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ORIGINAL RESEARCH ARTICLE

Exploring a treatment strategy for long-term increase of varroa tolerance on Marmara Island, Turkey

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Summary

We explored practical steps to implement a sustainable treatment against *Varroa destructor* which is adapted to common beekeeping situations, and applies conventional control but nevertheless exerts selection pressure towards increased mite tolerance in honey bees. This approach approximates conditions of natural selection in host-parasite systems, and is supported by evidence that the impact of *V. destructor* decreases when bee populations are overexploited by the parasites. However, instead of a "live or let die" approach to selection, which is not feasible for commercial beekeeping, death of highly infested colonies was mimicked by treatment and requeening. We established a feasible treatment threshold based on powder sugar shaking of worker bee samples in 250 colonies kept by four beekeepers on the island of Marmara, Turkey. We subsequently requeened heavily infested colonies with queens from lightly infested colonies using simple methods. We found that although one third of the colonies were routinely left untreated, it was possible to decrease mean mite infestation levels and maintain a stable bee population in our apiaries.

Exploración de una estrategia de tratamiento para el aumento a largo plazo de la tolerancia a varroa en la isla de Marmara, Turquía

Resumen

Hemos explorado medidas prácticas para aplicar un tratamiento sostenible contra *Varroa destructor* la cual está adaptada a situaciones de apicultura comunes y a la cual se aplica un control convencional, pero sin embargo ejerce presión de selección hacia el aumento de la tolerancia del ácaro en las abejas de miel. Este enfoque se aproxima a las condiciones de la selección natural en los sistemas de huésped-parásito, y se apoya en la evidencia de que el impacto de *V. destructor* se reduce cuando las poblaciones de abejas están sobreexplotadas por los parásitos. Sin embargo, en lugar de dar un enfoque de " vive o dejar morir" a la selección, que no es factible para la apicultura comercial, se simuló la muerte de colonias altamente infestadas por tratamiento y renovación de la reina. Hemos establecido un umbral de tratamiento viable basado en espolvorear azúcar en polvo en muestras de abejas obreras en 250 colonias mantenidas por cuatro apicultores de la isla de Marmara, Turquía. Posteriormente se reemplazaron las reinas en las colonias altamente infestadas con reinas de colonias poco infestadas usando métodos sencillos. Se ha encontrado que, aunque una tercera parte de las colonias fueron rutinariamente dejadas sin tratar, fue posible disminuir los niveles medios de infestación de ácaros y mantener una población de abejas estable en nuestros apiarios.

Keywords: *Varroa destructor, Apis mellifera*, treatment, selection for tolerance

Introduction

Since the early theoretical evaluations of Anderson and May (1981), virulence of parasites or pathogens is understood to increase or decrease over time depending on the interrelations among three main factors: host mortality, parasite reproduction, and method and ease of parasite dispersion among hosts. As with any disease, the parasite-host system of the mite *Varroa destructor* and the honey bee *Apis mellifera* is subject to evolutionary pressures, which in the long run determine the amount of damage inflicted on the host. *V. destructor* haplotypes, highly specialized for parasitizing on the eastern honey bee *Apis cerana* in a benign way, transcended the invasion barrier and managed to reproduce on the western honey bee *A. mellifera* (Anderson and Trueman, 2000; Dietemann *et al*., 2013) with devastating effects. It can readily be assumed that the mites and their new hosts were subject to continuous processes of selection, moulding the system according to the new circumstances. We may further assume that these processes, though now predominantly acting in a beekeeping environment, still are guided by the same main principles of natural selection, resulting in a background of "unconscious" selection as opposed to any conscious breeding efforts. There are reasons to presume that general beekeeping conditions and habits, such as high colony density or prevention of colony renewal through swarming, are likely to result in the development of higher pathogenicity (Fries and Camazine, 2001). Among others, one factor of major impact on the parasite-host system and its long-term development can be readily identified which is the current and recommended practice of control of *V. destructor*. In this, the short term gains of saving colonies by treatment may actually deter long term prospects of a more benign parasite-host relationship in which virulence is reduced.

There seem to be three apparent long-term scenarios, here outlined following the terminology introduced by I Fries (pers. comm.) as "the good", "the bad" and "the ugly" strategies. The "bad" strategy is the current main course taken, which is treating all colonies once or several times per year, without considering whether individual colonies are significantly affected. From the bee colony perspective, this removes any advantage of expressing defence mechanisms of any kind, known or unknown. Due to their costs, existing traits are likely to disappear over time and the host population will become even more dependent on the treatment. From the mite perspective, the parasites will be kept continuously in a situation of low population levels where fast reproduction is favoured (r-selection), as opposed to a situation, where populations are close to the carrying capacity of the host (K-selection) and mites may benefit from restricted reproduction to keep the host alive for a longer time. Thus, long term prospects of treating colonies all the time indicate an intensification of the problem.

The "ugly" strategy is not to treat at all. In this scenario mite populations would increase over time, until this, in combination with virus infections affecting brood development or influencing adult bee mortality outside the colony, effects colony breakdown (Rosenkranz *et al*., 2009). Here, colonies which express any defence mechanism which slows down mite reproduction will gain an advantage, remain stronger for longer times, and contribute more to the next generation by producing swarms, queens and drones. In a beekeeping context, they are more likely to contribute to colony splits. In colony breakdown, mites also perish, with the exception of those transported into other colonies by robbing or drifting. If chances for transportation are low, as may be the case in natural populations with spaced colonies as opposed to a close proximity within apiaries, this situation would also select for less virulent mites. There is a body of supporting evidence that this kind of "live and let die" selection (the "Bond Test") (Kefuss *et al*., 2003, 2004) does indeed affect the mite-bee parasite-host relationship towards a tolerance in the outlined way, both in natural and experimental populations (Allsopp, 2006; Büchler *et al*., 2002; Fries *et al.,* 2006; LeConte, 2004; Ritter, 1990; Seeley, 2007; Villa *et al*., 2008), which will be discussed in more detail later. However, it is not a viable option not to treat, as there is a high likelihood that affected colonies will die within one or two years, causing substantial losses to the bee industry, and, even more important, for pollination.

We thus propose a third strategy, which captures the essential features of the "ugly" live-and-let-die strategy, but avoids colony losses by selective conventional acaricide treatment. The basic features of this strategy are straightforward and simple. In a two-step process, colonies would first be rated according to infestation levels. Then treatment would be applied only to colonies exceeding an infestation threshold where they are unlikely to survive over the next treatment period (next year, in temperate climates). Second, high-infested treated colonies would be requeened from low-infested, not-treated colonies. From the bee perspective, requeening would amount to genetic death of the old colony. However, a continuous worker force for beekeeping and honey production is retained, which is gradually replaced by the selected new queen's offspring. From the mite's perspective, treatment penalises high population levels, thus favouring genotypes of lower reproductive potential. Preventing actual colony breakdown by treatment also curtails benefits to the mites from enhanced dispersion through drifting and robbing, thus removing incentives for rapid reproduction to increase horizontal transmission among colonies (Fries and Camazine, 2001).

In the current project we investigated how a "good strategy" schedule consisting of diagnosis followed by infestation-dependent treatment and requeening could be put into practice. As the method addresses general beekeeping and treatment routines, our prominent aim was to offer simplified steps that might be accepted by common beekeepers without predominant interest in bee breeding issues. This rules out any complex routines which require long-term tracking of colony or queen fate. This is a difficult task even in fairly well organised apiaries, but is impossible in common beekeeping where colonies are continuously transported, split, or requeened by supersedure and swarming. Ideally, such a method is evaluated in an isolated bee population with no transportation of colonies from outside into the area, no mating of queens outside the area, and by including all colonies in the treatment and requeening schedule. The bee population should consist of several hundred colonies for sufficient genetic variation and to avoid inbreeding problems (Büchler *et al*., 2013). Further, colonies should be kept according the established beekeeping routines of the area to explore any difficulties pertaining to the integration of the method into the pre-existing habits. The island of Marmara, Turkey, chosen for the evaluation came close to these requirements. We aimed to provide simple procedures for diagnosis and requeening which do not require a bee breeding background, and to assess the acceptance of the work steps by the beekeepers. We further wanted to define a safe infestation level for not treating and to find out whether leaving some of the colonies untreated and to requeen considerable parts of the population is a realistic option.

Materials and methods

Experiments were carried out on Marmara Island, Turkey (40° 37′N 27° 37′E) between autumn 2009 and summer 2012. The island has an area of 117 square km with a highest elevation of 709 m. Mean temperatures are 4.8^{0}C in January and 24.9^{0}C in July, and mean rainfall is lowest in July with 9.5 mm and highest in December with 95.8 mm. In particular mountain flanks along valleys are covered with Mediterranean shrub vegetation. Agriculture is sparse, predominantly vegetables and olive trees. There are approximately 500 bee colonies, kept by about 15 beekeepers. Honey yield is low (about 15 kg/colony per year), mainly from *Melia* spp. in spring, *Salvia* spp. in summer and *Arbutus* spp. in autumn, but sufficient to maintain the honey bee population. The colony development follows a typical south Mediterranean pattern with main brood periods in spring and autumn. Bees belong to the *A. m. anatoliaca* subspecies, with influences from mainland bee populations of western Black Sea and South Marmara as well as Southern Thrace. Conventional treatment for *V. destructor* is predominantly based on Amitraz preparations added to the smoker.

In autumn 2009, we started with 234 colonies in five apiaries belonging to four beekeepers. Colonies were transferred from old local and widely diverse bee hives into new Langstroth hives allowing more easy access for inspection. Colony status was rated in terms of presence of queens, numbers of frames covered by bees, and brood frames. To equalize starting mite populations to low levels, colonies were treated with Bayvarol®, and dead mites were counted on the bottom boards. Colonies were again inspected in Spring 2010, and colony infestation was assessed by washing bee samples in detergent water. Bottom inserts to register natural mite fall or dead mites after treatment were abandoned after Spring 2010. Different versions of Vaseline or oil covering, or Tangle-Trap® insect glue, were ineffective to prevent ants from carrying mites away in significant numbers, and were unrealistic in common beekeeping conditions (Büchler *et al.,* 2013). In comparison to natural mite fall measurements, worker bee infestation it is more adequate, as it measures colony infestation levels independent of colony sizes (Büchler *et al.*, 2010). Powder sugar shaking proved similarly efficient as detergent washing, as long as a sufficient quantity of dry powder sugar is used (Cakmak *et al.*, 2011; Poker *et al.*, 2011). The method proved to be fast and efficient and can be performed directly at the apiary in parallel to colony inspection. In particular, as bees survive seemingly unharmed and can be returned to their colonies it is more readily accepted by beekeepers.

In Summer 2010, 2011 and 2012, after honey had been extracted by the beekeepers, colony status was assessed and colony infestation was determined by bee samples shaken with powder sugar. Based on previous non-treatment experiments in Germany (Büchler *et al.*, 2010) and considering the mild Mediterranean climate with brood over most of the year, we left colonies untreated with < 1% infestation (= mites/100 bees), all others were treated with Varostop® containing flumethrin (in 2010 and 2011) or with CheckMite® containing coumaphos in 2012. Heavily infested colonies were requeened, but we retained a middle group including colonies of intermediate infestation, too weak for requeening (< 5 frames with bees) or newly requeened to reduce the risk of colony losses from requeening.

As sources for requeening, we chose a subset of 10 to 30 colonies from the non-treated group which were strong and healthy. In 2010, brood pieces with eggs and young brood were transferred from these donor colonies to the recipient colonies, in which queens had been caged six days before and remaining own queen cells had been removed. In summer 2011 and 2012, we produced queen cells in the donor colonies by removing their queens. We used 2-3 queen cells per recipient colony in which the own queens had been removed the day before. Between summer treatments, colonies were checked in autumn and spring to monitor colony development and to detect any disastrous developments in colony infestation levels. While colony status was determined in all colonies, mite levels were determined by sugar shaking in a subset of colonies, which included all non-treated colonies.

Notes were taken at each check on colony status and strength as well as infestation level for each of the consecutively numbered colonies at each of the checks. Data were evaluated using IBM SPSS Statistics 20 (2012) to determine infestation rates, colony development and survival.

Results

Assessment of infestation, treatment and requeening

Assessment of worker bee infestation by sugar shaking of 227 colonies distributed on five apiaries in the first days of August 2010 showed a mean infestation of 2.2 ± 0.15% (= mites/100 bees, here and hereafter mean ± SEM), with 50% of the colonies infested by less than 1.34 mites per 100 bees. However, some colonies were highly infested (maximum 13%) resulting in a skewed distribution of colony infestation typical in most investigations (Fig. 1). Based on this data, 48 (21%) colonies infested by less than 1% were considered low infested and were not treated, and 62 (27%) colonies considered high infested were treated using Varostop® and were requeened. The remaining 117 colonies (52%) of intermediate infestation or weak colony status (< 5 frames of bees) were only treated.

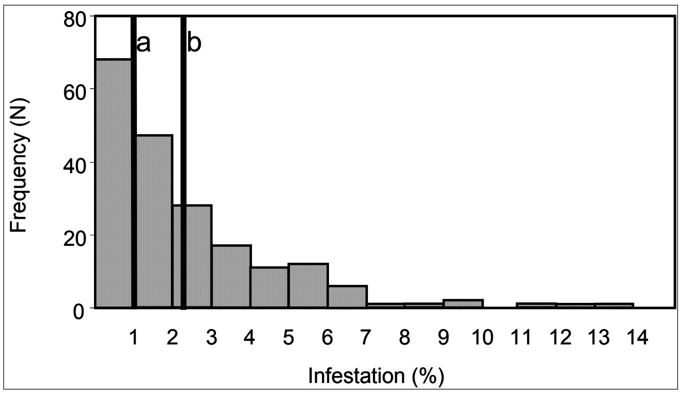

Fig. 1. Worker bee infestation by *Varroa destructor* mites derived from 169 samples of 300 bees by powder sugar shaking. Separation lines give limits for not treating (< a) and treating-requeening (> b).

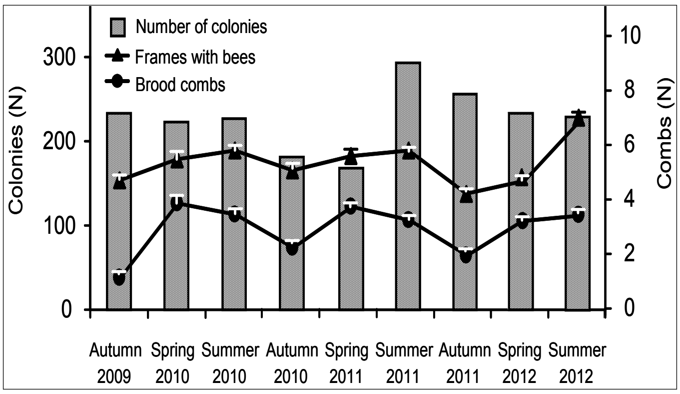

Fig. 2. Colony numbers (left ordinate) and colony strength as numbers of frames with bees and brood frames (means and SEM, right ordinate) over the course of the experiment.

Table 1. Treatment groups.

	Not treated	Treated only	Treated - requeened
2010	48 (21%)	117 (52%)	62 (27%)
2011	76 (26%)	110 (37%)	109 (37%)
2012	178 (69%)	25 (10%)	56 (21%)

Treatment groups in the following summers are given in Table 1. During the investigation, proportions of untreated colonies increased from 21% in 2010 (N = 295) to 69% in 2012 (N = 259), while the proportion of treated-only colonies decreased from 52% to 10%. The proportion of treated and requeened colonies was highest in summer 2011 and lowest in summer 2012, where infestation level was low. However, 17 out of the 76 colonies untreated in summer 2011 with infestation rates > 5% (mean 14.8%) were treated in autumn 2011 using Apivar®.

Requeening by transferring brood pieces with larvae from 26 selected low infested colonies to the high infested colonies resulted in a requeening success of > 85% in 2010. Requeening by transferring one to three queen cups from 22 selected low infested colonies to the high infested colonies resulted in a requeening success > 83% in 2011. Estimates were based on beekeeper’s records or on autumn checks where records were lacking.

Time course of colony numbers, strength, and infestation

Colony numbers and colony strength measures during the dates of inspection are summarized in Fig. 2. The average number of colonies between Autumn 2009 and Summer 2012 was 228.3 ± 11.9 (n = 9). There was an apparent annual pattern of peak values in summer followed by a decrease until spring of the following year, but there was no overall trend and the population remained stable over time (227 in summer 2010, 295 in summer 2011, and 230 in summer 2012). Similarly, colony strength in terms of frames covered by bees and brood frames showed seasonal variation but no significant overall trend.

Colony losses from summer treatment to next year’s summer are shown in Fig. 3 for the 3 treatment groups, with colony numbers during each summer treatment set as 100%. In total, losses were 29.1% and 30.5% from Summer 2010 to Summer 2011. Losses were not consistently related to colony strength or mite infestation in summer. In summer 2010, survival was lower in the treated-only group ($P < 0.005$, chi²-test), but in summer 2012, survival rates did not differ between the treatment groups. Each year losses were compensated by colony splits and swarms, predominantly between spring and summer, such that total numbers did not decline.

Mean worker bee infestation over time, as assessed by shaking bee samples with powder sugar, is shown in Fig. 4. In Autumn 2009, no powder sugar diagnosis was performed, but an average of 385 ± 9.4 (n = 234) dead mites was found on bottom inserts without apparent ant presence. In Spring 2010, no mites were discovered by powder sugar diagnosis in a subsample of 148 colonies. The average bee infestation of 2.2 ± 0.15% (n = 227) in Summer 2010 decreased to 0.74 ± 0.22% (n = 53) in Autumn 2010, due to the summer treatment of 79% of the colonies. It slightly increased until spring 2011 to 0.93 ± 0.16 (n = 81) and from there infestation reached a level of 4.5 ± 0.48% (n = 291) until summer 2011. Treating 74% of the colonies by then, the mean infestation dropped to 2.36 ± 0.18% (n = 142) until autumn. After treating further 17 high-infested colonies out of 76 colonies in the untreated group, average autumn infestation then was 0.69 ± 0.44 (n = 142), which was similar to the level recorded in the previous fall. Contrary to the previous year, infestation did not increase but decreased until next spring to a level of 0.26 ± 0.05% (n = 120), from which it increased to 0.86 ± 0.11% (n = 229) until Summer 2012. Thus, summer infestation had increased by 130% between Summer 2010 and 2011 but considerably decreased by 83% between Summer 2011 and 2012, mainly due to different mite population developments during the respective periods between autumn and spring.

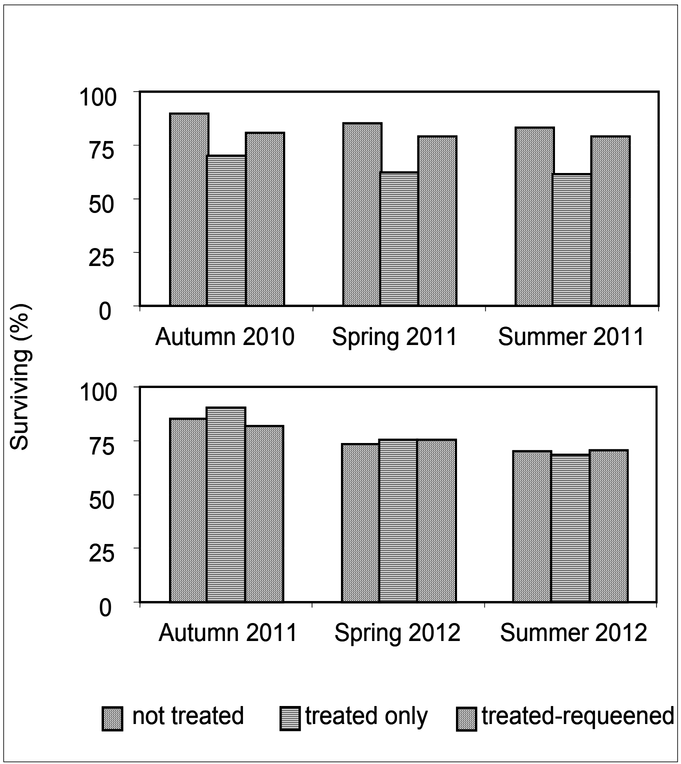

Fig. 3. Colony survival (%) in the treatment groups from the respective summer treatment (100%) until the following summer, for two treatment periods.

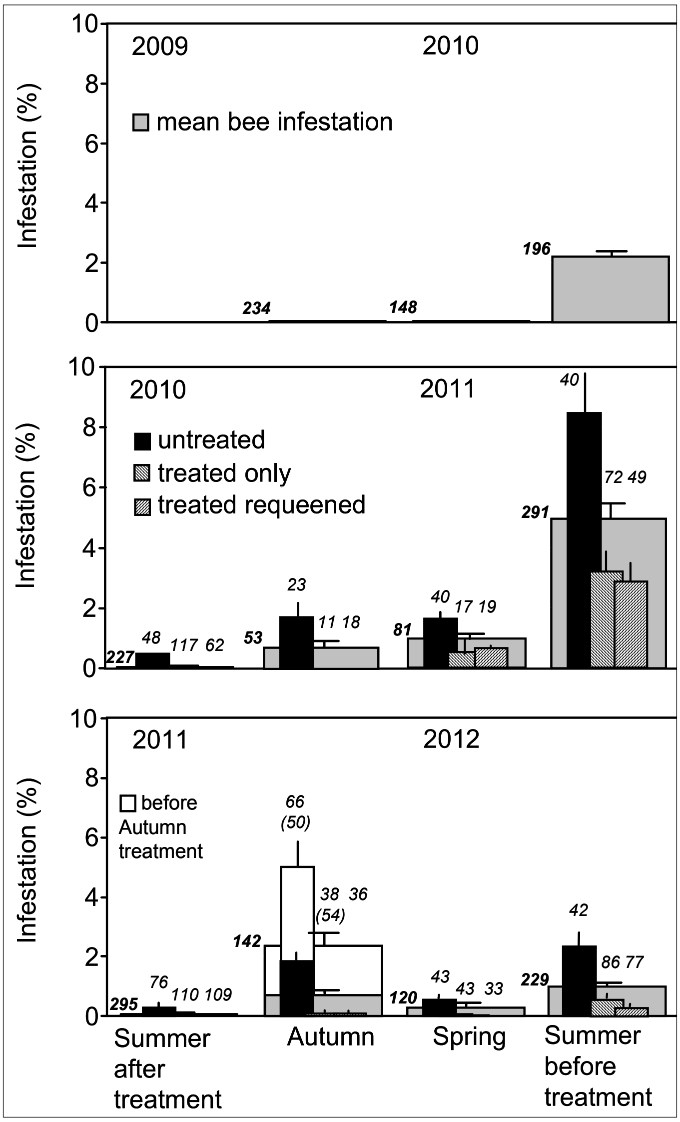

Fig. 4. Mean worker bee infestation with *V. destructor* assessed by powder sugar shaking of bee samples for the three seasons of investigation. Colonies were established in Autumn 2009 (no bee samples), and first treatment-requeening performed in summer 2010 (upper graph). The middle graph shows infestation after summer treatment 2010 until summer 2011, for all sampled colonies and for the sampled colonies from treatment groups established in summer 2010. The lower graph shows infestation after summer treatment 2011 until summer 2012, with the treatment groups established in summer 2011. Error bars show standard errors of the means, sample sizes are given in bold italics at the top left corner of columns for all colonies, and in plain italics above the columns for the treatment groups. In autumn 2011, means are given before and after treating 16 colonies from the not treated group.

Infestation differed between the treatment groups as defined by the previous summer group assignments. As expected the infestation was considerably higher in the untreated group and increased to a level of 8.5 ± 1.32% (n = 40) in summer 2010. However, no colony breakdown was reported up to then. Some of these non-treated colonies still had very low infestation (4 < 1%, 10 < 2% out of 40 colonies). After renewed grouping in summer 2011 (and additional autumn treatment of 17 untreated in summer), mean infestation of the untreated colonies in summer 2012 was only 2.17 ± 0.44% (n = 42), with 50% of the untreated colonies below treatment threshold (< 1% in 21 colonies). In the treated colonies, infestation was very low in autumn and spring (< 1% in both years), but still was below 4% in summer. Infestation was lower in the treated-requeened group compared to the treated-only group in both summers (2011: 25%. 2.97 ± 0.60, n = 49 and 3.96 ± 0.46, n = 72; 2012: 50%. 0.32 ± 0.06, n = 77; 0.64 ± 0.13, n = 86). However, this was significant only in 2012 (p = 0.036, t-test).

Discussion

The current evaluation method demonstrates several important points. For one, it demonstrates that a schedule in which about one third of the colonies are left untreated can be implemented over an extended period without apparent risk of losing inappropriately high numbers of colonies. It also shows that requeening a substantial portion (~30%) of the treated colonies did not unduly affect their survival chances. This supports the idea that a "good strategy" treatment schedule consisting of diagnosis followed by infestation-dependent treatment and requeening could be successfully applied. It further demonstrates that methods could be simplified to a degree which would make them applicable for ordinary beekeeping without unrealistic demands in terms of additional work or sophistication of methods.

After the initial overall treatment in Autumn 2009, colony numbers varied seasonally but in general slightly increased until Summer 2012, thus indicating a stable overall situation. Yet, colony losses of up to 30% between summer treatments and spring inspections indicated considerable colony turnover. Though these losses might seem unfeasibly high, they were not considered extraordinary by the beekeepers themselves. Their main cause was the type of Mediterranean beekeeping which involves maintaining very small colonies over dry summer and cold winter seasons, and regularly replacing losses by splits and swarms. Losses were similar or even lower in the untreated group compared to the treated and treated-requeened groups, supporting the view that not treating about one third of the colonies did not demonstrably add to colony losses.

Colony losses in the treatment groups, though roughly similar, seemingly differed in their causes. Non-treated colonies generally had higher mite loads and this probably affected colony strength though no major collapses due to *V. destructor* were reported. The treated-only group contained weaker high-infested colonies with lower prospects of overwintering successfully. In the treated-requeened group colonies were stronger, but colony losses were increased by requeening failures. As beekeeper's reports were incomplete, an upper limit estimate including all losses until autumn indicated reqeening losses well below 16%, considered acceptable under end-of mating season conditions and potential threats from bee-eaters at this time of the year.

Peak pre-treatment summer worker bee *V. destructor* infestation levels reached about 2%, 5% and 1% in 2010, 2011, and 2012, respectively, thus remaining below a level of immediate danger to the bee population. The high efficacy of the treatment is demonstrated by very low autumn infestation levels below 1% in both years in the treated-only and the treated-requeened groups. In the untreated group with less than 1% infestation the average infestation in the following summer was about 8% in 2011 and 2% in 2012 and no *V. destructor* related colony losses were apparent until then. However in Autumn 2011, 16 of the untreated colonies had dangerously high (<5%, maximum 35%) infestation and needed to be treated. This suggests that the level used for not-treating of < 1% in summer is adequate to ensure survival of the untreated colonies over a year until the next summer treatment. However, it is also concluded that an additional powder sugar infestation check is necessary. In a Mediterranean climate, attainment of a one year interval between treatments is a fairly favourable result. The necessity for one additional check in autumn or spring to detect single colonies which exhibit outstandingly high mite numbers, probably caused by re-infestation, seems acceptable, and might be not necessary in temperate climates, or under conditions without any re-infestation.

We did not detect or expect to detect a recognizable progress in mite tolerance in such a short time interval. However, a fairly low mean infestation in the second year can be seen as encouraging, though other reasons than an increase of tolerance might be responsible. The main difference was that between autumn and spring the mite infestation had increased in the first year but decreased in the second. Population development between spring and summer was similar. A further encouragement comes from the lower mite infestation in the treated-requeened as compared to the treated-only colonies, but again this might be based on effects of requeening as such rather than an increase in queen quality. Whether higher tolerance had already started to develop can be determined only in the long run, and finally comparisons with non-selected mainland queens will be required.

The selection scheme proposed here may appear, in comparison to organised high-tech queen breeding, fairly messy and inefficient. It needs to be emphasized that the proposed schedule is not intended to replace or compete with other efforts of breeding for mite resistance. There are certainly much more organised and efficient ways available in honey bee breeding, involving sophisticated methods focussing on resistance traits, pedigree based character evaluation methods, or using molecular markers (Büchler *et al.*, 2013). These methods, however, require scientific institutions and well-organised bee breeders, and a high degree of technical skills, organisation, book keeping and modern theory to progress in the fastest possible way. The current approach addresses a different and very basic organisational level, that of ordinary beekeepers, as a mandatory complementation. Even if resistant queen lines are produced by institutes and / or queen breeders, e.g. based on the promising hygienic behavioural traits (Spivak and Gilliam, 1998; Harris, 2007; Ibrahim *et al.,* 2007; but see Cakmak 2010), selling these to beekeepers will remain ineffective if habits at beekeepers level keep counteracting the resistance goal. All those good genes will be continuously lost again, and stabilising a tolerance level would necessitate a constant inflow of resistant queens. However, if current beekeeping adopts a scheme which, however slightly, selects for higher resistance, resistance traits originating either from this beekeeping level selection itself or from queens specifically bred for resistance can accumulate in the population.

But can a beekeeper-level selection work at all? The main support for this comes from evidence strongly indicating that natural selection working on this parasite-host system can indeed lead to reduced host damage under no-treatment situations. First, a low-damage host-parasite relationship of *V. destructor* has evolved in relation to its original host, *A. cerana,* based on a number of mechanisms not or only to a lesser degree existing in the *V. destructor / A. mellifera* relationship (Rath, 1999). Further, particularly in some tropical regions, *A. mellifera* colonies do survive without treatment. There is a solid body of research confirming reduced damage levels with a high local variation of responsible traits in South and Middle American Africanized (De Jong, 1996; Guzman-Novoa, 1999; Carneiro *et al*., 2007) and non-Africanized bee populations (De Jong and Soares, 1997, Ruttner *et al*., 1984). Early incidences of resistance after population breakdown was reported by Ritter *et al* (1990) in Tunesia, and recent reports from France. LeConte *et al*. (2007) provided data on considerably less susceptibility of bee strains exposed to *V. destructor* for a long time. Rinderer *et al.* (2001) imported *A. mellifera* queens from the Primorski region where exposure to *V. destructor* reached back about a century and indeed found them markedly less susceptible than local American strains. There is solid evidence that significant adaptation by natural selection can be attained within a time period of less than a decade. By comparing the impact of *V. destructor* on a feral bee population, Villa *et al.* (2008) reported initial bee population decline to about 60% followed by a recovery of the population to pre-infestation levels within 5 years. Seeley (2007) documented survival of a feral bee population in the Arnot Forest (New York State, USA) in spite of infestation with the parasite. Allsopp (2006) documented the impact of *V. destructor* after the parasite's arrival in South Africa in 1997, and observed the development of tolerance after initial damage in an unmanaged population of *A. m. capensis* within 4-5 years, and a significant decrease in susceptibility in *A. m. scutellata* within 7 years. In controlled experiments on the islands of Unije (Croatia) and Gotland (Sweden) populations of several hundred bee colonies were left to natural selection. While the Unije bee population succumbed to the parasite with no colonies left after four years (Büchler, 2002), in Gotland the population first declined but then stabilized with colonies surviving and reproducing after 5 years (Fries *et al*., 2003).

While various resistance traits have been identified in the bee (see Rosenkranz *et al.,* 2010), it is an open question whether *V. destructor* also responds to selection. Seeley (2007) concluded that in the Arnot Forest situation change in mite virulence led to co-existence, and mite adaptation was concluded to be the cause of lower reproduction in an Austrian selection experiment (Milani *et al*., 1999). However, Fries and Bommarco (2007) found no evidence for changes in the mites in the Gotland experiment. Nevertheless, rapid acaricide resistance in *V. destructor* populations proves that they are able to adapt to new conditions (Eltzen *et al*., 1998; Milani, 1994; Pettis, 2004), in spite of their clonal population origin and constraints on sexual gene mixing (Solignac *et al*., 2005).

Selecting on a parasite-host system level does not specify any particular mechanisms, nor select for specific mechanisms bringing about a higher tolerance level. Though this might work considerably more slowly than by selecting specific traits, it allows the possibility of the appearance of resistance factors not yet identified. Also, by selecting within local populations, their specific adaptations can be retained, which is important as bees found to be tolerant in one location have often not performed convincingly in other locations (Berg *et al*., 2005).

This first implementation of a "good strategy" treatment scheme for *V. destructor* also identified some specific difficulties to be encountered in broad-scale application. These are mainly on the human rather than bee level. We had chosen Marmara Island in order to be able to include virtually all bee colonies there in the trial, and to ensure isolation from mainland populations. It then became apparent that we had included only about half of the colonies, due to non-reported beekeeping operations, and a significant increase of colonies during our experiment. We also underestimated the degree of honey bee colony transport to or from the mainland, made easier by newly established ferry connections. We could counteract this by formal governmental prohibition, but only in the second year. Also we encountered some scepticism on the part of some beekeepers who did not join the project. In general, even beekeepers in the project were initially more interested in immediate returns as honey yield, reducing work load, and effective treatments and only slowly began to appreciate the possible long term rewards, such as increased mite tolerance. However, over the duration of the project more beekeepers became interested, mainly convinced by the better health of the with-in-project colonies, and beekeepers in the project became increasingly cooperative. These experiences underlined that a major part of the varroa problem or its solution is to induce changes in beekeeper habits and convictions.

Therefore a crucial component of this project's work is to provide uncompromisingly simple and straightforward methods to increase acceptance. Diagnosis by powder sugar shaking of bee samples proved to be a considerable simplification. Colony infestation can now be noted on the colonies during inspection together with their eligibility for breeding or requeening, allowing on the spot decisions without the need for list keeping. However, apart from simple labour-effective decision rules, social skills are needed to motivate, to provide incentives and to increase understanding which are indispensable to spread the application of these simple routines. Though progress on this side of the problem also takes a long time, the present positive trend is an encouragement to proceed with the "good strategy" project on Marmara, or possibly to adopt the strategy in other places as well.

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