

1 **Expression biomarkers used for the selective breeding of complex polygenic**  
2 **traits**

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## 27 **Abstract**

28           We present a novel way to select for highly polygenic traits. For millennia,  
29 humans have used observable phenotypes to selectively breed stronger or more  
30 productive livestock and crops. Selection on genotype, using single-nucleotide  
31 polymorphisms (SNPs) and quantitative trait loci (QTLs), is also now applied broadly in  
32 livestock breeding programs; however, selection on protein or mRNA expression  
33 markers have not been proved useful yet. Here we demonstrate the utility of protein  
34 markers to select for disease-resistant behaviour in the European honey bees (*Apis*  
35 *mellifera* L.). Robust, mechanistically-linked protein expression markers, by integrating  
36 cis and trans effects from many genomic loci, may overcome limitations of genomic  
37 markers to allow for selection. After three generations of selection, the resulting stock  
38 performed as well or better than bees selected using phenotype-based assessment of  
39 this trait, when challenged with disease. This is the first demonstration of the efficacy of  
40 protein markers for selective breeding in any agricultural species, plant or animal.

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## 42 **Significance statement**

43           The honey bee has been in the news a lot recently, largely because of world-  
44 wide die-offs due to the parasitic Varroa mite, which is becoming resistant to the  
45 chemical controls the bee industry uses. In this study, we show that robust expression  
46 biomarkers of a disease-resistance trait can be used, in an out-bred population, to select  
47 for that trait. After three generations of selection, the resulting stock performed as well or  
48 better than bees selected using the phenotypic best method for assessing this trait when  
49 challenged with disease. This is the first demonstration of an expression marker for  
50 selective breeding in any agricultural species, plant or animal. This also represents a  
51 completely novel way to select for highly polygenic traits.

52

## 53 Introduction

54 European honey bees are a keystone species in agriculture as many crops  
55 depend on them for pollination and increased yield<sup>4</sup>. Honey bee colonies have been  
56 dying at increased rates over the past decade, largely due to increased pressure from  
57 diseases and pests<sup>5</sup>. Since these pests and pathogens are continually evolving  
58 resistance to the synthetic chemicals used to treat them, the most sustainable, long-term  
59 solution for bee health is the development of selective breeding programs that can  
60 enrich natural disease resistance mechanisms. However, selective breeding in *A.*  
61 *mellifera* is particularly challenging because most traits are expressed at the colony  
62 level<sup>6</sup>, and due to the haplo-diploid sex determination system in bees, challenges in  
63 storing germplasm<sup>7</sup>, the requirement for heterozygosity at the complementary sex  
64 determination locus<sup>8</sup> that severely limits in-breeding, and the tendency for queens to  
65 mate with up to two dozen different drones. These factors mean that continual selection  
66 is required to maintain stock.

67 Bees do, however, have some effective disease-resistance traits, which also  
68 happen to be highly polygenic: one example is the social immunity function known as  
69 hygienic behaviour. Bees exhibiting hygienic behaviour are more efficient at removing  
70 dead, diseased, or dying brood from the hive<sup>9, 10</sup>, enabling them to resist or at least co-  
71 exist with pathogens such as American Foulbrood (*Paenibacillus larvae*) or parasites  
72 such as *Varroa* mites (*V. destructor*) that would otherwise kill the colony. A closely  
73 related but distinct trait known as *Varroa*-sensitive hygiene enables bees to detect and  
74 disrupt the life cycle of reproductive female *Varroa* mites<sup>11</sup>. Both hygienic behaviour and  
75 *Varroa*-sensitive hygiene are heritable<sup>12, 13</sup> and can therefore be selectively bred for;  
76 likewise, QTLs and SNPs have been linked to each behaviour<sup>14, 15</sup>, opening the door for  
77 their use in marker-assisted selective (MAS) breeding. Historically, genomic markers  
78 have been favoured for MAS because of their stability and reliability, whereas

79 expression markers such as the levels of transcripts or proteins, are typically thought to  
80 be too variable and dependent on environment for use in MAS.

81           However, even a closely linked DNA feature may not be sufficient for MAS in  
82 honey bees; *A. mellifera* has one of the fastest recombination rates (~32 cM/Mb) known  
83 among animals, and this will rapidly break down inter-allele linkage through repeated  
84 rounds of meiosis<sup>16</sup>. On the other hand, causally-linked expression markers should be  
85 more robust to recombination since their presence is required for the trait, even though  
86 they have historically been thought to be too dependent on environment. Here we use a  
87 panel of protein markers identified through a multi-generational study<sup>3</sup> to guide selective  
88 breeding of disease-resistance traits in honey bees through three generations. By the  
89 third generation, bee stocks selected through MAS were able to resist disease as  
90 effectively as bees raised through conventional selective breeding using standard field  
91 tests, with no detectable loss of other desirable traits such as honey production. This is  
92 the first successful use of expression markers for MAS that we are aware of.

93

## 94 **Results & Discussion**

95           We have previously identified seven proteins in adult worker bees' antennae  
96 whose expression is tightly linked to hygienic behaviour<sup>3</sup> (HB). To complete a  
97 comprehensive panel of markers for use in selective breeding, we added six more  
98 proteins derived from the same data, four that showed tight correlation with *Varroa*-  
99 sensitive hygiene and grooming behaviour, and two more also linked to hygienic  
100 behaviour. Of the latter, one was missing in an initial dataset and therefore failed to meet  
101 the inclusion criteria we used, and the other (Fig. 1a) had been 'lost' due to an  
102 unrealized change in accession number between protein database versions. These  
103 thirteen biomarkers were complemented by two 'housekeeping' proteins,  $\alpha$ -spectrin and  
104  $\beta$ -tubulin, that showed zero correlation with any behaviours (Supplemental Table 1) to

105 serve as loading controls. The biomarker panel had originally been discovered through  
106 untargeted, data-dependent liquid chromatography-tandem mass spectrometry but a  
107 less stochastic detection method was required for scanning hundreds of samples.  
108 Therefore, multiple reaction monitoring (MRM) assays<sup>17</sup> were developed for up to five  
109 peptides from each protein (Supplemental Table 1, Fig. 1b), with priority given to  
110 peptides we had observed in the discovery of these biomarkers<sup>3</sup>. The use of stable  
111 isotope-labelled standard peptides of known concentrations allowed quantitation of each  
112 peptide in protein extracts from worker bee antennae (Fig. 1c).

113 To identify a robust initial breeding population of honey bees that was not already  
114 enriched in disease resistance behaviours, we surveyed the hygienic behaviour (HB) of  
115 635 colonies from thirty-eight commercial beekeeping operations across western  
116 Canada in 2011. For this initial survey, we gave priority to beekeeping operations that  
117 bred their own bees so that the bees were well-adapted to the local climate<sup>18</sup> and  
118 representative of stock being bred and used in Western Canada. All beekeepers  
119 donated or sold a subset of the tested queens to incorporate into the selection program.  
120 The hygienic behaviour scores in this initial survey varied regionally and ranged from  
121 9.8% to 100%, with a median of 64% (Fig. 2a), matching levels of trait expression  
122 observed previously among unselected populations in a smaller survey within the same  
123 region<sup>19</sup>.

124 For selection using markers, nurse bees from 468 of these colonies were  
125 dissected for quantitation of peptide markers in their antennae (Supplemental Table 2).  
126 From the colonies surveyed, two to four colonies were randomly selected from each  
127 beekeeping operation (for a total of 100 queens) to serve as benchmark, unselected  
128 stock (Fig. 2a): these (BEN) were statistically indistinguishable from the wider surveyed  
129 population (ALL). In addition, we selected queens from an additional 100 colonies, each  
130 with the highest HB scores in their apiaries. Their HB scores ranged from 38% to 100%

131 with a mean score of 85%. We moved these queens to two breeding locations in  
132 Southern British Columbia and introduced them into colonies.

133 Over the next two years we reared three successive generations (F1 and F2 in  
134 2012, F3 in 2013) from this initial population using the response of parental colonies in  
135 each of two ways: (1) the classic freeze-killed brood assay<sup>9</sup> to quantify hygienic  
136 behaviour as a positive control (FAS, field-assisted selection), and (2) the levels of the  
137 best-performing subset of the peptide markers in Supplemental Table 3 (MAS, marker-  
138 assisted selection). For the selective breeding, the F1 and F2 queens were generated by  
139 instrumental insemination of virgin queens reared from the selected colonies using  
140 semen pooled from a random collection of drones from all the selected colonies in the  
141 appropriate stock. The same pooling of semen among selected was accomplished for F3  
142 queens by closed breeding in isolated mountain valleys in southeastern British Columbia  
143 where no known drone sources existed. In addition to these selective breedings,  
144 benchmark stock (BEN) was maintained through unselected open mating, as a control.

145 The freeze-killed brood field assay (FAS) is the gold standard for identifying  
146 hygienic behaviour<sup>9</sup> and colonies selectively bred using FAS showed the greatest  
147 enrichment of hygienic behaviour (Fig. 2b) over three generations. Notably, selective  
148 breeding based on the panel of protein markers (MAS) was also effective for enriching  
149 hygienic behaviour, demonstrating the potential of this technique in selective breeding.

150 By measuring hygienic behaviour in the colonies bred using MAS we could also  
151 monitor the specificity and sensitivity characteristics of the biomarker panels; while there  
152 was a statistically significant improvement detectable even in F1, by F2 there was a very  
153 marked improvement that was little changed in the F3 (Fig. 2c, d, e). The distribution of  
154 hygienic behaviour in the unselected BEN colonies, however, was unchanged between  
155 the starting group and the final population (Fig. 2b, left-most plot).

156 Hygienic behaviour can confer resistance to brood pests and pathogens that  
157 contribute to honey bee colony losses. We therefore evaluated how well colonies  
158 headed by selected queens performed under disease (American foulbrood (AFB), *P.*  
159 *larvae*) and mite (*V. destructor*) challenge conditions. Selected and benchmark stocks,  
160 as well as imported stock commonly used by Canadian beekeepers, were inoculated  
161 with either parasitic mites or AFB bacterial spores at levels that would normally result in  
162 high levels of colony mortality.

163 To test for the ability to survive with *Varroa* mites, in the early summer 2012 we  
164 pooled a large population of worker bees from colonies that were infested with *V.*  
165 *destructor* (about three hundred mites per colony, or a 3.5% infestation rate based upon  
166 mites per 100 bees) and aliquoted 8,600 bees (1 kg of bees) into individual colonies.  
167 Twenty-three F3 selected or unselected queens were then randomly introduced into  
168 these individual colonies and the colonies were left untreated until the following spring; a  
169 fall survey for mites measured infestation rates of  $23.0 \pm 1.4$  mites per 100 bees. For  
170 AFB, F3 colonies normalized for population received a frame with 225 cm<sup>2</sup> of brood  
171 comb with 30 to 54% of wax cells showing visible *P. larvae* disease symptoms on each  
172 side. We assessed the impact of the parasite and pathogen challenge on winter survival  
173 for the varroa challenge and for overall survival of asymptomatic colonies for the AFB  
174 challenge (Fig. 3).

175 To check that an independent but critical performance indicator of these stocks  
176 was not being degraded by too much focused selection on another trait, we also  
177 examined the ability of F3 colonies to collect honey in a separate study from the  
178 disease-challenge experiments. Reassuringly, honey production was not affected by  
179 selection for hygienic behaviour, regardless of which selection method was used (Fig.  
180 3c).

181           The results of these experiments were subsequently used to model the economic  
182 impact of integrating the use of marker-selected stock into a Canadian beekeeping  
183 operation. The marker-selected colonies' increased disease resistance and greater  
184 survival rates enhanced beekeeper profit. The economic modeling shows that when a  
185 beekeeper replaces his conventional colonies with MAS colonies selected for hygienic  
186 behavior we see up to a 5% increase in profit for a 40-colony apiary. This is likely an  
187 underestimate as we have not assumed any increase in colony productivity for the  
188 disease-resistant MAS colonies. The greatest economic value derived from MAS colony  
189 adoption was when resistance to traditional Varroa treatments was modeled in the  
190 apiary. To reduce the risk of economic loss associated with treatment resistance (or an  
191 equivalent ineffective/ lack of treatment), MAS colonies replaced 25%, 50% and 100% of  
192 traditional colonies within the apiary showing profit increases of over 300%, 600% and  
193 800% respectively compared to the no-MAS apiary.

194           The first genetic modification of bees has been reported<sup>21</sup> but industrial use of  
195 genetically modified bees is unlikely to be accepted by the public at this time. Therefore,  
196 tools that enable accelerated stock enrichment without resorting to genetic modification  
197 are highly desirable. While genetic markers for hygienic behaviour and *Varroa*-sensitive  
198 hygiene have been identified<sup>14, 15</sup>, they are unlikely to be linked tightly enough to be  
199 robust to the high recombination rate bees exhibit for more than a few generations. In  
200 addition, there may be several loci missing since both traits are highly polygenic<sup>22</sup>.  
201 Expression markers integrate many different *cis*- (e.g., transcriptional enhancer  
202 elements) and *trans*- (e.g., transcription factors) effects so if they are functionally linked  
203 to the trait in question then they should be robust to recombination. We have not yet  
204 shown that the markers we use here are functionally linked to hygienic behaviour,  
205 *Varroa*-sensitive hygiene, or grooming but they are tightly linked enough to enrich the  
206 trait as quickly as the best conventional methods available.



207           The most important pests and pathogens of bees are currently controlled with  
208 acaricides, antibiotics and antimycotics, but emerging resistance to these treatments  
209 may be partially responsible for the higher level of colony losses seen over the past  
210 seven years<sup>23</sup>. These exogenous treatments can leave residues in the hive<sup>24</sup> and honey  
211 though so disease-resistant stock is seen as the best solution, simultaneously reducing  
212 colony losses and the need for synthetic chemicals while ensuring food safety. Marker-  
213 assisted selection of honey bees will enable more sophisticated breeding of this critical  
214 agricultural service provider.

215           Selective breeding is a vital tool for improving yields and disease resistance in all  
216 plants and animals used in agriculture. Marker-assisted selection has the potential to be  
217 more precise and more robust to external influences; it has been widely used in certain  
218 plants<sup>25</sup> and animals<sup>26</sup>. To date, however, the markers used have been genomic loci  
219 exclusively, starting with restriction fragment length polymorphisms and leading up to  
220 single nucleotide polymorphisms. This is undoubtedly due, in part, to the availability of  
221 efficient genetic approaches for finding such markers. It is also a matter of focus though:  
222 researchers have spent more time looking for genetic loci than for expression markers  
223 (i.e., transcripts or proteins) because the latter have been hitherto considered to be too  
224 dependent on environment. Here we have shown that expression markers can be used  
225 to select for a very complex, polygenic trait. Even in this proof-of-principle with a first-  
226 generation panel of markers, MAS was as efficient at enriching disease-resistance as  
227 FAS methods: bees bred using marker-assisted selection could resist levels of disease  
228 that would typically kill 75% or more of unselected colonies. The data presented here  
229 have implications beyond bees: this is the first demonstration of marker-assisted  
230 selection in livestock using expression markers and it opens the door for molecular  
231 diagnostic approaches for selecting complex polygenic traits that are recalcitrant to  
232 genetic mapping methods<sup>27</sup>.

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249 **Author Contributions**

250 LJF, SFP, RWC, and MMG conceived the experiments. LJF, MMG, SFP, RWC, SEH,  
251 EH, AI, APM, and HH designed the experiments. EH and HH managed the selective  
252 breeding. RW and MMG developed the statistical treatment of the biomarkers and  
253 refined the prediction models. KMM oversaw the proteomic sample collection and  
254 processing. SEH, SFP, MMG and KMM analysed data from freeze-killed brood and  
255 MRM assays data to select breeding colonies. All authors except RW, MB, DD and CB  
256 helped with sample collection, hygienic behaviour testing, and general beekeeping  
257 activities. DD and CB developed and applied the multiple reaction monitoring assays.

258 SEH, AI, MP, SD, DM, SFP and RWC conducted the *Varroa*- and *P. larvae*-challenge  
259 experiments, as well as the evaluation of honey production. MEFB developed the  
260 economic model.

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## 264 **Figure Legends**

### 265 **Figure 1: Multiple reaction monitoring assays for markers of disease resistance. a)**

266 amino acid sequence of gi:110761334, Glycine-rich cell wall structural protein-like  
267 protein, one of the markers of hygienic behaviour. The two peptides identified in the  
268 initial discovery are highlighted in red; these same two peptides were targeted with  
269 multiple reaction monitoring assays here. b) overlaid chromatograms of the three  
270 selected transitions for the stable isotope-labelled forms of all fifty-five peptides listed in  
271 Table 1 for the fifteen proteins comprising the biomarker panel. c) Transitions for the  
272 stable isotope standard (SIS) and natural (NAT) forms of MGSIDEGVSK from Glycine-  
273 rich cell wall structural protein-like protein. The primary ( $1^\circ$ ) transition of each peptide  
274 was used for quantitation, while the secondary and tertiary transitions were used to  
275 confirm specificity.

276

### 277 **Figure 2: Starting distributions and enrichment of hygienic behaviour. BEN =**

278 benchmark, MAS = Marker-assisted selection, FAS = Field-assisted selection. (a) 90/10  
279 box-and-whisker plots of the hygienic behaviour scores from all colonies in initial survey  
280 across Western Canada, in British Columbia (BC), Alberta (AB), Manitoba (MB)(left  
281 section) (Means followed by the same letter are not different from each other Tukey  
282  $P < 0.05$ ), all colonies together (ALL), and the randomly selected starting benchmark  
283 population (BEN). 'All' is statistically identical to BEN ( $p = 0.21$ , Analysis of Means Test)

284 (b) The distribution of hygienic behaviour in the F1 and F3 generations of the benchmark  
285 population (left section, BEN, no statistical difference between F1 and F3,  $P=0.65$ ,  
286 contrast), the colonies selected by the biomarker panel (middle section, MAS,  $F3 > F1$ ,  
287  $p=0.03$ , contrast), and the freeze-killed brood assay (right section, FAS,  $F3 > F1$ ,  
288  $p=0.002$ , contrast). Within each generation, means followed by the same letter are not  
289 different from each other Tukey  $P=0.05$ ). Bottom: Receiver operating characteristics  
290 illustrating the performance of the F1 (c), F2 (d) and F3 (e) marker panels used for MAS.  
291

292 **Figure 3: Performance of selected stock.** IMP = imported stock, BEN = benchmark,  
293 MAS = Marker-assisted selection, FAS = Field-assisted selection. (a) Difference in  
294 winter survival of F3 generation colonies headed by queens from each stock type that  
295 were challenged with *Varroa* mites (*Varroa* challenge) (d.f. 3, Chi Sq 14.84  $p > \chi =$   
296 0.002). (b) Difference in symptom-free survival when challenged with American  
297 Foulbrood (*P. larvae*; AFB challenge:) (d.f. 3, Chi Sq 12.65  $p > \chi = 0.0054$ ). Horizontal  
298 lines represent Holm-Bonferonni adjusted single degree of freedom contrasts between  
299 MAS selected stock and the benchmark and imported stock controls. Siimilar results  
300 were found for FAS, with FAS survival higher than the BEN and IMP stocks for both the  
301 *Varroa* challenge ( $p=0.05$  and  $p=0.025$ , respectively) and AFB challenge experiments  
302 ( $p=0.025$  and  $p=0.013$ , respectively). Error bars represent the standard error of the  
303 binomial proportion. (c) Honey produced per colony for all stocks tested at three  
304 experimental sites in Alberta and Manitoba. There was no significant difference in honey  
305 production among the four stocks tested (d.f. 3,161;  $F=2.12$ ,  $p=0.099$ ).

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## 307 **References**

308

- 309 1. Larson, G. *et al.* Current perspectives and the future of domestication studies. *Proc.*  
310 *Natl. Acad. Sci. U. S. A.* **111**, 6139-6146 (2014).
- 311 2. Van Eenennaam, A. L., Weigel, K. A., Young, A. E., Cleveland, M. A. & Dekkers, J. C.  
312 Applied animal genomics: results from the field. *Annu. Rev. Anim. Biosci.* **2**, 105-139  
313 (2014).
- 314 3. Guarna, M. *et al.* A search for protein biomarkers links olfactory signal transduction to  
315 social immunity. *BMC Genomics* **16**, 63 (2015).
- 316 4. Klein, A. M. *et al.* Importance of pollinators in changing landscapes for world crops.  
317 *Proc Biol Sci* **274**, 303-313 (2007).
- 318 5. Evans, J. D. & Schwarz, R. S. Bees brought to their knees: microbes affecting honey  
319 bee health. *Trends Microbiol.* **19**, 614-620 (2011).
- 320 6. Bienefeld, K., Ehrhardt, K. & Reinhardt, F. Genetic evaluation in the honey bee  
321 considering queen and worker effects—a BLUP-animal model approach. *Apidologie*  
322 **31**, 77-87 (2007).
- 323 7. Cobey, S. W. & Tarpy, D. R. Standard methods for instrumental insemination of *Apis*  
324 *mellifera* queens. *J. Apicultural Res.* **52**, 1-18 (2013).
- 325 8. Woyke, J. What happens to diploid drone larvae in a honeybee colony. *J. Apicultural*  
326 *Res.* **2**, 73-76 (1963).
- 327 9. Spivak, M. & Gilliam, M. Hygienic behaviour of honey bees and its application for  
328 control of brood diseases and varroa. Part I. Hygienic behaviour and resistance to  
329 American foulbrood. *Bee World* **79**, 124-134 (1998).
- 330 10. Spivak, M. S. & Gilliam, M.  
331 Hygienic behavior of honey bees and its application for control of brood diseases  
332 and Varroa; Part II. Studies on hygienic behaviour since the Rothenbuhler era. *Bee*  
333 *World* **79**, 169-186 (1998).

- 334 11. Harbo, J. & Harris, J. Suppressed mite reproduction explained by the behaviour of  
335 adult bees. *J. Apicultural Res.* **44**, 21-23 (2005).
- 336 12. Harbo, J. R. & Harris, J. W. Heritability in honey bees (Hymenoptera: Apidae) of  
337 characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata:  
338 Varroidae). *J. Econ. Entomol.* **92**, 261-265 (1999).
- 339 13. Boecking, O., Bienefeld, K. & Drescher, W. Heritability of the Varroa-specific  
340 hygienic behaviour in honey bees (Hymenoptera: Apidae). *J. Anim. Breed. Genet.*  
341 **117**, 417-424 (2000).
- 342 14. Lapidge, K. L., Oldroyd, B. P. & Spivak, M. Seven suggestive quantitative trait loci  
343 influence hygienic behavior of honey bees. *Naturwissenschaften* **89**, 565-568  
344 (2002).
- 345 15. Tsuruda, J. M., Harris, J. W., Bourgeois, L., Danka, R. G. & Hunt, G. J. High-  
346 resolution linkage analyses to identify genes that influence Varroa sensitive hygiene  
347 behavior in honey bees. *PLoS One* **7**, e48276 (2012).
- 348 16. Liu, H. *et al.* Causes and consequences of crossing-over evidenced via a high-  
349 resolution recombinational landscape of the honey bee. *Genome Biol.* **16**, 15-014-  
350 0566-0 (2015).
- 351 17. Liebler, D. C. & Zimmerman, L. J. Targeted quantitation of proteins by mass  
352 spectrometry. *Biochemistry* **52**, 3797-3806 (2013).
- 353 18. Parker, R. *et al.* Ecological adaptation of diverse honey bee (*Apis mellifera*)  
354 populations. *PLoS One* **5**, e11096 (2010).
- 355 19. Pernal, S. F., Sewalem, A. & Melathopoulos, A. P. Breeding for hygienic behaviour in  
356 honeybees (*Apis mellifera*) using free-mated nucleus colonies. *Apidologie* **43**, 403-  
357 406 (2012).

- 358 20. Bixby, M. *et al.* A bio-economic model of Canadian honey bee colonies: marker-  
359 assisted selection (MAS) in queen breeding affects colony health and profits.  
360 *Economic Entomology Submitted* (2016).
- 361 21. Schulte, C., Theilenberg, E., Muller-Borg, M., Gempe, T. & Beye, M. Highly efficient  
362 integration and expression of piggyBac-derived cassettes in the honeybee (*Apis*  
363 *mellifera*). *Proc. Natl. Acad. Sci. U. S. A.* **111**, 9003-9008 (2014).
- 364 22. Rinderer, T. E., Harris, J. W., Hunt, G. J. & de Guzman, L. I. Breeding for resistance  
365 to *Varroa destructor* in North America. *Apidologie* **41**, 409-424 (2010).
- 366 23. Currie, R. W., Pernal, S. F. & Guzman-Novoa, E. Honey bee colony losses in  
367 Canada. *J. Apicultural Res.* **49**, 104-106 (2010).
- 368 24. Mullin, C. A. *et al.* High levels of miticides and agrochemicals in North American  
369 apiaries: implications for honey bee health. *PLoS One* **5**, e9754 (2010).
- 370 25. El Mohtar, C. A., Atamian, H. S., Dagher, R. B. & Abou-Jawdah, Y. Genotypes with  
371 the I-2 Gene for Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 2. *Plant*  
372 *Dis. J.* **91**, 758-762 (2007).
- 373 26. Ansari-Mahyari, S., Sorensen, A. C., Lund, M. S., Thomsen, H. & Berg, P. Across-  
374 family marker-assisted selection using selective genotyping strategies in dairy cattle  
375 breeding schemes. *J. Dairy Sci.* **91**, 1628-1639 (2008).
- 376 27. Blasco, A. & Toro, M. A. A short critical history of the application of genomics to  
377 animal breeding. *Livestock Sci.* **166**, 4-9 (2014).

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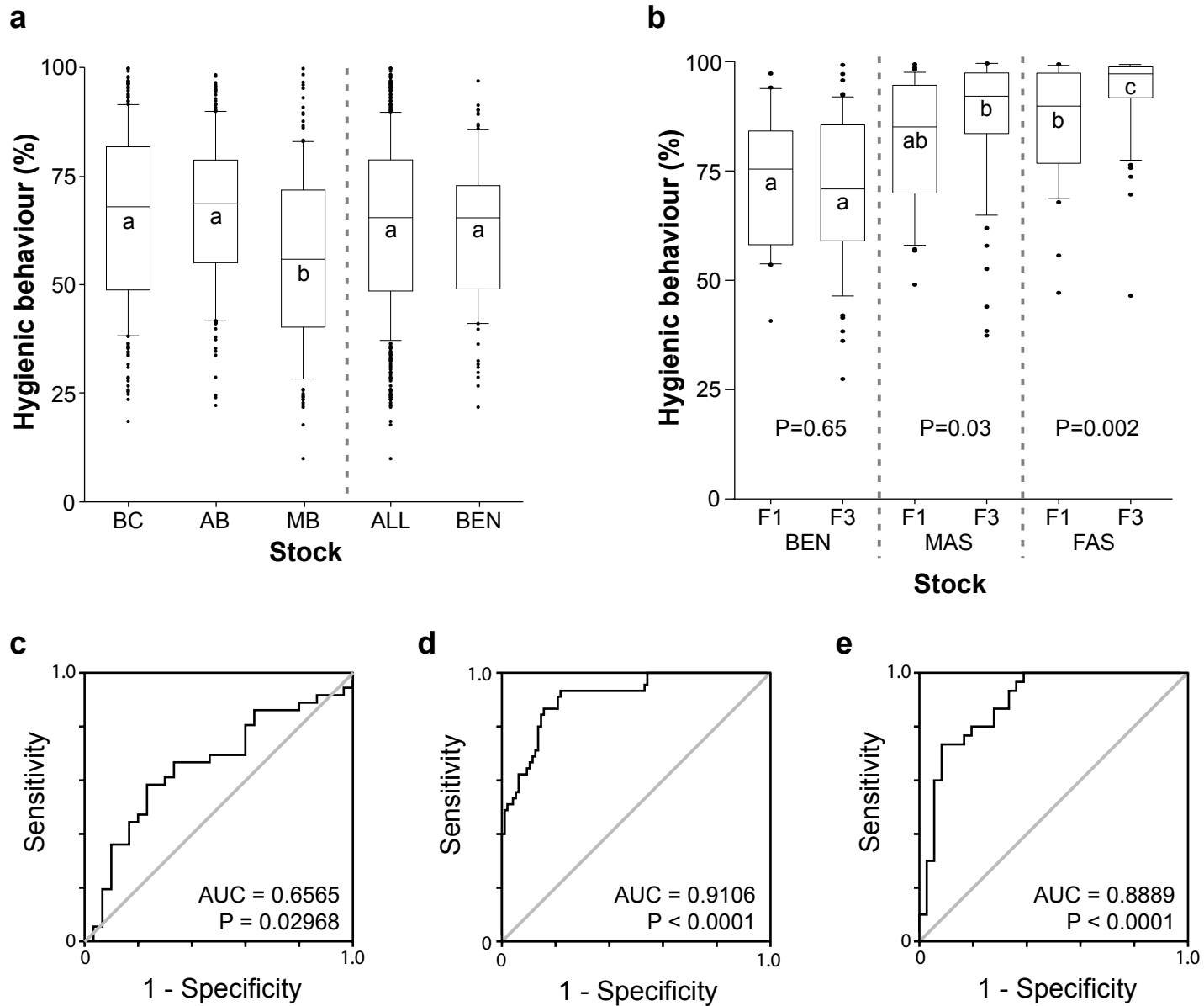


Figure 2, Guarna et al.

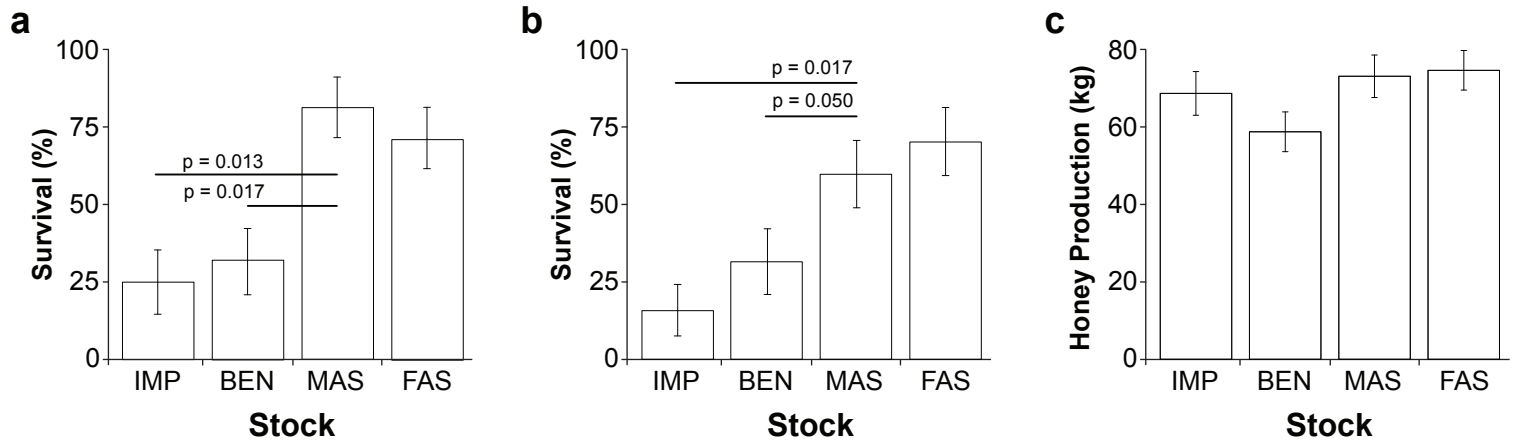


Figure 3, Guarna et al.