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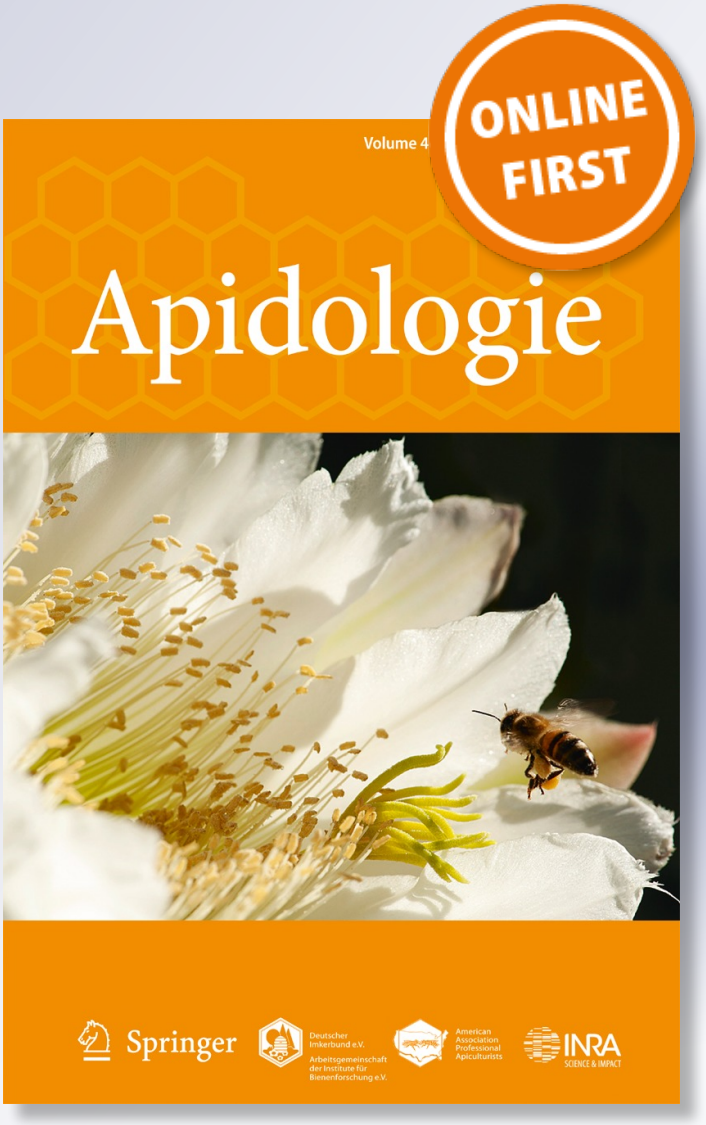

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Scientific note

Scientific note: varroa mite eradication, the strange case of Gorgona Island

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varroa mite eradication / DWV titer / honeybee health / monitoring program / small island

1. INTRODUCTION

Varroa mites are vectors for several bee viruses contributing also to their diffusion worldwide (Martin 2001; Di Prisco et al. 2011; Cersini et al. 2013). The tripartite relationship among bees, mites, and viruses is thought to be responsible for the loss of a large amount of colonies (Highfield et al. 2009; Berthoud et al. 2010; Francis et al. 2013).

Before the arrival of *Varroa destructor*, virus prevalence was lower (Martin et al. 2012). The mites have contributed to spread the viruses which could be correlated to the high number of mite-infested honey bee colony losses (Berthoud et al. 2010; Francis et al. 2013). Since the varroa mite arrival, control of varroosis has become necessary (Moritz et al. 2010). Such control is focused on the coexistence between honeybees and mites. A trial to eradicate the mite from isolated areas has not been successful. Experiments carried out to eradicate varroa mite by use of several coordinated treatments in Jersey, an island in the English Channel, did not succeed, showing how difficult it is to eradicate this ecto-parasite from an infested area (Sampson and Martin 1999).

The aim of this study is to report the disappearance of varroa mite from the island of Gorgona (43° 26′ N; 9° 54′ E) following several treatments in a single apiary kept in complete isolation. Furthermore, after the varroa disappearance, the honeybee viral load has been analyzed. Gorgona Island has been chosen because it provides suitable conditions to guarantee continuous monitoring and isolation to avoid new reintroduction of bee pathogens and pests.

2. MATERIAL AND METHODS

2.1. Study area and honeybees

Gorgona is a very small island (220 ha), 18 nautical miles from the Italian coast. It is located within the "Arcipelago Toscano" national park. It is very difficult for honeybees to fly over there, according to the farthest distances reported for this species (Beekman and Ratnieks 2000). The fact that the island is a prison site reinforces its isolation conditions.

In 1998, a single apiary of 17 colonies of *Apis mellifera* has been settled in the prison farm in Gorgona. The apiary has always been managed by the same technicians, also coauthors of this paper (RP and MG) with the supervision of the Bee Research Team of Pisa University (Gruppo di Apidologia di Pisa).

2.2. Treatments against varroa mite

Since 1998, all the colonies have been treated twice per year with oxalic acid and Apilifevar™.

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In 2009, only one treatment with Apistan™ was carried out in November. Such treatment is the one and only treatment with Apistan™ performed in the history of this apiary.

After this treatment, no other treatments with active ingredients have been carried out in the Gorgona apiary.

2.3. Monitoring of varroa mite

Since August of 2010, no symptoms related to varroa mite infestation, such as weakness of colonies with spotty brood pattern and specimens of mite detectable on pupa and adult (Sammataro et al. 2000), have been observed. From 2011 to December 2014, a bimonthly monitoring program started to detect dead or alive specimens of varroa mite. Three methods were used to detect *V. destructor* specimens: (a) using powdered sugar on a sample of 50 honeybees per hive, (b) opening 200 capped cells per hive (drone cells when possible) in brood presence, and (c) checking the diagnostic plate located at the bottom of hives.

In order to gain stronger evidence of no mite infestation of the apiary while maintaining the apiary treatment-free starting from 2010, only one colony was treated with Apilifevar in August 2011.

2.4. Monitoring of Deformed Wing Virus

In this study (2012 to 2014), we performed another monitoring program for deformed wing virus (DWV). A total of 95 forager bees were sampled and analyzed from a total of 5 hives.

Twenty foragers were collected from the apiary (four from each colony): in August 2012, in August 2013, and in February 2014, while in September 2014, 35 forager bees (7 from each colony) were collected. Analysis of dissected abdomen and head were performed on each bee (95 bees). Tissues were homogenized using a TissueLyser II (Qiagen, Hilden, Germany) and total RNA was extracted with Rneasy Mini Kit (Qiagen). RNA samples were tested by a One-Step TaqMan RT-qPCR protocol for DWV detection. Primers and probe are reported in our previous paper (Mazzei et al. 2014).

3. RESULTS AND DISCUSSION

Up to now, there are 17 colonies in Gorgona and since 2011 no colony death has been recorded. No varroa mite has been detected for a period of 3 years using different detection methods. Although each method individually might not be sufficient to confirm the absence of mites, the combination of three diagnostic tools over a long period gives strong evidence of the mite-free status. It is difficult to link the varroa eradication to the treatment only. A possible additional cause could be the small dimension of the island which may lead to a weak re-infestation rate from possible natural swarms.

According to Sampson and Martin (1999), one possible cause of failure of the eradication project on the island of Jersey is due to the size of that island (11,820 hectares), more than 50 times larger than Gorgona, and to the existence of other apiaries. The larger size of the island could allow a higher number of apiaries and of unmanaged feral swarms. The higher the number of apiaries and swarms, the higher the possibility of re-infestation of varroa mites.

The present study reveals that from 2012 to 2013, DWV was not detected from head and abdomen samples (40 honeybees). Unexpectedly, during February 2014, 16 abdomens out of 20 resulted positive, with viral load values close to the detection limit of the essay (mean value 35.3 s.d. 31.3 copies per microgram of RNA), whereas all heads resulted negative. In September 2014, 7 months after the previous check, samples of the abdomen and heads of 35 adult honeybees resulted all negative. Moreover, the negative results recorded in 2012 and 2013 and the low titer for DWV detected in February 2014, as well as the other negative results in September, are compatible with results reported for colonies varroa mite-free, between no detectable level (Highfield et al. 2009) and less than 10^3 DWV copies per bee (Martin et al. 2012), and always significantly lower than in colonies with varroosis (Natsopoulou et al. 2012). The disappearance of the varroa mite associated with a low viral load may suggest a leading role of "isolation" both on *V. destructor* and virus population.

DWV presence reported in this study confirms the data obtained by Mutinelli and colleagues in a monitoring study (APEnet project) performed in the same island, which reported the presence of DWV, during the years 2008–2010 (Mutinelli et al. 2011).

In the current investigation, varroa seems to be eradicated and the virus is seldom present and always with low titer and only in the bee abdomen. Further investigation is needed in order to better describe the dynamic of virus titer in the mite-free honeybee population.

The absence of colony mortality after the disappearance of *V. destructor* from Gorgona could be compared with data of Martin et al. (2012) that reported no case of CCD on Hawaii islands before the arrival of *V. destructor*. The link between colony mortality, virus titer, and varroa mite infestation seems to be evident, but it is difficult to measure and to detect any relationship. For this reason, Gorgona becomes an important model for this kind of studies.

4. CONCLUSION

Gorgona is at present a varroa-free island. Other territories are varroa-free because they were never reached by the mites. At the best of our knowledge, the varroa-free status of Gorgona is due to mite eradication. For this reason, the apiary of Gorgona could be considered an important experimental and monitoring station. Monitoring Gorgona Island is important (I) to guarantee the absence of varroa mite and (II) to study the dynamics of viral infection after *V. destructor* eradication.

It might be interesting trying to perform varroa mite eradication in other small islands or in closely isolated places, with a small honeybee population. We wish to suggest as a good candidate the island of Pianosa, in the same archipelago.

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Note scientifique sur l'éradication de l'acarien Varroa: le cas étrange de l'île Gorgona

Ausrottung der Varroamilbe: Der außergewöhnliche Fall der Insel Gorgona

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