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Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection

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Background: Managed, feral and wild populations of European honey bee subspecies, *Apis mellifera*, are currently facing severe colony losses globally. There is consensus that the ectoparasite mite *Varroa destructor*, that switched hosts from the Eastern honey bee *Apis cerana* to the Western honey bee *A. mellifera*, is a key factor driving these losses. For >20 years, breeding efforts have not achieved that European honey bee colonies survive infestations without the need for mite control. However, at least three populations of European honey bees have developed this by means of natural selection and have been surviving for >10 years without mite treatments. Reduced mite reproductive success has been suggested as a key factor explaining this natural survival. Here, we report a managed *A. mellifera* population in Norway, that has been naturally surviving consistent *V. destructor* infestations for >17 years. **Methods:** Surviving colonies and local susceptible controls were evaluated for mite infestation levels, mite reproductive success and two potential mechanisms explaining colony survival: grooming of adult worker bees and Varroa Sensitive Hygiene (VSH): adult workers specifically detecting and removing mite-infested brood. **Results:** Mite infestation levels were significantly lower in surviving colonies and mite reproductive success was reduced by ~30% compared to the controls. No significant differences were found between surviving and control colonies for either grooming or VSH. **Discussion:** Our data confirm that reduced mite reproductive success seems to be a key factor for natural survival of infested *A. mellifera* colonies. However, neither grooming nor VSH seem to explain colony survival. Instead, other behaviors of the adult bees seem to be sufficient to hinder mite reproductive success, because brood for this experiment was taken from susceptible donor colonies only. To mitigate the global impact of *V. destructor*, we suggest learning more from nature, i.e. identifying the obviously efficient mechanisms favored by natural selection.

2

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4 **infestations by means of natural selection**

5

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13

14 **Subjects:** Conservation Biology, Ecology

15 **Key words:** *Apis mellifera*, honey bees, mites, natural selection, parasites, *Varroa destructor*

17 **Abstract: (500 words max)**

18 **Background:** Managed, feral and wild populations of European honey bee subspecies,
19 *Apis mellifera*, are currently facing severe colony losses globally. There is consensus that the
20 ectoparasite mite *Varroa destructor*, that switched hosts from the Eastern honey bee *Apis cerana*
21 to the Western honey bee *A. mellifera*, is a key factor driving these losses. For >20 years,
22 breeding efforts have not achieved that European honey bee colonies survive infestations without
23 the need for mite control. However, at least three populations of European honey bees have
24 developed this by means of natural selection and have been surviving for >10 years without mite
25 treatments. Reduced mite reproductive success has been suggested as a key factor explaining this
26 natural survival. Here, we report a managed *A. mellifera* population in Norway, that has been
27 naturally surviving consistent *V. destructor* infestations for >17 years. **Methods:** Surviving
28 colonies and local susceptible controls were evaluated for mite infestation levels, mite
29 reproductive success and two potential mechanisms explaining colony survival: grooming of
30 adult worker bees and Varroa Sensitive Hygiene (VSH): adult workers specifically detecting and
31 removing mite-infested brood. **Results:** Mite infestation levels were significantly lower in
32 surviving colonies and mite reproductive success was reduced by ~30% compared to the
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34 grooming or VSH. **Discussion:** Our data confirm that reduced mite reproductive success seems
35 to be a key factor for natural survival of infested *A. mellifera* colonies. However, neither
36 grooming nor VSH seem to explain colony survival. Instead, other behaviors of the adult bees
37 seem to be sufficient to hinder mite reproductive success, because brood for this experiment was
38 taken from susceptible donor colonies only. To mitigate the global impact of *V. destructor*, we

39 suggest learning more from nature, i.e. identifying the obviously efficient mechanisms favored
40 by natural selection.

41 INTRODUCTION

42 The European honey bee, *Apis mellifera*, is an economically important insect, providing essential
43 pollination services for human food security as well as valuable hive products for the apicultural
44 sector (Morse & Calderone, 2000; Klein et al., 2007). A honey bee colony is considered a
45 superorganism and employs a series of social immunity strategies to optimize health and fitness;
46 individuals within the colony perform of hygienic behaviors to reduce risk of disease and
47 parasite invasion (Seeley, 1989; Cremer et al., 2007). However, major losses of managed and
48 feral *A. mellifera* colonies have been well documented in recent years (e.g. Kraus & Page 1995;
49 Neumann & Carreck, 2010; van Engelsdorp et al., 2011; Pirk et al., 2014). There is consensus
50 that the ectoparasitic mite, *Varroa destructor*, that originally infested the Eastern honey bee
51 *Apis cerana*, now infests *A. mellifera* near globally (Ellis & Munn, 2005) and is the main biotic
52 factor threatening *A. mellifera* colony survival (Neumann & Carreck 2010; Rosenkranz et al.,
53 2010). The mite is a very efficient vector of several honeybee viruses, generating a disease
54 epidemic within the colony, that will dwindle until it dies in 2-3 years (Neumann et al., 2012).
55 This, coupled with the exponential growth of mite populations sustained by developing host
56 worker brood throughout the year and additional seasonal male brood (Rosenkranz et al., 2010;
57 Dietemann et al., 2012).

58 Despite these drastic effects of *V. destructor* on *A. mellifera* host populations, there are reports of
59 managed and feral *A. mellifera* honey bee populations that survive mite infestations by means of
60 natural selection. These populations have now been documented for more than 10 years
61 (Avignon and Le Mans, France, Le Conte et al. 2007; Island of Gotland, Sweden, Fries et al.
62 2006; Arnot Forest, USA, Seeley 2007; reviewed by Locke 2016). In Gotland and Avignon,
63 reduced mite reproductive success has been observed (Locke 2016), which can contribute to

64 colony survival. Up until now, however, the mechanisms enabling the survival of mite-infested
65 colonies have not been identified.

66 Two behavioral mechanisms of social immunity have been suggested to contribute to
67 *V. destructor* survivability: one targets the mites at the phoretic stage, where it feeds on adult
68 host bees, and one targets the reproductive stage, when the mites are sealed in host brood. The
69 former occurs when adult worker bees remove phoretic mites from themselves and/or nestmates
70 via autogrooming and allogrooming (Guzman-Novoa et al. 2012). The latter describes adult
71 worker bees detecting and removing mite-infested brood and has been termed Varroa Sensitive
72 Hygiene (VSH). The removal of infested brood inhibits contribution of these mites to the next
73 generation and reduces the in-colony population (Harbo & Harris, 2009, Harris et al. 2010,
74 Harris et al 2012). Taken together, these two behaviors might explain reduced *V. destructor*
75 reproductive success and ultimately explain colony survival. However, data from natural
76 surviving populations remain scarce.

77 In 2014, it was reported that a managed population of honey bees had been surviving for >19
78 years with no mite treatment in the Østlandet region of Norway. Mite levels were anecdotally
79 low, despite the population being within sufficient distance of susceptible colonies to facilitate
80 horizontal parasite transfer. The aim of this study was to estimate mite infestation levels and mite
81 reproductive success in this surviving population with comparisons to a local and known-
82 susceptible population. It will also investigate the two aforementioned mechanisms for colony
83 survival by quantifying grooming and VSH in both surviving and susceptible colonies.

84 **METHODS**

85 Experiments were conducted in the Østlandet region, Norway, during local late summer and
86 early fall 2015. Surviving colonies were of a mixed origin (Buckfast) that had been kept without
87 any *V. destructor* treatments for 19 years prior to the study. After the last treatment against
88 *V. destructor* in 1997, mite levels seem to have increased and substantial losses of colonies
89 occurred. However, surviving and well-doing colonies were split and used to replace lost ones.
90 Over the last 10 years, colony losses have been lower than the national average of about 10%.
91 Susceptible local control colonies were located ~60km away from the surviving apiaries and
92 treated against *V. destructor* on a biannual basis.

93

94 **Mite infestation levels and proportions of damaged mites (grooming):**

95 Daily mite drops were considered a viable measure of population size (Flores et al., 2015) as
96 none of the experimental colonies in the year of study had been given treatment against
97 *V. destructor*. Rates were estimated using standard methods (Dietemann et al., 2013): The
98 bottoms of the colonies were equipped with a mesh divider separating the mite board from the
99 brood box and were prepared with paper towel soaked in vegetable oil to prevent scavenging of
100 fallen mite bodies by ants (Dainat et al., 2011). The boards were placed under the test colonies
101 and collected again six days later. Once the boards were collected, all mites were counted. The
102 total mite numbers were then divided by the number of days the boards were left out and
103 averaged across the colonies to give the mean daily drop rate for both surviving and susceptible
104 colonies.

105 The proportion of damaged mites was used to estimate levels of grooming within a colony. Up to
106 20 mites from each colony were examined under a dissecting microscope and damage to
107 carapace, ventral plate and legs was noted in line with methods used by Rosenkranz et al. (1997).

108 Each mite received a binary score of ‘damaged’ or ‘undamaged’ for the analysis and a proportion
109 of damaged mites was obtained for each apiary.

110

111 **Varroa-sensitive hygiene (VSH):**

112 One surviving and one control apiary were selected: Five colonies from each received 2 brood
113 frames from one of ten susceptible, local donor colonies in an apiary, which was geographically
114 distinct from that of the surviving and control test apiaries (~60 km) and similarly untreated that
115 year. Initial mite infestation levels in all test colonies were recorded two months prior. Only
116 worker brood was considered for this study as male sexual (drone) brood is generally scarce
117 during mite population peaks.

118 The ten susceptible donor colonies were chosen for their evidence of high mite loads. Each of
119 these external source colonies donated one worker brood frame to a surviving and a susceptible
120 receiver colony (N=2 in total). Prior to frame relocation, the queens of these colonies were caged
121 on each of the two empty frames for a period of two days to obtain defined age cohorts of brood.
122 Frames were removed from the source colonies as soon as the brood was capped. Brood patches
123 were then photographed and mapped on both sides to record brood patterns before being
124 transferred to the receiver colonies. Frames were placed into the center of the brood chamber and
125 left in the colonies for a period of 10 days to allow for a maturation point of ~24 hours prior to
126 adult emergence (Winston, 1991). After the allotted time, frames were removed and
127 photographed again before being transferred to storage at -20°C before examination.

128

129 Each cell opened was mapped on the printed photograph of the brood comb and marked
130 ‘infested’ or ‘uninfested’. If a cell had been cleaned and left empty by the bees this was also

131 marked, determined by comparing the new photographs to those taken before frames were
132 inserted into the test colonies. The number of empty cells was taken as a proportion of the total
133 number of cells examined on the frame. This measure together with the mite infestation rates
134 (Harris, 2007) were used to assess the level of VSH in surviving and susceptible control
135 colonies.

136

137 **Mite reproductive parameters**

138 Once a cell was opened, the bee pupae were removed using fine forceps. Mites clinging to the
139 body were brushed off with a small paint brush. The cell interior was also brushed carefully to
140 extract, but not damage the remaining mites and eggs. Once all contents had been removed from
141 the cell, the developmental stage of each mite was noted according to Martin (1994).

142 The measure of mite reproductive success was evaluated as the potential number of viable
143 female offspring produced per foundress mite. Offspring were only considered viable if they
144 were of an adequate stage to survive upon host emergence and if at least one male was present
145 within the cell (Corrêa-Marques et al., 2003, Locke et al., 2012).

146 All cells that did not have daughter mites meeting these requirements were given a value of zero.

147 The viable female offspring numbers were divided by the number of foundress mites contained
148 in each corresponding cell. For every colony, the average reproductive success per foundress was
149 found by averaging the number of viable female offspring produced in one cell and dividing it by
150 the number of foundresses in that cell. The brood stage was estimated based on a visual chart by
151 Martin (1994) and assigned a number from 7 to 12 loosely based on the number of days each
152 stage is commonly associated with. Brood younger than stage 7 (>170 hours capped) was not
153 considered.

154

155 **Statistics:**

156 R statistical analysis software (R core Team 2013) and the LME4 package (Bates et al., 2015)
157 were used to perform all statistical analyses. The daily mite drop of colonies was averaged for
158 surviving and susceptible groups separately and for surviving groups averages were taken in
159 August and again in October, both comparisons were made using a two-sample t-test. To
160 accommodate large outliers, data was log-transformed before statistical analyses were carried
161 out.

162 Proportions of damaged mites collected as well as the proportion of cells hygienically removed
163 were pooled by treatment and compared using a 2x2 chi-squared contingency test. General linear
164 mixed effects models and linear mixed effects models were performed for mite reproductive
165 parameters and infestation rates; models were fitted by maximum likelihood. Parameters were
166 averaged by frame and donor colony ID as well as receiver colony type were accounted for as
167 variables. Mite reproductive success is known to decrease with a higher number of foundresses
168 in a cell (Fuchs & Langenbach, 1989; Martin, 1995) and the potential offspring estimate is larger
169 in younger stages of brood (Locke et al., 2012). Both parameters were included as fixed effects
170 where necessary. Models were adjusted for count and binomial response variables using poisson
171 and binomial error structures respectively.

172

173 **RESULTS**

174 The average daily mite drop counts were significantly lower in surviving colonies compared to
175 susceptible ones (figure 1a. $t=2.8$, $df= 9$, $p= 0.02$). The overall average mite reproductive success
176 in surviving colonies was significantly reduced at 0.87 offspring per foundress whereas in

177 susceptible colonies it was 1.24. The reduction in mite reproductive success is ~30% (Figure 2.
178 $\chi^2= 4.09$, $p=0.027$).

179 There we no significant differences in the proportion of damaged mites between the surviving
180 and susceptible colonies (Figure 3a. $\chi^2= 0.12$, $df= 1$, $p=0.73$); ~40% of the mites collected were
181 damaged in both groups. Similarly, brood removal rates (VSH) were not significantly different
182 between the surviving and susceptible colonies (Figure 3b. $\chi^2= 1.88$, $df= 1$, $P= 0.171$) with rates
183 resting close to 5%.

184

185 **DISCUSSION**

186 Our data support the view that reduced *V. destructor* mite reproductive success is a prime
187 requisite for natural survival of infested *A. mellifera* honey bee colonies. Indeed, both mite
188 reproductive success and mite infestation levels were significantly lower in surviving Norwegian
189 colonies compared to local susceptible controls. Since we only used susceptible test brood in
190 both surviving and susceptible host colonies, traits of immature bees can be excluded to explain
191 our data. However, the proportions of damaged mites as a proxy for the efficacy of grooming
192 behavior and brood removal (VSH) by adult workers were not significantly different between
193 surviving and susceptible colonies, indicating that these two mechanisms are unlikely to explain
194 the natural survival of these mite-infested Norwegian honey bee colonies.

195 The mite infestations levels, as estimated by daily bottom board counts, were significantly lower
196 in surviving colonies compared to local susceptible controls. This result is well in line with
197 earlier findings for other surviving *A. mellifera* populations (Rosenkranz et al. 1999, reviewed by
198 Locke 2016). Lower mite infestation levels are an obvious explanation for colony survival and

199 can result from reduced mite reproductive success. Indeed, only about half of the mites in
200 Gotland colonies successfully produced viable mated daughter mites that contribute to the
201 colony's mite population, compared to ~80% in local susceptible colonies (Locke & Fries 2011).
202 Similarly, mite reproductive success in the Avignon mite-surviving population was also reduced
203 by 30 % compared to local mite-susceptible colonies (Locke et al. 2012). Finally, our data also
204 show a reduced mite reproductive success by ~30%, thereby strongly suggesting that such a
205 reduction is sufficient to enable colony survival. It appears essential therefore to understand the
206 mechanisms driving the reduced mite reproductive success.

207 Even though higher levels of grooming behavior have been shown to reduce *V. destructor*
208 infestations in *A. mellifera* (Guzman-Novoa et al., 2012), our results show no significant
209 differences for grooming or VSH between local surviving and susceptible Norwegian bees. This
210 agrees well with earlier findings for Gotland, where differences in hygienic and grooming
211 behavior were not apparent between the local surviving and mite-susceptible colonies (Locke &
212 Fries 2011). Reduced reproductive success in surviving Norwegian and Swedish colonies is not
213 likely due to a more sensitive grooming threshold nor a higher level of brood removal (VSH).
214 Neither of the tested traits seems to play a major role for local colony survival.

215 Since only susceptible donor brood was used for our experiments in both surviving and
216 susceptible host colonies, any traits of immature bees can safely be excluded to explain our data.
217 For example, changes in brood volatiles (Nazzi & Le Conte 2016) are therefore not a factor in
218 the results obtained. Instead, it appears that different adult behaviours are likely sufficient to
219 explain reduced *V. destructor* reproductive success and ultimately colony survival. These
220 behaviors need to be identified.

221 In conclusion, our data support that a reduced *V. destructor* mite reproductive success seems to
222 be a key factor for natural colony survival. However, grooming and VSH are unlikely for this
223 Norwegian case. Instead, yet unidentified behavioral traits of work bees seem sufficient to
224 explain reduced mite reproductive success. Therefore, the underlying mechanisms remain elusive
225 and should be a focus of future studies taking advantage of naturally selected survivors.

226 This Norwegian honey bee population, taken together with previously reported independent
227 cases (Locke 2016), clearly show that European honey bee subspecies can indeed develop traits
228 to overcome extreme *V. destructor* infestations by means of natural selection. It is therefore high
229 time we take advantage of these cases and gain a better understanding of natural host adaptations
230 (Fries and Bommarco, 2007) for a practical application in apiculture and honey bee conservation
231 worldwide.

232

233

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237

238 **ADDITIONAL INFORMATION AND DECLARATIONS**

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242 **Competing Interests**

243 The authors declare there are no competing interests.

244 **Author Contributions**

245 Melissa Oddie, Bjørn Dahle and Peter Neumann conceived and designed the experiments.

246 Melissa Oddie performed the experiments, analyzed the data and prepared figures.

247 Melissa Oddie and Peter Neumann wrote the paper.

248 Bjørn Dahle reviewed drafts of the paper.

249 **Data Availability**

250 The following information was supplied regarding data availability:

251 The raw data has been supplied as “.txt” files.

252

253

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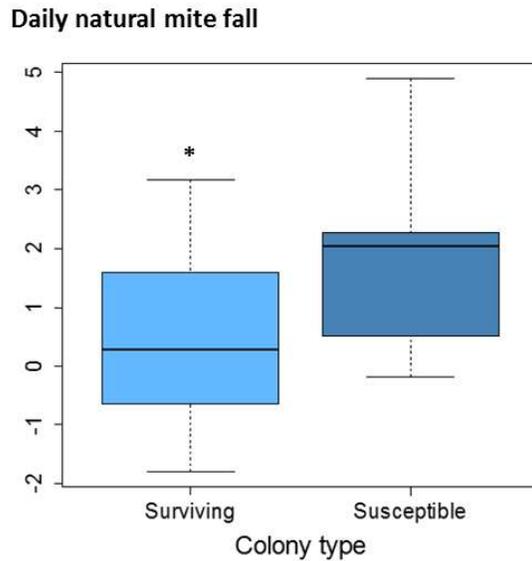
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362

363 **Figures**

364

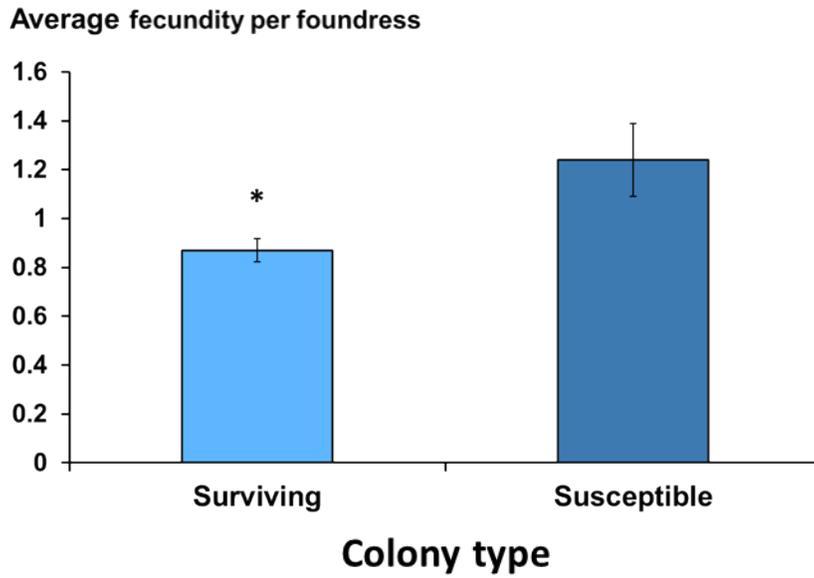


365

366 **Figure 1** Daily natural mite fall in surviving and susceptible colonies. Interquartile ranges and
367 medians of daily natural mite fall are shown. Values were log-transformed to accommodate
368 outliers. Natural mite fall was significantly lower in surviving colonies compared to susceptible
369 ones ($t=2.8$, $df=9$, $p<0.02$; * = $p<0.05$).

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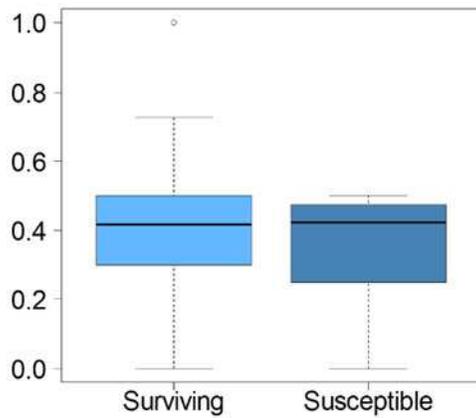
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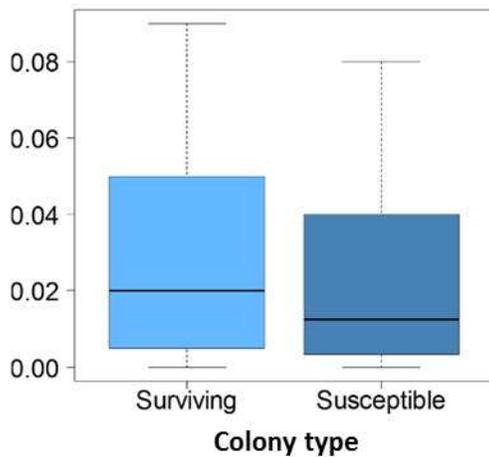
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373 **Figure 2** Viable female mite offspring per foundress in surviving and susceptible colonies. The
374 average number and standard errors are shown. The surviving colonies had a significant
375 decreased mite reproductive success by ~30% compared to susceptible ones ($\chi^2= 4.09$, $p=0.027$;
376 * = $p<0.05$).

a. Proportions of damaged mites



b. Proportions of removed brood



377

378 **Figure 3 a.** Proportions of damaged mites in surviving and susceptible colonies. Interquartile
379 ranges and medians are shown. There was no significant difference between the colony types
380 ($\chi^2= 0.12$, $df= 1$, $p=0.73$), **b.** Proportions of brood removed in surviving and susceptible colonies
381 over 10 days. Interquartile ranges and medians are shown. There was no significant difference
382 between surviving and susceptible apiaries ($\chi^2= 1.88$, $df= 1$, $P= 0.171$).