affect differential crop phenotypes is potentially greater than we estimate
289 here. First, we have not evaluated microbes' effect on crop development and how this might vary
290 between differential geographic communities and populations. This is apparent in some sense, as
291 different crops tend to suffer different levels of disease in different geographic areas; however the
292 subtler effects of microbes on crop development and quality are mostly not understood. Moreover,
293 many other species of fungi and bacteria contribute to the natural conversion of juice to wine and

294 many of these also significantly affect wine phenotype, and there is good evidence to show these
295 may synergistically interact[25,40]. Thus, the presence of regionally differentiated communities of yeast
296 and bacteria associated with ripe fruit, as has been demonstrated[1,15-17], may further affect
297 differences in wine phenotype over that we have revealed here, but this remains to be evaluated.
298 Here we conservatively remove both these effects as we use the same homogenized batch of grape
299 juice and examine the ability of differential populations of just one species to manipulate crop
300 produce. Even so, we provide evidence that different natural sub-populations of *S. cerevisiae*
301 deriving from different regions have the potential to significantly and differentially affect wine
302 phenotype.

303

304 The chemicals responsible for the differences between regions are not consistently from any
305 particular class (Supplementary Table S4), and thus the microbially driven signals for difference in
306 wine phenotype by region are complex, which makes intuitive sense. We attempted to evaluate the
307 impact of how humans might perceive these differences in wine phenotypes by standardizing
308 chemical concentrations with published OAVs[32,33]. This analysis again reported a significant effect of
309 regionally differentiated microbes on wine phenotypes; however, OAVs are subjective to an extent,
310 and interactions between chemicals that may lead to enhancement or masking of aromas are not
311 accounted for here[33]. Ultimately the inclusion of sensory trials in these kinds of studies would add an
312 extra layer to evaluate the extent that microbes play in the geographic differentiation of wine
313 phenotypes. In addition this study only employs microbes that were determined to differ by region
314 from just one year: how such population differentiation, and their resulting effects on crop
315 phenotypes, changes across multiple years remains to be tested.

316

317 Recently a handful of studies have shown that the communities and populations of microbes
318 associated with vines and wines vary by region[1,15-17], and these are the first demonstrations of

319 geographic variance in microbes associated with agriculture generally. Here we conduct a crucial
320 follow-on to these observations: to test whether the genetic variance in microbial populations
321 correlates with altered crop phenotypes. Geographic variance in crop physical and sensorial
322 signatures are well described, and have important economic and consumer preference
323 consequences[10], but the drivers behind these differences have not been objectively evaluated and
324 quantified. While we are not able to make any assertions regarding the temporal stability of these
325 results, these data show there is a quantifiable microbial aspect to *terroir,* thus revealing the
326 potential importance of microbial populations on the regional identity of wine, and may also extend
327 to other important agricultural commodities. With a better understanding of the forces driving
328 microbial population and community differentiation, food and agricultural sectors can develop
329 systems to better control and manage these communities to help conserve the regional identity of
330 products. More generally this finding indicates the importance of characterizing and understanding
331 biodiversity and the services it may provide. Together this suggests that the long-term
332 implementation of methods that maintain biodiversity may have tangible economic imperatives as
333 well as being driven by a desire to employ agricultural practices that increase responsible
334 environmental stewardship.
335
336

337 ## Methods

338 <u>Genotype selection</u>

339 Six genotypes were selected from six major wine growing regions in NZ to represent the genetic
340 diversity in each region (See Fig. 1 for geographic locations). Here we specifically employed
341 genotypes previously isolated from spontaneous ferments[17]. We used Bayesian analyses to select
342 one genotype from each region that harbored at least one allele that was unique to that region

343 while the remaining genotypes were selected to cover the diversity of ancestry profiles reported in
344 each regional population as reported in Knight and Goddard (2015)[17].
345

346 Micro-fermentation

347 The 126 ferments were conducted in three batches due to space constraints, and each batch
348 contained one replicate of every treatment. Each ferment contained 230 mL of Marlborough (NZ)
349 Sauvignon Blanc juice from the 2012 vintage (pH = 3.1, 22.1 °Brix) sterilized with 200 µL/L Dimethyl
350 dicarbonate (DMDC) and with the $SO_2$ level adjusted to 10 mg/L. Each *S. cerevisiae* genotype was
351 grown up independently in liquid YPD (1% yeast extract, 2% peptone, 2 % glucose) prior to
352 inoculation. The live cell concentration of each culture was determined using a haemocytometer
353 with methylene blue staining, and cells were inoculated to give a final concentration of $2.5 \times 10^6$
354 cells/mL. Regional co-ferments were performed by inoculating all six genotypes isolated from each
355 region in equal proportions to the same final concentration of $2.5 \times 10^6$ cells/mL. Triplicate un-
356 inoculated controls were included in each batch to control for weight loss via evaporation and to
357 identify potential contamination issues. This totaled 126 experimental ferments and 9 un-inoculated
358 controls. Ferments were conducted at 15 °C with 150 rpm shaking in 250 mL Erlenmeyer flasks with
359 air-locks. Fermentation progress was monitored by weighing the flasks daily[30] and ferments were
360 considered finished when the rate of weight loss was below 0.001 g/hr (after controlling for
361 evaporation as calculated from the controls) or when they reached 30 days. Ferments were
362 centrifuged at 6000 × *g* for 10 minutes to pellet cells and the supernatant was decanted and frozen
363 at -20 °C until chemical analyses were performed.
364

365 Blends

366 After fermentation, regional blends were constructed from the single genotype ferments. Equal
367 proportions of wine from ferments of each of the six genotypes from each region were

368 homogenized, creating triplicate regional blends for each of the six regions. This resulted in a total of
369 144 wine samples for chemical analyses.
370
371 Chemical analyses
372 Final ethanol concentration, pH, residual sugar, volatile acidity (VA) and titratable acidity (TA) were
373 quantified using FTIR (Fourier Transform Infrared Spectroscopy) with a FOSS WineScan™ FT120. The
374 varietal thiols 3MH, 3MHA and 4MMP were quantified using an ethyl propiolate derivatization and
375 analyzed on an Agilent 6890N gas chromatograph (Santa Clara, CA, USA) equipped with a 7683B
376 automatic liquid sampler, a G2614A autosampler and a 593 mass selective detector as outlined in
377 Herbst-Johnstone et al. (2013)[43]. Thirty-two esters, higher alcohols, terpenes, C6-alcohols and fatty
378 acids were quantified simultaneously using a HS-SPME/GC-MS method outlined in Herbst-Johnstone
379 et al. (2013a)[44]. Raw data was transformed with GCMSD Translator and peak integration was
380 performed using MS Quantitative Analysis, both part of the Agilent MassHunter Workstation
381 Software (Version B.04.00, Agilent Technologies).
382
383 Statistical Analyses
384 The sigmoid or altered Gompertz decay function described by Tronchoni et al. (2009)[45] was used to
385 build a model of fermentation kinetics for each ferment from the weight loss data to infer the lag
386 phase. The data was fitted using the non-linear least squares method implemented in the R package
387 *nlstools*[46]. Differences in the lag phase between batches were tested using a mixed linear model in
388 JMP (Version 10) accounting for genotype and stuck ferments as random factors.
389
390 Statistical tests for regional signal were performed on the chemical profiles for all datasets
391 separately using a PERMANOVA approach as implemented in the R package *vegan*[47]. Jaccard

392 distances were used to calculate pairwise distances in the model and 10 000 permutations of the
393 raw data constrained at the genotype level to account for the dependency between genotypes and
394 their replicates, were performed for the hypothesis tests (*F*- tests). Full factorial models were
395 implemented and subsequently reduced upon analysis of the results to obtain the model of best fit.
396 Pairwise PERMANOVA analyses were performed between all combinations of regions for the single
397 genotype ferments. Since *P*-values can be misleading when multiple comparisons are performed[34]
398 we follow the idea that more emphasis should be placed on the magnitude of effects[35] thus the *F*-
399 statistics from these comparisons are used as a measure of the strength of evidence for a regional
400 effect. Constrained Correspondence Analysis (CCA), implemented in the R package *vegan*, was used
401 to visualize the data. This is analogous to a Principle Component Analysis in that transformations of
402 the data are performed to provide components that allow the data to be visualized in 2-D plots. The
403 CCA additionally partitions these components into a part that is explained by the specified linear
404 model (in this case "region + batch") and a part that is residual to that model. The plot that is
405 produced rotates the data to the best orientation to observe the variation explained by the model.
406 This method allows the PERMANOVA model to be built into the visualization, providing the most
407 appropriate transformation and orientation of the data to visualize differences between the factors
408 of interest.

409

410 A Mantel test was performed in GenAlEx (Genetic Analyses in Excel) version 6.5[48,49] between a
411 chemical distance matrix calculated using the Jaccard similarity coefficient, and the genetic distance
412 matrix calculated using data from Knight and Goddard (2015)[17].

413

414

415

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547 ## Author contributions

548 S Knight designed and performed the research, analyzed the data and wrote the paper; S Klaere
549 analyzed the data; BF contributed reagents and analytical tools; MG designed the research, assisted
550 in data analyses and wrote the paper.

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553 ## Competing financial interests

554 The authors declare no competing financial interests

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566 ## Figure legends

567 **Figure 1:** A map of the regions the tested genotypes of *S. cerevisiae* were isolated from and the
568 strength of regional differentiation in the chemical profiles as indicated by *F*-statistics from pairwise
569 PERMANOVA analyses (Supplementary Table S3). Wider lines indicate weaker regional distinctions in
570 the chemical profiles produced (i.e. less distinct chemical profiles), while thinner lines indicate
571 stronger regional distinction (i.e. more distinct chemical profiles). The inset indicates the portion of
572 NZ highlighted in the larger map. The outline of the map of NZ was obtained
573 from www.spraypaintstencils.com, where it is freely available, and all modifications were performed
574 by the Authors in Microsoft Power Point.

575

576 **Figure 2:** CCA of the 105 single genotype ferments analyzed. (a) All sample points colored by region.
577 (b) Regional averages and 50 % ellipses.

578

579 **Figure 3:** Visualization of the chemicals that individually explain more than 25 % of the regional
580 variation as calculated by ANOVA analyses. (a) The direction and magnitude of all chemical loading
581 vectors, with labels for the chemicals that reported a magnitude above 0.25. The blue circles
582 represent the position of 0.1 and 0.25. (b) The chemical loading vectors in the CCA plot for those
583 that reported an $R^2$ value for region larger than 0.25 and an *F*-statistics larger than 5 in the ANOVA
584 analyses (Supplementary Table S4). The blue circles represent the position of 0.1 and 0.25. (c) The
585 same chemical loading vectors reported in *b* with respect to the regional centers of the chemical
586 profiles.

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590 ## Tables

591 **Table 1:** Summary of all PERMANOVA analyses.

| **Factors** | **Df** | ***F* Model** | **$R^2$** | ***P*-value** |
|---|---|---|---|---|
| *(a) Single strain ferments only* | | | | |
| **Region** | **5** | **2.056** | **0.100** | **0.001 ***** |
| Batch | 2 | 3.687 | 0.072 | 0.001 *** |
| Region*Batch | 9 | 0.860 | 0.076 | 0.093 |
| Residuals | 77 | | 0.752 | |
| Total | 93 | | 1 | |
| *(b) Single strain ferments only, with chemicals standardised by OAV* | | | | |
| **Region** | **5** | **2.758** | **0.127** | **0.002 **** |
| Batch | 2 | 4.302 | 0.079 | 0.001 *** |
| Region*Batch | 9 | 0.987 | 0.082 | 0.166 |
| Residuals | 77 | | 0.711 | |
| Total | 93 | | **1** | |
| *(c) Single strain ferments with strains with mixed ancestry removed* | | | | |
| **Region** | **5** | **3.176** | **0.198** | **0.006 **** |
| Batch | 2 | 3.092 | 0.077 | 0.005 ** |
| Region*Batch | 9 | 1.482 | 0.166 | 0.056 |
| Residuals | 45 | | 0.560 | |
| Total | 61 | | 1 | |
| *(d) Co-ferments and blends only, testing for effect of the type of ferment* | | | | |
| **Type** | **1** | **2.425** | **0.061** | **0.014 *** |
| Batch | 2 | 3.698 | 0.186 | 0.014 * |
| Residuals | 30 | | 0.753 | |
| Total | 33 | | 1 | |
| *(e) All co-ferment samples only* | | | | |
| **Region** | **5** | **1.555** | **0.346** | **0.073** |
| Batch | 2 | 2.364 | 0.210 | 0.073 |
| Residuals | 10 | | 0.444 | |
| Total | 17 | | 1 | |
| *(f) All blend samples only* | | | | |
| **Region** | **5** | **1.375** | **0.339** | **0.196** |
| Batch | 2 | 1.704 | 0.168 | 0.196 |
| Residuals | 10 | | 0.493 | |
| Total | 17 | | 1 | |

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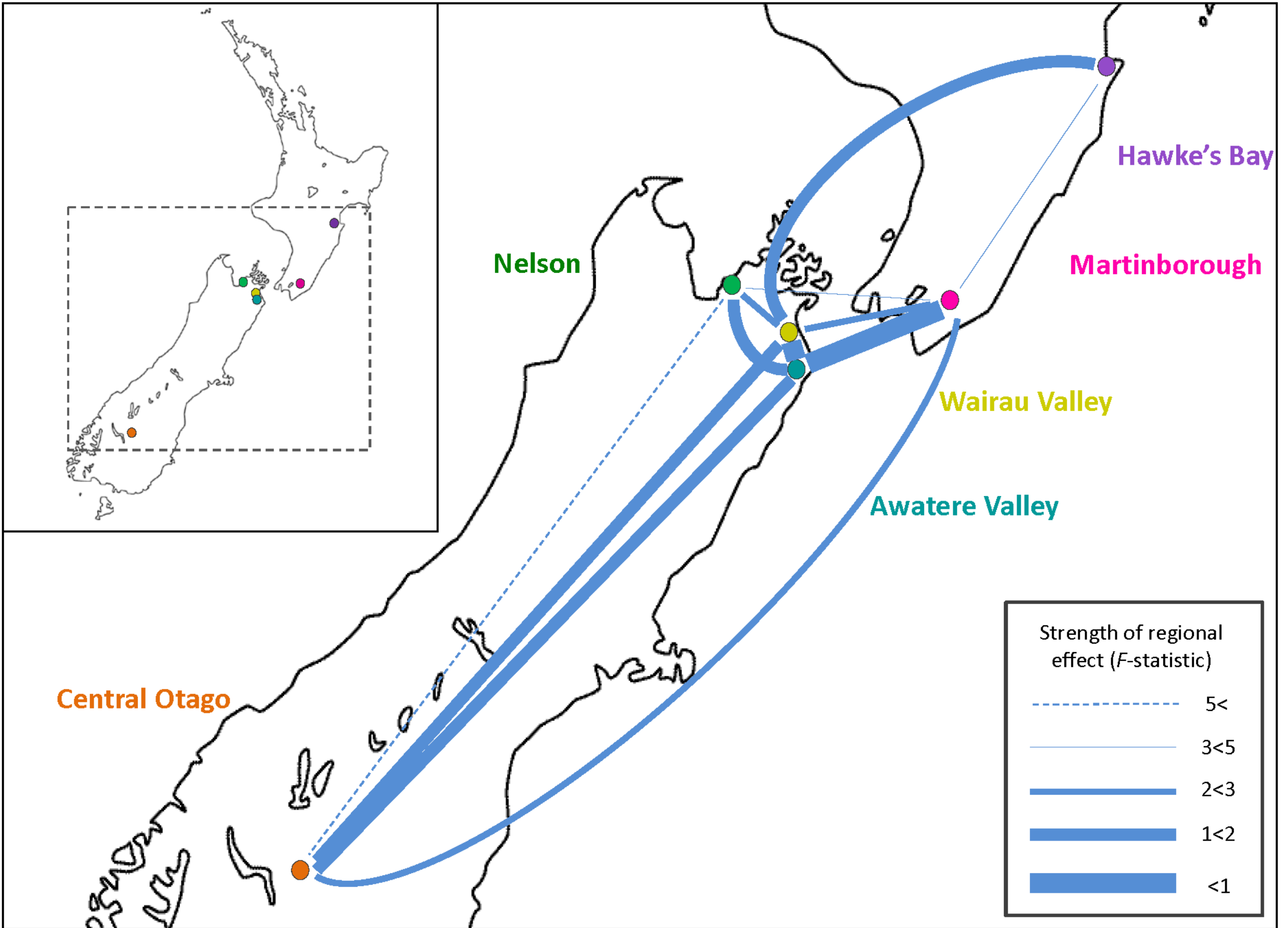
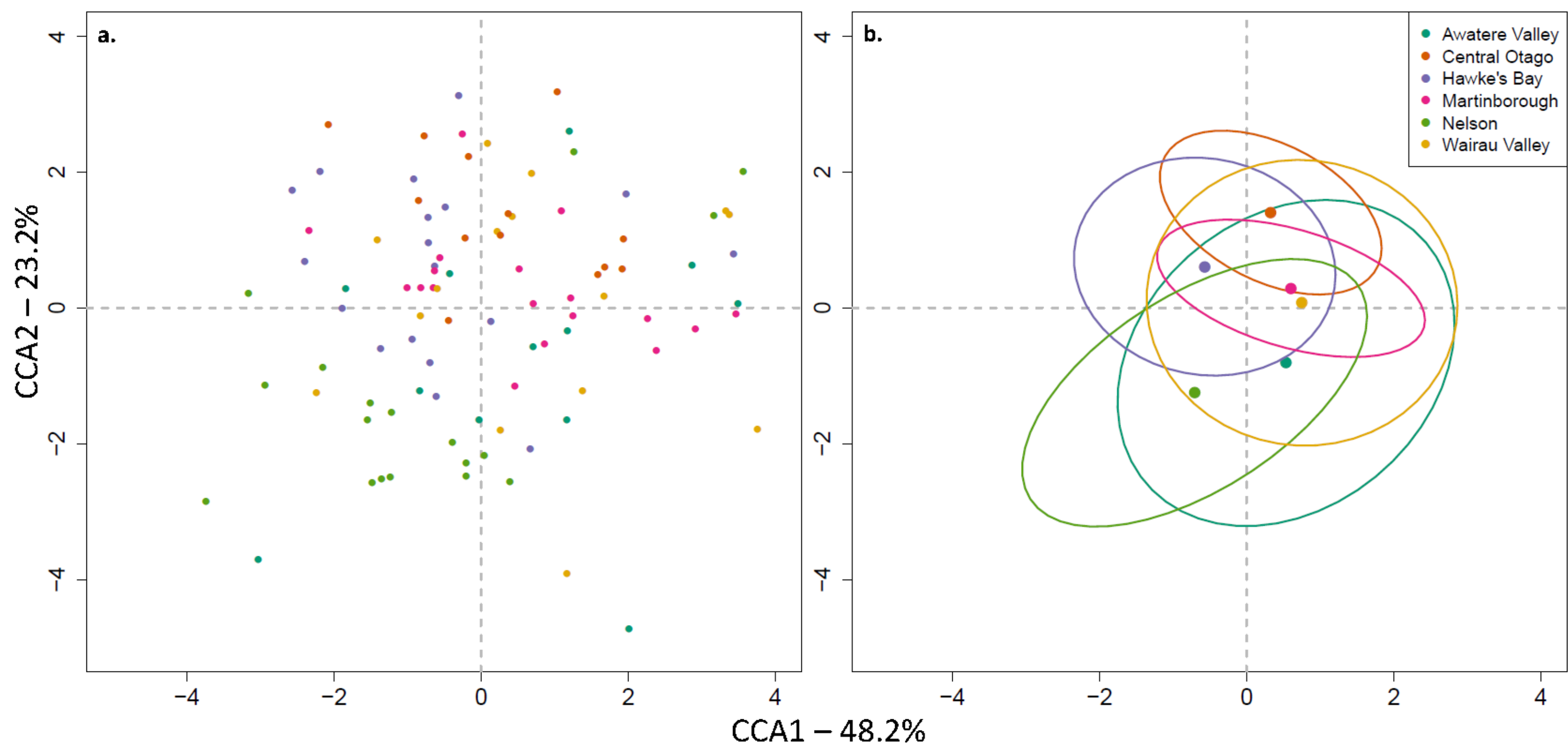
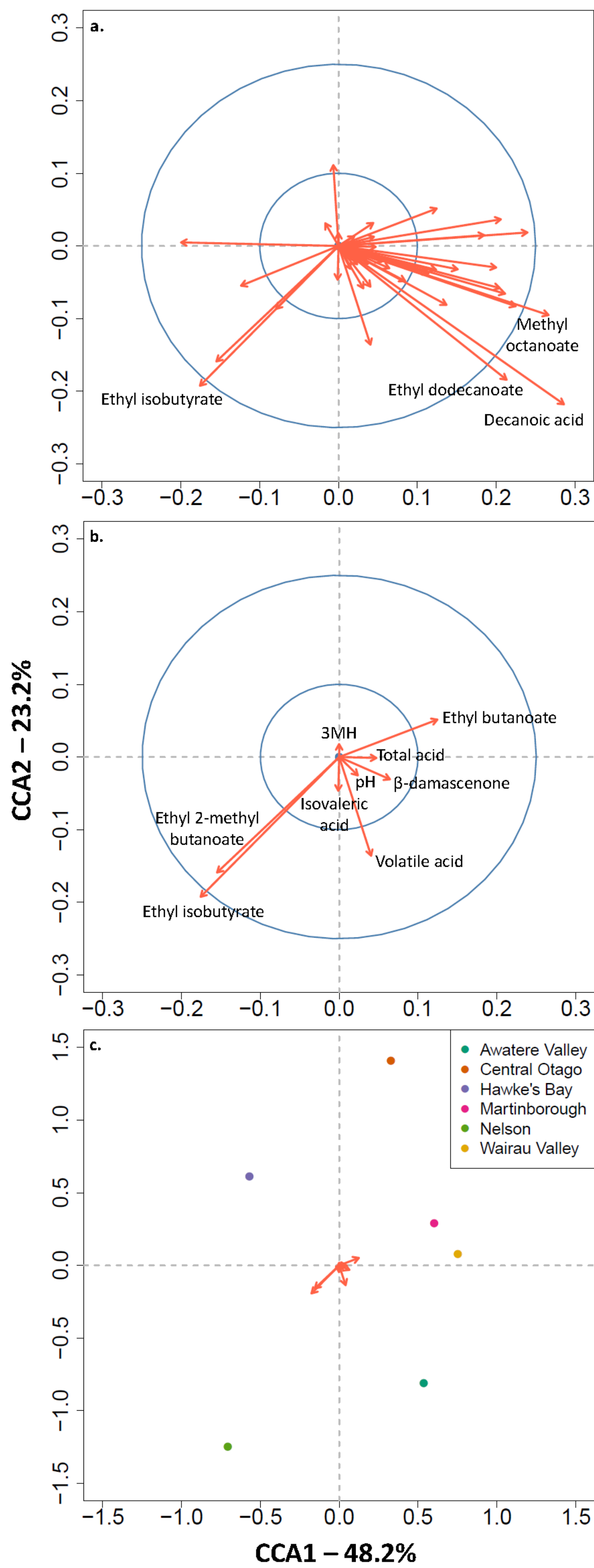

Hawke's Bay
Nelson
Martinborough
Wairau Valley
Awatere Valley
Central Otago
Strength of regional effect (F-statistic)
5<
3<5
2<3
1<2
<1


a.
b.
Awatere Valley
Central Otago
Hawke's Bay
Martinborough
Nelson
Wairau Valley
CCA2 – 23.2%
CCA1 – 48.2%
4
2
0
−2
−4
−4
−2
0
2
4


a.
Methyl octanoate
Ethyl dodecanoate
Decanoic acid
Ethyl isobutyrate
b.
3MH
Ethyl butanoate
Total acid
pH
β-damascenone
Isovaleric acid
Ethyl 2-methyl butanoate
Volatile acid
Ethyl isobutyrate
c.
Awatere Valley
Central Otago
Hawke's Bay
Martinborough
Nelson
Wairau Valley
CCA2 – 23.2%
CCA1 – 48.2%