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ORIGINAL RESEARCH ARTICLE

Investigating the influence of postcapping period on varroa mite infestation

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The aim of this study was to assess infestation levels of *Varroa destructor* in some honey bee colonies from the National breeding program in the central part of Iran, and the relationship between mite infestation levels and postcapping period (PCP) of worker brood cells. Shortening the PCP is an important parameter limiting the success of varroa mite reproduction in honey bee colonies. In the present study, four pure-bred line colonies from isolated areas were selected to investigate the brood capping durations and mite infestation levels. Cages were used to synchronize egg laying of the queens and the time of capping and emerging of the brood cells was recorded at two-hour intervals. The results did not show any significant differences on the average of PCP of brood cells and mite infestation levels among test colonies. In addition, non-significant correlations were found between mite infestation and the capping period during the experiment. A relatively shorter capping period of brood cells was observed in one of the provinces compared to others, corresponding with slightly lower mite levels in the same colonies, which might suggest that reducing capping duration may be one of the factors which can potentially affect mite populations in honey bee colonies and therefore should be considered in breeding programs.

Investigación de la influencia del período de operculado en la infestación de ácaros varroa

El objetivo de este estudio fue evaluar los niveles de infestación de *Varroa destructor* en algunas colonias de abejas melíferas de programa nacional de cría en la parte central de Irán, y la relación entre los niveles de infestación de ácaros y el periodo tras el operculado (PTO) de celdas de cría de obreras. Acortar el PTO es un parámetro importante que limita el éxito de la reproducción de los ácaros varroa en las colonias de abejas de miel. En el presente estudio, se seleccionaron cuatro colonias de líneas de raza pura de zonas aisladas para investigar la duración del operculado de la cría y los niveles de infestación de ácaros. Se utilizaron jaulas para sincronizar la puesta de huevos de las reinas y el tiempo de operculado y emergencia de las celdas de cría se registró a intervalos de dos horas. Los resultados no mostraron diferencias significativas en la media del período de operculado de las celdas de cría y los niveles de infestación del ácaro entre las colonias de prueba. Además, se encontraron correlaciones no significativas entre la infestación de ácaros y el período de operculado durante el experimento. Se observó un período de operculado relativamente corto en las celdas de cría en una de las provincias en comparación con las demás, lo que se corresponde con niveles ligeramente más bajos de ácaros en las mismas colonias, lo que podría sugerir que la reducción de la duración del periodo de operculado puede ser uno de los factores que pueden afectar potencialmente a las poblaciones de ácaros en las colonias de abejas de miel y, por tanto, deben ser consideradas en los programas de mejoramiento.

Keywords: *Varroa destructor*; mite; resistance mechanism; postcapping duration; *Apis mellifera*; breeding program

Introduction

The varroa mite is now among the most significant problems for beekeeping around the world. It causes several types of damage to honey bee adults and larvae, and can transmit viral diseases into honey bee colonies. In 1904, Oudemans reported this mite for the first time in Java, Indonesia as *Varroa jacobsoni*. From then, the mite spread almost all over the world. Anderson and Trueman (2000) found two morphological forms of this mite in Asia and the name of the mite on *Apis mellifera* was changed to *Varroa destructor*.

Selection methods for varroa resistance or tolerance in honey bee colonies have been well studied in the literature. Among them, active defense mechanisms such as grooming and hygienic behavior have been most commonly investigated (e.g., Boecking, 1992; Boecking & Spivak, 1999; Harbo & Harris, 1999; Harbo & Hoopingarner, 1997; Peng, Fang, Xu, & Ge, 1987). In grooming behavior, honey bee workers try to get rid of mites, either by grooming themselves or other nestmates. In hygienic behavior, honey bee workers uncap the infested brood cells and remove the dead or parasitized bees. These two tolerance mechanisms are known to be present to a larger degree in *Apis cerana* compared to *A. mellifera* colonies (Fries, Huazhen, Wei, & Jin, 1996). The presence of grooming behavior in *A. mellifera* colonies seems to be highly variable and some race-specific differences may exist in the level of its occurrence among honey bee colonies (reviewed by Rosenkranz, Aumeier, & Ziegelmann, 2010). By the removal behavior, honey bee workers also remove varroa mites from the opened brood cells; this leads to an

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interruption in the reproductive cycle of mites (Boecking & Spivak, 1999; Peng et al., 1987).

Shortening the postcapping period (PCP) of the sealed brood cells is also classified as one of the resistance mechanisms of honey bees to varroa, where the PCP is the time from sealing a brood cell till emerging an adult bee. The duration of capping brood cells in most *A. mellifera* subspecies is approximately 12 days (Bak, Siuda, & Wilde, 2012; Guerra, Gonçalves, & Jong, 2000; Jandricic & Otis, 2003; Wilde, Romaniuk, Siuda, & Bak, 2003). It was shown that *A. mellifera* strains, with a sufficiently shorter duration of the PCP, retarded the development of varroa populations (Garrido & Rosenkranz, 2003; Harbo & Harris, 2005; Rinderer, Harris, Hunt, & de Guzman, 2010; Siuda & Wilde, 2000).

A list of factors is given here among which the PCP is one of the most important parameters reducing varroa infestations (see below). The first factor for estimating varroa mite populations in honey bee colonies is reproduction and fecundity of the mites. Knowing more about life cycle of the mite will result in selecting better honey bee races for breeding programs (Carreck, 2011). Varroa mites prefer fifth instar larvae and enter approximately 15–20 h before capping of the worker cells and 40–50 h of the drone cells (Boot et al., 1997; Ifantidis, Thrashyvoulou, & Pappas, 1988). The mite can be attracted by bee larvae through chemical extracts from the larval cuticle (Martin et al., 2002), semiochemicals from larval food (Calderone & Lin, 2001; Donzé et al., 1998; Nazzi, Milani, & Della Vedova, 2004), and size of the brood cell. The difference between higher attractiveness in drone brood than worker cells is caused by both the effects of chemicals (Calderone & Lin, 2001; Martin et al., 2002) and size (Calderone & Kuenen, 2003).

Varroa mother mites start laying eggs 70 h (2.92 days) after cell capping (Ifantidis, Karamanidou, & Katikou, 1999; Steiner, Diehl, & Vlimant, 1995), while the first egg is unfertilized and the next eggs up to six eggs are laid in 30 h (1.25 days) intervals (Ifantidis, 1990; Martin & Kryger, 2002; Rehm & Ritter, 1989). It will take 5.8–6.6 days for the mite offsprings (female and male, respectively) to become adult (Donzé & Guerin, 1994; Ifantidis, 1990; Martin & Kryger, 2002; Rehm & Ritter, 1989).

Besides good reproduction of mite, other factors can affect mite population in bee colonies. These factors are mortality of the mite, availability (Calis, Fries, & Ryrie, 1999) and attractiveness of brood cells (Espinosa-Montano, Guzman-Novoa, Sanchez-Albarran, Montaldo, & Correa-Benitez, 2008), honey bee defense behaviors (reviewed by Boecking & Spivak, 1999), and climate, and nectar flow (Currie & Tahmasbi, 2008). Büchler, Berg, and Le Conte (2010) recently reviewed different factors affecting mite populations in honey bee colonies of which PCP was shown as an important factor used in breeding programs. The mite offspring were found to be negatively affected in some races with less than 12 days capping time (Boecking, 1992). The number of mite offspring was lower in *A. m. capensis,* which experienced a 2 day shorter capping period than *A. m. carnica* (Moritz, 1994). Büchler and Drescher (1990) suggested a positive correlation between capping period and mite infestation levels.

Importantly, this honey bee characteristic is genetically inherited to the next generation (Büchler & Drescher, 1990; Moritz, 1985). Similarly, studies in observation hives showed that the difference in capping period could be related to genetic background of the bees if other factors (e.g., temperature, quality and quantity of larval food, number, and the age of nursing bee) remain constant (Schousboe, 1986). Kralj and Otis (1999) referred to Harbo (1992), who noted a deduction of 5.4 h in capping period could be potentially used as a positive factor in honey bee breeding programs. The PCPs of 11, 10, and 9 days were estimated in some honey bee lines (Cobey & Lowrence, 1998) to result in 9, 35, and 100% reduction, respectively, in mite population (e.g., Allsopp, 2006; Medina, Martin, Espinosa-Montaño, & Ratnieks, 2002).

Few studies in the literature are available on the importance of the PCP in selecting resistant honey bee lines. In addition, the literature provides inconsistent support for a possible relationship between the duration of the PCP in honey bee development and of varroa mite reproduction, and after initial more optimistic scrutinizing the idea has been dropped from the list of promising resistance parameters. The present study represents new data again supporting such a relationship. Therefore, the aim of this study is to emphasize on this resistance mechanism in honey bee colonies, to evaluate its applicability in breeding programs, and to assess the relationship between varroa mite infestation level and the PCP.

Materials and methods

The experiments were carried out with honey bee colonies from the National breeding program, transferred to the Isfahan University of Technology research station (32° 32′ N, 51° 23′ E; Southwest Isfahan, Iran). The present study included honey bee colonies from four Iranian provinces: Isfahan, Markazi, Qazvin, and Tehran. The breeding program has been established for approximately 10 years, and the honey bees of each province have been pure-bred lines by isolated areas. The experiments were performed between February and September, with the air temperature ranging between 25 and 35 C during the day and between 5 and 25 C during the night and a relative humidity of less than 65%. In the last three months, the humidity was decreased to 25–30%. Colonies were maintained carefully for approximately two months to achieve homogenous characteristics such as quantity of honey, pollen, nursing bees, colony population, capability of queens for laying eggs, etc. (as described in Ardestani, Ebadi, & Tahmasbi, 2005). In the

latter study, the PCPs were measured for each province. In brief, specific cages (push-in) were used to synchronize egg laying of the queens. These cages are generally used to confine queens to a specific area of the comb. After 24 h, the caged-queens were released. Each test area containing approximately 250 brood cells was marked by placing a pin around the place of new eggs and considered for each replicate. For each province, four colonies (hives) and in each colony two replicates were investigated (n = 32).

On day 8 before cell capping, the frames with fifth instar larvae were monitored carefully at 2-h intervals and removed from each colony shortly after cell capping. The frames were transferred to a large incubator (34 ± 1 °C, 40–60% relative humidity). In order to determine the exact time of emergence, approximately 10 days later and from 24 h before emerging adult bees, the selected frames were inspected in the incubator. At 2-h intervals, the number and time of opening cells were recorded in each replicate. One-way analysis of variance (ANOVA) was used to compare the average PCP among different provinces.

To estimate infestation level of the varroa mites, every month two samples of approximately 200 worker bees were collected from each colony by brushing them from a random bee comb into a plastic container. By using approximately 150 ml detergent solution and a constant (slow) shaking, mites were separated at the bottom of the container. This procedure was done for the second time to collect additional mites from the same honey bees, and finally these bees were placed on a white surface to examine individually for probable remaining mites (De Jong, Roma, & Goncalves, 1982; Dietemann et al., 2013). Then, total number of mites was counted in each replicate. By dividing the total number of mites by the total number of honey bee workers in each replicate, the percentage of infestation level was calculated. Since including both samples (replicates) is a pseudo-replication, average infestation level for each test colony was used in the regression calculation and in the Figure 3. Using one-way ANOVA, the average infestation levels among different provinces and among different test colonies in each province were compared and the relationship between mite infestation level and capping period was investigated.

Unfortunately, no information was gathered about drone brood in the tested hives as well as the accurate measurement of reproduction of mites in the cells. It should be noted that mite infestation levels were brought to the same level (zero percent) after measuring initial infestation level in the colonies. Bromopropylate was used in its commercially available form, Fulbex VA (Ciba Geigy), as one fumigant strip per colony in mid-February. Treatment was given in the evening when almost all honey bee workers ceased flying into/from the hives.

All analyses were run in SPSS package 21.0 for Windows.

Results

The results of present study showed a small difference in the duration of capping period in the test colonies. The PCP varied between 278 and 294 h in different test colonies. The reported values for each province are the average of two replicates in each colony. Figure 1 shows the measurement of capping period among different colonies of each province (data are taken from Ardestani et al., 2005). On average, the PCPs (±SE) were 289.3 (±1.3), 285.8 (±2.8), 290.1 (±3.0), and 287.3 (±1.3) h in Isfahan, Markazi, Qazvin, and Tehran, respectively. An overall 5 h difference in the average of capping period is observed among the four provinces in this study. Statistical analysis did not show any significant differences between the average of capping period among provinces (ANOVA, p = .40).

Infestation levels varied between .3 and 22 percent in different colonies among four provinces. No significant differences were observed between the average of mite infestation levels among provinces (ANOVA, p = .24) and among different colonies in each province (ANOVA, p = .31). The level of infestation in the colonies was measured in the test colonies during the whole experiment. Mite infestation levels in all test colonies (all together) are shown in Figure 2. The initial mite infestation levels were not so high at the beginning of the test (approximately 14%). The results show that the infestation levels are lower in early spring and increases until the late summer (Figure 2). In September, the highest infestation level was observed.

Then, the relationship between mite infestation and the PCPs was obtained. Figure 3 shows this relationship at the end of the test (September). Average values for each colony was used for the calculation of regression (average of two replicates). Mite infestation level did not show a significant correlation with capping period of the broods cells (R^2 = .15, p = .14, n = 16). Since using the end data alone may fail to exploit the information from the other sampling times, average data for each sampling time were plotted against the average capping period and are shown in Supporting Material Figure S1 (A–G). In all cases, these correlations were not statistically significant. It should be mentioned that the average of capping period was measured once in the present study and assumed to be constant over time during the experiment.

Discussion

In the present study, an overall 5 h difference in the average of PCP was observed among different provinces from the Iranian National breeding program. These results are in line with the study of Le Conte, Bruchou, Benhamouda, Gauthier, and Cornuet (1994), who reported a 10 h difference among the averages of PCP in the selected *A. m. mellifera* worker broods. Büchler and Drescher (1990) also reported a 7 h difference

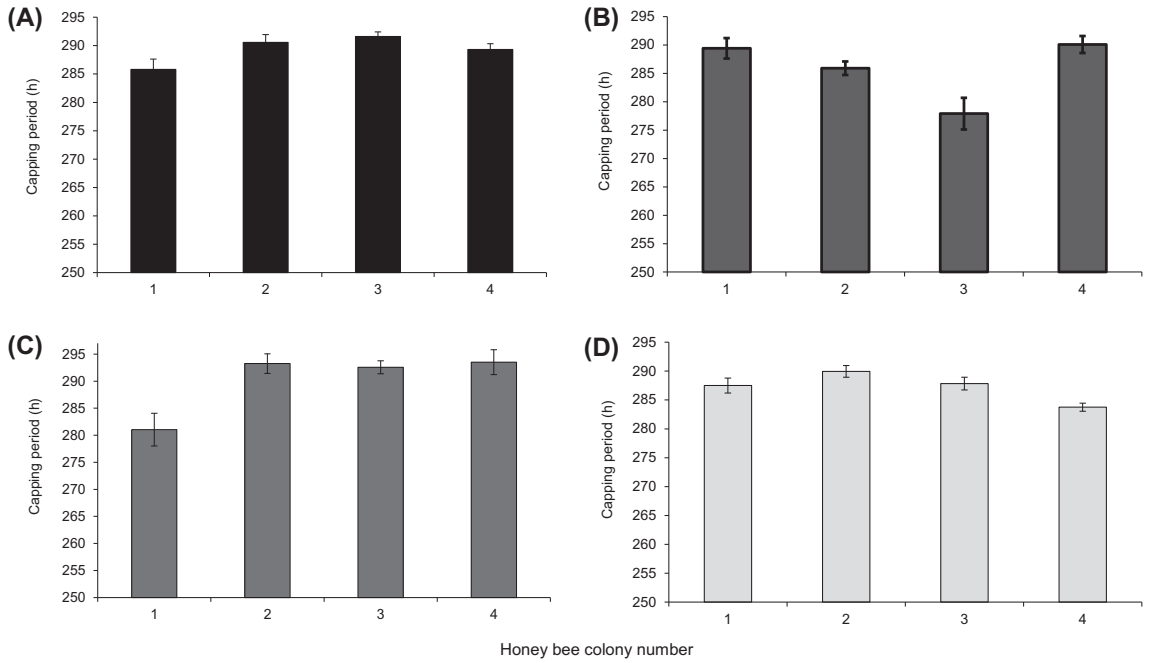

Figure 1. The average postcapping period in the test honey bee colonies from Isfahan (A), Markazi (B), Qazvin (C), and Tehran (D). Each province includes four colonies (numbers 1–4) and two replicates were considered in each colony. Each column shows the average of postcapping period in two replicates of each province colony and error bars show the variation between these two replicates (average ± SE). The data are taken from Ardestani et al. (2005) with modification.

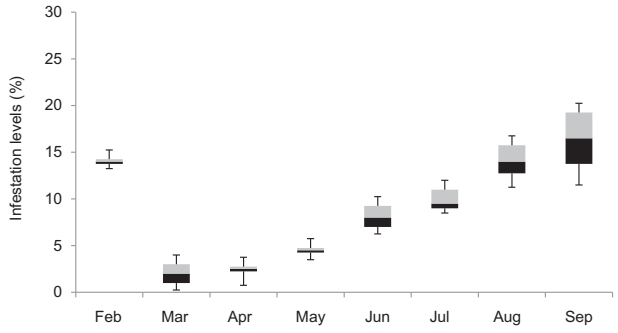

Figure 2. The level of infestation in the honey bee colonies of four provinces (Isfahan, Markazi, Qazvin, and Tehran) measured during the whole experiment. Each month, two samples were taken from each colony by taking approximately 200 honey bee workers. The *Varroa* mites were collected by using a detergent (see text for more details on collection method). At each sampling time, the average values (± SE) of all test colonies are shown.

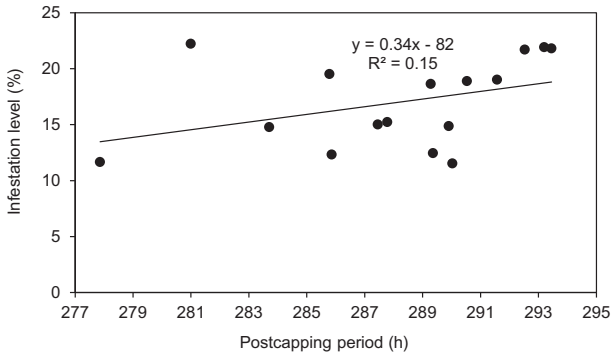

Figure 3. The correlation between *Varroa* mite infestation levels (%) and honey bee postcapping period (h) in honey bee colonies from four different provinces; Isfahan, Markazi, Qazvin, and Tehran (n = 16). For each province, four test colonies were considered.

between the average of capping period in early compared to late summer in their test colonies. In addition, they measured differences up to 9 h among strains and up to 19 h within individual colonies.

As discussed above, mite reproduction is related to the number of mated female mites on the emergence of the adult bee from cells. Therefore, the duration of capping stage of cells is an important factor for successful reproduction in the *varroa* mite (Rosenkranz et al., 2010). Regarding this statement, mite reproduction might be affected in the present study where slightly lower capping period was observed in some test colonies. In total, varroa mites need about 10.4 days to complete their life cycles inside the brood cells and come out of the cells when adult bees emerged. Thus, for some honey bee races such as *A. m. capensis* with shorter postcapping duration, this time becomes a limiting factor for the mites to complete their reproduction cycle. Büchler and Drescher (1990) concluded a 10% reduction in PCP can cause about 30% decrease in the mite populations.

Having lower infestation levels in some honey bee colonies in the present study indicates that the differences between capping periods might have led to a

positive effect on the mite population and could potentially increase the resistance of these colonies to the varroa mite. Although there was no significant difference between the average of the PCP among provinces, 5 h difference among provinces might be the reason for changes in mite populations. However, the latter conclusion should be drawn with caution, since there is no significant difference among provinces. The extension for further research on reproduction of mites inside brood cells will certainly help to confirm the decrease in mite population and its relationship with capping period in the test colonies used in the present study. Similar argument was also given by other studies; for example, Vandame, Colin, Morand, and Otero-Colina (2000) reported that low infestation levels in the colonies are the results of a reduction in mite fertility. Their results may reinforce the hypothesis that low mite reproduction may be associated with the genetic strain of mite rather than the strain of bees. This needs to be further investigated.

Previous work supports the idea that this mechanism can be a reason for lowering mite population levels. In agreement with Büchler and Drescher (1990), who found one hour reduction in capping period corresponded with 8.7% decrease in mite population, the results of present study showed approximately 9.7% decrease in mite infestation levels. The reason for a higher reduction of mite infestation for 1 h reduction of the capping period may be due to small sample size in this study and relatively low infestation levels in test colonies. If the mite does not have enough time to mature completely and produce enough offsprings in brood cells, its fecundity is likely to suffer and its offsprings will decline in number (Harbo, 1992). The results of this study are consistent with the capping period measured in brood cells of *A. m. mellifera* (European honey bee race) by Boecking (1992), who showed an overall 12 days of PCP in the tested honey bees.

As a consequence of the shorter developmental time of the worker brood in resistant lines, the mites' second offspring will not become adult in the brood cells (Martin & Kryger, 2002). In this case, cuticular hydrocarbons do not synthesize completely and female offsprings would not have sufficient time to complete tanning. Therefore, mites may be detected more easily by hosts and removed from their body by the active defense mechanisms such as grooming behavior (Nation, Sanford, & Milne, 1992).

The internal conditions of the colony, e.g., bee population, genetic background (Bienefeld & Zautke, 2007; Ron & Rosenthal, 1987), other resistance mechanisms (Ibrahim & Spivak, 2006; Peng et al., 1987), the application of acaricides (time of application and resistance of mites to acaricides) may have positive or negative effects on mite infestation level. This was in agreement with Moritz (1994) who concluded that the development of mite population in *A. m. capensis* was clearly host-dependent. They referred to resistance mechanisms of honey bees as well as shorter duration of capping period in *A. m. capensis* compared to other races. In our previous studies, however, grooming behavior was shown to exist to a somewhat lesser extent in similar honey bee colonies of the National breeding program (Ardestani, Ebadi, & Tahmasbi, 2011, 2002).

Using honey bee resistance mechanisms can decrease the application of acaricides in honey bee colonies and make their products safer for human health. Integrated management of varroa infestation in honey bee colonies was suggested by Delaplane (2011), with more emphasize on honey bees resistance behaviors rather than chemical control method. But, there are still some gaps in our knowledge on the biology and epidemiology of the varroa mite (Dietemann et al., 2012). Among resistance mechanisms, grooming and removal behaviors of honey bees have been most studied while the PCP can also provide more information regarding reproduction and fecundity of varroa mites inside the colonies. Further research based on innovative and challenging approaches is needed for the selection of resistant honey bee lines to varroa.

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Disclosure statement

No potential conflict of interest was reported by the author.

Supplementary material

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