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ORIGINAL RESEARCH ARTICLE

The effect of the 'Bee Gym™' grooming device on *Varroa destructor* mite fall from honey bee (*Apis mellifera*) colonies

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Grooming is a honey bee behavior that has the potential to minimize and manage the detrimental effects of *Varroa destructor*. Here we tested the efficacy of the Bee Gym™, a device hypothesized to increase honey bee auto-grooming and increase mite removal from colonies. Natural mite fall from 20 colonies was counted for 14 days, after which half the colonies were fitted with a Bee Gym and half with a control object. Mite fall and the proportion of damaged mites were then recorded for another 14 days. Total mite fall was generally higher over the second 14 days, but this increase was not significantly higher for the Bee Gym colonies than for the control colonies. There was also no difference in the proportion of damaged mites between the two treatments. Mite fall and damage to mites may be influenced by other factors, and this is discussed; however, given that we found no effect of the Bee Gym, we conclude that there is no evidence from this study of its efficacy as a management strategy for *V. destructor*.

Efecto del dispositivo de aseo 'Bee Gym ™' sobre la caída del ácaro *Varroa destructor* en colonias de abejas melíferas (*Apis mellifera*)

El aseo es un comportamiento de la abeja de la miel que tiene el potencial de minimizar y de manejar los efectos perjudiciales de *Varroa destructor*. Aquí hemos probado la eficacia del Bee Gym ™, un dispositivo que hipotéticamente aumenta el auto-aseo en la abeja de la miel y aumentar la eliminación de ácaros de las colonias. La caída natural de ácaros de 20 colonias se contó durante 14 días, tras lo cual la mitad de las colonias fueron equipadas con un Bee Gym y la otra mitad con un objeto de control. La caída de ácaros y la proporción de ácaros dañados se registraron durante otros 14 días. La caída total de ácaros fue generalmente mayor durante los segundos 14 días, pero este aumento no fue significativamente mayor para las colonias con Bee Gym que para las colonias de control. Tampoco hubo diferencias en la proporción de ácaros dañados entre los dos tratamientos. La caída de ácaros y el daño a los ácaros pueden estar influidos por otros factores, lo cual se discute; sin embargo, dado que no encontramos ningún efecto del Bee Gym, concluimos que no hay evidencia en este estudio de su eficacia como estrategia de manejo para *V. destructor*.

Keywords: *Apis mellifera*; Bee Gym; bottom boards; grooming; honey bee; mite fall; *Varroa destructor*

Introduction

Varroa destructor Anderson and Trueman 2000, is a major pest of *Apis mellifera* L. 1758, and is present in the majority of honey bee colonies worldwide (Rosenkranz, Aumeier, & Ziegelmann, 2010). *V. destructor* reproduce in honey bee brood cells (Boecking & Genersch, 2008), and directly harm the bees by attaching to and feeding from the haemolymph of the brood and the adults (Bowen-Walker & Gunn, 2001). They also act as vectors for several bee viruses (e.g., Bowen-Walker, Martin, & Gunn, 1999). These detrimental effects may act synergistically with other stressors and, without management, the majority of *V. destructor*-infested colonies will collapse (Guzmán-Novoa et al., 2010; Le Conte, Ellis, & Ritter, 2010; Rinderer et al., 2001).

There are several strategies available to beekeepers for managing *V. destructor* numbers (e.g., Calderone, 2005; Rademacher & Harz, 2006; and see Rosenkranz et al., 2010 for a review), although each has its limitations (e.g., Higes, Meana, Suárez, & Llorente, 1999; Lodesani, Colombo, & Spreafico, 1995; Martin, 2004). The effort and money required to treat *V. destructor* has likely been a factor in making beekeeping less attractive (Potts et al., 2010; Rosenkranz et al., 2010) and, given the current limits in treatment methods, there is a need for new solutions for managing infestations.

Some *A. mellifera* strains are resistant to the mites (Martin & Medina, 2004; Rinderer et al., 2001) giving hope for alternative approaches to managing *V. destructor*. Two behavioral traits have been identified that may contribute to the resistance: Varroa-sensitive hygiene and grooming (Boecking & Spivak, 1999; Rinderer, Harris, Hunt, & de Guzman, 2010). Varroa-sensitive hygiene is the ability of bees to remove infested brood (Evans & Spivak, 2010; Harris, 2007), and there has been some success breeding honey bee strains with this trait that have increased resistance to *V. destructor* (Rinderer et al., 2010). In contrast, evidence that grooming leads

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to increased resistance is poor for *A. mellifera*, and the breeding of high-grooming, mite-resistant lines of bees has not yet been successful (Rinderer, De Guzman, & Frake, 2013).

Despite this, many studies have demonstrated that *A. mellifera* are able to remove mites through grooming, even though they are not always very effective at doing so. Evidence for this has been shown both at the colony level (Arechavaleta-Velasco & Guzmán-Novoa, 2001; Guzmán-Novoa, Emsen, Unger, Espinosa-Montaño, & Petukhova, 2012; Mondragón, Spivak, & Vandame, 2005; Rinderer et al., 2001) and through assays inoculating individual bees with mites (Aumeier, 2001; Büchler, Drescher, & Tornier, 1992; Fries, Huazhen, Wei, & Jin, 1996; Guzmán-Novoa et al., 2012; Peng, Fang, Xu, & Ge, 1987). These are reviewed in detail by Rinderer et al. (2013), and also see Pritchard (2016).

Grooming behavior can be split into auto-grooming, where a bee grooms itself, and allo-grooming, in which a bee is groomed by others, often initiated through a recruitment dance (Evans & Spivak, 2010; Land & Seeley, 2004). In *A. mellifera*, auto-grooming seems to be the most frequently observed behavior in response to mites (Aumeier, 2001; Fries et al., 1996). This typically involves bees wiping their body with their legs, but can include the bees grasping mites with their mandibles (Boecking & Spivak, 1999; Guzmán-Novoa et al., 2012).

More substantial evidence that grooming is potentially important for mite resistance comes from *Apis cerana* Fabricus 1793, the natural host of *V. destructor*. *A. cerana* are in a balanced host-parasite relationship with the mites and, although other factors may play a part, grooming is believed to contribute to this (Boecking & Spivak, 1999; Rosenkranz et al., 2010). Notably, the grooming behavior of *A. cerana* in response to the mites is stronger and more effective than in *A. mellifera* (Boecking & Spivak, 1999; Büchler et al., 1992; Fries et al., 1996; Peng et al., 1987; Rosenkranz et al., 2010). This suggests that methods to enhance grooming in *A. mellifera*, either through selecting high-grooming lines or through improving the effectiveness of the behavior in other ways, could increase mite removal and colony resistance.

The Bee Gym™ (http://www.beegym.co.uk/) is a device proposed to effect mite removal from bee colonies by increasing auto-grooming behavior. It consists of a 110 × 117 mm plastic frame with a range of rough protrusions such as spikes and semi-rigid flaps (Figure 1(a)). It is hypothesized to work by functioning as a "scratching post", allowing adult workers to actively groom themselves against the protrusions, increasing mite removal from the colony. It is designed to be placed in hives throughout the year, either directly underneath or above the brood frames (Stuart Roweth, Personal Communication, 2014). As a potential management strategy that is non-chemical, cheap, and easy to implement, the Bee Gym could be a valuable addition to the catalog of methods for managing *V. destructor*.

Here we tested the function of the Bee Gym in an apiary trial by recording mite fall from honey bee colonies. If the Bee Gym is effective then, following its introduction to a hive, mite fall should increase above the level expected from normal mite population growth. Mite fall was recorded in 10 hives over two periods of 14 days, before and after Bee Gym treatment. As a control, mite fall was recorded for the same duration in a further 10 hives with no treatment. As a second measure to determine the Bee Gym's efficacy, the proportion of damaged mites was recorded over the second 14 days.

Materials and methods

Hives and study sites

The trial was completed with 20 honey bee colonies, with a range of *V. destructor* infestation levels. All colonies were in National hives, and were spread across 4 apiaries: Impington (IM; 10 hives), King's College (KC; 2 hives), Trinity College (TC; 4 hives) and Cambridge University Botanic Garden (BG; 4 hives). These were all within a 6 km radius of the centre of Cambridge, UK, and thus experienced similar environmental conditions throughout the trial.

Mite counting

All hives were fitted with open mesh floors. The number of mites falling out of the hive was recorded using sticky drop/bottom boards placed beneath the mesh floor at the bottom of the hives (Dietemann, Nazzi, & Martin, 2013). These were coated with a film of petroleum jelly to prevent mites escaping. The number of mites on a board was recorded with the aid of a counting grid. After counting, the drop boards were scraped clean and recoated with petroleum jelly before reinsertion into hives. Mite numbers were counted on average every 2 days, although, as it was rarely possible to count mite fall from all hives on a single day, hives sometimes had 1 or 3 days between counts.

Mite fall was counted for 14 days with no treatment (7 counts per hive). The drop boards were then removed, and, on day 15, the hives received either a Bee Gym (10 hives), or a control frame (10 hives). These were placed onto the mesh floor at the bottom of the hive. The control frame was the same size and material as the Bee Gym but with all rough edges and protrusions removed or sanded down (Figure 1(b)). The hives were given a day to settle following disturbance, with drop boards replaced on day 16. Mite numbers were then recorded for the following 14 days (7 counts per hive). For the second 14 days, counted mites were classified as undamaged or damaged with the aid of a 10 × hand lens. Mites were classed as damaged if they had missing legs or mouthparts or if there were

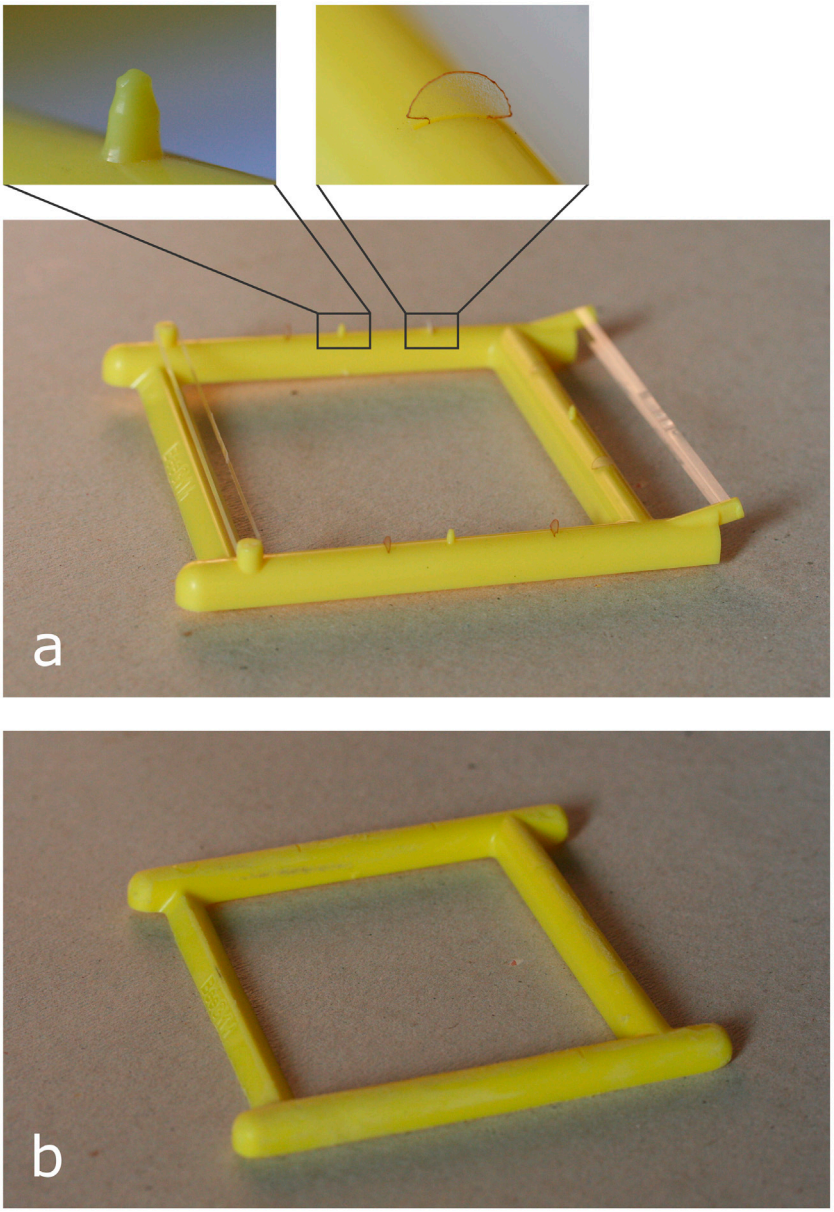

Figure 1. (a) The Bee Gym, with close-ups of two of the nine grooming protrusions. These vary in stiffness. (b) The Control Frame, a Bee Gym with all grooming protrusions removed or sanded to be smooth.

sections of the dorsal shield (idiosoma) or ventral shield missing. Mites with a dented idiosoma were not included as damaged as this has been shown to be ontological (Davis, 2009). All living mites were included in the undamaged category. Mite counting started on 30 June and finished on 30 July 2014.

Randomization process and exclusion criteria

In addition to the 20 colonies that completed the trial, a further 11 colonies initially present were removed. Five colonies were removed during the first two weeks. Four colonies had a very low mite infestation and one underwent a colony merger.

The 26 colonies that entered the second two weeks of the trial were assigned the treatment or control randomly, with the following constraints: (1) The number of treatment and control hives was constrained to be equal within apiaries with an even number of hives (IM and BG) and equal across the combined hives from the apiaries with odd numbers of hives (TC and KC) (ensuring within these apiaries that the disparity between control/treatment hives was not greater than one). (2) The treatment was randomised separately for four hives at IM that had low mite fall (again with equal numbers assigned control/treatment). The counting was performed blind with respect to which hives had the Bee Gyms or

control frames; the person recording mites did not know the treatment assignments of each hive.

Brood presence/absence was recorded for all colonies during the trial, as this can have significant effects on mite fall (Branco, Kidd, & Pickard, 2006; Lobb & Martin, 1997). As six colonies had brood absent for a portion of the trial, these were removed from the analyzes, leaving the final 20 colonies that completed the trial.

Statistical analysis

Mite fall was summed for each colony for the 14 days pre-treatment and 14 days with-treatment and adjusted for the actual number of hours each drop board was in place, giving a mean fall per 14 days per colony. The data were analyzed considering both absolute and proportional change in mite fall rate. A two-sample Wilcoxon test was used to test for a difference in the proportional change in mite fall between the Bee Gym and control colonies from the first 14 days to the second 14 days. To investigate absolute changes in mite fall, the data were modeled using a linear regression with mite fall after as response and mite fall before and treatment as predictors. Fall after and before were log transformed using natural logarithms to account for non-constant variance of residuals. To test whether there was a higher proportion of damaged mites in the mite fall from the Bee Gym colonies than the control colonies for the second 14 days, the proportion of damaged mites was modeled using a generalized linear model with a binomial error structure. For two colonies which had a very high daily fall, mites were not split into the two categories due to time restrictions of the recording schedule. This left 18 colonies which were analyzed for damage to mites. All statistics were carried out using R version 3.1.3 (R Core Team, 2015).

Results

For the 20 analyzed colonies, 18,566 mites were counted over the 28 days. Mite fall varied considerably between colonies. The colony with the highest fall had a mean daily fall rate of 212 mites per day, and seven further colonies had daily fall rates higher than 30 mites per day. In contrast, nine colonies had a mean daily fall rate lower than 10 mites per day. The remaining three colonies had daily fall rates between 10 and 15 mites per day. The mean fall rate for the 20 colonies was 34 mites per day and median 11 mites per day with interquartile range 5.1–38.9. Mite fall also varied considerably from day to day within colonies, particularly in the colonies with a lower fall rate (Figure 2).

Proportional changes in mite fall

The majority of colonies showed an increase in mite fall from the first 14 days (fall before) to the second 14 days (fall after). The median proportional increase in fall from before to after was 0.73 for the Bee Gym colonies (interquartile range 0.22–1.24) and 1.25 (0.89–1.45) for the control colonies, and there was a larger variability in proportional increase for the Bee Gym colonies (Figure 3(a)). There was no significant difference in proportional increase in mite fall between the Bee Gym and control colonies (Two-sample Wilcoxon test, $W = 35$, $p = 0.28$).

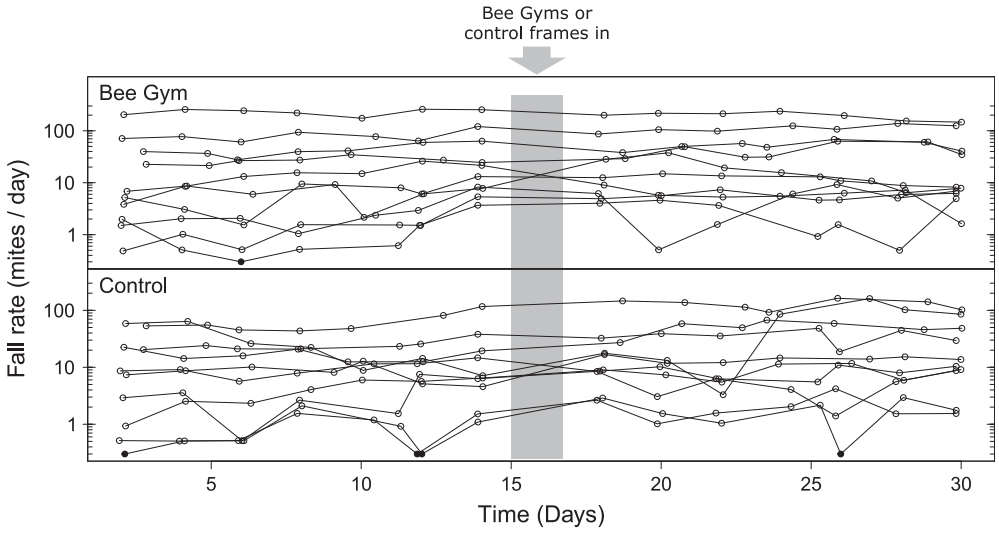

Figure 2. Mite fall rate (mites per day) at each count over 30 days with 1 count approximately every 2 days for the Bee Gym treatment colonies ($n = 10$) and the control colonies ($n = 10$). Bee Gym or control frame treatment started on day 15. Counts where the fall rate was 0 are plotted as solid circles, and have been given a nominal value of 0.3 mites per day, to allow plotting on the log axis.

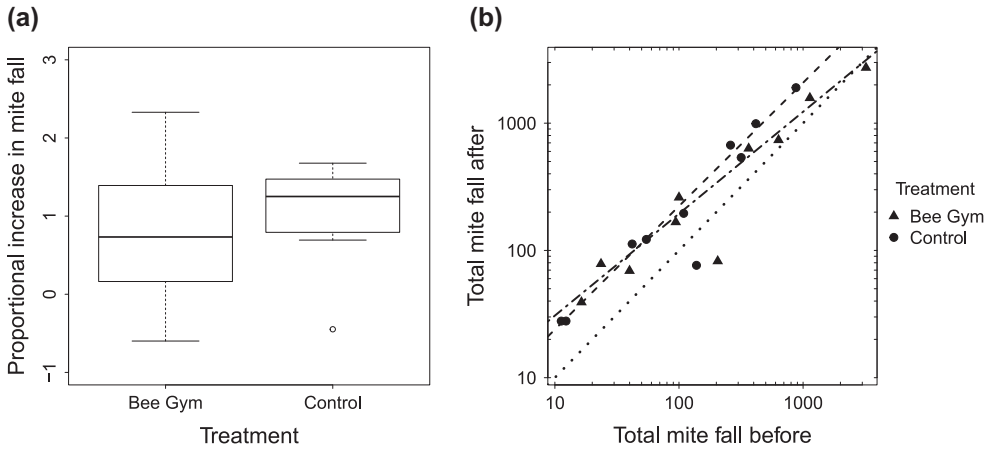

Figure 3. The change in mite fall for the Bee Gym and control colonies represented as: (a) Proportional increase in mite fall from the first 14 days to the second 14 days for the Bee Gym (n = 10) and control colonies (n = 10), giving medians (central line), and interquartile ranges (boxes); whiskers include all observations within 1.5 interquartile ranges of the boxes, (b) Total mite fall after Bee Gym/control frame insertion vs. total mite fall before insertion for Bee Gym (triangles) (n = 10) and control (solid circles) colonies (n = 10). Mite fall was adjusted for the number of hours the drop boards were in place, giving non-integer values. Fitted regression lines are for the data with the two outliers removed (dashed line = control hives; dashed and dotted line = Bee Gym hives); the dotted line is $y = x$ representing no difference from before to after.

Absolute changes in mite fall

Mite fall after was significantly correlated with fall before for the log-transformed data (slope = 0.848, CI 95% = [0.695; 1.001], intercept = 1.28, CI 95% = [0.49; 2.07], adjusted R^2 = 0.88, F = 136, $p < 0.0001$) (Figure 3(b)). If the Bee Gym significantly increased mite fall for all colonies (regardless of the level of mite fall), this would be reflected in a significant increase in intercept of the regression model, assuming no significant change in slope. If the Bee Gym increased mite fall differently between colonies depending on their fall rate, this would be reflected in a significant change in the slope of the model. There were no significant differences between the Bee Gym and control colonies in slope (t = 1.04, p = 0.31) or intercept (t = 0.762, p = 0.46) of the regression fit.

Two colonies had a markedly smaller fall after than fall before (Figure 3(b)), one with Bee Gym treatment and one control colony, and these two colonies are notable outliers in the model fit with large residuals. As it is possible that a change in colony state could have caused the low fall after in these hives, the data were remodeled with these colonies removed (giving N = 18). For this new regression fit for the Bee Gym colonies there was a small significant decrease in the slope ($\beta = -0.169$, CI 95% [−0.294; −0.044], t = 2.90, p = 0.012), and marginally significant increase in intercept (β = 0.640, CI 95% [0.004; 1.276], t = 2.16, p = 0.049) compared to the regression fit for the control colonies (Figure 3(b)). Thus mite "fall after" in high-infestation colonies was slightly lower for the Bee Gym hives than for the control colonies.

Mite damage

Mites that fall from a hive as a result of grooming activities may be more likely to be damaged, thus mite fall was split into damaged and undamaged mites. There was no significant difference between the percentage of mites which were damaged for the Bee Gym and the control colonies (z = 0.272, p = 0.79) with respective model-predicted means 34.0% CI 95% [31.8; 36.2] and 34.3% CI 95% [32.7; 35.9].

Discussion

The effect of the Bee Gym on honey bee grooming of *V. destructor* was tested with two measures of mite fall out of a colony: total fall, and the proportion of damaged mites. Mite fall generally increased from the 14 days pre-treatment to the 14 days with Bee Gym or control treatment, as expected given typical mite population growth over the summer when brood are being raised (Boecking & Genersch, 2008). If bees were able to remove more mites through grooming in the hives with the Bee Gym fitted, we would expect to see an increase in the mite fall for the 14 days with treatment over that observed in the control colonies. However, there was no difference in the mite fall increase between the Bee Gym treatment and the control colonies. There was also no difference between the percentage of damaged mites between the Bee Gym and control colonies.

The significant difference of the slope of the regression line between treatment and control colonies when the data were remodeled with the two outliers removed implies that the Bee Gym colonies with high initial fall had a lower fall after, compared to the control hives of similar initial fall. This is contrary to the expected effect if the Bee Gym was effective as a grooming device. The coincident increase in intercept is small and only marginally significant and is not well

reflected in the actual values for Bee Gym colonies with low fall (Figure 3(b)). Moreover, the significant difference in slope appears to be the result of a few high-leverage points, corresponding to high fall colonies, affecting the fit of the regression line (Figure 3(b)) and should be treated with caution. We conclude that this change in slope is not evidence of a significant positive effect of the Bee Gym. Hence, there is no clear evidence from this study that the Bee Gym increases mite fall through enhanced grooming by the bees.

The presence of brood has a large effect on mite fall (Branco et al., 2006; Lobb & Martin, 1997). When brood is present, the majority of mites on drop boards are those associated with emerging brood (Lobb & Martin, 1997), with hygienic behavior and phoretic mites making up the remainder. As the Bee Gym is predicted to target phoretic mites, any effect would be to increase this portion of mite fall only. Many of the colonies had comparatively high *V. destructor* infestation, given their observed daily fall rates (Figure 2). If the Bee Gym only had a small effect on increasing mite fall through increased grooming, this may have been undetected because of the large numbers of mites associated with emerging brood in these high infestation colonies.

Mite fall was highly variable within colonies from count to count (Figure 2). Although it is possible that this variability could also obscure any increase in the portion of mite fall comprised of groomed phoretic mites, here mite fall was summed over two-week periods. Mite fall counted over two weeks has been shown to have a good correlation with colony mite population (Branco et al., 2006) averaging out day-to-day variability. For the majority of the colonies, fall before showed a good correlation with fall after in the regression fit of log-log data (Adjusted $R^2 = 0.88$, $F = 136$, $p < 0.0001$) (Figure 3(b)), suggesting that brood or other effects on mite fall were relatively constant when averaged over the recording period.

Recording the proportion of damaged mites may avoid the problem of the source of the fallen mites, by more directly considering that portion of mite fall related to grooming (Andino & Hunt, 2011). The observed percentages of damaged mites recorded here, 34.0% for the Bee Gym and 34.3% for the control colonies, are similar to results from previous studies; for example Rinderer et al. (2013) recorded 29% mites as damaged. There was no significant difference observed between the Bee Gym and control colonies.

There is, however, some debate about the reliability of using damaged mites for determining grooming level of a colony (Guzmán-Novoa et al., 2012; Rinderer et al., 2010). Damaged mites may result from causes other than grooming by adult bees (Rinderer et al., 2010). It is also not known whether mites removed through grooming using an external object would show similar damage to those removed by auto- and allo-grooming. It has been suggested that damage to mites from bees using their mandibles may mainly result from allo-grooming (Invernizzi, Zefferino, Santos, Sánchez, & Mendoza, 2015). If this were the case here, then mite damage would not be a reliable indicator of efficacy of the Bee Gym. Different honey bee strains may differ in their ability to remove mites through grooming (Bahreini & Currie, 2015; Guzmán-Novoa et al., 2012). Here, the genetic background of the colonies was not controlled, and a grooming-dependent treatment might only be effective in high-grooming honey bee strains.

Although the drop boards were covered with petroleum jelly to both trap the mites and prevent removal of trapped mites by other insects, ants were observed on the drop boards of some hives. It is possible that these affected the counts of mite fall (Dainat, Kuhn, Cherix, & Neumann, 2011). Petroleum jelly is suggested as a preventative measure to stop ant predation of *V. destructor* from drop boards (Dietemann et al., 2013) but we suggest that more substantial measures, such as those used by Dainat et al. (2011), should be taken to prevent ant predation when measuring *V. destructor* fall.

Grooming is a promising area for improving *V. destructor* management (Rinderer et al., 2013); however, from this investigation we find no detectable effect of the Bee Gym grooming device on *V. destructor* removal in honey bee colonies over a 2 week period. The measures used for assessing grooming efficacy are still under debate, and it is possible that an effect could be missed, or obscured by other confounding factors. Nevertheless, to our knowledge, there is currently no evidence that bees use external objects to groom. If further work is carried out on this as a potential treatment method, we therefore suggest that a sensible initial step would be to directly test the potential of grooming devices in assays with individual bees.

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Underlying research materials

The underlying research materials for this article can be accessed at http://dx.doi.org/10.17863/CAM.6364.

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