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DISSERTATION / DOCTORAL THESIS

Titel der Dissertation /Title of the Doctoral Thesis

„Breeding Carnolian bees *Apis mellifera carnica* (Pollmann 1879) (Hymenoptera: Apidae) **on different comb cell sizes and analysing the effect of the different cell sizes on Varroa** *Varroa destructor* (Anderson & Trueman 2000) (Mesostigmata:Varroidae) **infestation rates."**

verfasst von / submitted by

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This thesis is dedicated to my family and my honeybees.

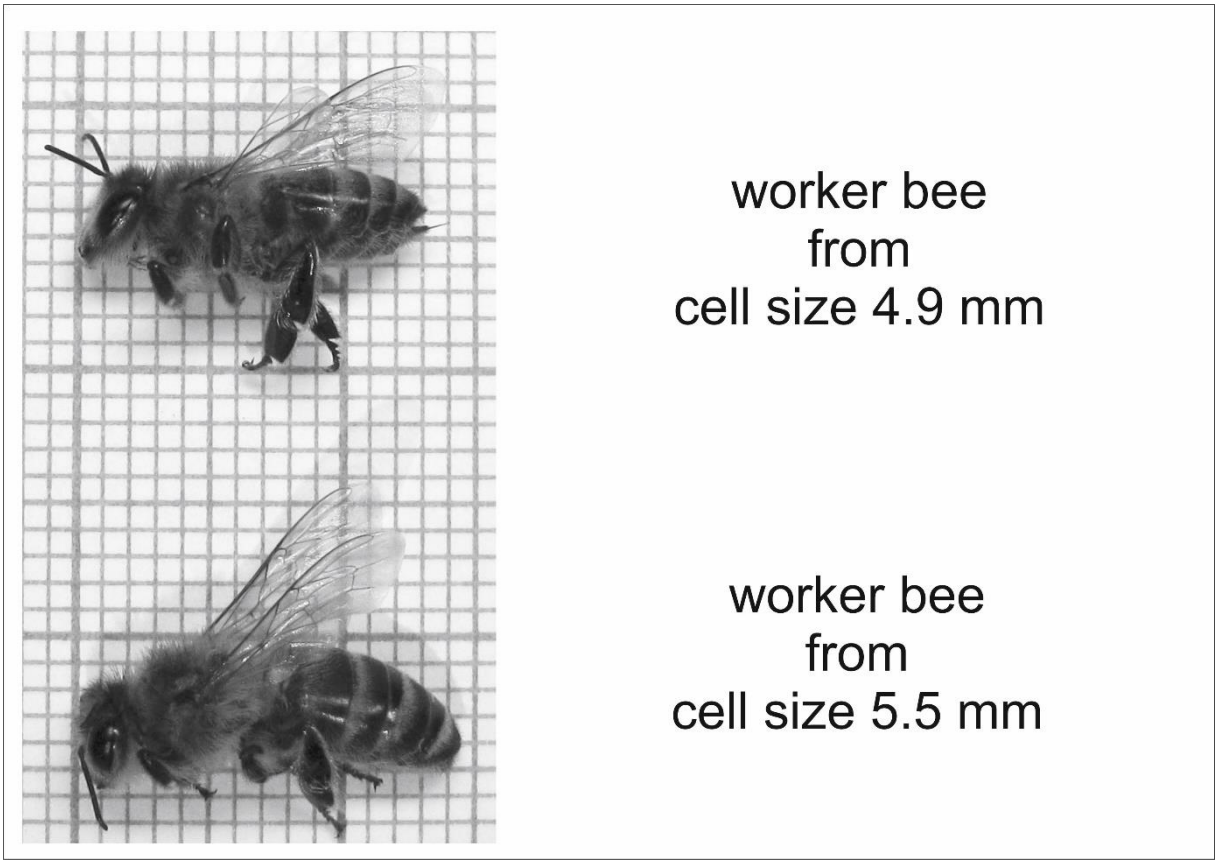

Photo: Singer

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Firstly, I would like to thank **Ed** und **Dee Lusby** for their idea that honeybees on smaller cell size can survive with Varroamites without varroa treatment under the condition of Arizona.

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Breeding Carnolian bees *Apis mellifera carnica* (Pollmann 1879) (Hymenoptera: Apidae) **on different comb cell sizes and analysing the effect of the different cell sizes on Varroa** *Varroa destructor* (Anderson & Trueman 2000) (Mesostigmata:Varroidae) **infestation rates.**

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Abstract

English:

Worldwide on *Apis mellifera* the “new” ectoparasite *Varroa destructor* is considered to be a major cause of bee colony-losses.

In 1997, after the arrival of the varroa mite (*Varroa destructor* Anderson & Trueman, 2000) in Arizona, Ed and Dee Lusby observed a better survival rate of their colonies on honey combs with a small cell size (5.08 mm). Nowadays the western honeybees are commonly kept on a cell size of 5.4mm to 5.6 mm. In this study I analyzed whether small cells (4.9 mm) compared to standard cell sizes (5.5 mm) have a negative effect on the population development of varroa mites.

First I had to search in my Carnolian (*Apis mellifera carnica* Pollmann, 1879) closed breeding population for colonies which could build small cells. I then created 2 groups of test colonies consisting of young sister queens and 1.5 kg varroa-infested bees on small cell size (4.9 mm) and large cell size (5.5 mm). To prevent any contamination with residues from miticides, new hives and organic-certified foundation were used. The bottom boards were equipped with mesh-protected drawers to collect the mortal mites (natural mites fall).

The data of 3 years, analysis of 494 test-colonies, have shown a positive result: the colonies on small cells had a slower varroa-mite population development.

For 2 additional years, the varroa reproduction parameters were examined.

Four lines with 80 new colonies on large cells and small cells were created with small sized bees. From each colony a comb with elderly sealed brood was analyzed for reproductive success of varroa.

The following year, 2 lines with small cells queens and bees were used for creating 20 new test-colonies. Each was fitted with 4 small cell and 4 large cell drawn combs alternately positioned. The infested brood cells were analyzed again.

The collected data allowed to calculate the VSH parameter (= Varroa Sensitive Hygienic) which is the quotient of brood cells with non-reproductive varroa divided per the total number of infested brood-cells.

Comparing both cell sizes in one colony has shown that there is a higher level of VSH for infested brood-cells on small cells in the selected Carnolian bee population.

The heritable traits "ability to construct small cell size comb" plus "VSH" results in a reduced varroa population growth in those colonies with both traits present. Smaller cell size combs in combination with corresponding genetically breed bees (cell size **and** VSH) can be one part of an integrated sustainable treatment concept for varroa control.

It remains unclear why the VSH-activity in the same colony differs and is more intense on small cell size infested combs as on large cell size infested combs.

Short abstract in English:

In beekeeping differences in cell sizes are often proposed to be one of the factors regulating varroa population growth. An in depth research of the relationship between these factors has been undertaken using the 'Carnica Singer population'. The acceptance of foundation with a specific cell size appears to underlay selection. Smaller cell size (4.9 mm) foundations/combs reduce the varroa-population growth compared to 5.5 mm. This reduction is also dependent on the presence of the VSH- behaviour trait in the worker bee population within the colony. Smaller cell size combs in combination with corresponding genetically breed bees (cell size **and** VSH) can be one part of an integrated sustainable treatment concept for varroa control.

Deutsch:

Der Einsatz von Mittelwänden mit kleinen Zellen wird auch als Varroa- Gegenwehr propagiert. In dieser Studie ist diese Problematik gezielt analysiert worden. Die Frage der Annahme anderer Zellgrößen unterlag in der untersuchten Carnica Singer Population einer

Selektion. Völker auf Waben mit kleineren Zellen (4.9 mm) haben gegenüber Völkern mit gleicher Abstammung, auf größeren Zellen (5.5 mm), eine geringere Varroamilbenvermehrungsrate. Dies gilt jedoch nur dann, wenn die Völker auch über die VSH-Eigenschaft verfügen. Kleine Zellen kombiniert mit der passenden Genetik (Zellgröße **und** VSH) kann als Teil eines integrierten Varroabehandlungskonzeptes dienen.

NL:
Translated by Jacob Peter van Praagh:

Het gebruik van kunstraat met kleine cellen wordt gepropageerd als hulpmiddel in de strijd tegen Varroa. In deze studie worden experimenten en resultaten rondom deze vraag beschreven. Om kunstraat met kleine cellen bruikbaar uit te laten bouwen, bleek in de onderhavige Carnica Singer populatie een selectie noodzakelijk. In experimentele volken is de reproductie van Varroa in de broedcellen is op raat met kleine cellen (4,9 mm) significant geringer als op raat met grotere cellen (5,5 mm). Dit geldt echter alleen in volken die ook VSH-gedrag vertonen. Kleine cellen gecombineerd met de juiste genetica (celgrootte en VSH) kan worden gebruikt als onderdeel van een geïntegreerd varroa behandelingsconcept.

Keywords: *Apis mellifera carnica* (Pollmann 1879) – *Varroa destructor* (Anderson & Trueman 2000) – integrated Varroa treatment - natural comb cell size – small cell size – selection - small cell foundation – survival – VSH (Varroa sensitive hygienic behaviour) – Varroa population growth

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1 Introduction

Worldwide the ‘new’ ectoparasite *Varroa destructor* (Anderson & Trueman 2000) is considered to be the main cause of colony-losses.
One possible remedy that is considered to help bee colonies to better cope with those parasites is a small cell size foundation. Beekeepers are experimenting with different cell sized foundations as it is contemplated to be a valuable tool in the fight against varroa.

In the past, the natural cell sizes of European-honeybees (*Apis mellifera*) were smaller [Zeissloff 2007]. In 1941 Zander, a zoologist and beekeeper discovered in Tula (Central Russia) bee colonies which had cell sizes of 4.74 mm up to 5.0 mm. [http://www.bienenarchiv.de/veroeffentlichungen/2003_zellengroesse/zellengroesse.htm].
One of the reasons why beekeepers adapted the cell size of combs was because they wanted to have more productive bees. The main advocate that initiated that switch in cell sizes and introduced the use of specially produced foundations was the Belgian professor Baudoux. He propagated larger cell sizes for colonies with the aim to increase the performance of bees and to have a greater honey crop. He emphasized that the use of large cells led to bigger bees, which he considered to be an advantage when it came to harvesting and storing honey. [Baudoux 1933].

Currently the company Rietsche GmbH produces equipment for the production of wax foundations. The conventional cell size of the mill is about 5.4 mm which is the equivalent of 800 cells per dm^2. On request, against a surcharge, various sized combs, ranking from 4.8 mm to 5.6 mm, can be manufactured. [http://www.rietsche.de/index.php/kunstwabenmaschinen/4-rietsche-vollautomatische-kunstwabenmaschine].

In 1989 Dee and Ed Lusby observed a better survival chance against *Acarapis woodii* (Rennie 1921) in colonies on 5.1 mm cell size combs. At that time commercial foundation varied between 5.3 to 5.7 mm in their cell size. They started the same kind of experiments after the arrival of *Varroa destructor* in 1997 [Lusby 1996 a, b.]. In the years afterwards many papers on cell size and varroa were published.

A various number of studies exist, detailing the negative influences of smaller cell sizes on *Varroa destructor* population growth: [Message & Goncalves 1995; Martin & Kryger 2002; Kober 2003; Piccorillo 2003; Forsman et al. 2004; Johnsen 2005; Kleinfeld 2006; Maggi 2009].
On the other hand scientific trials reported no-effect or even larger populations comparing small versus large cell sizes and varroa-development: [Fries 2004; Berg 2004 & 2005; Dreher 2007; Dreher and Liebig 2007; Liebig and Aumeier 2007; Ellis 2008; Taylor et al 2008; Berry 2009; Berry et al 2010; Coffey et al 2010; Seeley et al 2011; Khoei 2015].

Beekeepers are using and propagating cell sizes of 5.1 mm and 4.9 mm to support the colonies to better cope with the ectoparasite.
A standardised cell size for *Apis mellifera* foundation does not exist. In Austria the standard foundation that is offered to bees is a cell size of 5.5 mm.

Since 2004 the removal of mite infested brood by adult bees is described as an inheritable behaviour trait that suppresses the mite reproduction [Ibrahim and Spivak, 2004, 2006; Harbo and Harris, 2002]. This mite resistant trait is known as 'Varroa Sensitive Hygiene' or VSH as it appears to be a form of hygienic behaviour [Harris 2007].

To measure or analyse the presence and expression of the VSH-trait in a colony, the varroa infestation on a comb of sealed brood prior to emergence is used. A standardised number of infested cells is opened and the reproductive success of the mites gets recorded.

VSH gets calculated as the quotient of cells with non-reproductive varroa/ total number of infested brood-cells (=50). The theory behind VSH behaviour as a factor in the tolerance of honeybees to varroa shows that an active interruption of the reproductive cycle of a mature varroa (reproductive phase) reduces the population growth. The active interruption is executed by worker bees shortly before the young adult bees emerge (elderly pupae) [Harris 2007].

2 Material & Methods

2.1 Wax

To secure the wax foundations from any kind of contamination, only organic-certified and residue-free foundation were used in the trial. This was critical since varroa treatment ingredients accumulate in wax. Normally insecticides, herbicides and fungicides used in agriculture as well as in varroa treatment appliances disperse and enrich themselves in wax. In 2010 an American study was conducted and released on that topic, examining the impact of used plant protection products on honeybees. In that study, they traced 121 substances and metabolites in wax, pollen and bees [Johnson 2010].
These residual compounds diffuse from the wax into the honey and to the feeding juice of the bee larvae [Wallner. 2017].
To secure the trials from these kinds of falsifications and to prevent it from unnecessary enhanced varroa mortality, it was essential to use checked, residue-free foundation with the desired cell sizes of 4.9 mm and 5.5 mm.
In general, there are two different kinds of wax foundation: milled and poured ones.
Milled foundations are mould pressed while poured ones are formed through a pouring motion. In this process, liquid bee wax gets poured into cold wax moulds. As a consequence of that pouring motion the wax tends to be less dense, which furthermore means it can be quicker drawn out by bees. Both types of foundation have different advantages.
Poured foundation combs are more stable when it comes to high temperatures inside the hives, while milled ones would start to corrugate at that point. On the contrary poured foundations are less elastic when exposed to low temperatures.

2.1.1 Measuring brood comb sizes

Cell sizes were measured using a digital ruler (calliper). Measurements of ten cells across the foundation were taken along the three axes, displaced at 60° and the cell sizes were

expressed in a mean linear distance between two parallel sides of the hexagonal cell-imprinted per cell size [Coffey et al. 2010].
http://www.bienenarchiv.de/veroeffentlichungen/2003_zellengroesse/zellengroesse.htm

2.1.2 Foundations

Milled foundations get torn in the direction they are pulled from the wax roll. This happens due to a difference in pressure. In order to stabilize the roll, the moulding pressure declines towards the edges. Hence the cell mill roll is convex, which causes a distortion of the cells towards the edges and in the direction of pulling. In those areas, the cell sizes alternate and are bigger than the actual mould cells of the roll.
It is necessary to establish a mean value to evaluate the average cell size of the foundation. Otherwise the calculated, ordinary size of the cells would vary in every of the three directions measured.
Poured foundations combs are equally sized, which means the measured average cell size is exactly the same- regardless of the direction of measurement.
I used milled foundations from the companies Kieweg (Germany), Muhr (Germany) and poured foundations from Ver (Hungary).

2.2 Hives

The bees seal their hives with wax, which has the tendency to penetrate wood deeply. They use propolis, a mixture of wax, saliva and different secretions, to disinfect and seal all elements and cracks in the hive. Correspondingly I was forced to use only brand new parts for my trials. The bee hives were designed by myself and are therefore called ‘Singer Hives’.
The bee spaces were in all parts of the hive equally casted, with a gap of 8 mm to 10 mm.
The hives consisted of different parts: a varroa diagnosis board, a brood chamber, a queen excluder, a super, a feeding magazine and an aluminium lid.
To avoid interferences, I didn’t use any synthetic materials, like a cover sheet.

The purpose of the varroa diagnosis board is to collect the natural mites fall. It is made of stainless steel and looks like a collecting drawer. To suppress the building of drone cells, which is normally observed when using higher bottom boards, I used a low bottom.
In Austria, the common bottom board is a high one. This allows the bees to build drone cells accordingly to their desires. In natural bee habitations there are about 10% drone brood when pre-settings aren't present.
In my trials I wanted to avoid that phenomenon. Drone cells favour varroa mites and are causing higher varroa population growths. The reason for the increased varroa reproduction is the extended time of hatching. While worker bees require a period of 21 days, drones emerge after 24 days. Therefore, varroa mites have another 3 days to reproduce and mature.
The brood chamber is a squared based magazine and can hold 12 frames. The height of the frames is equivalent to the size of 2 honey storage frames plus bee-space. If proven necessary, they could be moved up to the magazine. The exterior height of the frames is 32.8 mm, the exterior width 42.7 mm. The brood chamber is designed to hold a volumetric capacity of 66.8 litres which provides the bees with plenty of space to enlarge their nest.
Frames with a cell size of 4.9 mm cover a surface of 1.200 cm^2 per frame and hold about 11.400 cells each, which approximately means there are about 136.800 cells present in the brood chamber.
However, frames with 5.55 mm exhibit 9.000 cells per frame, so 108.000 cells in total. The volume of those large cells is 36% bigger.
The queen excluder separates the brood chamber from the super. It is made of stainless steel and consists of round bars, which are embedded in a wooden frame. The excluder is positioned on top of the brood chamber and is compliable with the bee space.
The super holds 10 frames with an exterior height of 16 cm. The bee spaces between the frames are larger. Instead of 12 frames there are only 10. This makes the honey extraction later on easier.
The wooden honey-comb frames are equipped with plastic foundations, which are made of polyethylene. Those plastic foundations were plunged in residue-free bee-wax before usage. The size of the combs is about 5.1mm to 5.2 mm. The bees accepted those foundations and drew them out perfectly.
The feeding magazine holds a volume of 10 l and is equipped with a central ascending tube that is closable with a cap on top. The tube allows the bees to climb on top and reach the liquid food without drowning. It is covered up with an interior lid.

Inside the interior lid is an insulating plate that is made of soft fibres. Above the feeding magazine is an exterior lid with an aluminium cap to secure the hive from weather damages. The hives were coated with boiled linseed oil on the outside and were painted over 2 times with ochre-coloured pigments. The interior of the hives remained untouched in that process. The coating of the hives was tested during a 14-days period to assess whether it would trigger an increased bee-lethality. Therefore, the coating was applied twice on 10 wooden queen-cages, which are typically used for transmissions, and was placed in an incubator with 35 degree Celsius and a humidity of 70%. Inside each cage were 13 bees and some sugar paste. The outcome of the trial was unremarkable. There were no differences between the painted and unpainted cages regarding mortality.

2.2.1 Bee race used in the thesis

The worker bees of the mother lines were chosen by morphological criterions [Ruttner 1991]: "cubital-index", felt tie width and armature colour by "breed survey master" Harald Singer and Elisabeth Singer. The probed bees were descended from the Carnica race.
The bees were also selected by DNA-analysis [Soland-Reckeweg 2006]. The findings were presented by Soland-Reckeweg in her speech at the OEIB meeting of the professional beekeepers of Austria in 2007 in Graz (Austria). The bees and queens that were used for the DNA-tests, were typical Carnolian ones.

2.2.2 Bee colony, value in Euros €

A colony consists of a queen, worker bees and frames with combs.
The value of a colony depends on the strength and the time of season. In spring, the price in Middle Europe for a colony (including the queen) with 10 or more frames (where at least 6 ones are brood frames) is about 200,-- €. For pollination in spring (3 weeks) the price is 160,-- €. A nuc with 6 frames costs 120,-- € in spring time.
Queens, inseminated on a mating station with selected drones cost between 50,-- and 100,-- €. Artificial inseminated queens cost 200,-- €.

A shook swarm (consisting of a mated queen and 1.5 kg bees) costs 160,-- €
A detailed price list can be required by e-mail.

Giving this overview of colony prices, I want to point out the immense costs for carrying out the trials and bring the magnitude to one's awareness, of how much monetary value is consumed by the varroa mites worldwide on a yearly base.

For my dissertation, I used 764 shook swarms in total. (2002: 160; 2003: 205; 2004: 221; 2005: 78; 2007: 80; 2008: 20) The fix cost of a shook swarm is 120,-- € per unit. The overall value of the bee material used in my tests add up to 91.680,-- € (This amount doesn't include feeding, foundations, hives, boxes, etc.)

2.3 Population build-up, Selection of queens

2.3.1 Basic requirements

Honeybees which can genetically built 4.9 mm cell sizes.
The size of the honeybee depends on the genotype and phenotype. Small bees can build smaller cell sizes as well as greater cell sizes. On the contrary larger bees are solely able to build large cell sizes and are incapable to build small cells.
In order to test Ed and Dee Lusby's hypotheses objectively I had to use small bees which were able the build both cell sizes.

2.3.2 Initiating testing series- Searching for the right bees

In 2002, 1.287 colonies from the Carnolian closed breeding population (Carnica Harald Singer; >1000 colonies) were selected. In this population, the standard cell size of the foundations that was given to the colonies was the large cell size (LCS) of 5.5 mm. The foundations were milled in Austria by the company Altmann with a Rietche-machine that used a common cell size of 5.5 mm to 5.6 mm.

In order to have the bees of the selected 1.287 colonies in the right physiological conditions ("summer bees"), I proceeded after the cherry flowering as followed:

Step 1. During a 10 days interval, three empty frames were positioned in the 12 frames OE-Breitwaben (wide frames)- hive type at '2', 'centre' and '12' to allow the bees to build free combs without prescribing a cell size. This was to allow the worker bees to show the 'natural cell size preference' of the colony and let them build worker and drone combs.

Step 2. At day 11 the centre frame was measured and replaced with a small cell size (SCS) foundation of 4.9 mm.

Step 3. 10 to 21 days later the SCS -combs were checked; 9.3 % = 120 colonies showed acceptable drown-out cell patterns and were given a frame with a 'small cell size start-up foundation' (5 cm wide).

Step 4. 79 of the 120 colonies were selected as being capable to draw SCS combs in an acceptable way with regular brood patterns.

Step 5. To see their real acceptance to SCS it was necessary to offer the SCS foundation twice; with a 40-day interval between the two experimental set-ups (1.5 generation of worker bees).

Step 6. The queens of 8 colonies out of the 79 group were selected to become foundresses for 8 new lines. For each of the 8 lines 20 daughter queens were artificially inseminated with drones from their own mother colony.

Step 7. The next generations were mated on an isolated mating yard with the 79 group as males. I used 2 mating yards. Each captured an area of 5 km and was unoccupied by other bee colonies. The mating yards were located in the Alps (northern Kalkalpen), south of Mariazell: One was embedded in Aschbach (850 meters above sea level), the other one in Seewiesen (1.000 meters above sea level). Close to that area are various mountains -e.g.: Veitsch with 1.981 m, Hochschwab with 2.277 m.

Due to the distance between the bee yards alien drones from the outside weren't able to reach those colonies.

Steps 8. & 9. 2003 and 2004 more foundresses from the 79 group were used to create 8 more lines. The young daughter-queens were brought to the mating yard (79 group males).

In total 16 SCS lines were analysed (8 from 2002, 5 from 2003, and 3 from 2004).

2.3.3 Artificial insemination of queens

I acquired the skills of artificial insemination of queens from parental business. My mother, Elisabeth Singer, is inseminating queens since 1966. Moreover I attended further educational courses like "introduction to the artificial insemination" at the higher federal teaching and education institute of Beekeeping and Viticulture with Institute of Beekeeping, Department of Beekeeping in Lunz / See, Austria and at the Bee Institute Dol, Výzkumný ústav včelařský, s. r. O., Máslovice - Dol 94, 252 66 Libčice nad Vltavou, Czech Republic.
Professional inseminators (Dr. Wilde, Poland, DI Titera, Czech Republic) have been inseminating Carnica queens in my breeding farm since 1994. The inseminations for my inbreeding lines were carried out by myself and my mother.
The model "Vesely" (= Insemination Apparatus for Bee-Queens, model Vesely) was used as insemination apparatus.

2.3.3.1 Breeding of queens

With the "Swiss queen transferring spoon", the smallest round larvae were grafted into artificial wax-worm cups in breeding frames and put into queen-less populations for breeding. After capping, the cells (6th - 11th day) were dislocated and kept until further hatching in the incubator at 35^0C and 60-70% humidity for further development. Before the hatch, the queens are put in a hatching cage with 5 young bees which take care of the young queens. The queens are checked and marked after hatching. Subsequently, mating nucs, 3-comb Singer boxes made of polystyrene and polyethylene, are filled with ¼ l young bees, lined with 300 ml of sugar water (1: 1) and 3 combs with foundations. After creating the copulation units, the nucs

are stored for 3 days in dark at approx. 15^0C. On the evening of the 3 rd day, the nucs are placed on the mating station and the entrance is opened.
To prevent the queens that are meant for artificial insemination, from flying away, the right wing is shortened by about 1/3 with a nail scissor. These nucs are placed in Aschbach next to the drone colonies.

2.3.3.2 Breeding of drones

On the mating stations, every father colony has 2 combs for drones (empty frames without foundation) which are built for drones by the bees. In case of a lack of honey flow, honey is fed so that drones are constantly produced and are available in large quantities for mating.
For the artificial insemination, capped combs with drone brood are taken from selected hives, marked, and put between the honeycombs in the super above the queen excluder with sealed bee breed combs beside the honeycombs for hatching. A lid with a flight hole is placed on the super. On top of this is a drone flying cage, which has a barrier (queen excluder) on one side. Here the bees can escape, the drones are held back. These flight cages keep the drones in an ideal mood during the dissemination of semen.

2.3.3.3 Bee hives performance survey

Beyond the strength of the hives, the honey output, the placidity, the swarm tendency, the vitality and the winterizing strength of the colonies were examined.

2.3.3.4 Inbreeding lines

The only criterion that was taken into account when working with the mother hives-to-be (the foundresses for further colonies) was their ability to build small cell size combs.

The elected queens (breeding mothers) were put in artificial swarm boxes (shook swarms), which were fitted with a 1 l container of sugared water (in a 1:1 ratio).
Then the shook swarm boxes were set up in a dark room, were I kept them for 2 days at an approximate temperature of 15 degrees Celsius. In this period, the bees started to build natural combs (burr combs) on the lid of the shook swarm boxes, with at least 3 comb pieces around the cage of the queen. The comb spaces were determined by measuring the cell size of the natural combs with a digital calliper. After that final procedure, the queens were chosen to be foundresses for further lines.

In the F-1 generation, clear signs of inbreeding damages were noticeable.
The honey output was in regard of the quantity of bees less than the honey crop of natural inseminated queens.
Due to a high varroa infestation rate, the strength of the colonies couldn't be objectively assessed.
The signs of vitality (behaviour of defence in a case of intrusion) were highly pronounced, which might have been coherent with the high pressure of varroa mites in the hives.
The tendency to swarm was not present.
Despite of the inbreeding there were no indications of laid diploid drone eggs. So the relationship between the males that each queen was mated with was not too close. A too close relationship causes the egg laid by the queen by chance to be fertilized with a sperm that has the genetically ident information by decent at the sex-locus. These eggs give raise to diploid drones. Young larvae with that same genetical background are removed by adult worker bees.

2.3.3.5 Queens inseminated on a mating yard

In spite of high varroa infestation rates, the colony strength of these hives was, along with the honey output, quite high. The signs of vitality were more pronounced. The winterizing strength was ordinary considering the varroa pressure that the hives were under. Swarm tendencies were not present. When being under immense varroa pressure, isolated cases of queen changes were noticeable.
In general, the hives required one super, on rare occasions though they needed up to two supers.

2.3.3.6 Differences between small cell size and large cell size

2.3.3.6.1 Comb spaces

There were no considerable differences noticeable between the natural combs (burr combs) of the shook swarm boxes from the foundresses and the ones of the mini plus hives, which were built without frames with a cell size of 5.5 mm.
However beekeeping literature claims that bees on small cell sizes have a minor comb distance. This needs to be further examined in studies with more suitable bees.
In my trials the bees used a comb distances of 33 mm to 35 mm. After monitoring the mini plus hives, which weren't equipped with any frames, I observed that the bees tended to prefer a small comb distance near the entrance of the hive, when fitted with high bottoms under the building restriction zone.
In the honey storage areas (like the feeding chamber in case of storage shortage), a comb distances of 35 mm to 36 mm were used from both kind of bees. The ones from small cell sizes and the ones from large cell sizes utilized the same comb distance here autonomously and despite of their cell size derivation.

2.3.3.6.2 Brood nest temperatures

Beekeeping literature and beekeeper claims that bees on large cell sizes of 5.5 mm have a lower brood nest temperature [Kober 2002, pers. communication].
When breeding bees on small cell sizes of 4.9 mm, a higher brood nest temperature is noticeable due to the compactibility of the nest.
I own 11 wireless transmitting scales from the company CAPAZ (Germany): http://www.bienenwaage.de/, which are equipped with temperature sensors.
With those scales I established the weight, precipitation, humidity and the brood nest temperature of the hives. Every day the collected data were transmitted to my computer (or optionally sent to my cell phone). The temperature records were essential to me to choose the right time for the oxalic acid application (remaining mites treatment).
The colonies were brood-free when the brood temperature dropped from 34^0C to 18^0C or 14^0C (dependent were the temperature sensors were located in the hives, if they were

positioned in the middle or in the outer parts of the hives). That was the perfect time for the remaining mites' treatment.
10 wireless transmitting scales were utilized to measure differences in the brood nest temperature in the centre part of the hives. From those scales, 5 were used to record the brood nest temperature in 4.9 mm cell size colonies, the other 5 scales were put in 5.5 mm cell size colonies to keep track of the brood temperature within those. All monitored colonies were positioned at the same bee yard. I couldn't detect any difference between small and large cell size colonies. However since I only used 10 scales, a precise statement regarding brood nest temperature and cell size cannot be made. Further examinations are needed on that field.

2.3.3.6.3 Amount of brood cells on large and small cell size combs

Common scientific beekeeping magazines claim that small cell size colonies have more brood cells. I measured the surface of the brood area from sibling-queens, which were put on small and large cell size combs [Imdorf 1987; Delaplane 2013].
When measuring the surface of the combs, there were no differences between the number of cells when factoring in that small cells are 26% smaller than conventional cells.
There were no differences in the number of brood cells when putting the same genetic line on different sized combs and locate on the same bee yard, (depending on colony strength and bee composition- young bees, collecting bees).
The degree of the varroa infestation rate distorts those data.
Hives with higher varroa infestation rates try to compensate the loss of their bee-population with an extensive breeding behaviour. Differences in the number of brood cells between genetic lines are observable.

2.3.3.6.4 Earlier bee eclosion in small cell size combs

Beekeepers assume that the earlier period of hatching in small cell sized combs is a possible reason for the noticed lower varroa infestation rate in small cells. Small cell size bees hatch 18 to 20 hours prior than large cell sized bees. A shorter capping time means that the mites

have less time to reproduce and develop which results in a reduced mite population. Through selection of specific bee races, no reduced capping time could be established (pers. communication Wilde).
Other bee races are genetically smaller and have a shortened capping period, which consequently correlates with a diminished varroa infestation rate. This includes the African bee races, oriental races and the "killer bees" in South and Central America.
In order to get an objective answer to this open question, further research needs to be done (like documenting the egg disposal of the queen within the cell and monitoring the period of hatching hourly with a video recorder).

2.3.3.6.5 Hatching weight of bees from small cell size and large cell size

Bees from small cell sizes have a lower hatching weight than bees from larger cell sizes. [Kleinfeld 2006].

2.4 Estimating varroa reproduction parameter

2.4.1 Incubator tests

To find out the ideal time to count mites and their offspring, I put sealed brood frames from colonies with high varroa infestation rates in the incubator. I opened sealed brood cells to establish the time at what bees were fully developed and ready to hatch.
One day before hatching the eyes of the bees get coloured and they start to move with their extremities. I acquired the skills to properly count mother mites and their infants.
Developed young female mites are brown in colour; undeveloped female ones are light brown and white. Male mites are white or light yellow coloured, but newer brown.
In the cells are set up structures for mite excreta and dead mites. I used cold light (from my artificial insemination model) to gain insights into the structures within those cells.
Surprisingly I noticed a high number of dead mites. When I checked the temperature sensor of my new incubator (model Dol), I found out that the temperature was 2^0C above the norm.

Instead of 35^0C, the incubator had a temperature of 37^0C. I adjusted the temperature and put new sealed varroa infected brood combs in the incubator. On the next day, the mites were still alive. I concluded that the defect temperature sensor must have led to the high mite mortality. Beside I noticed that mite offspring, which weren't coloured, died within 10 minutes after opening the sealed combs. This probably happened because their chitin wasn't fully developed which caused them to dry out.

2.4.2 Estimating varroa reproduction parameter in the colonies

2.4.2.1 Varroa population growth on LCS and SCS

As one of the basic requirements I needed some shook swarms that were infested with varroa mites. As I was an organic beekeeper (from 1996 until 2014) I only utilized organic acids to protect my bees from varroa mites. Hence I had enough mites in my colonies left for further trials.

From 2003 onwards, groups of colonies (see table 1 for details) were built using young queens with 1.5 kg of worker bees (shook swarm technique). Each 20 kg shook swarm contained varroa infested bees. The worker bees for the shook swarms came from SCS colonies. In the initial varroa mite population it is important to keep in mind that 40-60% of the mites, which are artificially added later on, diminish within the first 48 hours [Liebig 2002, pers. communication, mailpost]. Hence it is essential that the initial varroa mite population is created naturally.
Of each 20 kg bees swarm, samples were taken to collect data on the infestation-levels. The test shook swarms contained between 196 and 492 varroa mites per swarm (mean number of test shook swarms: 268 mites / swarm). I treated the test swarms with "Varidol" (http://www.beedol.cz/varroaza/) from the Beeinstitut Dol, CR. To obtain equal starting conditions, the later-on created groups had the same initial mite population. From each 20.kg shook swarms, 13 colonies were formed: 6 colonies on small cell sizes, 6 colonies on large cell sizes and 1 test colony. Both groups had the same genetic line (sister queens) and were placed on secure bee yards with minor re-infestation rates. Due to collapsing colonies, a re-infection (which revealed itself in an increased mites fall into the varroa diagnosis boards)

was observed on the bee yards. Table 1 visualizes the re-infection cases in the recorded drop-out rate.

In the 2003 season, 205 new colonies were built up. For each line two groups (SCS & LCS colonies) were created and randomly divided on 7 bee yards in the 'Nationalpark Donau- & March-Auen'; south-east of Vienna. To prevent any contamination with miticide-residues, new hives and bio-certified wax foundations were used. The bottom-boards were equipped with mesh-protected drawers to collect the natural mites-fall [Dietemann 2013].
During the active season (May - September) the drawer-contents were collected every 10 days. In this period of time, between 312 to 562 developed mites were detected in the varroa diagnosis board per colony and sample, .
Dependent on bee yard and colony, the quantity of counted varroa mites showed great variability between colonies and bee yards. In total (when adding up the finding of every 10 days) 5.011 to 14.557 mites per hive were detected. The threshold of collapse was reached between 8.000 and 12.000 mites. Once a colony hit that critical mass, it deceased. The point of collapse depended on colony, re-infection and bee yard. Colonies with more than 12.000 mites didn't survive the upcoming winter.
According to Ritter, the common level of collapse is around 15.000 mites per colony [Ritter 2001, pers. communication]. At an average start population of 260 mother mites per swarm, the threshold would be reached in my hives after 5 reproduction cycles (8.320 mites excluding the cases of re-infection). 2016 the threshold of collapse was reached after 5.000 mites per colony.

In August the colony strength was judged using the standard procedures (as described) by using a screen frame [Imdorf 1987; Delaplane 2013].

Colony management was undertaken according to "good beekeeping praxis"- by a bee master, but without varroa-treatment. All established colonies were overwintered to analyse their winter-survival rate.

In principle, the Kefuss way of selection "Bond Test – live and let die" was used [Mc Neil 2010]. This selection process allowed an additional way of determining differences in varroa resistance between the SCS and LCS groups.

In the season 2004, 221 new colonies were created. To avoid varroa infections from former colonies, the surviving colonies from 2003 were brought to a separate bee yard.
5 new lines were selected from the 79 group and from the 4 old lines. These 9 lines were randomly divided on 7 bee-yards.
The new mean value of the total amount of mites was lower than the one in 2003.

The surviving colonies from 2004 were again collected and transferred to a separate bee yard (like the ones in the year before). The 2005 experimental group consisted of 3 new lines from the 79 group plus the 4 lines (daughters from 2003 and 2004 colonies). In the season 2005, a total amount of 78 colonies were created from the shook swarms and were analysed. The quantity of mites in the varroa diagnosis boards were less than the number counted in 2003 and 2004. One possible impact, that might have shaped that outcome, could be the different weather conditions. It is known that humidity levels above 75% repress the development of mites [Kraus 1997]. Data regarding the humidity in the hives were transmitted from the set up scales in Capaz. At the end of the 2005 bee-season, all colonies (including the surviving colonies - 6 from 2003 plus 126 from 2004) were treated with the oxalic acid application Varrox®-Andermatt [Andermatt BioVet AG 2012]. At that time the colonies were without sealed brood.

From 2006 onwards around 400 colonies on SCS were used to keep the 7 lines as described. Two mating-periods allowed the use of two different male groups; the surviving queens born in 2003 and their daughter-queens (in total from 4 lines). The second male group consisted of the surviving queens born in 2004 and their daughter queens (4 lines).

2.4.2.2 Estimating varroa reproduction parameter

From the 7 lines kept, four showed significant differences in varroa-development when comparing LCS & SCS colonies.
In May 2007 from these four lines, 20 new colonies per line on LCS and SCS with SCS-bees were created as described (2.3: shook swarm technique); 4 pairs of sister queens; the 80 young queens were all mated on one mating station.

In the middle of August, from each colony a comb with elder sealed brood was analyzed for the reproductive success of varroa- opening cells aged latest one day before emergence [Harbo & Harris 2009; Harris 2007; Dietemann 2013].

From each colony, a number of brood-cells were opened, searching for 50 infested cells. From these 50 infested cells the contents were registered according to the following classification:

A- Cell with one dead varroa

B- Cell with one living varroa

C- Cell with one living varroa plus 1 young varroa

D- Cell with one living varroa plus 2 young varroa

E- Cell with one living varroa plus 3 young varroa

These values were used to estimate VSH for each colony.

For each of the 80 colonies the VSH-value was calculated as the quotient of the cells with non-reproductive varroa divided per the total number of infested brood-cells (n=50). (A+B)/ (A+B+C+D+E)

In May 2008 from the two-2007 lines (1/2007 & 3/2007) the SCS queens and bees were used to produce 20 new colonies. Each fitted with 4 LCS and 4 SCS drawn combs alternatively positioned. Due to supersedure in 2007, 10 original queens and 10 daughters (open mated) were tested.

From each colony 400 infested brood cells were analyzed; 200 from SCS and 200 from LCS combs. These data were used to compare the expression of the VSH-trait in each colony and on each cell size within that colony.

2.4.3 Quantity of varroa mites

Counted varroa mites (data collection):

2003:	1.325.715	n=205	
2004:	1.351.812	n=278	
2005:	715.056	n=249	
2005 OX:	1.178.740		
2004 total:	1.893.796		
2007:	9.933 c	n=80	50:436 (436 sealed brood cells needed to be opened to find 50 infected ones)
2008:	10.082	n=20	
Total:	4.591.338	(1.275 hours; 1 mite/sec.)	

*Supplementary note: OX= oxalic acid application

2.5 Statistical analysis

All statistical analyses were performed using IBM SPSS v20. Data are summarized as mean and standard deviation. A general linear model (GLM) was used to analyse the impact of factors such as cell size, line, bee yard and year on natural mite drop per brood area and total mites fall after oxalic acid application followed by post hoc tests using Bonferroni's alpha correction procedure. In addition the difference between the two cell sizes was analysed separately for every year using t-tests for independent samples. This was done with and without the influence of the line of the mother. The odds ratio (OR) was calculated to evaluate the survival chance according to cell size. The assumption of normal distribution was tested using the Kolmogorov-Smirnov-test. The parameters natural mite drop per brood area and total mites fall after oxalic acid application were log transformed to fit the normal distribution. For all statistical analyses a p-value below 5% ($p < 0.05$) was seen as significant.

3 Results

3.1 Varroa development

Describing the varroa population growth by means of the observed dead mites in the bottom-board drawer led to following results (Table 1).

Table 1. Mean number of dead mites on varroa-board/brood area/year

Cell size	*Year*	*Mean*	*SD*	*N*[a]
4.9 mm	2003	1416.4	886.9	97
	2004	787.3	653.7	123
	2005	688.3	663.7	39
	Total	1008.0	813.0	259
5.5 mm	2003	1721.5	916.9	99
	2004	991.9	680.1	98
	2005	859.5	824.3	38
	Total	1277.9	892.8	235
total	2003	1570.5	912.7	196
	2004	878.0	671.7	221
	2005	772.8	747.3	77
	Total	1136.4	861.7	494

N[a] = number of colonies in test

Figure 1 shows SCS colonies compared to LSC colonies. The colonies on SCS show a significant (p= 0.001) slower development of varroa populations. This effect is independent of year, line and bee yard. Year, line and bee yard are considered as factors in the statistical analysis. (Data from the years 2003, 2004 and 2005)

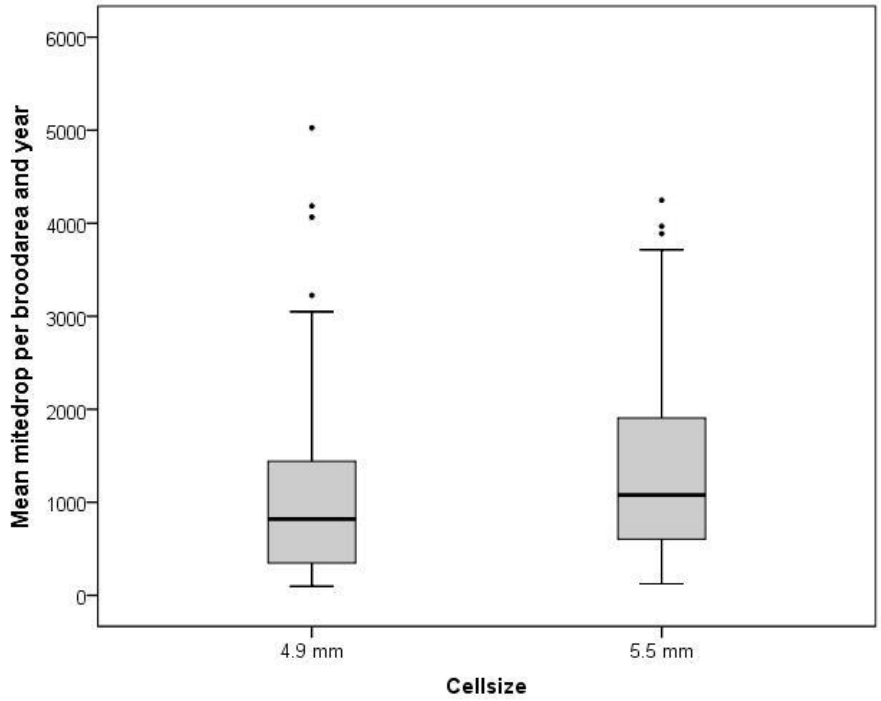

Figure 1. SCS colonies compared to LSC colonies (2003, 2004, 2005)

The drop-outs in this figure are colonies, which imported high numbers of mites by robbing weaker colonies that were about to collapsed due to the high varroa infestation rate in their hives. The hostile bees dragged the mites with them when they robbed those other infested colonies of their honey.
This could be noticed due to the fact that the dead mites-fall on the bottom board increased at the end of season when other colonies collapsed. Moreover a gain of weight (honey from dead colonies) was noticeable in those particular hives.

Table 2 shows the results from the GLM (model I). All main effects and interactions except cell size x year remain significant on mite drop. A clear difference of overall varroa population growth between the LCS and SCS colonies could be observed between lines ($p=0.001$). The difference between cell sizes among years is not different. Varying differences between cell sizes among years and mother line can be noticed as well as between mother line and bee yard among years. This means, that varying mite drop can be observed with a difference between cell sizes which remains constant over the years. Year and bee yard, as they influence colony growth, showed to be significant factors with $p<0.05$. These results indicate the complexity and variability of honey bee colonies as a result of individual queen

genetics and the individual genetic of males mated with (interaction between mother-line, bee yard and year).

Table 2. Results of the mixed models for all four factors

Source	*Df*	*F*(*df*, 611)	*P*
cell size	1	11.0	0.001
year	2	21.5	< 0.001
line	12	13.2	< 0.001
bee yard	9	20.7	< 0.001
cell size x year	2	1.4	0.246
cell size x line	11	2.5	0.005
year x line	17	1.9	0.014
year x bee yard	10	42.9	< 0.001

In a second analysis, the factors mother line and bee yard were excluded from the model (results shown in Table 3). As seen in model I the main effects (year and cell size) remain significant. The interaction between cell size and year now disappeared ($F(2, 670) < 1$; $p = 0.931$). This means that the natural mites-fall differs among years but the difference between cell sizes remains the same. Small cell size combs have a lower mites' drop rate, whereas large cell size combs feature a higher one.

Table 3. Influence of cell size and year per mean number of dead mites in varroa-board/brood area/year (Simplification of analysis above)

Factor / Interaction	*Df*	*F*(*df*. 670)	p-value
cell size	1	19.3	< 0.001
Year	2	38.1	< 0.001[a]
cell size x year	2	.1	.931

Supplementary note: [a]*Sign. differences (p < 0,0) existed between the years 2003 and 2004 as in 2003 and 2005 (Bonferroni corrected).*

Table 4 shows differences in mean number of dead mites on varroa-board/brood area/year between the two cell sizes per each year. Colonies on large cell size combs show a higher infestation rate as colonies on small cell size combs. Figure 2 illustrates these results.

Table 4. Difference in mite infestation separated per year (results of t-tests)

Year	*T*	*df*	*p*-value
2003	-2.166	194	0.032
2004	-2.933	267	0.004
2005	-2.601	209	0.01

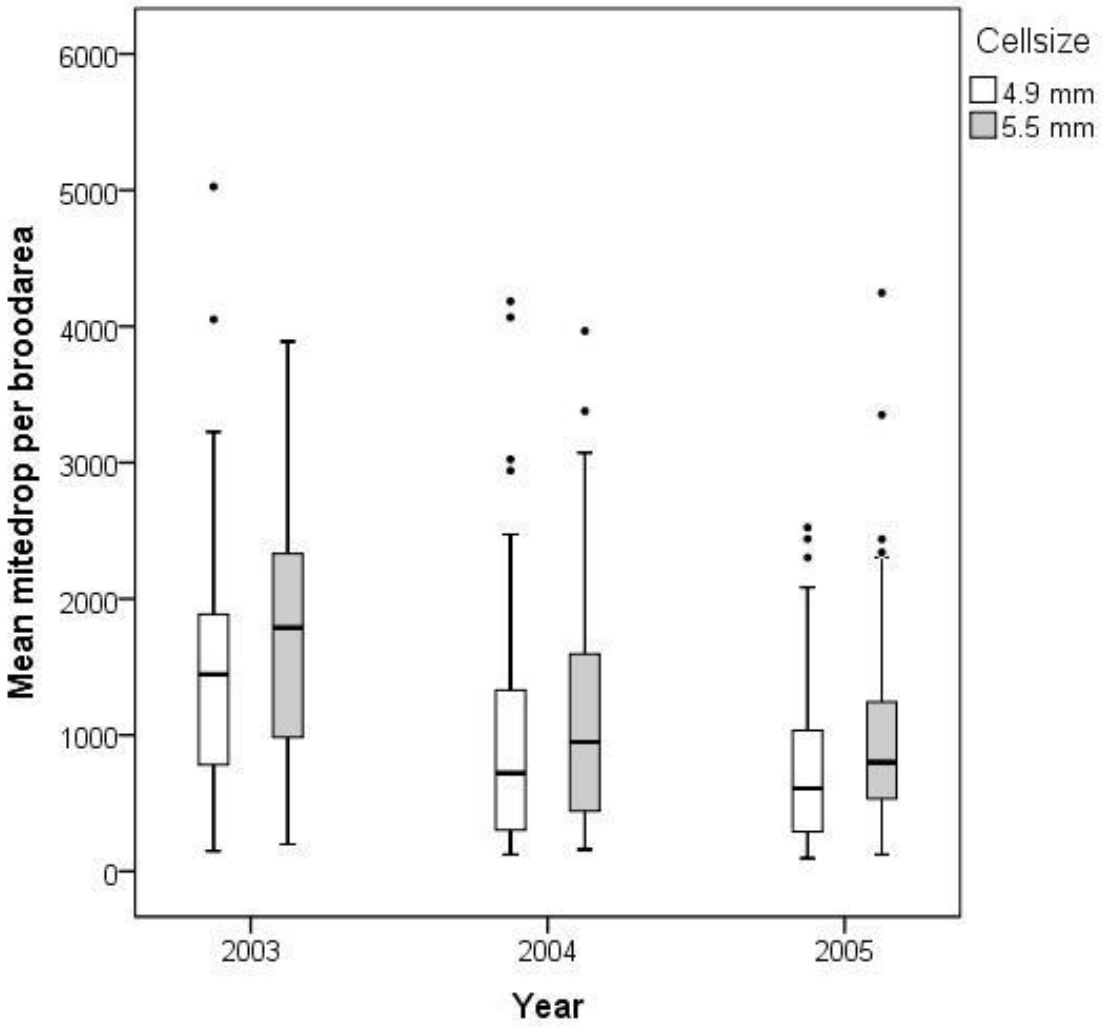

Figure 2. Mean number of varroa for LCS & SCS per year

Table 5 shows the mean number of dead varroa on the bottom-boards per brood area, separated after line and cell size.

Table 5. Mean number of dead Varroa on the bottom-boards per brood area, separated after line and cell size (2003, 2004, 2005)

Line	SCS (4.9 mm)		LCS (5.5 mm)				
	N	*M* ± SD	*N*	*M* ± SD	*T*	*df*	*p*
1	75	1090.8 ± 630.5	59	1558.6 ± 892.9	-2.94	126	0.004
2	29	1517.5 ± 697.4	34	1602.9 ± 622.4	-0.63	58	0.534
3	11	3015.2 ± 1077.9	10	2275.8 ± 552.2	1.81	17	0.087
4	34	1644.2 ± 644.2	26	1977.6 ± 776.7	-1.75	55	0.085
5	37	379.0 ± 300.4	36	511.4 ± 450.9	-1.51	69	0.135
6	47	360.5 ± 315.1	8	1138.4 ± 1146.2	-2.05	8	0.075
7	16	1427.3 ± 554.2	16	1858.2 ± 488.4	-2.52	26	0.018
8	21	1477.3 ± 469.2	31	1467.2 ± 926.3	1.16	46	0.250
9	7	825.8 ± 308.8	8	908.9 ± 384.9	-0.34	13	0.739
10	35	818.1 ± 463.7	35	955.3 ± 694.3	-0.79	67	0.431
12	10	1041.3 ± 493.1	10	1040.3 ± 530.7	0.03	18	0.976
15	32	494.3 ± 273.9	32	493.3 ± 241.4	-0.16	62	0.875
16	17	415.3 ± 272.1					

The lines 1 and 7 were used for further analysis of their significant lower mite development on SCS combs, based on their genetic background. The lines 2 and 6 showed significant lower development in at least one of the three years' period and were also used for the detailed analysis of the varroa reproduction parameters.

Table 6 shows differences in the mean number of dead mites on varroa-board/brood area/year for each genetic line on small and large cell sizes. The genetic line 1 and 7 show significant differences. As illustrated in table 5 the mean values of both genetic lines show a minor varroa infestation rate on small cell sizes as compared to large cell sizes.
Figure 3 depicts those results.

Table 6. Differences in mite fall using genetic line as factor in the analysis (Results of t-test)

Genetic line	*T*	*df*	*p*-value
1	-2.94	126	.004
2	-.63	58	.534
3	1.81	17	.087
4	-1.75	55	.085
5	-1.51	69	.135
6	-2.05	8	.075
7	-2.52	26	.018
8	1.16	46	.250
9	-.34	13	.739
10	-.79	67	.431
12	.03	18	.976
15	-.16	62	.875

Supplementary note: $*p < 0.05$.

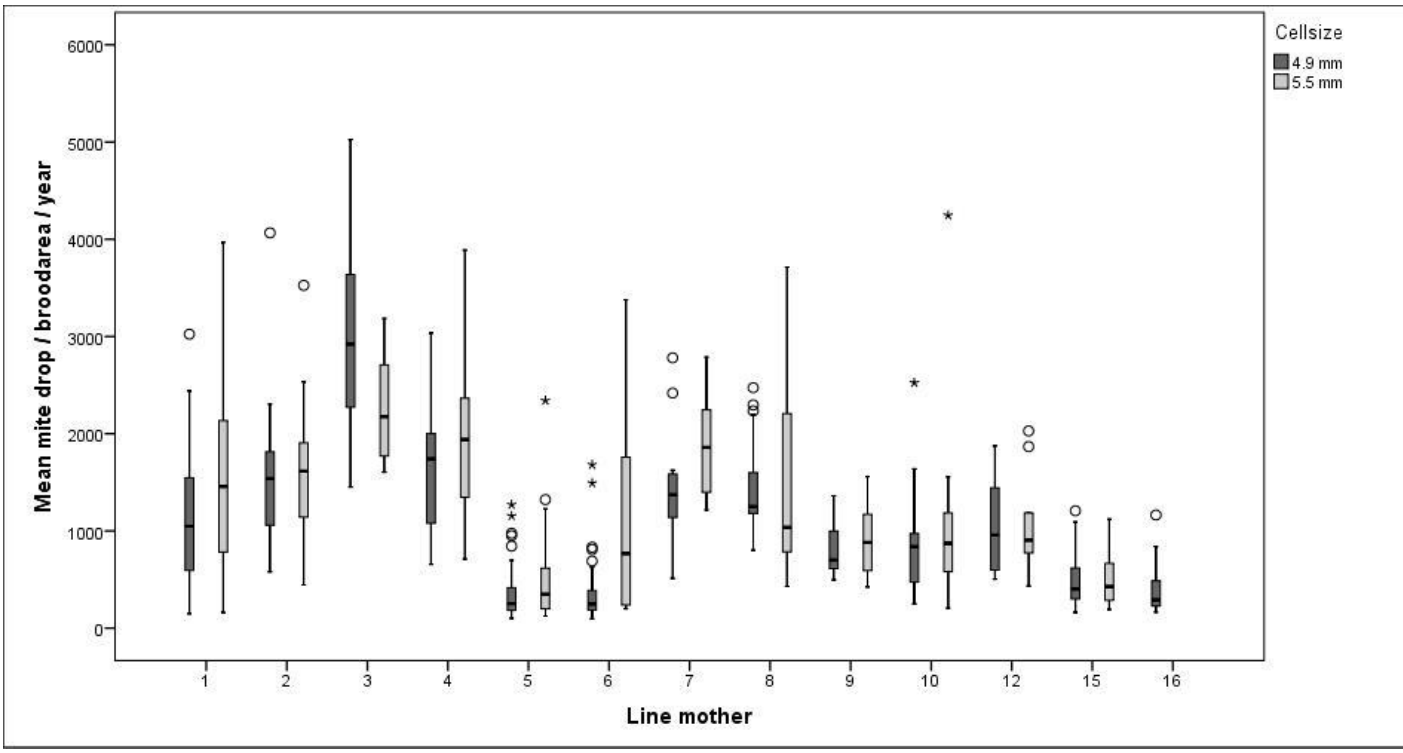

Figure 3. Mean number of dead mites on varroa-board/brood area/year considering cell size and genetic line

Table 7 shows the mean number of dead mites on varroa-board/brood area/year separated by bee yard and cell size.

Table 7. Mean number of dead mites on varroa-board/brood area/ year separated by bee yard and cell size

Bee yard	4.9 mm			5.5 mm		
	N	*M*	*SD*	*N*	*M*	*SD*
1	62	1204.5	793.2	54	1529.3	1018.1
2	12	2658.1	1030.6	12	2597.7	713.8
3	46	1524.2	748.9	35	1753.0	661.1
4	33	1268.8	844.7	37	1378.7	882.7
5	83	814.4	456.0	77	1023.8	654.1
6	16	562.0	525.6	13	1181.5	1242.3
7	22	1186.1	721.0	23	1460.6	657.1
12	55	474.1	342.5	33	661.8	381.9
19	21	389.3	186.6	9	707.2	233.7
20	21	567.8	218.0	12	679.3	246.3

Supplementary note: The grey underlays indicate bee yards, which depict differences in cell size as a factor.

Table 8 shows differences in the mean number of dead mites on varroa-board/brood area/year for both cell sizes on each bee yard. Bee yards 5, 12 and 19 show significant differences. Comparing the mean values of table 7, we can recognize that all three bee yards have less varroa mites on small cell size colonies compared to large cell size ones.
Figure 4 captures these results graphically to give a closer overview.

Table 8. Differences in mite infestation rates separated by bee yard (*results of t-test*)

Bee yard	*T*	*Df*	*p*-value
1	-1.1	114	.289
2	.0	22	.989
3	-1.6	79	.103
4	-.6	68	.531
5	-2.1*	158	.038*
6	-1.8	27	.081
7	-1.7	43	.103
12	-2.8*	86	.006*
19	-3.5*	28	.002*
20	-1.4	31	.181

Supplementary note. *$p < 0.05$.

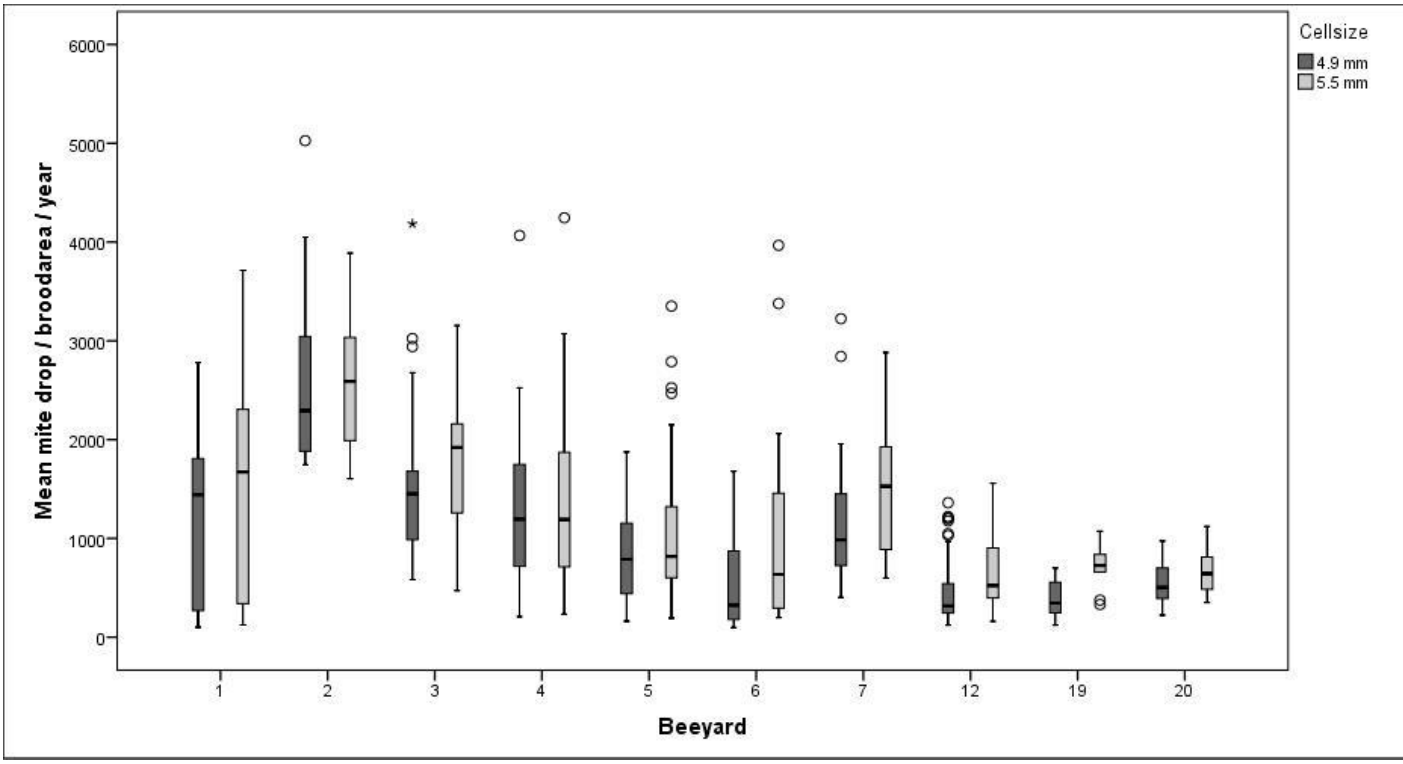

Figure 4. Mean number of dead mites on varroa-board/brood area/year considering cell sizes and bee yards

Figure 5 shows the mean number of dead mites on varroa-board/brood area/year considering cell sizes, genetic line and bee yard.

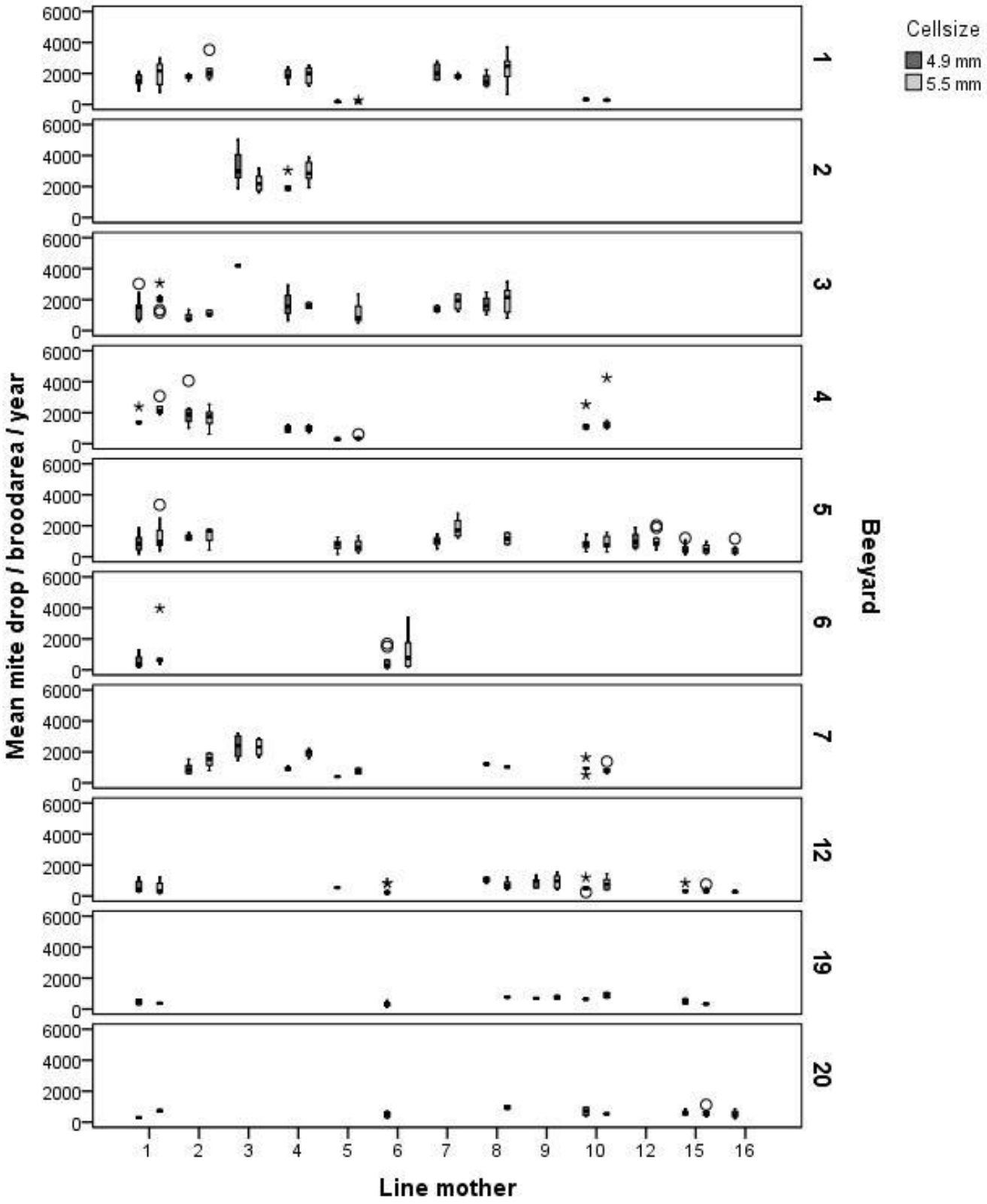

Figure 5. Mean number of dead mites on varroa-board/brood area/year considering cell sizes, genetic line and bee yard

3.2 Wintering

The results below are dealing with the drop-out rates caused by varroa mites. The survival rates on different cell sizes are compared for every year using chi square tests.
In table 9 the survival rates between the two cell sizes separated by year are compared.

Table 9. Comparison of the survival rate between the two cell sizes separated by year

Year			colony		total	X^2	p-value
			survived	dead			
2003	cell size	4.9 mm	29	68	97	1.9	0.163
		5.5 mm	21	78	99		
	Total		50	146	196		
2004	cell size	4.9 mm	85	66	151	5.1	0.023
		5.5 mm	50	68	118		
	Total		135	134	269		
2005	cell size	4.9 mm	107	16	123	1.0	0.302
		5.5 mm	72	16	88		
	Total		179	32	211		

Supplementary note: Odds Ratios (OR) with 95% confidence interval: 2003: OR= 1.58 [0.8; 3.0]. 2004: OR= 1.75 [1.1; 2.8] 2005: OR= 1.48 [0.7; 3.2]

In 2004 a significant difference between cell sizes can be observed (X2 = 5,1; p = 0,023). With an odds ratio (OR) = 1.75 colonies with smaller cell size are more likely to survive the first winter after build up. The OR is similar with 1.58 in 2003 and 1.48 in 2005, but not significant.
Among all years, the difference between cell sizes is significant (X^2 = 10.8; p = 0.001) with an OR = 1.69. Thus, bees on small cell size combs have a greater chance to survive then bees on large cell size combs.

Table 10 shows the comparison of the survival rate of colonies on SCS and LCS.

Table 10. Comparison of the survival rate of colonies on SCS and LCS (summarized for all years)

		colony		total	X^2	p-value
		survived	dead			
cell size	4.9 mm	221	150	371	10.8	< 0.001
	5.5 mm	143	162	305		
Total		364	312	676		

Supplementary note: Odds Ratio (OR) with 95% confidence interval: OR= 1.69 [1.3 ; 2.3].

In table 11 the comparison of the survival rate on both cell sizes separated by bee yard is figured.

Table 11. Comparison of the survival rate on both cell sizes separated by bee yard

bee yard			colony		total	X^2	p-value
			survived	dead			
1	cell size	4.9 mm	20	42	62	0.1	.929
		5.5 mm	17	37	54		
	Total		37	79	116		
2	cell size	4.9 mm		12	12		
		5.5 mm		12	12		
	Total			24	24		
3	cell size	4.9 mm	15	31	46	8.7	.003
		5.5 mm	2	33	35		
	Total		17	64	81		
4	cell size	4.9 mm	17	16	33	0.1	.832
		5.5 mm	20	17	37		
	Total		37	33	70		
5	cell size	4.9 mm	68	15	83	1.5	.227
		5.5 mm	57	20	77		
	Total		125	35	160		
6	cell size	4.9 mm	12	4	16	3.9	.047
		5.5 mm	5	8	13		
	Total		17	12	29		
7	cell size	4.9 mm	15	7	22	2.8	.095
		5.5 mm	10	13	23		
	Total		25	20	45		
12	cell size	4.9 mm	42	13	55	1.6	.200
		5.5 mm	21	12	33		
	Total		63	25	88		
19	cell size	4.9 mm	18	3	21	5.5	.019
		5.5 mm	4	5	9		
	Total		22	8	30		
20	cell size	4.9 mm	14	7	21	0.2	.632
		5.5 mm	7	5	12		
	Total		21	12	33		

Even when examining specific bee yards, significant differences are noticeable (table 11). Significant bee yards are highlighted in grey. In bee yard 3, 6 and 19 the survival rate significantly favours small cell size colonies. The correlated OR are displayed in table 12.

Bee hives with small cell sizes have an 8 times higher chance of survival as those with large cell sizes.

Table 12. ***Odds Ratios (OR) of the survival chance which is depict in table 11, separated after bee yard***

Bee yard	OR	95% confidential interval	
		lower	upper
1	1.04	0.5	2.3
3	7.98	1.7	37.8
4	0.90	0.4	2.3
5	1.59	0.7	3.4
6	4.80	1.0	23.5
7	2.79	0.8	9.4
12	1.85	0.7	4.7
19	7.50	1.2	45.2
20	1.43	0.3	6.2

Supplementary note: A OR > 1 means that the survival rate for small cell size is higher than the one for large cell size. In bee yard 3 the chance of survival is for small cell size colonies (4.9 mm combs) 7.98 times higher than the rate of survival for large cell size colonies (5.5 mm combs). Bee yard 4 is the only location where large cell size colonies have a better chance of survival with an OR= 1.11, disfavouring small cell sizes ($1.11 = 0.90^{-1}$).

Within SCS, 150 out of 371 colonies (40.4%) were lost, compared to 162 (53.1%) out of 305 colonies within LCS, which is significant ($X^2=10.8$; $p < 0.001$).

SCS colonies are significantly more likely to survive the first winter after build-up (OR=1.69 with a 95% confidence interval ranging from [1.3 - 2.3]).

3.3 Varroa reproduction parameter

Table 13 and 16 give a detailed overlook of the descriptive results.
In table 13 and 14 the mite production is reviewed. Table 15 and 16 display the calculated

reproduction parameter. Since the studies were differently designed in the years 2007 and 2008, table 13 and 15 break down the parameters for the year 2007, whereas table 14 and 16 illustrate the parameters for the year 2008.

Table 13. Overview of mite production in brood cells in the year 2007, using genetic line and cell size as factors in the analysis. ***N per line and cell size = 10***

		Cell size									
		4.9 mm					5.5 mm				
Line	Parameter	N	min	Max	M	SD	N	Min	max	M	SD
1	cells with dead mites	10	0	8	2.1	3.1	10	0	3	.6	1.3
	mother mites only	10	4	22	10.2	5.8	10	2	14	5.6	3.7
	1 offspring	10	11	32	19.9	7.0	10	14	34	21.8	7.5
	2 offspring	10	13	23	18.4	3.6	10	6	22	17.0	5.7
	3 offspring	10	0	6	1.1	2.3	10	0	9	3.8	3.7
	total mites	10	89	138	110.2	15.7	10	92	134	115.4	12.6
	all offspring	10	39	88	60.2	15.7	10	42	84	65.4	12.6
	offspring / infested cells	10	.78	1.76	1.2	.3	10	.84	1.68	1.3	.3
2	cells with one dead mite	10	0	2	.3	.7	10	0	2	.3	.7
	mother mites only	10	0	8	3.8	2.6	10	0	7	3.4	2.6
	1 offspring	10	7	33	16.8	8.1	10	5	25	13.0	6.7
	2 offspring	10	10	40	30.0	7.9	10	24	38	29.0	4.6
	3 offspring	10	0	5	2.0	2.1	10	0	12	5.3	3.5
	total mites	10	101	154	135.4	14.8	10	122	151	137.6	9.9
	all offspring	10	51	104	85.4	14.8	10	72	101	87.6	9.9
	offspring / infested cells (50 or 200)	10	1.02	2.08	1.7	.3	10	1.44	2.02	1.8	.2
3	cells with one dead mite	10	0	1	.1	.3	10	0	1	.1	.3
	mother mites only	10	0	10	4.6	2.5	10	0	10	3.3	3.0
	1 offspring	10	5	21	12.3	5.3	10	3	18	9.0	4.9
	2 offspring	10	25	35	30.8	3.6	10	21	43	34.6	6.0
	3 offspring	10	0	8	3.2	3.2	10	0	7	3.0	2.4
	total mites	10	122	149	134.4	8.4	10	117	155	137.9	11.4
	all offspring	10	72	99	84.4	8.4	10	67	105	87.9	11.4
	offspring / infested cells (50 or 200)	10	1.44	1.98	1.7	.2	10	1.34	2.10	1.8	.2

4	cells with one dead mite	10	0	2	.2	.6	10	0	5	1.2	1.8
	mother mites only	10	0	15	8.1	4.4	10	3	22	10.9	6.5
	1 offspring	10	14	37	26.4	7.3	10	14	37	28.0	7.4
	2 offspring	10	6	24	15.7	6.5	10	6	15	10.5	3.4
	3 offspring	10	0	8	2.2	3.0	10	0	4	1.8	1.3
	total mites	10	93	142	116.8	16.4	10	82	121	105.6	12.1
	all offspring	10	43	92	66.8	16.4	10	32	71	55.6	12.1
	offspring / infested cells (50 or 200)	10	.86	1.84	1.3	.3	10	.64	1.42	1.1	.2
total 2007	cells with one dead mite	40	0	8	.7	1.8	40	0	5	.6	1.2
	mother mites only	40	0	22	6.7	4.7	40	0	22	5.8	5.1
	1 offspring	40	5	37	18.9	8.5	40	3	37	18.0	9.9
	2 offspring	40	6	40	23.7	8.8	40	6	43	22.8	10.8
	3 offspring	40	0	8	2.1	2.7	40	0	12	3.5	3.0
	total mites	40	89	154	124.2	17.6	40	82	155	124.1	18.1
	all offspring	40	39	104	74.2	17.6	40	32	105	74.1	18.1
	offspring / infested cells (50 or 200)	40	.78	2.08	1.5	.4	40	.64	2.10	1.5	.4

Table 14. Overview of the mite production in brood cells in the year 2008, using genetic line and cell size as factors in the analysis. *N per line = 10*

			Cell size								
			4.9 mm					5.5 mm			
Line	Parameter	N	Min	Max	M	SD		Min	Max	M	SD
1	cells with one dead mite	10	0	26	7.1	8.9		0	11	2.6	3.5
	mother mites only	10	6	41	23.9	12.0		2	21	11.4	6.9
	1 offspring	10	36	84	56.2	18.1		40	73	53.6	10.0
	2 offspring	10	14	91	39.5	22.2		24	73	46.4	19.4
	3 offspring	10	0	1	.1	.3		0	7	2.7	1.9
	total mites	10	169	452	255.2	81.8		199	362	270.2	52.3
	all offspring	10	71	266	135.5	55.1		103	223	154.5	39.4
	offspring/ infested cells	10	.36	1.33	.7	.3		.52	1.12	.8	.2
3	cells with one dead mite	10	0	4	.6	1.3		0	1	.1	.3
	mother mites only	10	2	31	12.0	7.7		2	11	4.6	2.8
	1 offspring	10	14	58	31.0	14.8		11	41	28.0	9.9
	2 offspring	10	33	81	55.8	16.4		28	76	55.0	15.1
	3 offspring	10	0	3	.9	1.1		0	7	3.0	2.7
	total mites	10	184	294	245.0	32.8		185	280	237.8	34.8
	all offspring	10	112	186	145.3	24.2		106	184	147.0	26.3
	offspring/ infested cells	10	.56	.93	.7	.1		.53	.92	.7	.1
Total 2008	cells with one dead mite	20	0	26	3.9	7.0		0	11	1.4	2.7
	mother mites only	20	2	41	18.0	11.5		2	21	8.0	6.2
	1 offspring	20	14	84	43.6	20.7		11	73	40.8	16.3
	2 offspring	20	14	91	47.7	20.8		24	76	50.7	17.5
	3 offspring	20	0	3	.5	.9		0	7	2.9	2.3
	total mites	20	169	452	250.1	60.9		185	362	254.0	46.3
	all offspring	20	71	266	140.4	41.7		103	223	150.8	32.8
	offspring/ infested cells	20	.36	1.33	.7	.2		.52	1.12	.8	.2

Table 15. Overview of the reproductive factors in brood cells in the year 2007, using genetic line and cell size as factors in the analysis. ***N per line and cell size = 10***

		Cell size									
		4.9 mm					5.5 mm				
Line	Parameter	N	Min	Max	M	SD	N	Min	Max	M	SD
1	infestation : wholesome	10	.08	.17	.13	.03	10	.09	.24	.14	.05
	degree of infestation (%)	10	.08	.14	.11	.02	10	.08	.19	.12	.04
	offspring / infested cells (50 or 200)	10	.78	1.76	1.20	.31	10	.84	1.68	1.31	.25
	ratio non-reproductive / reproductive (VSH)	10	.09	.81	.35	.25	10	.04	.35	.15	.10
	percentage of non-reproductive mother mites (%)	10	.08	.45	.24	.13	10	.04	.26	.13	.07
	non-reproductive mites	10	4	26	12.30	7.15	10	2	14	6.20	3.71
	reproductive mites	10	30	52	39.40	7.44	10	37	49	42.60	4.20
2	infestation : wholesome	10	.17	.35	.24	.06	10	.16	.30	.22	.05
	degree of infestation (%)	10	.14	.26	.19	.04	10	.14	.23	.18	.03
	offspring / infested cells (50 or 200)	10	1.02	2.08	1.71	.30	10	1.44	2.02	1.75	.20
	ratio non-reproductive / reproductive (VSH)	10	.00	.16	.09	.05	10	.00	.16	.08	.06
	percentage of non-reproductive mother mites (%)	10	.00	.14	.08	.05	10	.00	.14	.07	.05
	non-reproductive mites	10	0	8	4.10	2.56	10	0	7	3.70	2.58
	reproductive mites	10	43	54	48.80	3.71	10	41	52	47.30	3.53
3	infestation : wholesome	10	.15	.28	.22	.04	10	.19	.31	.26	.04
	degree of infestation (%)	10	.13	.22	.18	.03	10	.16	.23	.20	.02
	offspring / infested cells (50 or 200)	10	1.44	1.98	1.69	.17	10	1.34	2.10	1.76	.23
	ratio non-reproductive / reproductive (VSH)	10	.00	.22	.10	.06	10	.00	.27	.07	.08
	percentage of non-reproductive mother mites (%)	10	.00	.18	.09	.05	10	.00	.21	.07	.06
	non-reproductive mites	10	0	10	4.70	2.58	10	0	11	3.40	3.27
	reproductive mites	10	43	51	46.30	2.41	10	41	53	46.60	3.53

4	infestation : wholesome	10	.10	.17	.12	.02	10	.09	.18	.12	.03
	degree of infestation (%)	10	.09	.14	.11	.02	10	.08	.15	.10	.02
	offspring / infested cells (50 or 200)	10	.86	1.84	1.34	.33	10	.64	1.42	1.11	.24
	ratio non-reproductive / reproductive (VSH)	10	.00	.41	.19	.12	10	.06	1.13	.34	.31
	percentage of non-reproductive mother mites (%)	10	.00	.29	.15	.08	10	.06	.53	.23	.14
	non-reproductive mites	10	0	17	8.30	4.76	10	3	27	12.10	7.53
	reproductive mites	10	39	51	44.30	3.74	10	24	48	40.30	7.01
Total 2007	infestation : wholesome	40	.08	.35	.18	.07	40	.09	.31	.18	.07
	degree of infestation (%)	40	.08	.26	.15	.05	40	.08	.23	.15	.05
	offspring / infested cells (50 or 200)	40	.78	2.08	1.48	.35	40	.64	2.10	1.48	.36
	ratio non-reproductive / reproductive (VSH)	40	.00	.81	.18	.17	40	.00	1.13	.16	.20
	percentage of non-reproductive mother mites (%)	40	.00	.45	.14	.10	40	.00	.53	.12	.11
	non-reproductive mites	40	0	26	7.35	5.58	40	0	27	6.35	5.73
	reproductive mites	40	30	54	44.70	5.72	40	24	53	44.20	5.45

Table 16. Overview of the reproductive factors in brood cells in the year 2008, using genetic line and cell size as factors in the analysis. *N per line = 10*

			Cell size								
			4.9 mm					5.5 mm			
Line	Parameter	N	Min	Max	M	SD		Min	Max	M	SD
1	infestation : wholesome	10	.12	.28	.19	.05	10	.13	.25	.18	.04
	degree of infestation (%)	10	.11	.22	.16	.03	10	.12	.20	.15	.03
	offspring / infested cells (50 or 200)	10	.36	1.33	.68	.28	10	.52	1.12	.77	.20
	ratio non-reproductive / reproductive (VSH)	10	.06	1.18	.40	.33	10	.02	.41	.15	.12
	percentage of non-reproductive mother mites (%)	10	.05	.54	.26	.15	10	.02	.29	.12	.08
	non-reproductive mites	10	6	67	31.00	18.04	10	2	31	14.00	8.88
	reproductive mites	10	57	175	95.80	34.55	10	76	136	102.7	18.57
3	infestation : wholesome	10	.15	.36	.22	.07	10	.15	.28	.23	.04
	degree of infestation (%)	10	.13	.26	.18	.04	10	.13	.22	.18	.03
	offspring / infested cells (50 or 200)	10	.56	.93	.73	.12	10	.53	.92	.74	.13
	ratio non-reproductive / reproductive (VSH)	10	.03	.38	.14	.09	10	.02	.15	.06	.04
	percentage of non-reproductive mother mites (%)	10	.03	.27	.12	.07	10	.02	.13	.05	.03
	non-reproductive mites	10	2	31	12.60	7.88	10	2	11	4.70	2.87
	reproductive mites	10	70	99	87.70	9.49	10	71	96	86.00	9.82

Total 2008	infestation : wholesome	20	.12	.36	.21	.06		.13	.28	.21	.05
	degree of infestation (%)	20	.11	.26	.17	.04		.12	.22	.17	.03
	offspring / infested cells (50 or 200)	20	.36	1.33	.70	.21		.52	1.12	.75	.16
	ratio non-reproductive / reproductive (VSH)	20	.03	1.18	.27	.27		.02	.41	.10	.10
	percentage of non-reproductive mother mites (%)	20	.03	.54	.19	.13		.02	.29	.09	.07
	non-reproductive mites	20	2	67	21.80	16.51		2	31	9.35	8.00
	reproductive mites	20	57	175	91.75	25.0		71	96	94.35	16.8

Table 17 shows the results of the variance analysis, which were conducted in the year 2007. The effect of the line (distinction between the genetic lines) is significant when it comes to reproductive parameters. Line 1 and 4 quite differ from line 2 and 3. Cell size plays no significant factor for these reproduction parameters.
There are 3 significant factors: VSH, the percentage of non-reproductive mother mites and the total contingent of non-reproductive mites.

That means, that the VSH trait doesn't primary rely on cell size, but on the genetic line. VSH differs vastly between the lines, not between cell sizes. However, the degree of VSH can vary within a line between small and large cell size.
Hence the statement, that small cell size exhibit a higher VSH trait, is only right for certain genetic lines, but not for all. Lines that miss the trait of course do not demonstrate the effect of cell size on the expression of the trait.

Table 17. Influence of genetic line and cell size on the reproduction parameter of varroa mites in the year 2007. (Results of variance analysis)

QdV	Parameter	df	F(df. 72)	p-value
Line	total of mites	3	23.6	< 0.001[a]
	infestation : wholesome	3	45.3	< 0.001[a]
	degree of infestation (%)	3	48.2	< 0.001[a]
	offspring / infested cells	3	23.6	< 0.001[a]
	ratio non-reproductive / reproductive (VSH)	3	8.2	< 0.001[a]
	percentage of non-reproductive mother mites (%)	3	11.2	< 0.001[a]
	non-reproductive mites	3	10.2	< 0.001[a]
	reproductive mites	3	9.9	< 0.001[a]
Cell size	total mites	1	< 1	0.979
	infested : wholesome	1	< 1	0.488
	degree of infestation (%)	1	< 1	0.508
	offspring / infested cells (50 or 200)	1	< 1	0.979
	ratio non-reproductive / reproductive (VSH)	1	< 1	0.590
	percentage of non-reproductive mother mites (%)	1	< 1	0.387
	non-reproductive mites	1	< 1	0.342
	reproductive mites	1	< 1	0.639
Line x Cell size	total mites	3	1.7	0.177
	infested : wholesome	3	1.7	0.180
	degree of infestation (%)	3	1.6	0.204
	offspring / infested cells (50 or 200)	3	1.7	0.177
	ratio non-reproductive / reproductive (VSH)	3	4.1	0.010*
	percentage of non-reproductive mother mites (%)	3	3.9	0.013*
	non-reproductive mites	3	3.8	0.014*
	reproductive mites	3	2.0	0.116

Supplementary note: [a]Post hoc analysis using Sheffe's alpha correction showed significant differences between line 1 and line 2 and between line 1 and line 3. Line 4 was different from line 2 and line 3. *$p < 0{,}01$.

Table 18 and 19 show the p-values as a result of the mixed model analyses for the year 2008.

Table 18. Results of the mixed model for the year 2008, *illustrating the p-value*

	total	infestation : wholesome	degree of infestation (%)	RI	VSH (non-reproductive : reproductive)	VSH II (%) (non reproductive : total)	non-reproductive mites	reproductive mites
Line	0.222	0.023*	0.023*	0.925	0.006*	0.002*	< 0.001*	0.069
Cell size	0.821	0.976	0.982	0.399	0.007*	0.002*	< 0.001*	0.695
Line x Cell size	0.521	0.650	0.608	0.480	0.167	0.289	0.198	0.518

Supplementary note: The highlighted grey segments indicate significant results. *$p < 0.05$.

The mixed model analysis shows no direct correlation between genetic line and cell size. However a significant effect of the line on ratio and degree of infestation was detectable. In line 3 a higher degree of infestation occurs than within line 1 (see table 16). The cell size was significant for VSH. Other significant factors were the ratio of non-reproductive mites and the quantity of non-reproductive mites. Within line 1 and 3, higher (better) values were observed when using small cell size.

Table 19. Results of the mixed model for the year 2008, illustrating the f-values from the table above

	total	infested : wholesome	Degree of infestation (%)	RI	VSH (non-reproductive : reproductive)	VSH II (%) (non reproductive : total)	non-reproductive mites	reproductive mites
Line	1.5	5.7	5.7	< 1	9.4	12.0	16.2	3.6
Cell size	< 1	< 1	< 1	< 1	8.6	11.4	13.1	< 1
Line x Cell size	< 1	< 1	< 1	< 1	2.0	1.2	1.7	< 1

Figure 6 to 19 show the most important reproduction parameter. In each year, genetic line and cell size were compared with each other.

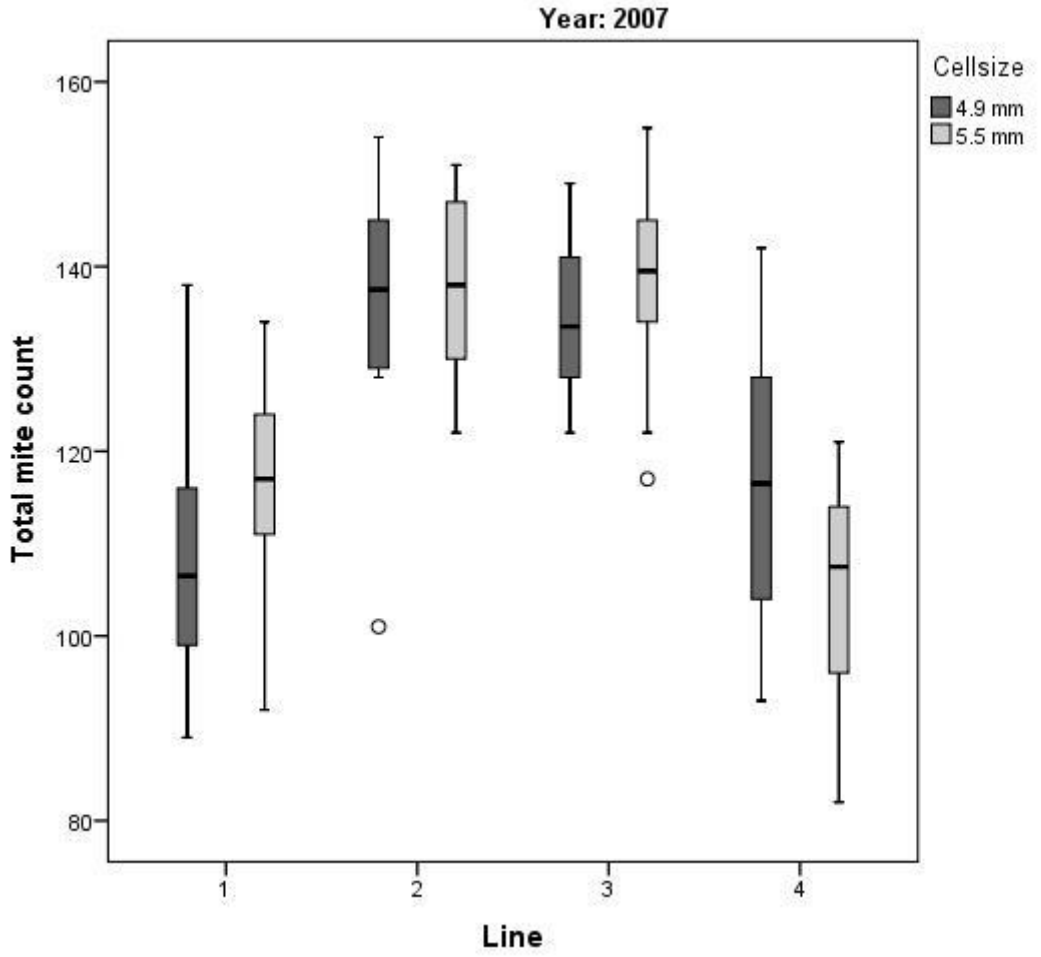

Figure 6. Total amount of mites per 50 infested brood cells, using genetic line and cell size as factors in the analysis

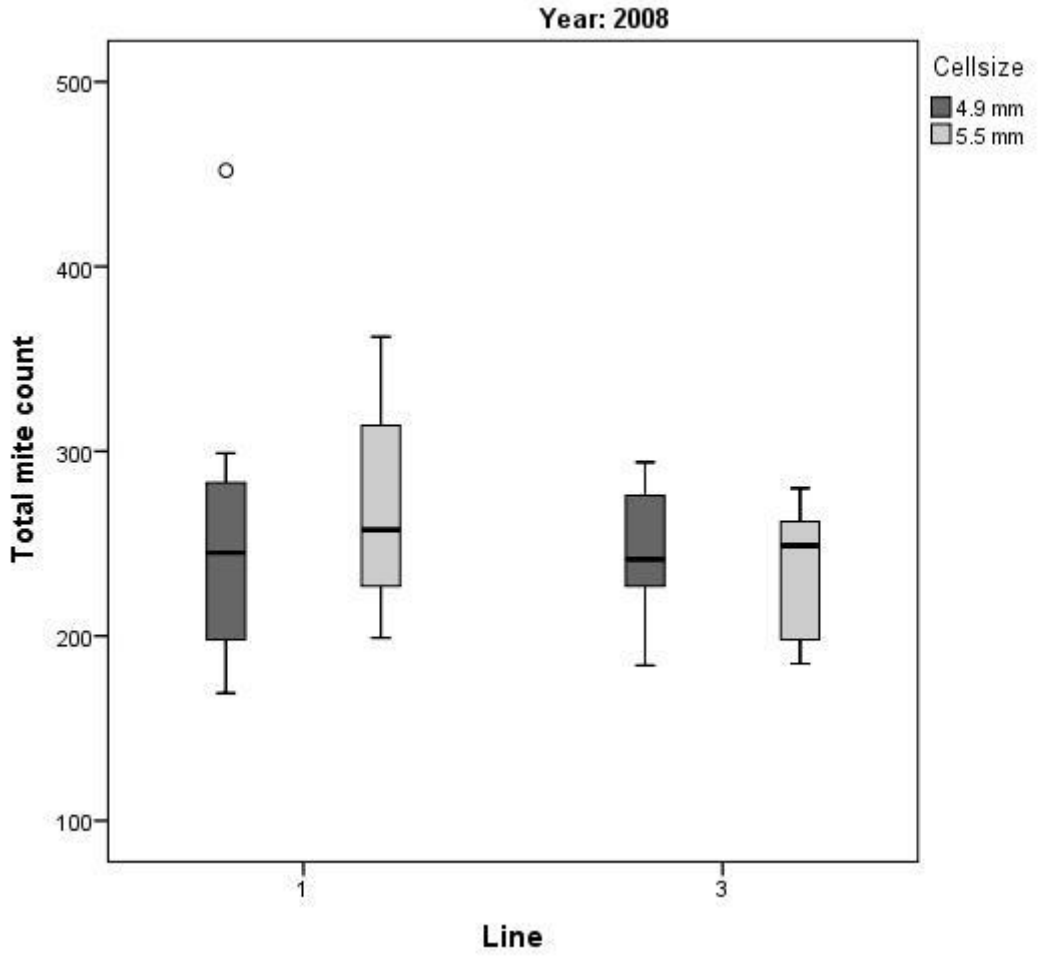

Figure 7. Total number of mites per 200 infested brood cells, using genetic line and cell size as factors in the analysis

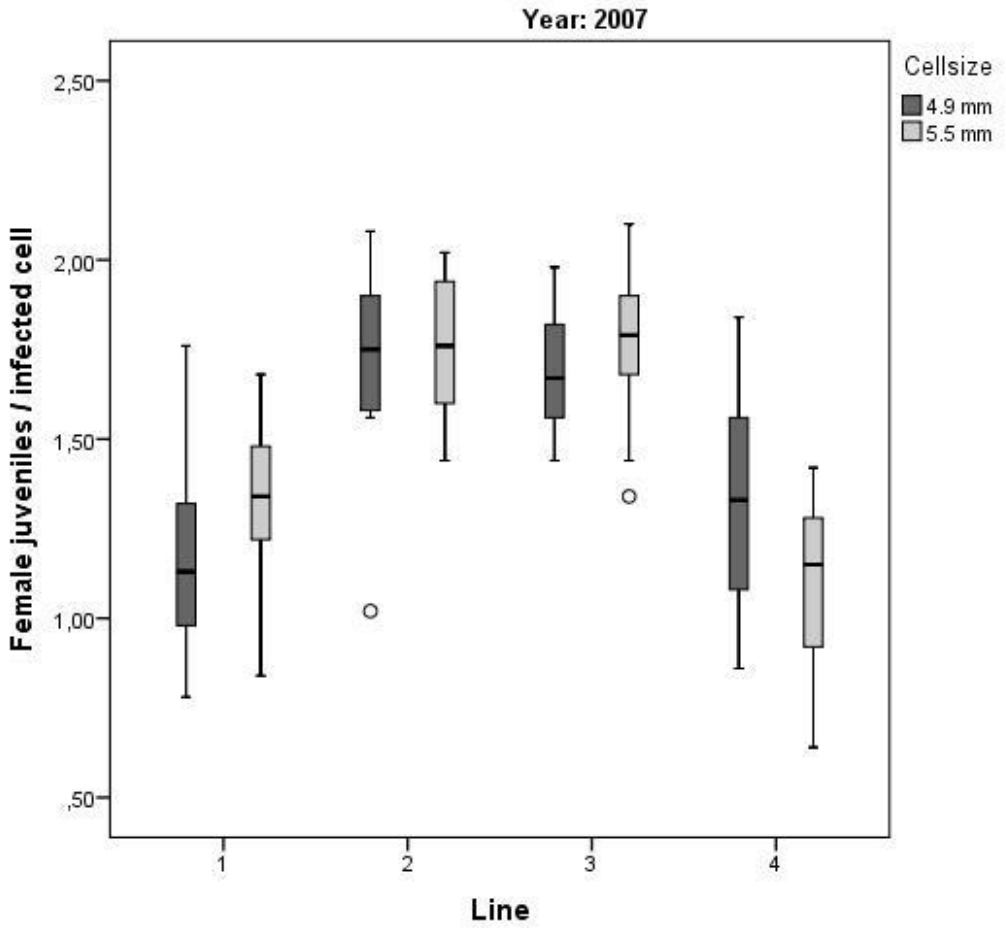

Figure 8. Number of offspring per 50 infested brood cells, using genetic line and cell size as factors in the analysis

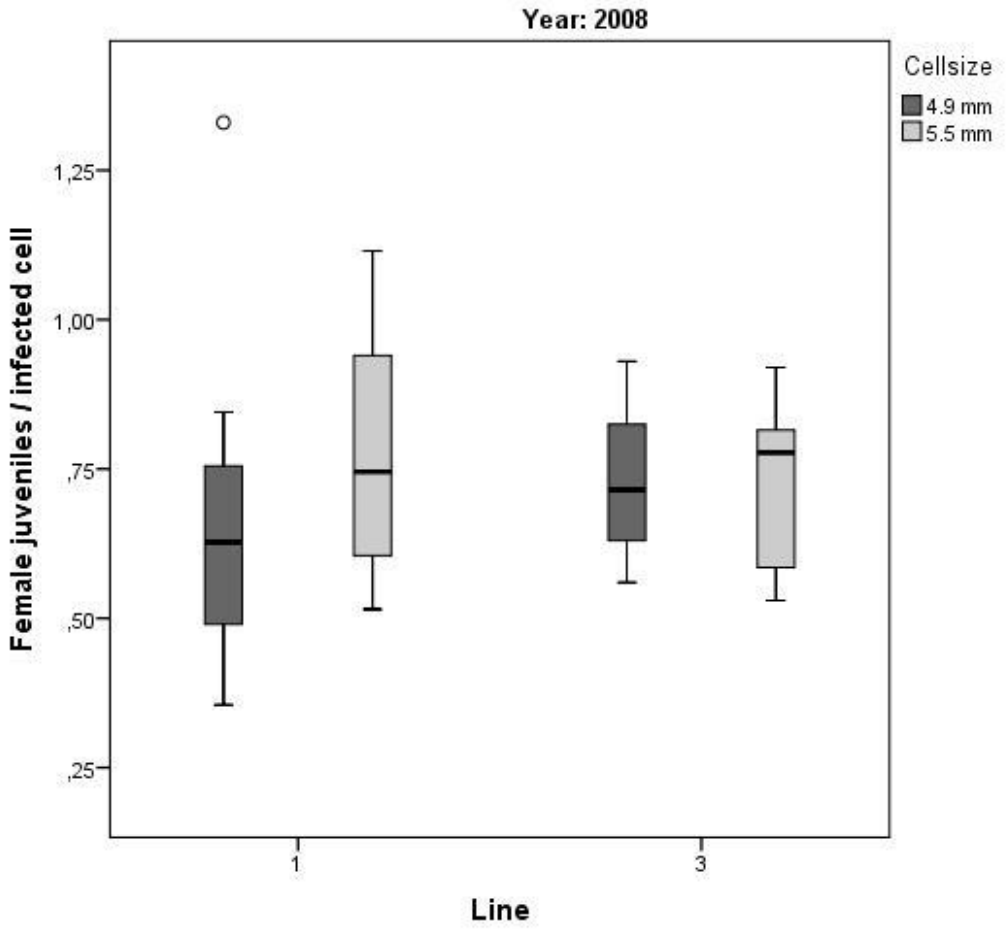

Figure 9. Number of offspring per 200 infested brood cells, using genetic line and cell size as factors in the analysis

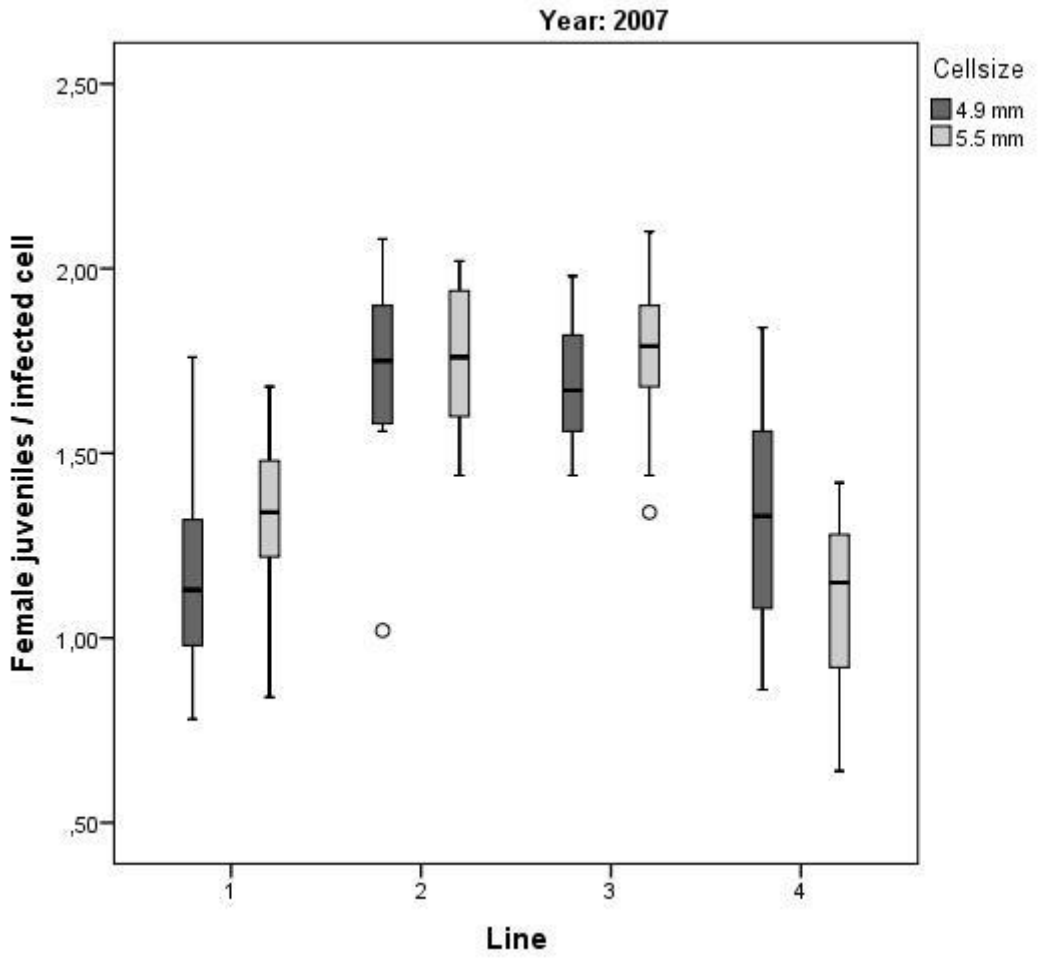

Figure 10. Ratio of infestation in the year 2007, using genetic line and cell size as factors in the analysis

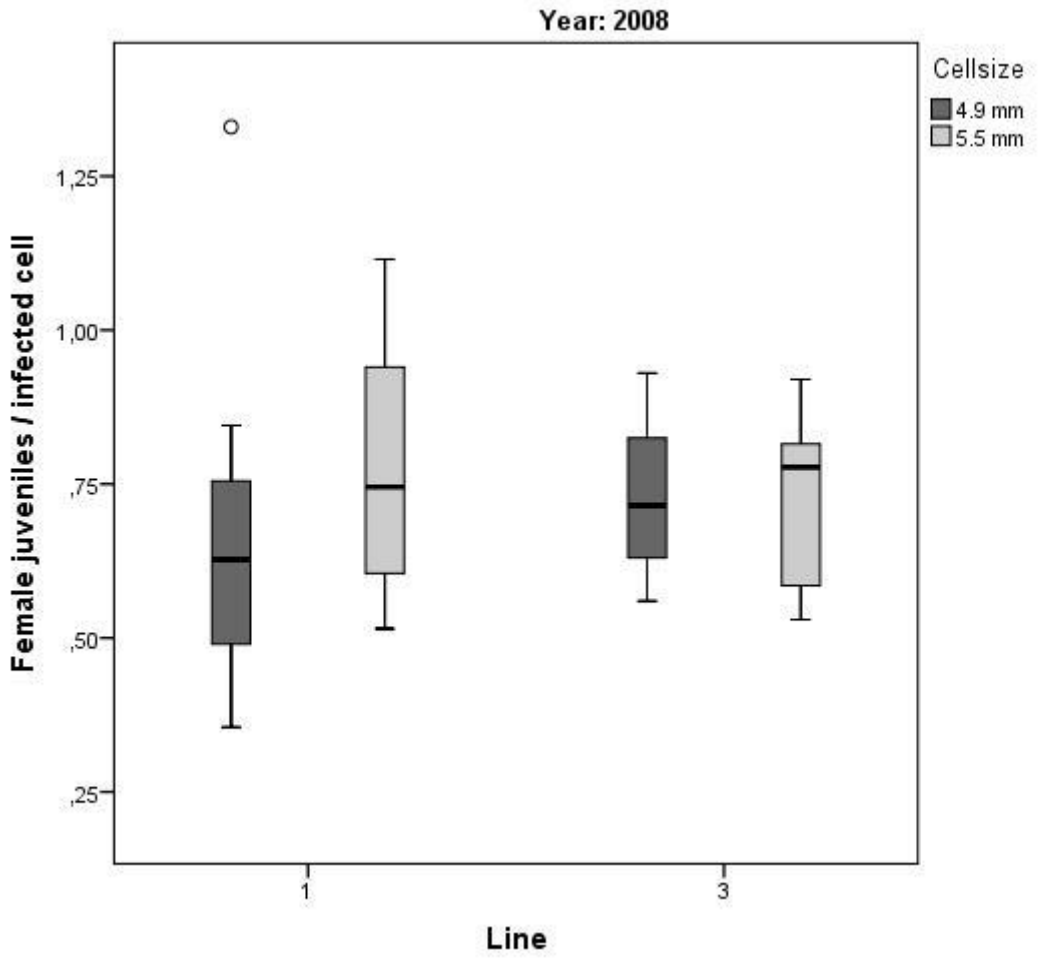

Figure 11. Ratio of infestation in the year 2008, using genetic line and cell size as factors in the analysis

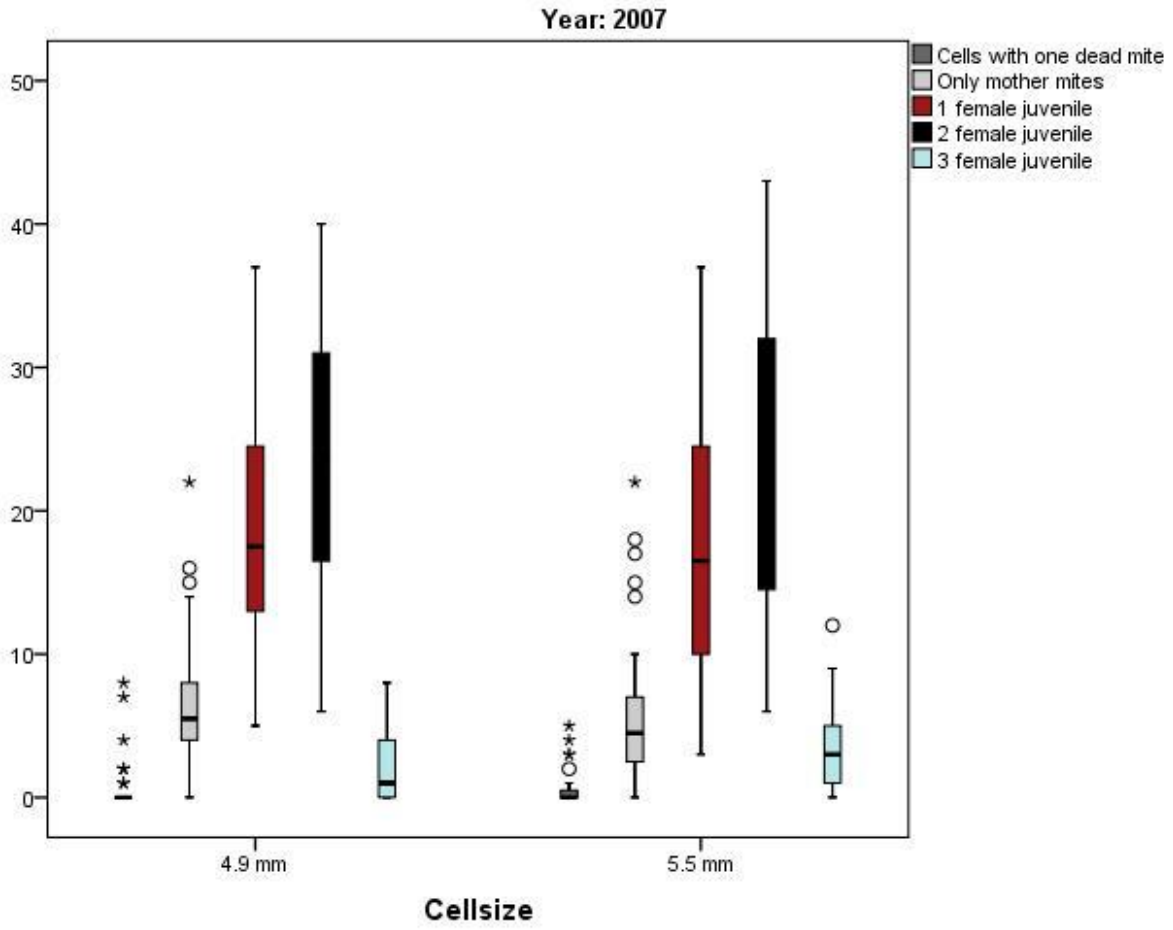

Figure 12. Ratio of infestation in the year 2007, using genetic line and cell size as factors in the analysis

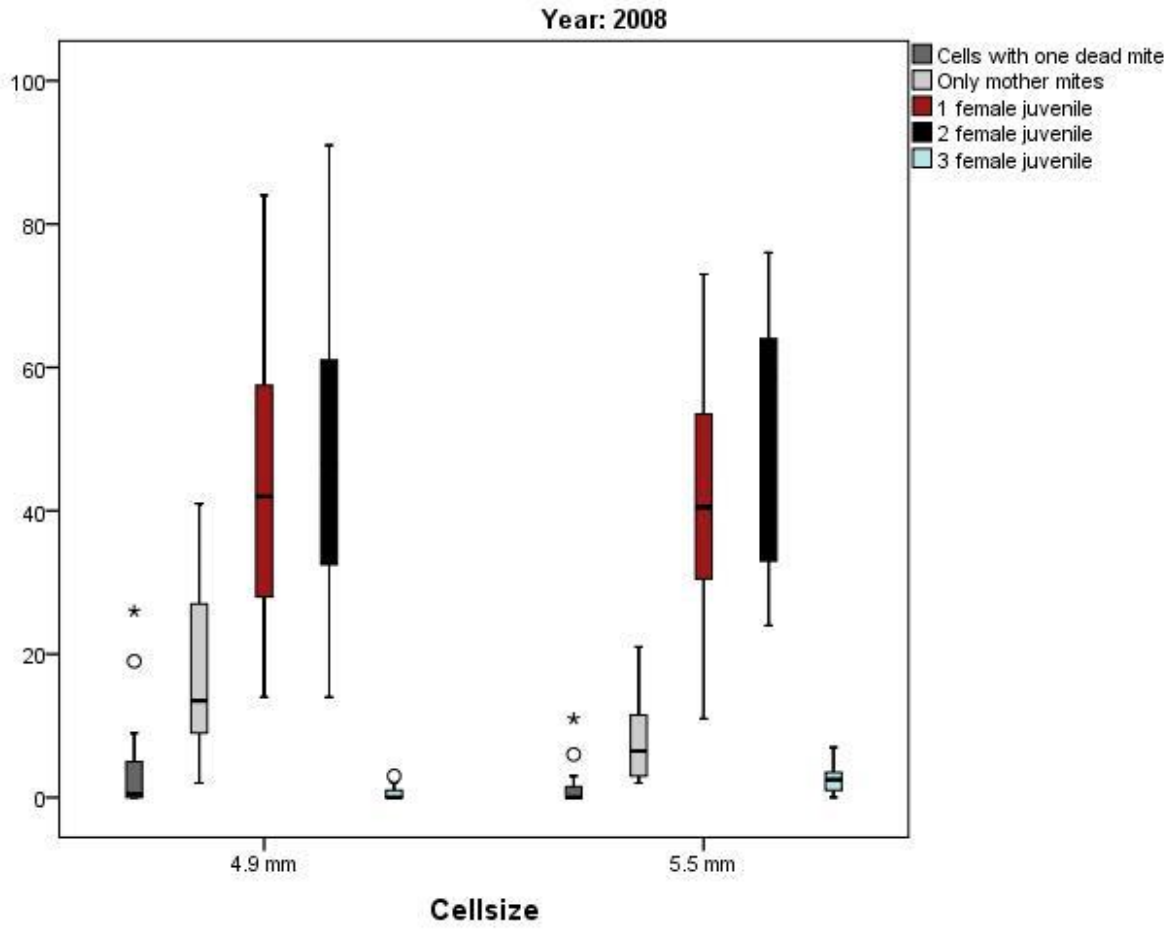

Figure 13. Degree of infestation in the year 2008, using genetic line and cell size as factors in the analysis

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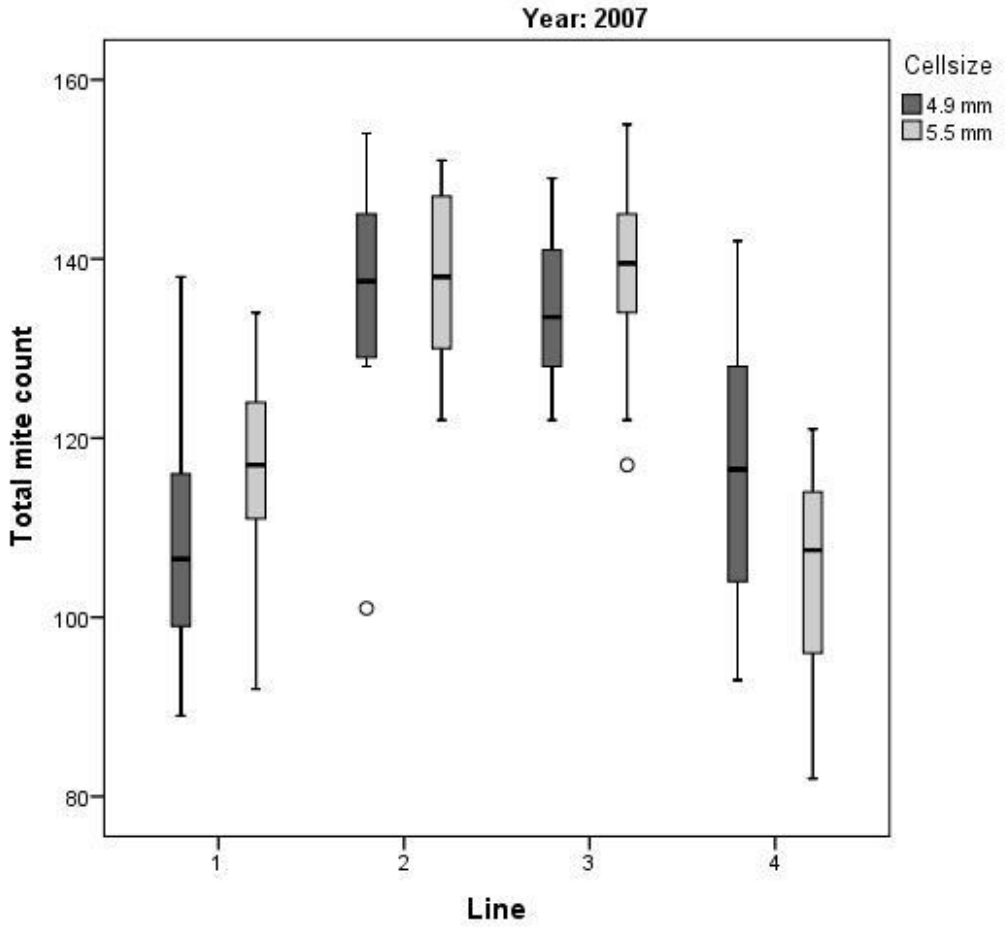

Figure 14. Reproduction index (RI) in the year 2007, using genetic line and cell size as factors in the analysis

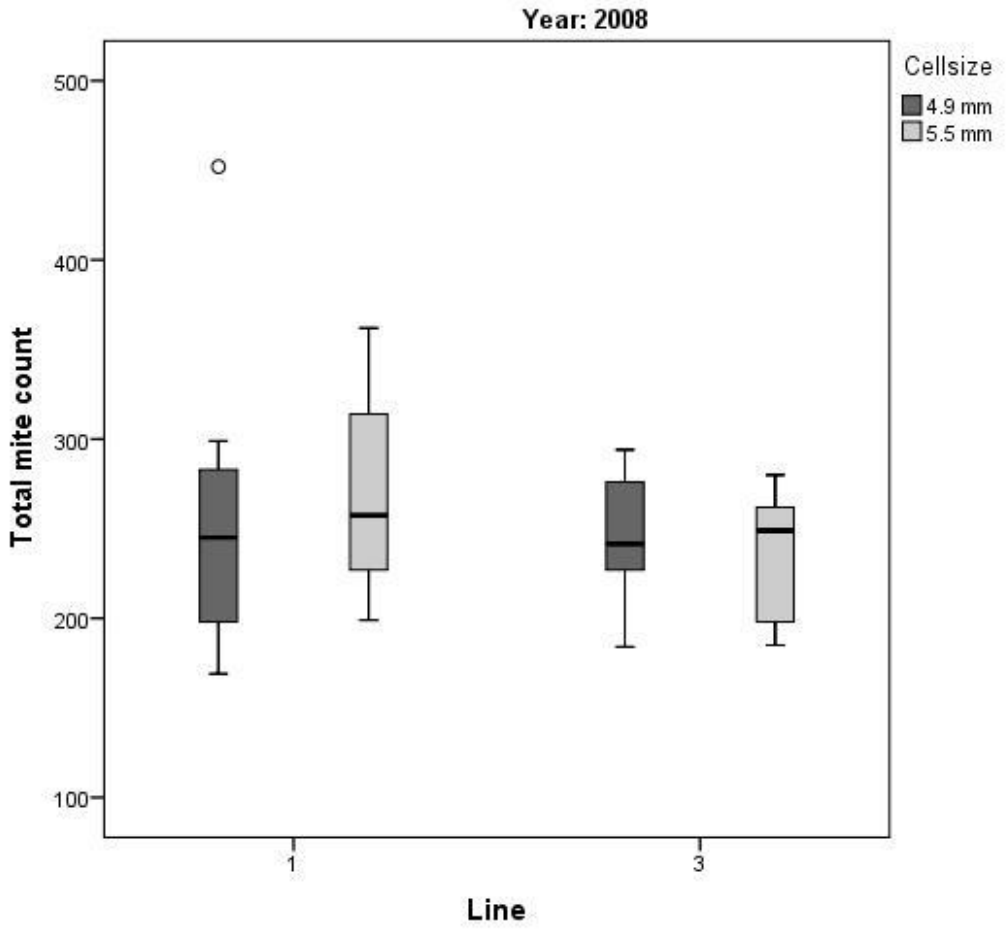

Figure 15. Reproduction index (RI) in the year 2008, using genetic line and cell size as factors in the analysis

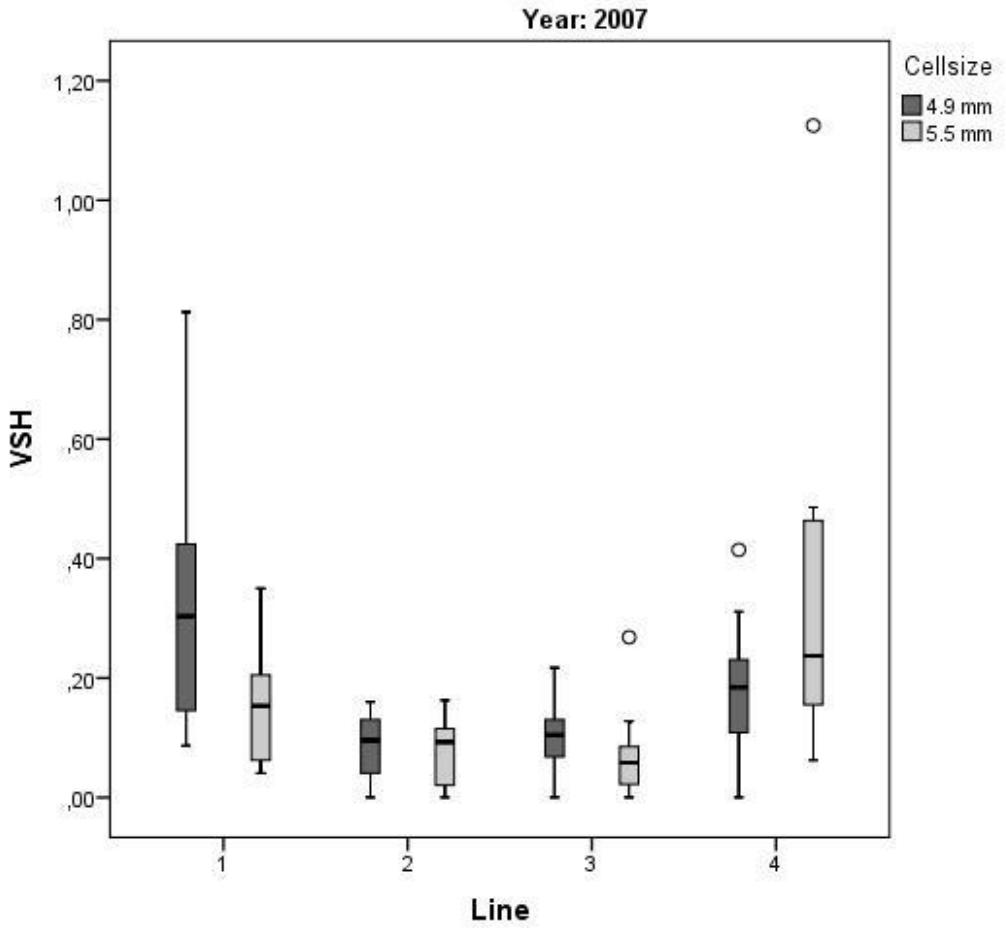

Figure 16. VSH in the year 2007, using genetic line and cell size as factors in the analysis

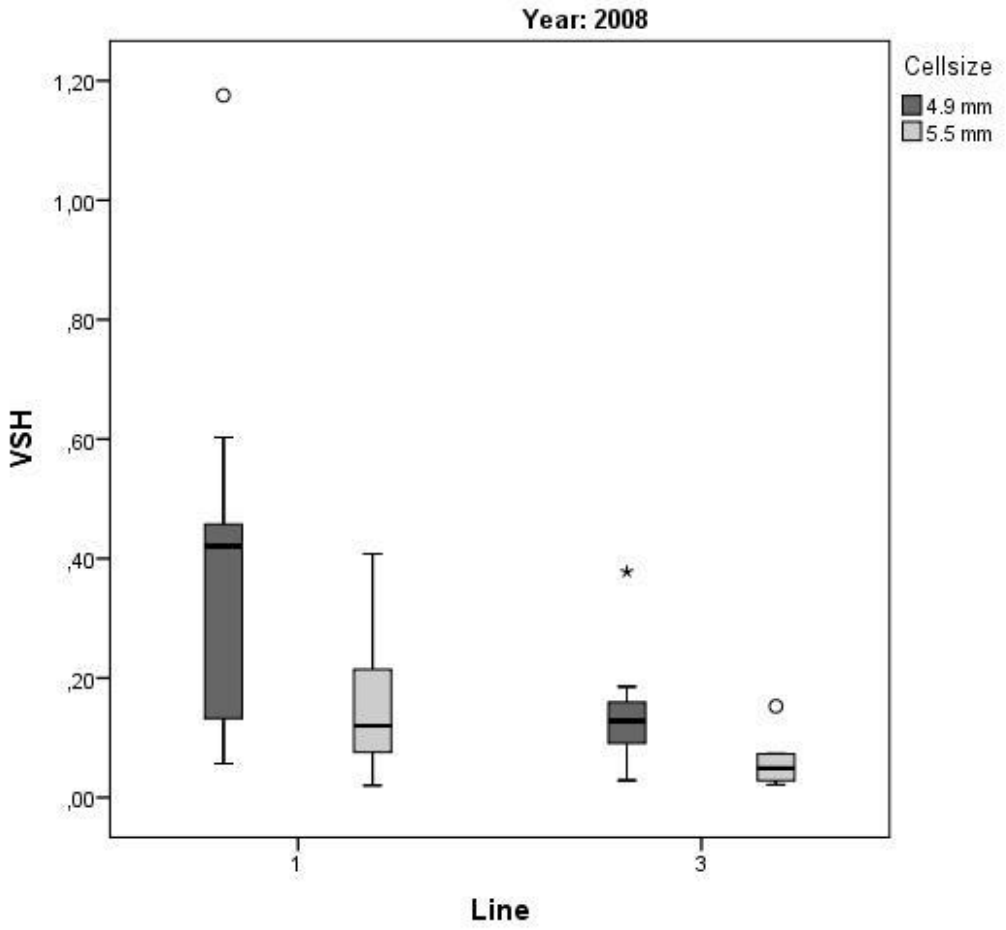

Figure 17. VSH in the year 2008, separated per genetic line and cell size

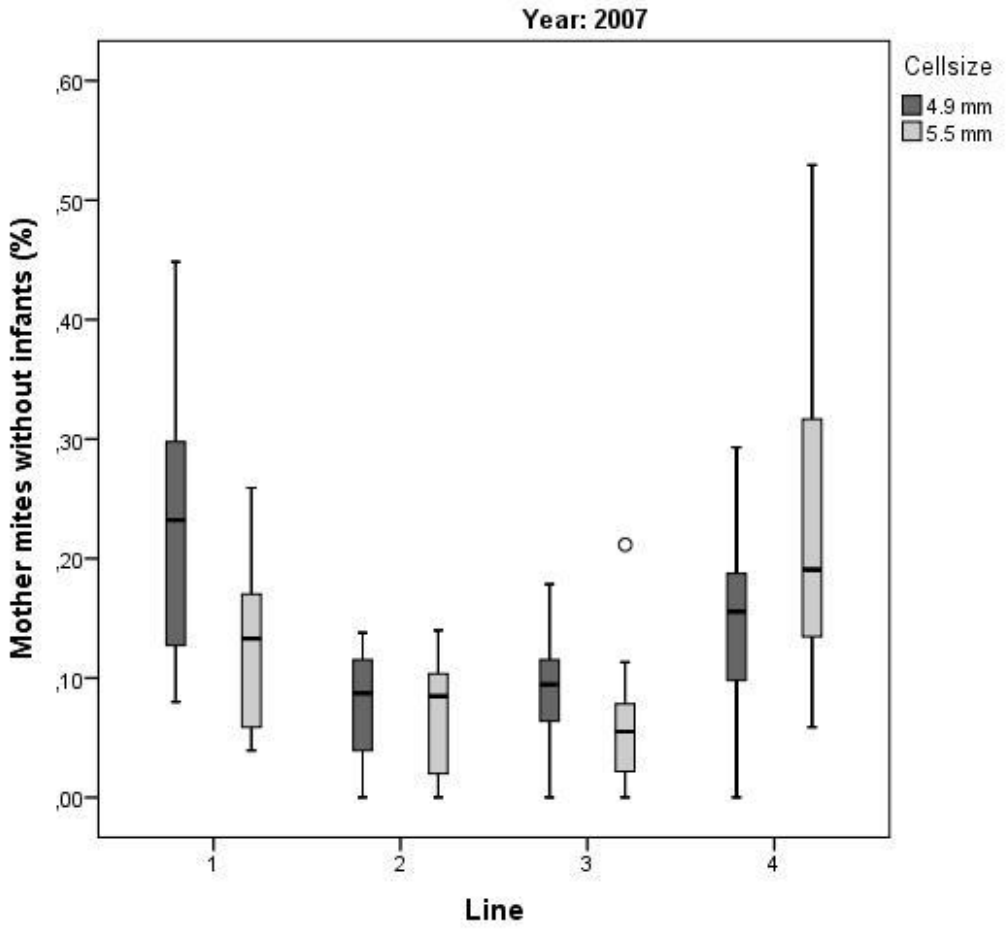

Figure 18. Ratio of non-reproductive mother mites in the year 2007, separated per genetic line and cell size

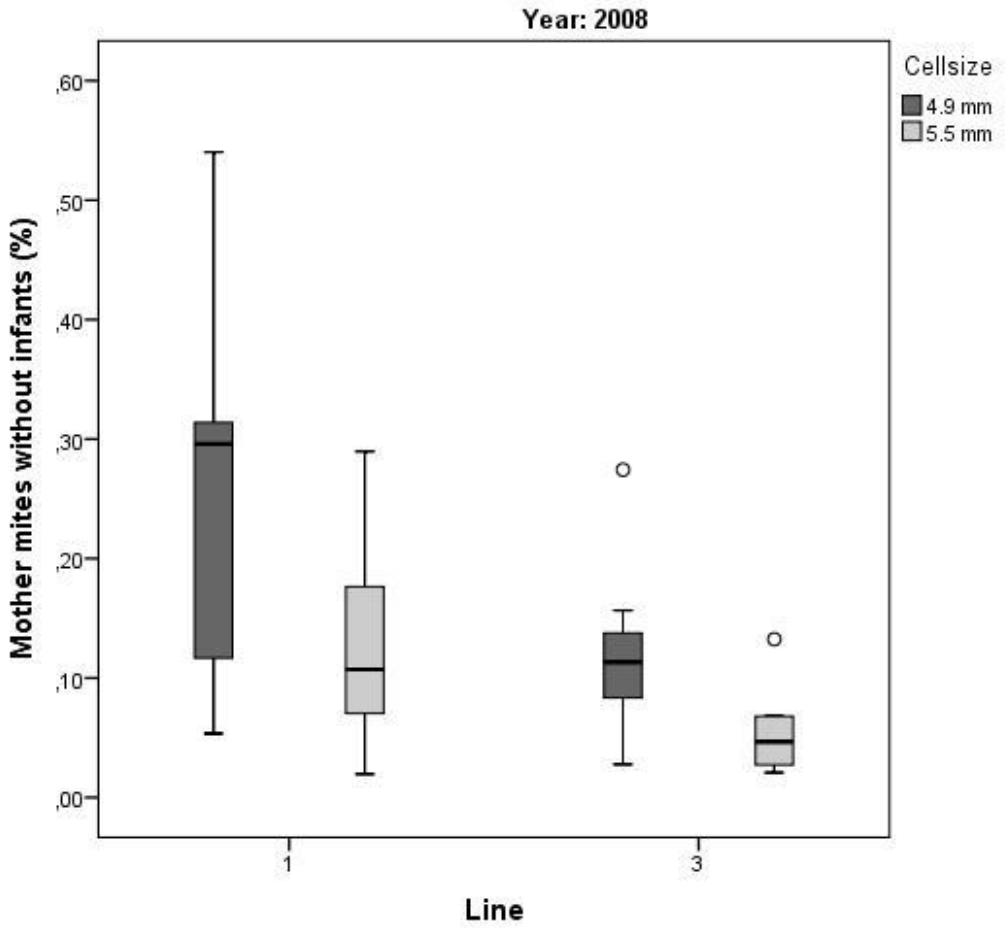

Figure 19. Ratio of non-reproductive mother mites in the year 2008, using genetic line and cell size as factors in the analysis

The VSH-values of the 4 lines can be seen in table 20.

Table 20. VSH-values of the 4 lines

year 2007	SCS (4.9 mm)				LCS (5.5 mm)			
Line	N	max	N-VSH ≥0.2	Min	N	max	N-VSH ≥0.2	Min
L1[a]	10	0.44	60 %	0.08	10	0.26	40 %	0.04
L2 [b]	10	0.14	0 %	0	10	0.14	0 %	0
L6 [b]	10	0.17	10 %	0	10	0.21	10 %	0
L7 [a]	10	0.29	60 %	0	10	0.53	70 %	0.06

Post Hoc analysis (∝-corrected using Scheffé's procedure) showed a significant difference between L1 and L2 ($p<0.01$); based on 50 infested cells per colony.

The VSH calculation for the individual colonies in the test (2007) showed that - using the arbitrarily criteria VSH > 0.2 (e.g. in 20% of the infected cells no reproduction could be observed) - a clear difference between the lines existed, but not between cell-sizes.

Table 21 illustrates the VSH values of 5 colonies per group, with SCS & LCS combs in each colony.

Table 21. VSH values of 5 colonies per group, with SCS & LCS combs in each colony

year 2008	SCS - 200 infested cells each hive				LCS - 200 infested cells each hive			
Line	N	Max	N-VSH ≥0.2	Min	N	max	N-VSH ≥0.2	Min
L1	5	0.54	5	0.29	5	0.29	2	0.09
L1 (F1)	5	0.38	2	0.03	5	0.21	1	0.02
L6	5	0.16	1	0.03	5	0.07	0	0.02
L6 (F1)	5	0.27	1	0.08	5	0.13	0	0.02

Within each line, inside the colony on the SCS infested combs a more pronounced VSH treatment is observed. Even in the line L3 with low VSH activity.

Table 22 shows the VSH of single queens and their daughters locally mated 2007.

Table 22. VSH of single queens and their daughters locally mated 2007; details of L1 & L1(F1) and L3 & L3(F1) in 2008 (Table 21)

number	L1 (2007)	L1(F1) (2008)	+/-	L3 (2007)	L3(F1) (2008)	+/-
1	0.45	0.38	-	0.1	0.14	+ +
2	0.16	0.06	- -	0.09	0.13	+ +
3	0.13	0.19	+	0.18	0.27	+ +
4	0.08	0.12	+	0.06	0.08	+
5	0.09	0.05	- -	0.12	0.14	+

The measured VSH of the original colonies and each daughter colony as measured in 2007 and 2008 to show the natural “drift” of the VSH-trait when queens mated freely.

4 Discussion

4.1 Cell size

For over 30 generations of the Carnica Harald Singer population, the bees were forced to build combs on wax foundations with a 5.5 mm cell size. Choosing a certain cell size for a honeybee population which underwent selection results in a dependency on that given cell size to draw impeccable combs. Mc Mullan & Brown registered the same not-intended result for the original "wild" *Apis mellifera mellifera* (Linnaeus, 1758) bee population in Dublin, after they were kept by humans [Mc Mullan , Brown 2006 ; pers. comm.]. To induce colonies under selection to accept more than one cell size, a selection in the population appeared to be necessary.

Only 16 mother queens out of 1.287 (= 1.2 %) fulfilled the criteria "accepting foundation with 4.9 mm and 5.5 mm". Apparently this group (N=1287) had unintendedly undergone a selection on the cell size 5.5 mm for at least approximately 40 years.

Despite selection, there are still colonies existing which can genetically build smaller cell sizes when foundations aren't given (natural burr) [Reisenberger 2014].

It can be argued that some of the research done on the relationship between cell size and varroa as put forward by A. & D. Lusby just failed to replicate their results because of this fact [Lusby 1996].

Dreher used a bee strain which could not built SCS foundations correctly [Dreher 2007].

Taylor used *Apis mellifera ligustica*. The bees were not able to construct small cell sizes correctly. No effects of cell sizes were described in her findings [Taylor 2008]. In her tests, the used foundations were not produced from the same company. This is important though as it can influence the mite migration.

Ellis used two different bee strains from different apiaries in his trials. One bee strain descended from a LCS- apiary and one from a SCS apiary. No effect between LCS and SCS was recognized [Ellis 2008]. The reason for the indifferent results may lay in the two different bee strains, which could not be compared.

Berry found higher numbers of varroa mites in SCS brood. Hence, he concluded that small cell size combs had a higher infestation rate [Berry 2010]. However, Barry did not mention anything about his used bee strain in the US. As we know, the cell size coheres with the bee race and in the US there are many different bee strains. Another factor that might be taken into account is a possible re-infection of varroa mites in his trials. I described this phenomenon in figure 1 (drop-out rate).

On the other side, Message reported that varroa mites preferred LCS [Message 1995]. He used an Africanized honey bee strain, which builds SCS.

Mc Mullan tested in Irland his *Apis mellifera mellifera* strain and these bees easily accept SCS [Mc Mullan 2006].

A considerable correlation between bee strain and cell size, as my tests 2002 show, exists.

Coffey used a non-HYG subset of their population and as they describe it: none of the colonies were opening infested cells, and they did not see any effect of cell size on varroa reproduction in undisturbed varroa-infested cells [Coffey et al 2010]. This population showed no signs of the SMR-behavior as described by Harbo and Harris [Harbo & Harris 2002 & 2005]. They already pointed out that HYG and SMR/VSH target different in-cell brood-diseases, which might be very well due to different types of cell cap handling behaviour [Rothenbuhler 1964, Masterman et al. 2000]; each with different functional properties and probably involving different parts of the honeybee genome [Mondet et al. 2015].

Just being able to draw small size combs does not automatically result in a slower growing varroa population on SCS (lines 3/2002 and 12/2003 in my tests).

4.2 SCS effects on colonies

According to common scientific beekeeping papers and their authors (like Thomas Kober), colonies with small cell sizes have a

- quicker colony development
- honey crop increased

- no secondary infections (including the ones transmitted by varroa mites)
- no significant varroa mites damages

[Kober 2002, pers. communication]. My work doesn't confirm these general claims. When it comes to the enlisted characteristics above, bee genetic is more fundamental than cell size.

4.3 Selection

To see differences in varroa reproduction, a high varroa pressure is needed. This pressure is simply created by not treating SCS and LSC groups, which automatically results in a higher varroa infestation rate.

Most lines showed a higher number of survivors in the SCS group of the line under Kefuss way of selection "live and let die" [Mc Neil 2010], [Khoei 2015] ; 12 from 16 lines passed the live and let die selection which was applied on SCS and LCS. Analyzing the varroa-development, 4 lines do not show any influence of the factor: cell-size; 2 lines showed a higher development of varroa in the SCS colonies. The genetics of 6 lines out of the 16 confirmed the Lusby hypotheses [Lusby D. & Lusby A. 1996a].

The step by step selection, which was applied on 1,287 genetically different colonies, demonstrates the existence of a variability in dealing with cell size and varroa. The results help to explain the controversial results found in literature. It clearly shows the genetic variability in a honeybee breeding-population after a long time of classical selection on honey-production for traits, not selected for. In this actual project the combination of the traits varroa-pressure-survival and differences in cell-sizes are compared.
The lines L1 (2002), L2 (2002), L6 (2002) and L7 (2003) show a clear differentiated varroa development in dependence of cell size; slower development on SCS-combs being observed.

The negative influences of smaller cell-sizes on varroa-population growth is published by: [Martin and Kryger 2002; Kober 2003; Piccorillo 2003; Forsman et al. 2004; Johnsen 2005; Kleinfeld 2006; Maggi 2009].

No effects comparing cell size and varroa-development are described by: [Fries 2004, Berg, 2004, 2005; Dreher 2007; Liebig and Aumeier 2007; Ellis 2008; Taylor 2008; Berry 2010; Coffey 2010; Seeley 2011].

4.4 Mechanism

As not all lines showed the positive effect on varroa reproduction for the colony survival on SCS, the varroa-reproduction within the sealed brood cells had to be analyzed.

4.4.1 Estimating the varroa reproduction parameter

The division of the young mated daughter-sister queens across the cell size groups was always randomized. A difference by chance of a relevant trait to only appear in one of the colonies of a specific group (SCS or LSC) within each line can be excluded. The suggested superiority of SCS caused by the not randomized division of a specific trait can be excluded.

Looking at lines 3 and 12, they could potentially lack parts of a relevant trait, as the mother-queens of all lines are first-generation-inbreed-descendants of a single artificial inseminated “foundress” queen [Janousek, 1992 Brno, personal communication]. They can genetically differ. In the whole Carnica Harald Singer group, each generation consists of 5 or more related groups (”lines”) always mated with a high number of non-sister-queens-as-fathers, after the old Singer tradition. From generation to generation 7 till 15 selected mother queens are control-mated on isolated mating yards with drone-producing colonies from the whole population. So differences in the genotype can be expected. [Singer 1976; Praagh, van 2015; Ebersten 1996].

The daughter-generation from line 3/2002 do not exhibit the traits, that help SCS to be a favorable varroa reproduction-inhibitor; three generations were openly mated with the same male pool as the queens of the lines 1/2002, 2/2002, 4/2002, 5/2002, 6/2002, 7/2003 and 8/2003. These 7 lines all exhibit the traits. They all show a reduced varroa reproduction in the SCS parts of the lines compared to their "LSC-sisters". (table 5.)

Not all lines after the "Bond" selection showed a difference in varroa-population growth depending on cell-size. The lines 1/2002, 2/2002, 4/2002, 5/2002, 6/2002, 7/2003 and 8/2003 confirm the hypothesis, that small cell size is a tool against varroa population growth. Line 3/2002, 12/2005, 15/2005 (table 5) markedly have a high level of varroa-development and no differentiation of this level due to cell-size. These lines apparently miss the VSH-traits. We must conclude that those lines that do not show the VSH-trait, clearly demonstrate that cell-size per se does not influence varroa-population growth under the experimental conditions.

As group of father queens producing the drones, the four mother colonies (2005, 2006) were used plus the still available sister colonies of each line, building a POOL of father queens [Praagh, 2015]. Doing so, the probability of "saving" the bigger part of the genotype of the whole selection is given. The results show, that this way of bee breeding prevented the loss of the hardly understood genetics of VSH behavior.

At a first glance, the expression of the trait showed significant differences between lines. But statistically the expression was not significant in the test comparing daughter queens on the different cell sizes. Due to the genetic make-up of a colony: the relationship between sister queens is only 25% [Praagh, van 1994]. The 4 used lines showed clear differences in varroa reproduction between cell sizes. The expression of the worker-bee trait inside each colony within each line was estimated on 50 infested cells only. It led to the conclusion that the clear differences found in earlier experiments which were based on varroa reproduction (10 days natural mites-fall) couldn't be explained in this experimental make up just by measuring it the older classical way. Different worker composition per colony must be expected. In 2008, using experimental colonies containing both comb types per line, the inevitable variability between workers per colony were overcome. The same kind of experimental set-up was used by Message and Gonçalves [Message & Gonçalves 1995].

Offering the different comb-sizes at each side of the bee-spaces gave significant proof, that comb-size influences the observed reproduction rate of varroa.

Due to an unknown underlying mechanism, the rate of the VSH cleaning behavior is higher on the smaller cell size side of a "bee-space".

Comparing the infestation rates on each cell size per colony, supposing the reproduction on the LSC being undisturbed, allows to estimate the % per cells without present reproducing parasites (= varroa reproduction being disturbed) on the SCS, supposing the original percentage of non-reproducing parasites on both comb types were the same.

Using the data, a 2.6 times higher chance for SCS varroa infected cell to be cleaned was calculated, compared to the infected LCS cell in the same colony.

Supposing the LCS brood offers the parasite a better chance to reproduce compared to SCS without the VSH trait being present in the worker bee population of the line is unrealistic. The lines (L 2 & L 6) showed no difference in reproduction rate of the parasite between SCS and LCS; a clear indication that the invasion rate of the parasite is not influenced by cell-size, but the more intensive active disruption of the reproduction (VSH) on SCS must be the mechanism that makes SCS support the colony survival under varroa-pressure.

This difference in cleaning behaviour explains the observed divergence in reproduction of varroa.

Only colonies headed by mated queens which produce worker bees that are able to build 4.9 mm cell size combs (SCS-able) **and** possess the VSH trait, have a chance to survive without treatment in an undisturbed environment. Nevertheless, being SCS-able & VSH as colony offers no re-invasion protection. Protection against re-invasion should be a next selection goal, e.g. by selection of intensive guarding as trait.

Dreher and Liebig, Ellis and Berry used genetically unspecified queens (and bees) and demonstrated clearly that cell size as such does not influence varroa population growth [Dreher & Liebig 2007; Ellis et al. 2008; Berry et al. 2009]. Dreher, Liebig and Berry even found a higher number of infested cells in colonies with 4.9 mm as compared to 5.3 mm cell size [Dreher & Liebig 2007; Berry et al. 2009]. This supports the view, that cell size **plus** defined genetics can influence varroa population growth.

The VSH trait was unobserved present in the Carnica Harald Singer population [Fries 2004; Berg 2004 & 2005; Dreher 2007; Dreher and Liebig 2007; Ellis 2008; Taylor 2008; Berry 2010; Coffey 2010; Seeley 2011].

Piccirillo and De Jong offered africanised Mellifera colonies three cell-sizes (4.84 mm "African"; 5.16 mm "Italian" and 5.27 mm "Carnolian"). They reported a significantly higher infestation level in the largest cell size as compared to the other two sizes and suggest:" the use of unnaturally large comb cell size should be re-examined in the light of its effect on parasite levels." They also mention a 60% higher infestation rate of adult bees observed in colonies with two cell sizes (4.84 mm and 5.16 mm) as compared to the feral colonies with only 4.84 mm cell size [Piccirillo and De Jong 2003]. This was already observed in Brazil [Goncalves et al.1982]. Piccirillo and De Jong 2003 presented the first experimental data on effects of cell size on varroa infested brood cell rates [Piccirillo and De Jong 2003]. Our data on surviving colonies show that for the bee population used, the colonies on smaller cell size (2003-2005) have a 1.3-2.3 times higher survival expectation rate.

Cell size can influence the active reproduction of varroa inside capped cells using worker larvae in worker and drone-cell of *Apis mellifera* & *Apis cerana* (*Apis cerana* Fabricius 1793). The reproduction appeared to be disturbed in the larger cell-types [Zhou,Yao, Huang, Huang, 2001].

A study on the mite reproduction related to available space in the cell was done by Martin and Kryger. The authors used scutellata (*Apis mellifera scutellata* Lepeletier, 1836) colonies invaded by a capensis pseudo-clone (*Apis mellifera capensis* Eschscholtz, 1821). The capensis larva occupies more of the cell space. In normal filled cells (Scutellata pupae) the measured varroa reproduction rate was higher. They suggest, that the male, as egg laid in the upper part of the cell, cannot reach the feeding site on the pupae and the moulting site in the lower part of the cell, if the cell is "overfilled" by a capensis pupae. The phenomenon as described by Martin & Kryger cannot explain the reduced varroa reproduction rates I found in the SCS group. [Martin & Kryger 2002]

As it is known that in A*pis mellifera* the phenotype body size is regulated by the cell size, the emerging worker from SCS are expected to be smaller compared to those emerging from LCS [Daly et al 1988; Mc Mullan, & Brown 2006].

This influence on varroa reproduction cannot hold for the results obtained with the lines 3/2002, 12/2003. Here we

could not find the reduced reproduction of varroa in the SCS group as compared with their LCS group of sister queens. These two lines lacked the VSH trait. This meant that the factor of 2.7 on reduction of varroa reproduction due to VSH was not available in the genetics of the lines 3/2002 and 12/2003.

The seasonal appearance of out-drops in figure 1. and 2. are most probably caused by a reinvasion, due to active robbing and absconding of weaker colonies. (= varroa carried into a colony by drifted bees).
I suggest that the high numbers of mites within the SCS are the result of active robberies of weak colonies which gives foreign mites the opportunity to be transferred to healthy colonies under consideration.

For the population under consideration I found cell size can be used as a management tool for varroa treatment during the active season (in "Wiener Becken" Vienna Valley). The used *Apis mellifera carnica* Singer population readily accepts the CS after the selection, as shown by the actual commercial used population of >1250 colonies wintered in 2014/2015. 2010 the commercial Carnica Harald Singer population was completely on SC >1000 colonies. Not all "wild mated" (F1) queens produce colonies that readily accept SC; another indication that cell size is a genetically controlled trait. About 10% of the F1-colonies struggled with the correct comb building.

Using the varroa population growth as a selection parameter caused the VSH trait to be kept (or improved) during the process of selection (2003-2005). The 2007 & 2008 results clearly show this trait was not explicitly available in all lines. As those lines were not scanned for the trait I can only speak about different expression of the trait between the lines. I consider the traits cell size and VSH to be genetically independent. The analysis of the 2007 and 2008 experiments for VSH showed differenced levels of VSH to be correlated with cell size. The results of 2008 - both cell sizes in a colony - show higher level of VSH for infested brood

cells on small cells. The data are statistically convincing - a behavioural explanation is missing.

Though it´s a fact that one of the major factors for varroa mite infestation rates is the genetic beside the bee yard (figure 4), year (figure 2), environment, nectar flow and period of bee season.

5 Conclusion

I selected my bee colonies to determine which ones could build SCS. After establishing that, I switched the common cell size in my apiary from LCS to SCS. This process took up more than 10 years due to other selectional criteria (e.g. like honey, etc.).

Results of my trials regarding the thesis:

1. less natural mites fall on SCS
2. less winter losses on SCS colonies
3. varroa reproduction parameter is highly dependable on line but correlates with SCS

Small cell size combs plus VSH lead to a minor varroa infestation rate.

Based on the empiric data of the underlying investigations this is strongly suggested as a remedy for the existing varroa crisis.

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7 Appendices

Supplementary tables to the results Part I

Table 23. Mean number of natural mite fall/ brood area/ year separated by bee yard, genetic of mother line and cell size.

Bee yard	Mother line	4.9 mm					5.5 mm				
		N	Min	Max	M	SD	N	Min	Max	M	SD
1	1	13	869.7	2136.8	1534.6	420.1	12	806.5	2989.8	1962.2	753.2
	2	6	1538.0	1960.3	1773.6	154.9	6	1576.8	3525.8	2195.8	698.9
	4	10	1323.7	2395.3	1871.9	349.8	10	1173.3	2513.4	1889.5	513.4
	5	17	101.6	274.4	188.4	46.9	11	123.6	277.8	172.0	41.8
	7	4	1579.0	2780.7	2090.5	605.9	3	1648.2	2041.0	1826.1	199.0
	8	8	1152.4	2235.3	1563.3	425.7	8	641.2	3714.3	2310.8	916.0
	10	4	290.2	357.6	324.6	33.8	4	205.3	351.5	277.4	78.6
2	3	6	1874.8	5026.0	3251.3	1120.3	6	1604.0	3185.3	2258.4	586.2
	4	6	1745.8	3036.3	2064.9	486.9	6	1926.8	3888.0	2937.0	707.8
3	1	18	582.3	3023.5	1374.3	673.3	12	1164.2	3064.4	2011.7	469.3
	2	3	644.2	1355.7	907.0	390.5	5	916.0	1316.2	1109.5	184.8
	3	1	4185.0	4185.0	4185.0						
	4	11	655.8	2940.8	1656.7	783.5	2	1448.6	1794.3	1621.5	244.4

	5						3	471.1	2341.0	1205.1	997.6
	7	6	1195.3	1624.3	1399.2	184.5	6	1216.5	2350.6	1863.3	475.7
	8	7	1042.8	2473.0	1693.0	548.6	7	794.8	3153.3	1946.9	910.4
4	1	5	1278.2	2377.7	1555.5	462.0	5	1870.3	3072.0	2292.2	477.0
	2	10	1007.3	4066.0	2002.7	827.6	13	620.0	2534.5	1644.2	608.3
	4	4	718.5	1220.8	949.5	230.2	4	712.3	1191.8	982.4	210.6
	5	8	207.8	415.7	294.3	73.4	8	233.3	609.3	371.5	113.7
	10	6	919.5	2524.0	1318.7	600.6	7	988.5	4246.0	1610.5	1174.0

Table 23. (continuation) Mean number of natural mite fall/ brood area/ year separated by bee yard, genetic of mother line and cell size

Bee yard		4.9 mm					5.5 mm				
		N	Min	Max	M	SD	N	Min	Max	M	SD
5	1	24	171.3	1847.0	881.1	509.6	18	389.4	3351.5	1257.0	781.9
	2	4	1059.0	1578.3	1253.8	226.7	4	445.2	1779.5	1388.7	631.4
	5	9	182.8	1270.2	790.0	345.3	12	273.5	1322.0	699.2	373.2
	7	6	511.0	1465.3	1013.3	329.9	7	1234.3	2788.0	1867.7	627.7
	8						2	805.4	1533.8	1169.6	515.1
	10	9	323.0	1474.0	836.6	351.3	9	311.3	1553.5	967.3	446.0
	12	10	504.0	1874.0	1041.3	493.1	10	433.0	2028.0	1040.3	530.7
	15	14	162.0	1207.0	513.0	355.1	15	193.4	982.8	516.0	259.2
	16	7	164.8	1163.8	445.2	352.8					
6	1	7	148.5	1264.4	550.4	459.0	5	374.3	3966.8	1250.4	1523.6
	6	9	96.8	1680.4	571.1	599.8	8	198.0	3377.8	1138.4	1146.2
7	2	6	578.0	1548.0	933.7	370.9	6	802.0	1905.0	1474.1	433.3
	3	4	1451.4	3224.4	2368.5	809.8	4	1672.2	2880.6	2301.8	583.5
	4	3	794.3	1078.5	924.2	143.7	4	1598.8	2208.3	1932.2	251.1

	5	2	402.0	416.6	409.3	10.3	2	599.0	942.3	770.7	242.8
	8	2	1186.8	1250.8	1218.8	45.2	1	1036.5	1036.5	1036.5	
	10	5	518.5	1638.7	997.8	403.3	6	619.3	1380.0	872.5	269.9
12	1	5	265.3	1220.8	626.9	432.6	4	160.5	1238.8	524.4	486.0
	5	1	537.3	537.3	537.3						
	6	20	122.5	832.3	281.6	196.6					
	8	4	801.3	1202.3	1057.0	183.6	8	429.2	1215.5	718.6	264.9
	9	5	497.2	1361.0	879.2	361.2	6	424.0	1557.0	956.5	438.3
	10	6	249.3	1205.5	579.4	325.2	6	379.8	1406.0	795.3	417.2
	15	9	264.0	845.0	371.9	182.1	9	227.8	758.8	386.8	168.5
	16	5	194.7	340.3	265.3	56.1					

Table 23. (continuation) Mean number of natural mite fall/ brood area/ year separated by bee yard, genetic of mother line and cell size

Bee yard		4.9 mm					5.5 mm				
		N	Min	Max	M	SD	N	Min	Max	M	SD
19	1	2	288.1	623.5	455.8	237.1	1	375.5	375.5	375.5	
	6	14	121.3	554.8	302.3	125.3					
	8						3	716.6	837.9	775.9	60.7
	9	2	683.1	701.0	692.1	12.6	2	660.1	872.0	766.1	149.8
	10	1	647.9	647.9	647.9		2	725.9	1073.0	899.4	245.5
	15	2	346.2	652.8	499.5	216.8	1	330.6	330.6	330.6	
20	1	1	301.1	301.1	301.1		2	676.9	782.7	729.8	74.9
	6	4	285.4	689.6	484.4	198.0					
	8						2	838.5	1077.7	958.1	169.2
	10	4	391.6	973.1	694.8	295.3	1	541.1	541.1	541.1	
	15	7	459.3	847.6	612.7	150.7	7	351.9	1120.6	604.9	262.9
	16	5	224.1	840.2	523.6	248.8					

Ancestry line: (marking the foundresses with the number of bottom board and letter of the bee yard)

2002: 79 colonies (gen-pool SCS)

Tested colonies

lines:		artificial inseminated F		VSH	SCS:LCS (varroa)	lines:	2003	2004	2005	2007	2008
1		E612	Lusby	yes		**1**	**x**	**x, F2**	**x**	**x**	**x**
2		P485	Lusby	yes	No	**2**	**x**	**x, F2**	**x, F2**	**x**	
3		S649		no		3	**x**	**x**			
4		S481		yes		4	**x**	**x**	**x**		
5		SA1		yes		5	**x**	**x**	**x**		
6		P10	Lusby	yes	No	**6**	**x**	**x, F2**	**x, F2**	**x**	**x**
7		R70	Lusby	yes		**7**	**x**	**x, F2**		**x**	
8		R80		yes		8	**x**	**x**			
2003 (5 lines)						2003 (5 lines)					
9		106				9		**x**	**x**		
10		467				10		**x**	**x, F2**		
11		14	failed Bond Test			11					
12		862		no		12		**x**	**x, F2**		
13		222	failed Bond Test			13					
2004 (3 lines)						2004 (3 lines)					
14		862	failed Bond Test			14			**x**		
15		PS		no		15			**x**		
16		P018	failed on LCS			16			**x**		
colonies in test							205	221	78		

drone colonies					
2003	2004	2005	2006	2007	
A: 79 group	A: 79 group	S: 1,2,6,7	S: 2,6,10,12	S: F1/ 2003: 1,2,6,7	
		A: 2,6,10,12			

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Picture:

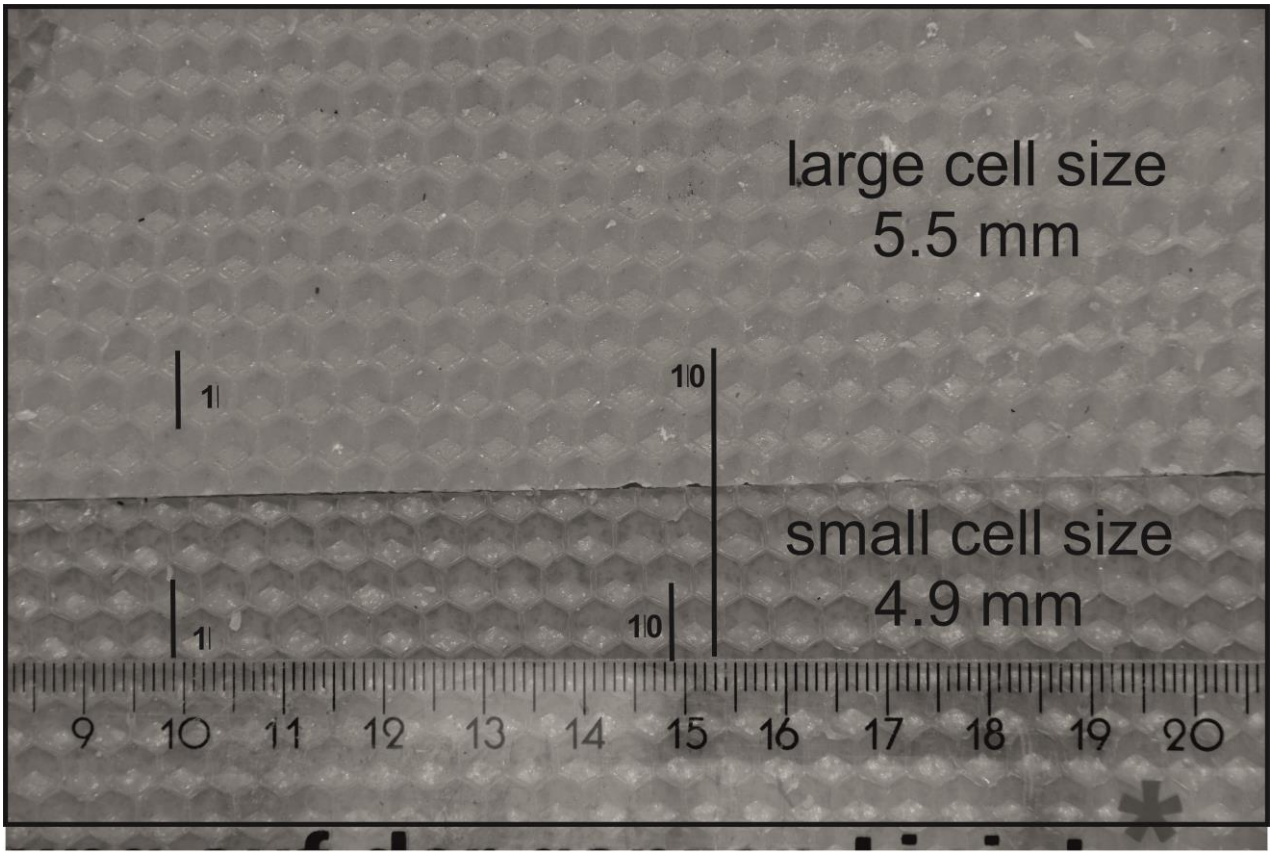

Photo: Job van Praagh

Description: Comparing 10 cells of small cell size with 10 cells of large cell size.

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MANUSCRIPT

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Titel:

Breeding Carnolian bees *Apis mellifera carnica* (Pollmann 1879) (Hymenoptera: Apidae) **on different comb cell sizes and analysing the effect of the different cell sizes on Varroa** *Varroa destructor* (Anderson & Trueman 2000) (Mesostigmata:Varroidae) **infestation rates.**

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Abstract

English:

Differences in cell sizes used in beekeeping are often proposed to be one of the factors regulating Varroa population growth. An in depth research of the relationship between these factors has been undertaken using the ‘Carnica Singer population’. The acceptance of foundation with a specific cell size appears to underly selection. Smaller cell size (4.9 mm.) foundation/combs reduces the Varroa-population growth compared to 5.5 mm. This reduction

is also dependent on the presence of the VSH- behaviour trait in the worker bee population within the colony. Smaller cell size combs in combination with breeding for the 'right genetics' (cell size **and** VSH) can be one part of an integrated sustainable treatment concept for Varroa control.

Deutsch:

Der Einsatz von Mittelwänden mit kleinen Zellen wird auch als Varroa-Gegenwehr propagiert. In diese Studie ist diese Problematik gezielt analysiert worden. Die Frage der Annahme anderer Zellgrößen unterlag in der untersuchten Carnica Singer Population einer Selektion. Völker auf Waben mit kleineren Zellen (4.9 mm.) haben gegenüber Völker mit gleicher Abstammung auf größeren Zellen (5.5 mm.) eine geringere Varroamilbenvermehrungsrate. Dieses gilt jedoch nur dann, wenn die Völker auch über die VSH-Eigenschaft verfügen. Kleine Zellen kombiniert mit der passende Genetik (Zellgröße **und** VSH) kann als Teil eines integrierten Varroabehandlungskonzeptes dienen.

NL:

Het gebruik van kunstraat met kleine cellen wordt gepropageerd als hulpmiddel in de strijd tegen Varroa. In deze studie worden experimenten en resultaten rondom deze vraag beschreven. Om kunstraat met kleine cellen bruikbaar uit te laten bouwen, bleek in de onderhavige Carnica Singer populatie een selectie noodzakelijk. In experimentele volken is de reproductie van Varroa in de broedcellen is op raat met kleine cellen (4,9 mm.) significant geringer als op raat met grotere cellen (5,5 mm.). Dit geldt echter alleen in volken die ook VSH-gedrag vertonen. Kleine cellen gecombineerd met de juiste genetica (celgrootte en VSH) kan worden gebruikt als onderdeel van een geïntegreerd varroa behandelingsconcept.

Keywords: *Apis mellifera carnica* (Pollmann 1879) – *Varroa destructor* (Anderson & Trueman 2000) – integrated Varroa treatment - natural comb cell size – small cell size – selection - small cell foundation – survival – VSH (Varroa sensitive hygienic behavior) – Varroa population growth

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1 Introduction

Worldwide the ‘new’ ectoparasite *Varroa destructor* (Anderson & Trueman 2000) is considered the main cause of colony-losses.
Cellsize of the foundation is one of the possible solutions beekeepers are experimenting with to help bee colonies to better cope with this parasite.

In the past the natural cell sizes of European-honeybees (*Apis mellifera*) were smaller [Zeissloff 2007]. However beekeepers wanted more productive bees and Baudoux propagated larger cell sizes for colonies to increase performance, introducing the use of specially produced foundation [Baudoux 1933].

In 1989 Dee and Ed Lusby observed a better survival of colonies against *Acarapis woodii* (Rennie 1921) on colonies with combs with cell size 5.1 mm. At that time commercial foundation varied between 5.3-5.7 mm. in their cell size. They started the same kind of experiments after the arrival of *Varroa destructor* in 1997 [Lusby 1996 a, b.]. In the years afterwards many papers on cell size and Varroa were published.
A number of studies exist detailing the negative influences of smaller cell sizes on *Varroa destructor* population growth: [Message & Goncalves 1995; Martin & Kryger 2002; Kober 2003; Piccorillo 2003; Forsman et al. 2004; Johnsen 2005; Kleinfeld 2006; Maggi 2009].
On the other hand scientific works reported no-effect or even larger populations comparing small versus large cell sizes and Varroa-development: [Fries 2004; Berg 2004 & 2005; Dreher 2007; Dreher and Liebig 2007; Liebig and Aumeier 2007; Ellis 2008; Taylor et al 2008; Berry 2009; Berry et al 2010; Coffey et al 2010; Seeley et al 2011; Khoei 2015].

Beekeepers are using and propagating cell sizes of 5.1 mm. and 4.9 mm. in order to support the colony to cope better with the ectoparasite.
A standardised cell size for *Apis mellifera* foundation does not exist. In Austria the standard foundation offers the bees a cell size of 5.5 mm. to draw their combs.

Since 2004 the removal of mite infested brood by adult bees is described as an inheritable behaviour trait that suppresses the mite reproduction [Ibrahim and Spivak, 2004, 2006; Harbo

and Harris, 2002]. This mite resistant trait is known as ‘Varroa Sensitive Hygiene’ or VSH as it appears to be a form of hygienic behaviour [Harris 2007].

To measure or analyse the presence and expression of the VSH-trait in a colony, the Varroa infestation on a comb of sealed brood prior to emergence is used. A standardised number of infested cells is opened and the reproductive success of the mite is scored.

Calculating VSH as the quotient of cells with a non-reproductive Varroa/ total number of infested brood-cells (=50). The theory behind VSH behaviour as a factor in the tolerance of honey bees to Varroa shows that an active interruption of the reproductive cycle of a mature Varroa (reproductive phase) reduces the population growth. The active interruption is done by worker bees shortly before the young adult bee emerges (elderly pupae) [Harris 2007].

2 Material & methods

2.1 Measuring brood comb sizes

Cell sizes were measured using a digital ruler (calliper). Measurements of ten cells across the foundation were taken along the three axes displaced at 60°, and the cell sizes were expressed in mean linear distance between two parallel sides of the hexagonal cell-imprint per cell size [Coffey et al. 2010].

2.2 Population build-up, Selection of queens

In 2002 1,287 colonies from the Carnolian closed breeding population (Carnica Harald Singer; >1000 colonies) were selected. In this population, the standard cell size of the foundation given to the colonies was the large cell size (LCS) of 5.5 mm.

In order to have the bees in the right physiological condition (“summer bees”) I offered after the cherry flowering the selected 1,287 colonies:

Step 1. During a 10 days interval three empty frames were positioned in the 12 frames OE-Breitwaben- hive type at '2', 'center' and '12' to allow the bees to build free combs without prescribing a cell size. This was to allow the worker bees to show the 'natural cell size preference' of the colony and let them build worker and drone combs.

Step 2. At day 11 the center frame was measured and replaced by the foundation with a small cell size (SCS) size of 4.9 mm.

Step 3. 10 to 21 days later the SCS -comb was checked; 9.3 % = 120 colonies showed acceptable drown-out cell patterns and were given a frame with a small 'SC start-up foundation' (5 cm wide).

Step 4. 79 of the 120 colonies were selected as being capable to draw SCS combs in an acceptable way with a regular brood pattern.

Step 5. To see their real acceptance of the SCS it was necessary to offer the SCS foundation twice; with a 40 day interval between the two (1.5 generation of worker bees).

Step 6. The queens of 8 colonies out of the 79 group were selected to become foundresses for 8 new lines. For each of the 8 lines 20 daughter queens were artificially inseminated with drones from their own mother colony.

Step 7. The next generations were mated on an isolated mating yard with the 79 group as males.

Steps 8. & 9. 2003 and 2004 more foundresses from the 79 group were used to create 8 more lines. The young daughter-queens were brought to the mating yard (79 group males).

In total 16 SCS lines were analysed (8 from 2002, 5 from 2003, and 3 from 2004).

2.3 Varroa population growth on LCS and SCS

From 2003 onwards, groups of colonies (see Table 1. for details) were built using young queens with 1.5 kg of worker bees (shook swarming technique). Each 20.kg shook swarm contained Varroa infested bees. The worker bees for the shook swarms came from SCS colonies. Samples of each 20 kg bees stocks were taken in order to collect data on the infestation-levels. In the 2003 season 205 new colonies were built up. For each line two groups, SCS & LCS colonies, were created and randomly divided on 7 bee-yards in the 'Nationalpark Donau- & March-Auen'; south-east of Vienna. To prevent any contamination with miticide-residues, new hives and bio certified wax foundations were used. The bottom-boards were equipped with mesh-protected drawers to collect the natural mite-fall [Dietemann 2013].
During the active season (May - September) the drawer-contents were collected every 10 days.

Colony strength was judged once in August using the standard procedures as described by [Imdorf 1987; Delaplane 2013].

Colony management was undertaken according to 'gute imkerliche Praxis'- by an 'Imkermeister' beemaster), but without Varroa-treatment. All established colonies were overwintered to analyse their winter-survival.

In principle this means the Kefuss way of selection "Bond Test – live and let die" [Mc Neil 2010]. This selection process allows an additional way of determining differences in Varroa resistance between the SCS and LCS groups.

In the season 2004 221 new colonies were created. 5 new lines were selected from the 79 group and 4 old lines; breeding daughters from the 4 2003 surviving colonies. These 9 lines were at random divided on 7 bee-yards.

The 2005 experimental group consisted of 3 new lines from the 79 group plus 4 lines (daughters from 2003 and 2004 colonies). In the 2005 season a total amount of 78 colonies developed out of shook swarms were analysed. At the end of the 2005 bee-season all colonies (including the surviving colonies - 6 from 2003 plus 126 from 2004) were treated with an

oxalic acid sublimation using, the Varrox®-Andermatt [Andermatt BioVet AG 2012]. At that time the colonies were without sealed brood.

From 2006 onwards around 400 colonies on SCS were used to keep the 7 lines as described. Two mating-periods allowed the use of two different male groups; the surviving queens born 2003 and their daughter-queens (in total from 4 lines). The second male group consisted of the surviving queens born 2004 and their daughter queens (4 lines).

2.4 Estimating Varroa reproduction parameter

From the 7 lines kept, four showed significant differences in Varroa-development when comparing LCS & SCS colonies.
In May 2007 from these four lines 20 new colonies per line on LCS and SCS with SCS-bees were created as described (2.3 - shook swarms); 4 pairs of sister queens; the 80 young queens were all mated on one mating station.
In the middle of August from each colony a comb with elder sealed brood was analyzed for reproductive success of Varroa, opening cells aged latest one-day-before-emergence [Harbo & Harris 2009; Harris 2007; Dietemann 2013]. From each colony a number of brood-cells were opened, searching for 50 infested cells. From these 50 infested cells the contents was registered according to the following classification:
A- Cell with one dead Varroa
B- Cell with one living Varroa
C- Cell with one living Varroa plus 1 young Varroa
D- Cell with one living Varroa plus 2 young Varroas
E- Cell with one living Varroa plus 3 young Varroas

These values were used to estimate VSH for each colony.

For each of the 80 colonies the VSH-value was calculated as the quotient of cells with a non-reproductive Varroa per total number of infested brood-cells (n=50). (A+B)/(A+B+C+D+E)

In May 2008 from two 2007 lines (1/2007 & 3/2007) the SCS queens and bees were used to produce 20 new colonies. Each fitted with 4 LCS and 4 SCS drawn combs alternatively positioned. Due to supersedure in 2007 10 original queens and 10 daughters (open mated) were tested.
From each colony 400 infested brood cells were analyzed; 200 from SCS and 200 from LCS combs. These data were used to compare the expression of the VSH-trait in each colony and on each cell size within that colony.

2.5 Statistical analysis

Data were analyzed using IBM SPSS v20. Data are presented as mean and standarddeviation. To analyze the impact of factors such as cell size, year, line and bee-yard on mite drop per brood area, a linear mixed model was applied followed by post hoc tests using Sheffe's alpha correction procedure. The assumption of normal distribution was tested using Kolmogorov-Smirnov-test. Differences between SCS and LCS in frequency distribution (eg winter losses) were analyzed using chi-square tests. The odds ratio (OR) was calculated to evaluate the survival chance according to cell size. For all statistical analyses a p-value below 5% ($p < 0.05$) was seen as significant.

3. Results

3.1 Varroa development

Describing the Varroa population growth by means of the observed dead mites in the bottom-board drawer gives the following results (Table 1).

Table 1: Mean number of dead mites on varroa-board/broodarea/year.

Cellsize	*Year*	*Mean*	*SD*	*N*[a]
4.9 mm	2003	1416.4	886.9	97
	2004	787.3	653.7	123
	2005	688.3	663.7	39
	total	1008.0	813.0	259
5.5 mm	2003	1721.5	916.9	99
	2004	991.9	680.1	98
	2005	859.5	824.3	38
	total	1277.9	892.8	235
total	2003	1570.5	912.7	196
	2004	878.0	671.7	221
	2005	772.8	747.3	77
	total	1136.4	861.7	494

N[a] = number of colonies in test

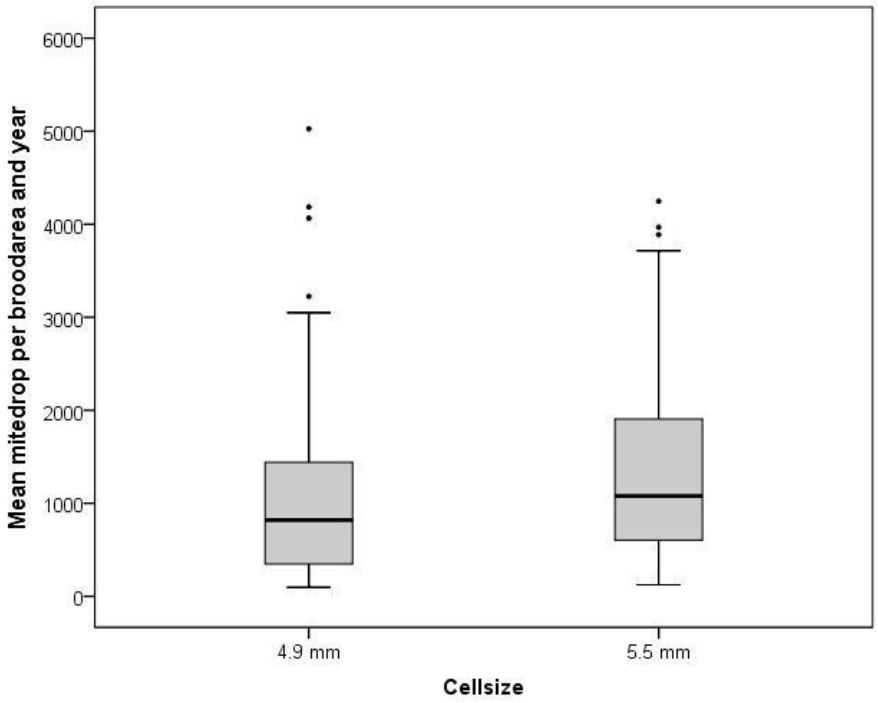

Figure 1 shows SCS colonies compared to LSC colonies. The colonies on SCS show a significant (p= 0.001) slower development of the Varroa populations. This effect is based on year, line and bee-yard. Year, line and bee-yard are considered as factors in the statistical analysis.

Table 2: Results of the mixed model for all four factors.

Source	*Df*	*F*(*df*, 611)	*p*
cellsize	1	11.0	0.001
year	2	21.5	< 0.001
line	12	13.2	< 0.001
bee yard	9	20.7	< 0.001
cellsize x year	2	1.4	0.246
cellsize x line	11	2.5	0.005
year x line	17	1.9	0.014

year x bee yard	10	42.9	< 0.001

A clear difference of overall Varroa population growth between the LCS and SCS colonies could be observed between lines (p=0.001). The magnitude of growth difference (LCS & SCS) varied between 33% and 0% within lines. Year and bee yard, as they influence colony growth, showed to be significant factors at p<0.05.

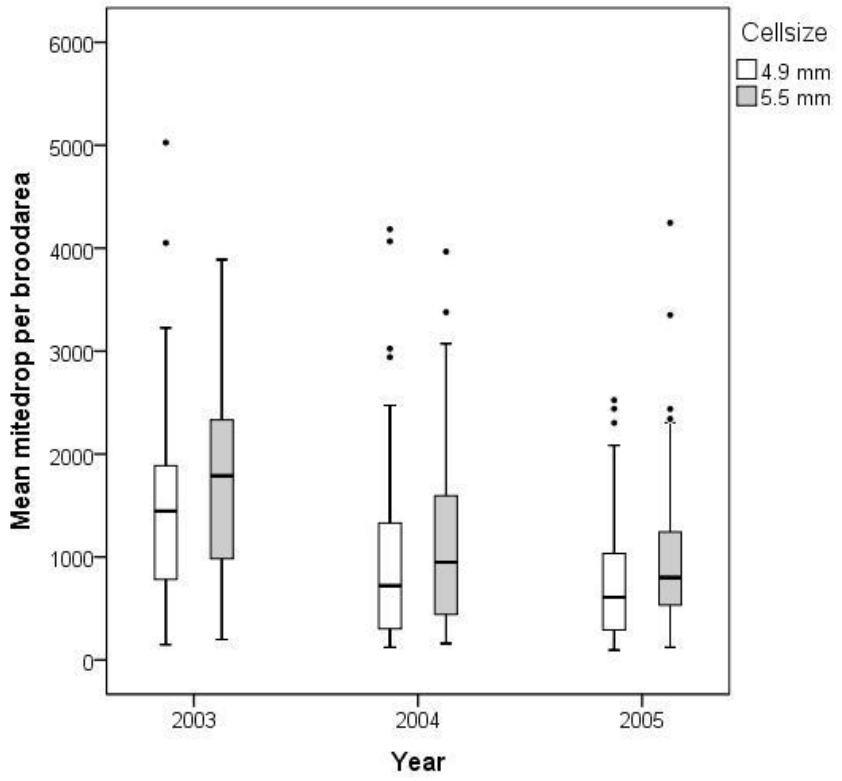

Figure 2: Mean number of Varroas for LCS & SCS per year.

Table 3: Mean number of dead Varroa on the bottom-boards per broodarea, separated for line and cell size.

Line	SCS (4.9 mm)		LCS (5.5 mm)				
	N	*M* ± SD	*N*	*M* ± SD	*T*	*df*	*p*
1	75	1090.8 ± 630.5	59	1558.6 ± 892.9	-2.94	126	0.004
2	29	1517.5 ± 697.4	34	1602.9 ± 622.4	-0.63	58	0.534

3	11	3015.2 ± 1077.9	10	2275.8 ± 552.2	1.81	17	0.087
4	34	1644.2 ± 644.2	26	1977.6 ± 776.7	-1.75	55	0.085
5	37	379.0 ± 300.4	36	511.4 ± 450.9	-1.51	69	0.135
6	47	360.5 ± 315.1	8	1138.4 ± 1146.2	-2.05	8	0.075
7	16	1427.3 ± 554.2	16	1858.2 ± 488.4	-2.52	26	0.018
8	21	1477.3 ± 469.2	31	1467.2 ± 926.3	1.16	46	0.250
9	7	825.8 ± 308.8	8	908.9 ± 384.9	-0.34	13	0.739
10	35	818.1 ± 463.7	35	955.3 ± 694.3	-0.79	67	0.431
12	10	1041.3 ± 493.1	10	1040.3 ± 530.7	0.03	18	0.976
15	32	494.3 ± 273.9	32	493.3 ± 241.4	-0.16	62	0.875
16	17	415.3 ± 272.1					

The lines 1 and 7 were used for further analysis of their significant lower mite development on SCS combs, based on their genetic background. The lines 2 and 6 showed significant lower development at least in one of the three years period and were also used for the detailed analysis of the Varroa reproduction parameters.

3.2 Wintering

Within SCS 150 out of 371 colonies (40.4%) were lost, compared to 162 (53.1%) out of 305 colonies within LCS, which is significant ($X^2=10.8$; $p < 0.001$).

SCS colonies are significantly more likely to survive the first winter after build-up (OR=1.69 with a 95% confidence interval ranging from [1.3 - 2.3]).

3.3 Varroa reproduction parameter

Table 4: VSH-values of 4 lines

year 2007	SCS (4.9 mm)				LCS (5.5 mm)			
line	N	max	N-VSH ≥0.2	min	N	max	N-VSH ≥0.2	min
L1[a]	10	0.44	60 %	0.08	10	0.26	40 %	0.04
L2 [b]	10	0.14	0 %	0	10	0.14	0 %	0
L6 [b]	10	0.17	10 %	0	10	0.21	10 %	0
L7 [a]	10	0.29	60 %	0	10	0.53	70 %	0.06

Post Hoc analysis (∝-corrected using Scheffé's procedure) showed a significant difference between L1 and L2 ($p<0.01$); based on 50 infested cells per colony.

The VSH calculation for the individual colonies in the test (2007) showed that - using the arbitrarily criteria VSH > 0.2 (eg. in 20% of the infected cells no reproduction could be observed) - a clear difference between the lines exists, but not between cell-sizes.

Table 5: VSH values of 5 colonies per group with SCS & LCS combs in each colony.

year 2008	SCS - 200 infested cells each hive				LCS - 200 infested cells each hive			
line	N	max	N-VSH ≥0.2	min	N	max	N-VSH ≥0.2	min
L1	5	0.54	5	0.29	5	0.29	2	0.09
L1 (F1)	5	0.38	2	0.03	5	0.21	1	0.02

L3	5	0.16	1	0.03	5	0.07	0	0.02
L3 (F1)	5	0.27	1	0.08	5	0.13	0	0.02

Within each line, inside the colony on the SCS infested combs a more pronounced VSH treatment is observed. Even in the line L3 with low VSH activity.

Table 6: VSH of single queens and their daughters locally mated 2007; details of L1 & L1(F1) and L3 & L3(F1) in 2008 (Table 5.)

number	L1 (2007)	L1(F1) (2008)	+/-	L3 (2007)	L3(F1) (2008)	+/-
1	0.45	0.38	-	0.1	0.14	+ +
2	0.16	0.06	- -	0.09	0.13	+ +
3	0.13	0.19	+	0.18	0.27	+ +
4	0.08	0.12	+	0.06	0.08	+
5	0.09	0.05	- -	0.12	0.14	+

The measured VSH of the original colonies and each daughter colony as measured in 2007 and 2008 to show the natural “drift” of the VSH-trait when queens mate free.

4. Discussion

4.1 Cellsize

For over 30 generations of the Carnica Harald Singer population the bees were forced to build combs on wax foundation with a 5.5 mm. cell size. Choosing a certain cell-size for a honeybee population under selection results in a dependency of the population under control on that cell size to draw impeccable combs. Mc Mullan & Brown showed the same non-intentioned result of keeping bees by humans for a "wild" *Apis mellifera mellifera* (Linnaeus, 1758) population in Dublin [McMullan , Brown 2006 ; pers. comm.]. To make colonies under selection accept more than one cell size a selection in the population appeared to be necessary.

Only 16 mother queens out of 1.287 = 1.2 % fulfilled the criteria "accepting foundation with 4.9 mm. and 5.5 mm". Apparently this group (N=1287) had unintendedly undergone a selection on the cell size 5.5 mm. for at least approximately 40 years.

It can be argued that some of the research done on the relationship between cell size and Varroa as put forward by A. & D. Lusby just failed to replicate their results because of this fact [Lusby 1996] [Dreher 2007]. Coffey used a non-HYG subset of their population and as they describe it: none of the colonies were opening infested cells, and they did not see any effect of cell size on Varroa reproduction in undisturbed Varroa-infested cells [Coffey et al 2010]. This population showed no signs of the SMR-behavior as described by Harbo and Harris [Harbo & Harris 2002 & 2005]. They already pointed out that HYG and SMR/VSH targeting different in-cell brood-diseases, might very well be different types of cell cap handling behaviour [Rothenbuhler 1964, Masterman et al. 2000]; each with different functional properties and probably involving different parts of the honeybee genome [Mondet et al. 2015].

Just being able to draw small size combs does not automatically results in a slower growing Varroa population on SCS. (Lines 3/2002 and 12/2003)

4.2 Selection.

To see differences in Varroa reproduction a high Varroa pressure is needed. Keeping SCS and LSC groups without any Varroa treatment brings this high infestation pressure.

Most lines showed a higher number of survivors in the SCS group of the line under Kefuss way of selection "live and let die" [Mc Neil 2010]; 12 from 16 lines passed the live and let die

selection applied on SCS and LCS. Analyzing the Varroa-development 4 lines do not show any influence of the factor cell-size; 2 lines showed a higher development of Varroa in the SCS colonies. The genetics of 6 lines out of the 16 confirmed the Lusby hypotheses [Lusby D. & Lusby A. 1996a].

The step by step selection applied on 1,287 genetically different colonies demonstrates the existence of a variability in dealing with cell-size and Varroa. The results helps to explain the controversial results found in literature. It clearly shows the genetic variability in a honeybee breeding population after a long time of classical selection on honey-production for traits, not selected for. In this actual project the combination of the traits surviving Varroa-pressure and differences in cell-sizes are compared.
The lines L1 (2002), L2 (2002), L6 (2002) and L7 (2003) show a clear differentiated Varroa development in dependence of cell size; slower on SCS-combs being observed.

The negative influences of smaller cell-sizes on Varroa-population growth is published by: [Martin and Kryger 2002; Kober 2003; Piccorillo 2003; Forsman et al. 2004; Johnsen 2005; Kleinfeld 2006; Maggi 2009].

No effects comparing cell size and Varroa-development are described by: [Fries 2004, Berg, 2004, 2005; Dreher 2007; Liebig and Aumeier 2007; Ellis 2008; Taylor 2008; Berry 2010; Coffey 2010; Seeley 2011].

4.3 Mechanism

As not all lines showed the positive effect on Varroa reproduction for the colony survival on SCS, the Varroa-reproduction within the sealed brood cells had to be analyzed.

4.3.1. Estimating the Varroa reproduction parameter

The division of the young mated daughter sister queens across the cell size groups was always randomized. So a difference by chance of a relevant trait just to appear only in a colony of a specific group (SCS or LSC) within each line can be excluded. The suggested superiority of SCS caused by the not at random division of a specific trait can be excluded.

Looking at the lines 3 and 12 they could eventually miss parts of a relevant trait, as the mother-queens of all lines are the first generation inbred descendants of a single artificial inseminated “foundress” queen [Janousek, 1992 Brno, personal communication]. They can be genetically different. In the whole Carnica Harald Singer group, each generation consist of 5 or more related groups (”lines”) always mated with a high number of non-sister queens as fathers, in the old Singer tradition. From generation to generation 7 till 15 selected mother queens are control-mated on isolated mating places with male-producing colonies from the whole population. So differences in the genotype can be expected. [Singer 1976; Praagh, van 2015; Ebersten 1996].

The daughter-generation from line 3/2002 do not exhibit the traits, that help SCS to be a favorable Varroa reproduction-inhibitor; three generations were open mated with the same male pool as the queens of the lines 1/2002, 2/2002, 4/2002, 5/2002, 6/2002, 7/2003 and 8/2003. These 7 lines all exhibit the traits. They all show a reduced Varroa reproduction in the SCS parts of the lines compared to their “LSC-sisters”. (Table 5.)

Not all lines after the “Bond” selection showed a difference in Varroa-population growth depending on cell-size. The lines 1/2002, 2/2002, 4/2002, 5/2002, 6/2002, 7/2003 and 8/2003 confirm the hypthesis. Line 3/2002, 12/2005, 15/2005 (Table 5) markedly have a high level of Varroa-development and no differentation of this level due to cell-size. These lines apparently miss the VSH-traits. We must conclude that those lines that do not show the VSH-trait, clearly demonstrate that cell-size per se does not influence Varroa-population growth under the experimental conditions.

As group of father queens producing the drones, the four mother colonies (2005, 2006) were used plus the still available sister colonies of each line, building a POOL of father queens [Praagh, 2015]. Doing so, the probability of “saving” the bigger part of the genotype of the whole selection is there. The results show, that this way of bee breeding prevented the loss of the hardly understood genetics of VSH behavior.

At a first glance the expression of the trait showed significant differences between lines. But statistically the expression was not significant in the test comparing daughter queens on the different cell sizes. Due to the genetic make-up of a colony: The relationship between sister

queens is only 25% [Praagh, van 1994]. The 4 lines used showed clear differences in Varroa reproduction between cellsizes. But expression of the worker-bee trait inside each colony within each line was estimated on 50 infested cells only. Leading to the conclusion that the clear differences found in earlier experiments and based on Varroa reproduction (10 days natural mite-fall) could in this experimental make up not be explained just by measuring the difference the classical way. Different worker composition per colony has to be expected. In 2008, using experimental colonies containing both comb types per line the inevitable variability between workers per colony, were overcome. The same kind of experimental set-up was used by [Message & Gonçalves 1995].

Offering the different comb-sizes at each side of the bee-spaces gave significant proof, that comb-size influences the observed reproduction rate of Varroa.

Due to an unknown underlying mechanism, the rate of the VSH cleaning behaviour is higher on the smaller cell size side of a "bee-space".

Comparing the infestation rates on each cell size per colony supposing the reproduction on the LSC being undisturbed, allows to estimate the % per cells with reproducing parasites missing (=Varroa reproduction being disturbed) on the SCS supposing the original percentage non-reproducing parasites on both comb types were the same.

Using the data we calculate a 2.6 x higher chance for a SCS Varroa infected cell to be cleaned, compared to the infected LCS cell in the same colony.

Supposing the LCS brood offers the parasite a better chance to reproduce compared to SCS without the VSH trait being present in the worker bee population of the line is unrealistic. The lines (L 2 & L 6) showed no difference in reproduction rate of the parasite between SCS and LCS; a clear indication that the invasion rate of the parasite is not influenced by cell-size, but the more intensive active disruption of the reproduction (VSH) on SCS must be the mechanism that makes SCS support the colony survival under Varroa-pressure.

This difference in cleaning behaviour explains the observed difference in reproduction of Varroa.

Only colonies headed by mated queens producing worker bees able to build 4.9 mm. cell size combs (SCS-able) **and** having the VSH trait have a chance to survive without treatment in an undisturbed environment. Nevertheless being SCS-able & VSH as colony offers no re-

invasion protection. Protection against re-invasion should be a next selection goal, eg. by selection of intensive guarding as trait.

Dreher and Liebig, Ellis and Berry used genetically unspecified queens (and bees) and demonstrated clearly that cellsize as such does not influence Varroa population growth [Dreher & Liebig 2007; Ellis et al. 2008; Berry et al. 2009]. Dreher and Liebig, Berry even found a higher number of infested cells in colonies with 4.9 mm. as compared to 5.3 mm. cellsize [Dreher & Liebig 2007; Berry et al. 2009]. This supports our view, that cellsize **plus** defined genetics can influence Varroa population growth.

The VSH trait was unobserved present in the Carnica Harald Singer population [Fries 2004; Berg 2004 & 2005; Dreher 2007; Dreher and Liebig 2007; Ellis 2008; Taylor 2008; Berry 2010; Coffey 2010; Seeley 2011].

Piccirillo and De Jong offered Africanised mellifera colonies three cell-sizes (4.84 mm. "African"; 5.16 mm. "Italian" and 5.27 mm. "Carnolian"). They reported on a significantly higher infestation level in the largest cell size as compared to the other two sizes and suggest: "the use of unnaturally large comb cellsize should be re-examined in the light of its effect on parasite levels." They also mention a 60% higher infestation rate of adult bees observed in colonies with two cell sizes (4.84 mm. and 5.16 mm.) as compared to the feral colonies with only 4.84 mm. cell size [Piccirillo and De Jong 2003]. This was already observed in Brazil [Goncalves et al.1982]. Piccirillo and De Jong 2003 presents the first experimental data on effects of cellsize on Varroa brood cell infestation rates [Piccirillo and De Jong 2003]. Our data on surviving colonies show that for the bee population used the colonies on smaller cell size (2003-2005) have a 1.3-2.3 times higher survival expectation rate.

Cell size can influence the active reproduction of Varroa inside capped cells using worker larvae in worker and drone-cell of *Apis mellifera* & *Apis cerana* (*Apis cerana* Fabricius 1793). The reproduction appeared to be disturbed in the larger celltype [Zhou,Yao, Huang, Huang, 2001].

A studie on the mite reproduction related to available space in the cell was done by Martin and Kryger. The authors used scutellata (*Apis mellifera scutellata* Lepeletier, 1836) colonies invaded by a capensis pseudo-clone (*Apis mellifera capensis* Eschscholtz, 1821). The capensis larvae occupies more of the cellspace. In normally filled cells (Scutellata pupae) the Varroa reproduction rate measured was higher. They suggest, that the male, as egg laid in the

upper part of the cell, can not reach the feeding site on the pupae and the moulting site in the lower part of the cell, if the cell is "overfilled" by a capensis pupae. The phenomenon as described by Martin & Kryger can not explain the reduced Varroa reproduction rates we found in the SCS group. [Martin & Kryger 2002]

As it is known that in A*pis mellifera* the phenotype bodysize is regulated by the cell size the worker emerges from the SCS cells we expect them to be smaller compared to those emerging from LCS [Daly et al 1988; Mc Mullan, & Brown 2006].

This influence on Varroa reproduction can not hold for the results obtained with the lines 3/2002, 12/2003. Here we could not find the reduced reproduction of Varroa in the SCS group as compared with their LCS group of sister queens. These two lines lacked the VSH trait. This meant that the factor of 2.7 on reduction of Varroa reproduction due to VSH was not available in the genetics of the lines 3/2002 and 12/2003.

The seasonal appearence of outlyers in Figure 1., 2. are most probably caused by a reinvasion, due to active robbing and absconding of weaker colonies. (= Varroas carried into a colony by drifting bees).
We suggest the high numbers of mites within the SCS as being the result of actively robbing weak colonies and given foreign mites the opportunity to be transferred to a healthy colony under consideration.

For the population under consideration we found CS can be used as a management tool for Varroa treatment during the active season (in "Wiener Becken"). The used *Apis mellifera carnica* singer population readily accepts the CS after the selection, as shown by the actual commercial used population of >1250 colonies wintered 2014/2015. 2010 the commercial Carnica Harald Singer population was completely on SC >1000 colonies. Not all "wild mated" (F1)queens produce colonies that readily accept SC; another indication that cell size is a genetically controlled trait. Actually about 10% of the F1-colonies show problems by the correct comb building.

Using the parameter Varroa population growth as a selection parameter caused the VSH trait to be kept (or improved) during the selection (2003-2005). The 2007 & 2008 results clearly

show this trait was not explicitly available in all lines. As those lines were not scanned for the trait we can only speak about different expression of the trait between the lines. We consider the traits cellsize and VSH to be genetically independent. The analysis of the 2007 and 2008 experiments for VSH showed differenced levels of VSH to be correlated with cell size. The results of 2008 - both CS's in a colony - show higher level of VSH for infested brood cells on small cells. The data are statistically convincing - a behavioral explanation is missing.

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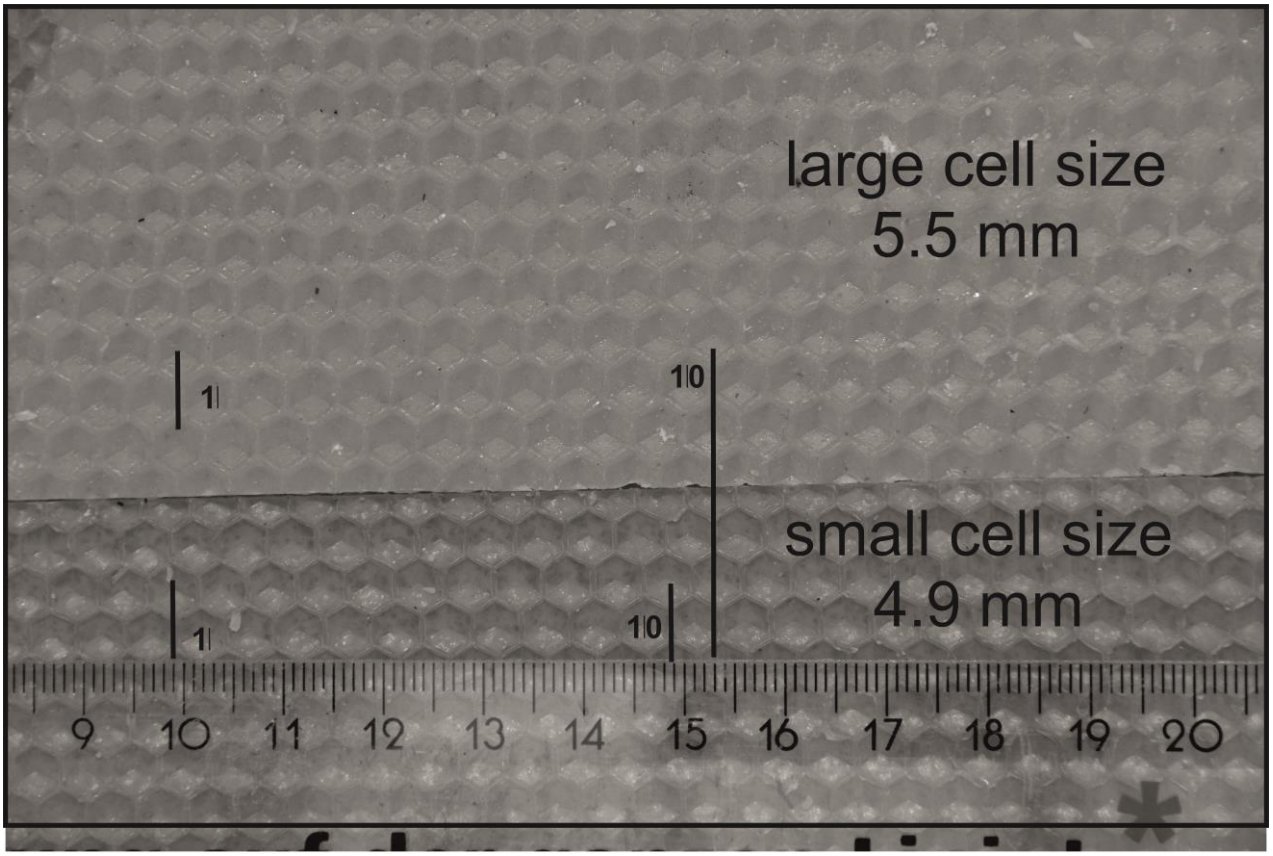
2 Pictures for the journal:



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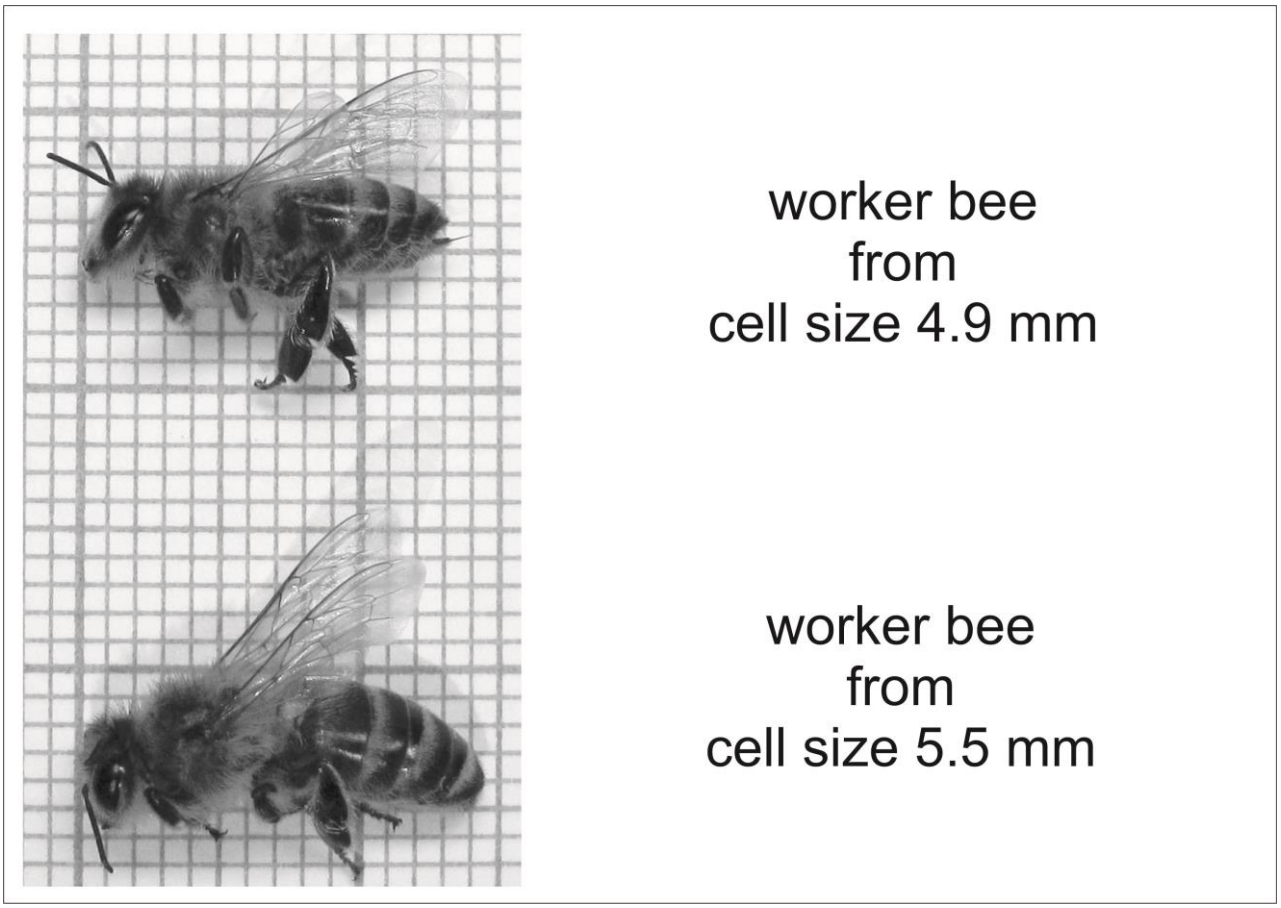

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