

1

2 **A small shift in VSH-gene frequency instead** 3 **of rapid parallel evolution in bees. A** 4 **comment on Oddie et al. 2018**

5

6

By

7

8

Jacques J. M. van Alphen^{1,2,3} and BartJan Fernhout³

9

10

11

12

13

14

15

16

17

18

19

20

21

¹Institute for Biodiversity and Ecosystem Dynamics,
University of Amsterdam, P.O. Box 94248
1090 GE Amsterdam
The Netherlands

26

²Naturalis Biodiversity Centre
P.O. Box 9517, 2300 RA Leiden
The Netherlands

29

³Arista Bee Research Foundation
Nachtegaal 2
5831 WL Boxmeer
The Netherlands

33

34 Introduction

35

36 Recently (Oddie et al., 2018) claimed that parallel evolution in four European populations of
37 honeybees has resulted in a not previously reported behavioural defense mechanism of the bees
38 against the parasitic mite *Varroa destructor*, the ability of uncapping/recapping to reduce mite
39 reproductive success. Their study does not provide the data to support this claim, it does not
40 consider a more plausible alternative interpretation of the results (the reduced mite reproduction
41 through Varroa Sensitive Hygiene) and lacks experimental evidence to distinguish between both
42 hypotheses.

43

44 Background

45 *Varroa destructor* is an external parasitic mite of honeybees that shifted from its original host
46 *Apis cerana*, the Asian hive bee to *Apis mellifera*, the European honeybee. The parasite spread
47 rapidly and colonized western Europe and North America in the early eighties, where it has been
48 the major mortality factor of honeybees ever since. *Varroa* mites are vectors of several bee
49 viruses and at high mite densities these viruses cause colony collapse.

50 On its original host, the mite is an innocuous parasite. One reason why *Varroa* is so
51 virulent on *A. mellifera* is that it can breed in worker brood and so obtain a much longer
52 reproductive season, while in *A. cerana*, mite-infected pupae are always removed from worker
53 cells (Lin et al., 2016) and breeding is restricted to the short season when drones are produced
54 (Boot et al., 1999).

55 In western Europe and North America, hives are frequently treated with acaricides,
56 natural acids or essential oils to control *Varroa*, or *Varroa* reproduction is disrupted by other
57 apicultural measures (Rosenkranz, Aumeier, & Ziegelmann, 2010). Moreover, a large proportion
58 of the hives are regularly requeened with non-resistant pure-bred queens. These practices are
59 thought to prevent natural selection from selecting for resistance against *Varroa*.

60 The traits that provide resistance against *Varroa* in *A. cerana*, are also present in
61 European *A. mellifera* populations, albeit in low frequency: auto- and allo-grooming can results
62 in the removal of phoretic adult mites and inflict mortality among them (Guzman-Novoa et al.,
63 2012). In addition, the uncapping of *Varroa*-infected cells and the subsequent removal of
64 parasitized pupae, together described as “hygienic behaviour” (Spivak, 1996; Page et al., 2016;
65 Lin et al., 2016) and more precisely as “*Varroa* sensitive hygiene” (VSH) (Harbo &
66 Hoopingarner, 1997; Harbo JR & Harris JW, 2005; Harris, Danka, & Villa, 2012; Harris, Danka,
67 & Villa, 2010; Harris, 2007), results in the removal of mite offspring before they have been able
68 to reproduce successfully. Bees with the alleles for VSH recognize cells containing reproducing
69 *Varroa* (Mondet et al., 2015; Mondet et al., 2016). They uncap these cells from which the pupae
70 are subsequently removed, thus interrupting the reproductive cycle of the *Varroa*. The proportion
71 of workers in a colony expressing the VSH behaviour is positively correlated with the proportion
72 of non-reproducing mites in the brood¹. This is because VSH bees preferentially attack cells
73 with reproducing mites (Harris et al., 2012). The subsequent removal of reproducing mites

74 results in an increase of the proportion non-reproducing mites (Harbo JR & Harris JW, 2005;
75 Harbo & Harris, 2009).

76 Natural selection for these characters would be possible in groups of colonies that are
77 neither treated against *Varroa* nor requeened. A number of natural (Seeley, 2007; Seeley &
78 Smith, 2015) and designed experiments (Fries et al. 2003; Oddie, Dahle, & Neumann, 2017;
79 (Locke et al. 2012) have reported survival of such colonies and suggested that the surviving bees
80 have developed tolerance or resistance against *Varroa*. Some of these studies started more than
81 17 years ago and reported bee colonies surviving *Varroa* already more than ten years ago (Le
82 Conte, et al. 2007; 22. Given the magnitude of the *Varroa* problem and the associated economic
83 losses, this begs the question why *Varroa* surviving bees have not become widely available to
84 apiculture.

85 Now, M. Oddie et al. (2018) suggest that parallel evolution by natural selection in some
86 of these populations (two French, Avignon and Sarthe (Locke, 2016), a Norwegian (Oddie et al.,
87 2017) and a Swedish population, Gotland (Locke et al., 2012)), has resulted in a common and not
88 previously reported behavioural mechanism in the European honeybee to reduce reproductive
89 success of the mites.

90 They claim that the bees in the four populations (1) recognize cells with reproducing *Varroa*-
91 mites, (2) that they open these cells and then (3) close them again. (4) That the brief period
92 during which the cells are open interrupts the *Varroa* breeding cycle and kills the offspring. (5)
93 That this behaviour is the mechanism that causes the lower reproductive rate of *Varroa* in
94 comparison with that of control colonies from the same geographical areas not exposed to natural
95 selection. (6) They also claim that this behaviour is superior to the alternative of removing
96 infested pupae (*i.e.* VSH). The first two behavioural steps of the mechanism that they suggest are
97 identical to those of VSH behaviour.

98 Oddie et al. (2018) did not measure VSH and did not exclude it as a cause for their
99 results. As we will show, the data they supply can better be explained as caused by VSH
100 behaviour, hence, Oddie et al. (2018) provide no evidence for the existence of an opening-
101 recapping strategy. Moreover, we will argue that an opening recapping-strategy would not be not
102 superior to VSH behaviour in terms of survival of colonies as suggested by Oddie et al.
103 (2018). We also will show that the *Varroa* resistance of these four populations is incomplete and
104 that beekeepers' practices help the populations to survive.

105

106 Recapping or VSH?

107 Oddie et al. (2018) found a higher frequency of recapping in surviving colonies when compared
108 to *Varroa*-susceptible ones. This easily can be explained by the fact that surviving colonies have
109 a larger proportion of bees that express VSH. Therefore, more infested cells are opened and
110 hence there is more opportunity for workers that do not express VSH behaviour to recap cells
111 (Harris et al., 2012). Thus, the observation cannot serve as evidence for an "opening-recapping"
112 strategy.

113 Oddie et al. (2018) found no significant difference between recapped and undisturbed
114 cells in the proportion of non-reproductive mites, in contrast to what they expected. They argue

that bees preferentially open cells with reproducing *Varroa* (a preference that is typical for VSH (Harris, 2007; Mondet et al. 2016; Harbo & Harris, 2009)) thus interrupting reproduction of the mites, making that the difference between undisturbed and opened cells would disappear. To test this hypothesis they compared experimentally uncapped cells with undisturbed cells. The reproductive rate of *Varroa* was lower in the uncapped cells than in the undisturbed cells. However, this does not prove that uncapping-recapping was instrumental in lowering the reproductive success of *Varroa*, as the frames with uncapped and undisturbed cells were placed back into hives in which bees with VSH genes were present at an unknown frequency. The VSH behaviour of bees in these colonies will have resulted in the removal of pupae with reproducing *Varroa* mites from the uncapped cells. This would leave opened cells with non-reproductive mites more often untouched and available for recapping, thus accounting for the lower reproductive rate in recapped cells as compared to undisturbed cells. Hence, there is neither proof that uncapping-recapping results in mite mortality, nor in disruption of mite reproduction and hence no evidence that it reduces reproductive success in *Varroa*.

129

130 **Recapping explained**

131 The genes for VSH occur in all European honeybee populations at low frequencies. Moreover, there is evidence for the Norwegian population that it expresses VSH (Oddie et al., 2017) and for all populations for suppressed mite reproduction, a trait typically associated with VSH (Locke et al. 2012; Le Conte & Mondet, 2017; Martin & Medina, 2004) .

135 Oddie et al. (2018) do not consider that the worker bees differ in genetic make-up, because the queens of the four populations mated naturally and worker bees in each colony originate from 10 to 20 different drones.

138 Therefore, only a part of the workers express VSH genes. They uncap cells and remove larvae infested with reproducing *Varroa* mites. Another part of the workers lack copies of the VSH alleles. When they encounter an uncapped cell, they will recap it, thus counteracting the actions of the VSH bees.

142 Support for this interpretation comes from a study (Harris et al., 2012) that used an experimental design that allowed to discriminate between the effects of VSH behaviour and recapping. It compared bee colonies that expressed VSH for about 70%, and control colonies that expressed VSH for about 25%. A much higher incidence of recapping occurred in the high VSH colonies. The frequency of infertile mites in recapped cells was not significantly different between the two types of bees, suggesting that uncapping and recapping of brood cells is not a major cause of infertility of mites, while the VSH-behaviour resulted in a reduction of 70% of *Varroa*-infested cells. Evidence that non-hygienic bees recap cells opened by hygienic bees comes from Spivak & Gilliam (1998). When they added young non-hygienic bees to hygienic colonies, it suppressed the hygienic behaviour. In a different experiment they showed that non-hygienic bees tended to recap partially uncapped cells containing dead brood, whereas hygienic bees never recapped those cells. More evidence comes from Boecking & Spivak, (1999), who found that bees from pre-selected non-hygienic colonies tended to recap partially uncapped cells that contained freeze-killed brood and from

Arathi, Ho, & Spivak (2006) who demonstrated that in mixed colonies, as compared to a colony of hygienic bees, a higher proportion of uncapped cells were subsequently recapped, resulting in delayed removal of dead brood. Hence, recapping by non-hygienic bees counteracts the activity of the hygienic workers and can reduce their efficacy against *Varroa*.

A Cost-Benefit analysis of VSH and Opening and Recapping

Oddie et al. (2018) claim that the costs of VSH exceed those of opening and recapping cells. They do not provide any data to support this claim but simply state that colonies would not be able to sustain high rates of killing of their own offspring. They even suggest that VSH could be instrumental in the destruction of a colony, when workers are lost at a faster rate than they are being replaced, but do not explain how the dynamics of the interaction between bees and *Varroa* could ever produce such an effect.

The proper way to compare the two strategies and predict which of the two would be favoured by natural selection, would be to measure both costs (in number of workers killed) and benefits (in number of mite offspring killed) of the two strategies and to determine how these affect the population dynamics of the mite-bee interaction. We give three reasons why VSH is likely to be the better strategy.

(1) The costs of VSH (and that of recapping) increase with *Varroa* infestation rate, but since colonies that show a high rate of VSH have very low *Varroa* infestation rates, the costs in numbers of workers lost are negligible, once a colony is resistant.

(2) The probability of interrupting the reproductive cycle of *Varroa* is 1.0, in VSH, while mites often reproduce successfully after opening-recapping[®]. Hence, the probability of interrupting the reproduction is much smaller than 1.0. for opening-recapping.

(3) Even when uncapping results in disruption of the reproduction of the *Varroa* mites, host bees surviving after recapping would already be infected with viruses such as DWV and be instrumental in dispersing the virus in the colony. As these viruses ultimately cause the collapse of the colony, killing the infested pupae should be better. This is why VSH may have long-term benefits that more than compensate for the costs.

The presence of VSH in these populations creates the logical problem why the two strategies would co-exist. It is difficult to see how a mixed strategy would be stable, as all worker genotypes would benefit from the strategy that most efficiently reduces reproduction of *Varroa*. Selection would thus remove the less efficient strategy.

Resistance to *Varroa*

It is clear that *Varroa* mites have lower reproductive rates in the four surviving populations than in the control populations they were compared with. The most plausible cause for this reduced *Varroa* reproduction is VSH behaviour. A bee colony is fully resistant against *Varroa*, when the population growth rate of *Varroa* in the colony is zero. Values of W , (= the number of fertilized daughters per breeding cycle) exceeding 0.7 result in growing *Varroa*

197 populations. $W = 0.9$ already results in exponential growth (Martin & Medina, 2004). The W
198 value for Norway is 0.87 (Oddie et al., 2017), those for Avignon and Gotland have not been
199 published but data on the *Varroa* reproduction in these populations indicate that they are higher
200 than that of the Norwegian population (Locke et al. 2012). Hence, the suppression of mite
201 reproduction in these populations alone is not strong enough to prevent the populations of
202 *Varroa* from growing and eventually causing colony collapse. Other heritable traits, like
203 resistance to viruses could play an additional role (Le Conte & Mondet, 2017; Locke, Forsgren,
204 & De Miranda, 2014). In addition non-heritable traits like small colony size and swarming
205 frequency (Fries & Bommarco, 2007; Seeley, 2007; Seeley & Smith, 2015) could explain the
206 survival of these colonies, while bee-keeping practices in the four populations also play an
207 important role (Büchler et al., 2014; Le Conte & Mondet, 2017). As example a quotation on the
208 Avignon and Sarthe bees (Le Conte & Mondet, 2017) “*What has happened to these bees since*
209 *we published those results in 2007? Once every two years, we graft queen larvae from the three*
210 *best colonies in each apiary (west and south of France) to get 20 colonies. The queens are*
211 *naturally mated by local drones. About 30–35% of the colonies die within 18 months, but the rest*
212 *of the colonies are good candidates for surviving to the mite, so the stock still survives*
213 *efficiently*”. This quote shows that mortality rate of the surviving colonies is not different from
214 that of *Varroa*-sensitive ones. It also shows that beekeeping practices interfere with natural
215 selection, by creating a large number of new queens and by artificial selection. Nevertheless, the
216 bees do not perform any better than *Varroa*-sensitive ones, as was shown in a large comparative
217 study (Büchler et al., 2014). The Avignon colonies did not survive any longer than *Varroa*-
218 sensitive strains.

219

220 Conclusions

221

222 The results of Oddie et al. (2018) *i.e.* (1) a reduction in *Varroa* mite reproductive success
223 and (2) a higher frequency of recapping behaviour in surviving colonies and (3) a higher
224 proportion of non-reproductive mites in recapped cells can easily be explained by incomplete
225 VSH behaviour in these colonies.

226 The observed reduction in *Varroa* mite reproduction rate W , is not enough to allow the
227 colonies to survive. Survival of the populations is partly due to apicultural practices. In fact,
228 these colonies do not survive better than *Varroa*-sensitive colonies. A modest reduction in
229 *Varroa* mite reproduction in a period of almost 20 years is not exceptional and should not be
230 called “rapid parallel evolution”.

231 For more than 35 years *Varroa* has been the major threat for apiculture. The scale of the
232 damage and the costs of its control make it a very urgent problem. The study of “surviving”
233 colonies has, so far, not resulted in a lasting solution for the beekeeping community. It seems
234 time for the research field to shift its attention to more efficient ways of obtaining *Varroa*-
235 resistant bees.

236

237 References

- 238 Arathi, H. S., Ho, G., & Spivak, M. (2006). Inefficient task partitioning among nonhygienic
239 honeybees, *Apis mellifera* L., and implications for disease transmission. *Animal Behaviour*,
240 72(2), 431–438. <https://doi.org/10.1016/j.anbehav.2006.01.018>
- 241 Boecking, O., & Spivak, M. (1999). Behavioral defenses of honey bees against *Varroa jacobsoni*
242 Oud. *Apidologie*, 30(2–3), 141–158. <https://doi.org/10.1051/apido:19990205>
- 243 Boot, W. J. *et al.* (1999). Natural selection of *Varroa jacobsoni* explains the differential
244 reproductive strategies in colonies of *Apis cerana* and *Apis mellifera*. *Ecol. Evol. Acari* **23**,
245 349–357.
- 246 Büchler, R. *et al.* (2014). The influence of genetic origin and its interaction with environmental
247 effects on the survival of *Apis mellifera* L. colonies in Europe. *J. Apic. Res.* **53**, 205–214.
248 (2014).
- 249 Conte, Y. Le & Mondet, F. (2017). Natural Selection of Honeybees Against *Varroa destructor*.
250 in *Beekeeping- From Science to Practice* Eds. RH Vreeland & D. Sammartoro Springer 2017
251 189–194 (2017). doi:10.1007/978-3-319-60637-8.
- 252 Fries, I. & Bommarco, R. (2007). Possible host-parasite adaptations in honey bees infested by
253 *Varroa destructor* mites. *Apidologie* **38**, 525–533
- 254 Fries, I., Hansen, H., Imdorf, A. & Rosenkranz, P. (2003). Swarming in honey bees (*Apis*
255 *mellifera*) and *Varroa destructor* population development in Sweden. *APIDOLOGIE* **34**, 389–
256 397
- 257 Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montano, L. G. & Petukhova, T. (2012).
258 Genotypic variability and relationships between mite infestation levels, mite damage, grooming
259 intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees
260 (*Apis mellifera* L.). *J. Invertebr. Pathol.* **110**, 314–320
- 261 Harbo J. R. & Harris J.W. (2005). Suppressed mite reproduction explained by the behaviour of
262 adult bees. *J. Apic. Res.* **44**, 21–23.
- 263 Harbo, J. R. & Harris, J. W. (2009). Levels of Varroa Sensitive Hygiene. *J. Apic. Res.* **48**, 156–
264 161.
- 265 Harbo, J. R. & Hoopingarner, R. A. (1997). Honey Bees (Hymenoptera: Apidae) in the United
266 States That Express Resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Economic*
267 *Entomol.* **90**, 893–898
- 268 Harris, J. (2007). Bees with Varroa Sensitive Hygiene preferentially remove mite infested pupae
269 aged ≤ five days post capping. *Journal of Apicultural Research and Bee World* 46(3): 134–139
- 270 Harris, J. W., Danka, R. G. & Villa, J. D. (2012). Changes in Infestation, Cell Cap Condition,
271 and Reproductive Status of *Varroa destructor* (Mesostigmata: Varroidae) in Brood Exposed to
272 Honey Bees With Varroa Sensitive Hygiene. *Ann. Entomol. Soc. Am.* **105**, 512–518.
- 273 Harris, J. W., Danka, R. G. & Villa, J. D. Honey Bees (Hymenoptera: Apidae) With the Trait of
274 Varroa Sensitive Hygiene Remove Brood With All Reproductive Stages of *Varroa* Mites
275 (Mesostigmata: Varroidae). *Ann. Entomol. Soc. Am.* **103**, 146–152 (2010).
- 276 Le Conte, Y., V. G. De, Didier, C., François, J. & Anderson, V. Honey bee colonies that have
277 survived Varroa destructor *. **38**, 566–572 (2007).
- 278 Lin, Z. *et al.* Go east for better honey bee health: *Apis cerana* is faster at hygienic behavior than
279 *A. mellifera*. *PLoS One* **11**, 1–10 (2016).
- 280 Locke, B. Natural Varroa mite-surviving *Apis mellifera* honeybee populations. *Apidologie* **47**,
281 467–482 (2016).

- 282 Locke, B., Le Conte, Y., Crauser, D. & Fries, I. Host adaptations reduce the reproductive success
283 of *Varroa destructor* in two distinct european honey bee populations. *Ecol. Evol.* **2**, 1144–1150
284 (2012).
- 285 Loftus, J. C., Smith, M. L. & Seeley, T. D. How honey bee colonies survive in the wild: Testing
286 the importance of small nests and frequent swarming. *PLoS One* **11**, 1–11 (2016).
- 287 Martin, S. J. & Medina, L. M. Africanized honeybees have unique tolerance to *Varroa* mites.
288 *Trends Parasitol.* **20**, 112–114 (2004).
- 289 Mondet, F. *et al.* Antennae hold a key to Varroa-sensitive hygiene behaviour in honey bees. *Sci.*
290 *Rep.* **5**, (2015).
- 291 Mondet, F. *et al.* Specific Cues Associated with Honey Bee Social Defence against *Varroa*
292 *destructor* Infested Brood. *Sci. Rep.* **6**, 1–8 (2016).
- 293 Oddie, M. A. Y., Dahle, B. & Neumann, P. (2017). Norwegian honey bees surviving *Varroa*
294 *destructor* mite infestations by means of natural selection. *PeerJ* **5**, e3956
- 295 Oddie, M., Büchler R., Dahle, B., Covacic, M., Le Conte Y., Locke B., De Miranda J.R., Mondet
296 F., Neumann P. (2018). Rapid parallel evolution overcomes global honey bee parasite.
297 Scientific Reports 8:7704 1–9. doi:10.1038/s41598-018-26001-7
- 298 Page, P. *et al.* (2016). Social apoptosis in honey bee superorganisms. *Sci. Rep.* **6**, 10–15
- 299 Rosenkranz, P., Aumeier, P. & Ziegelmann, B. (2010). Biology and control of *Varroa*
300 *destructor*. *J. Invertebr. Pathol.* **103**, S96–S119.
- 301 Seeley, T. D. (2007). Honey bees of the Arnot Forest: a population of feral colonies persisting
302 with *Varroa destructor* in the northeastern United States. *Apidologie* **38**, 19–29.
- 303 Seeley, T. D. & Smith, M. L. (2015). Crowding honeybee colonies in apiaries can increase their
304 vulnerability to the deadly ectoparasite *Varroa destructor*. *Apidologie* **46**, 716–727.
- 305 Spivak, M. (1996). Honey bee hygienic behavior and defense against *Varroa jacobsoni*.
306 *Apidologie* **27**, 245–260.
- 307 Spivak, M., & Gilliam, M. (1998). Hygienic behaviour of honey bees and its application for
308 control of brood diseases and *Varroa*: Part II. Studies on hygienic behaviour since the
309 Rothenbuhler era. *Bee World*, 79(4), 169–186.
310 <https://doi.org/10.1080/0005772X.1998.11099408>
- 311
- 312
- 313 **Author Contributions Statement**
314 J.v.A and B.J.F. wrote the main manuscript text. Both authors reviewed the manuscript."
- 315 **Competing interests**
316 The author(s) declare no competing interests.