Research Network for Sustainable Bee Breeding

Version 2017-09-01

Screening for low Varroa mite reproduction (SMR) and recapping in European honey bees

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Introduction

In European honey bee subspecies usually 5-20% of the Varroa mites remain infertile after invading worker brood cells (see review by Rosenkranz et al., 2010). In addition, some mites show a delayed egg laying in relation to the brood development or have only male offspring due to unfertilized eggs. These occurrences limit the reproductive success of Varroa which can be measured as the number of adult daughters per foundress mite at the moment of brood emergence.

In the 1990s, Harbo and Harris identified high levels of non-reproduction as an inherited character of worker bees which they named **"Suppression of mite reproduction - SMR"**. Later on, specific experiments suggested that the low proportion of fertile mites, at least in the US stock, mostly was due to preferential removal of reproducing mites by worker bees, so the trait was renamed **"Varroa sensitive hygiene - VSH"**. However, SMR in a colony can potentially result from mechanisms other than VSH behavior (there is evidence that brood may be able to prevent mite reproduction). One of the potential mechanisms is uncapping and recapping of (infested) brood cells. At the right time of brood development, opening of infested cell for some time can have a negative impact on success of mite reproduction (Kirrane et al., 2011).

High levels of mite resistance were achieved by strong selection for SMR. Increased proportions of non-reproducing mites (40 – 50 %) have also been shown as a significant trait of some naturally selected mite resistant A. mellifera populations in Europe ((Locke et al., 2012). Novel research on surviving populations indicate that recapping plays a very important role in resistance. However, we don´t have much information about the distribution of the SMR trait in different European subspecies and its variability due to seasonal and environmental effects. Therefore, RNSBB partner institutes are running a joint investigation to gain more information on the variability of the rate of non-reproducing mites in different European honey bee populations and in different locations.

Sampling

The test colonies should have an undisturbed brood development for at least 30 days prior to sampling (no brood interruption, no queen exchange). The sampled brood should mainly contain elder pupae (pink eyes stage to moult completed, corresponding to 7-12 days post capping).

As the efficiency of the brood analysis depends on high infestation levels the sampling date may be postponed until Varroosis treatment needs to be started. According to our experience, the bee infestation should be at least about 2 % at the time of brood sampling for SMR control.

The brood samples will immediately be evaluated or stored in freezers (-18°C) until examination.

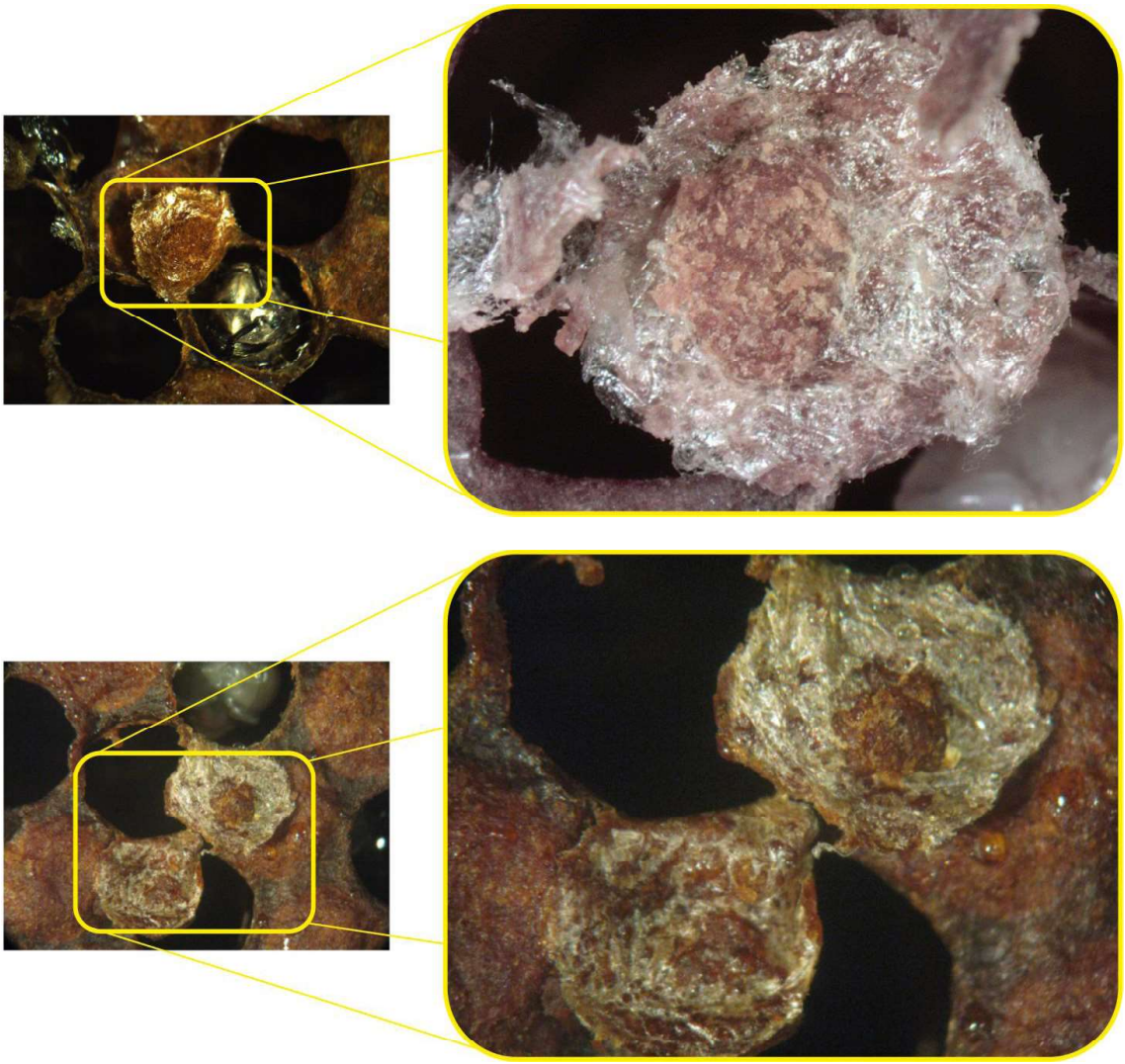
The pedigree of the test colonies should be documented as far as possible. Furthermore, 50 young workers and 10 young drones per colony should be sampled for a potential molecular-genetic analysis of individual test colonies in accordance with the attached sampling protocol.

Brood examination

Examining brood cells requires some care and attention: a stereomicroscope or a magnification lamp (5X is ideal) does support identification of the presence of Varroa mites and their offspring. Fine forceps, a scalpel, and a small paintbrush can aid investigation of the brood cell, together with LED illumination.

Recapping

Recapping activity on a brood cell can be simply determined by checking the inner surface of the cell capping. With the tip of the forceps, or by cutting the edges of the cell cap with a scalpel, the capping is gently detached and turned upside down. It is important to preserve the whole cap during this manipulation. Recapped cells lack part of the pupa's spun cocoon and this can be noticed as a matte hole in a normally glossy cap. The hole can be different in size, ranging from just a small part of the capping to the complete extent of the cap. This indicates that bees have opened the cell and than recapped it again.



Recapping activity can be noticed as a matte wax hole on the inner side of the caping (Photos by M. Kovačić (upper) and C. Costa (lower))

Mite reproduction investigation

For the investigation of mite reproduction, only single infested brood cells (one foundress mite) in the developmental stages between "purple eyes" (7 days post capping) and "pupal moult completed" (12 days post capping) are evaluated. In the purple eye stage (7-9 days post capping), normally reproducing mites have at least one deutonymph and one male. On pupae with black eyes (10-12 days post capping), normally reproducing mites have at least one adult daughter mite and one adult male. The main criteria to differentiate between day 9 and 10 in terms of bee developmental stage is the presence of grey wing pads at day 10. Infested pupae without any offspring (infertility), with only younger stages of Varroa offspring (delayed reproduction) or without a male are therefore counted as containing non-reproductive mites.

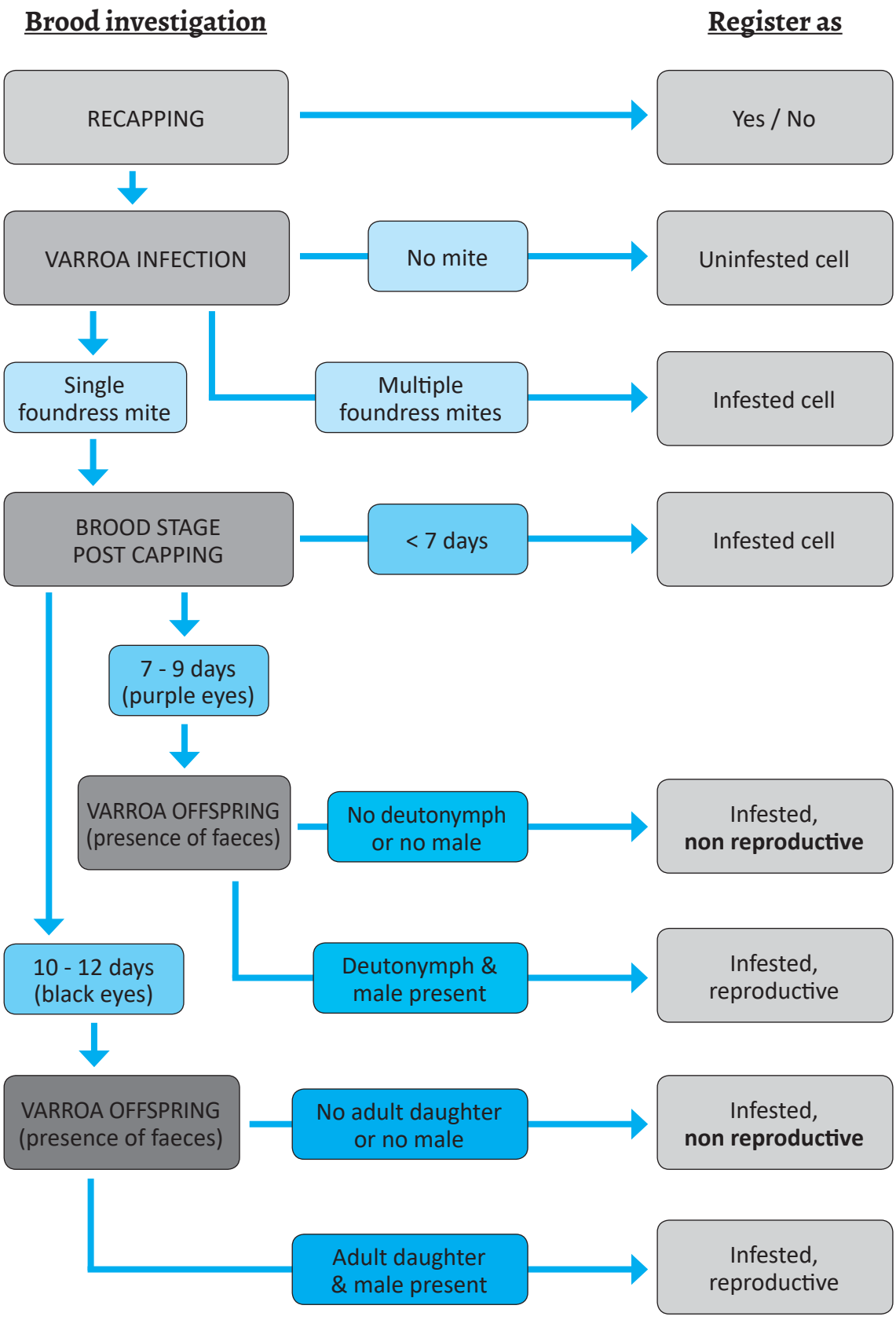
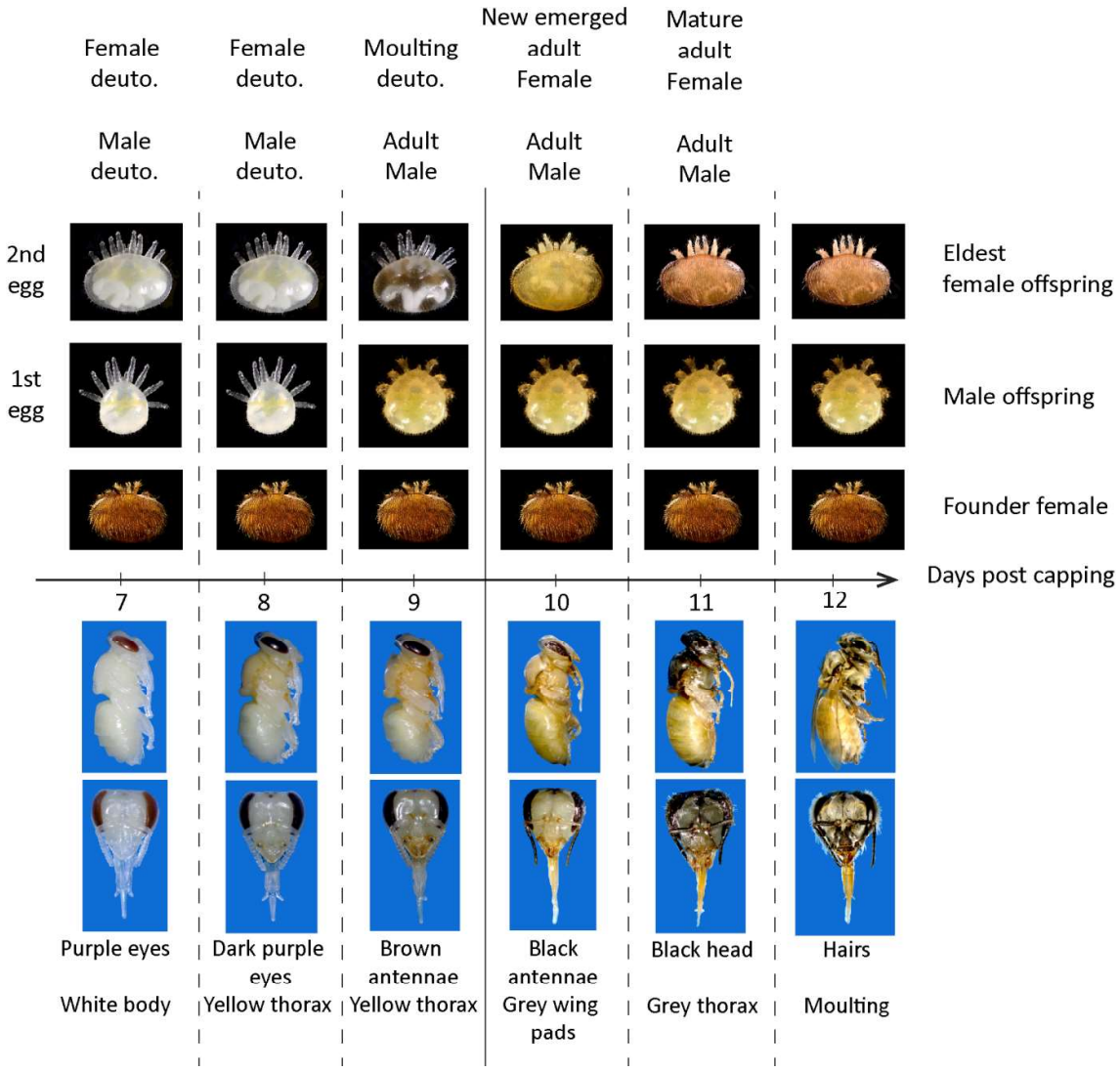


Diagram of inspection of a brood cell for assessment of recapping and Varroa reproduction

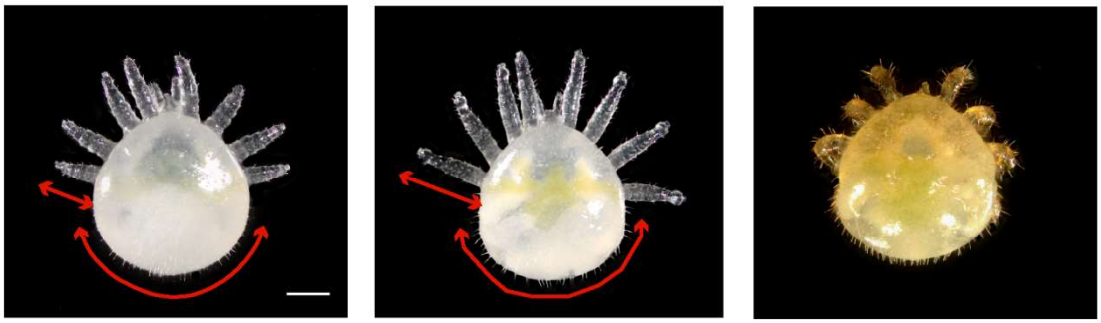


Comparative development of A.m. honey bees (bottom) and Varroa destructor mites (first two eggs) (top), 7 to 12 days post-capping of brood cells (photos by F. Mondet)

Photographs show the average appearance of development of mite progeny (first two eggs) in relation to bee pupae stage. For bees, the main characteristics used to determine each stage are indicated below the photographs. For mites, the normal expected stage of eldest female and male offspring are indicated above the photographs. If the eldest found progeny is at a younger stage than the illustrated one, for a given bee stage, then the founder mite will be classified as NON-REPRODUCING. The solid line placed between day 9 (bees with coloured thorax, but no grey wing pads) and 10 (bees with grey wing pads) post capping separates the period before and after which we should expect adult female varroa offspring.

The differentiation of male Varroa offspring from female protonymphs can be difficult and time-consuming and also needs some experience. It is therefore not demanded for this European wide survey but still recommended as an additional informative parameter.

If observation is performed on day 12 the adult daughter may have assumed the same colouring as the foundress mite.



Key characteristics used to differentiate female protonymphs from young (protonymph or deutonymph) males in the mite Varroa destructor (photos by F. Mondet)

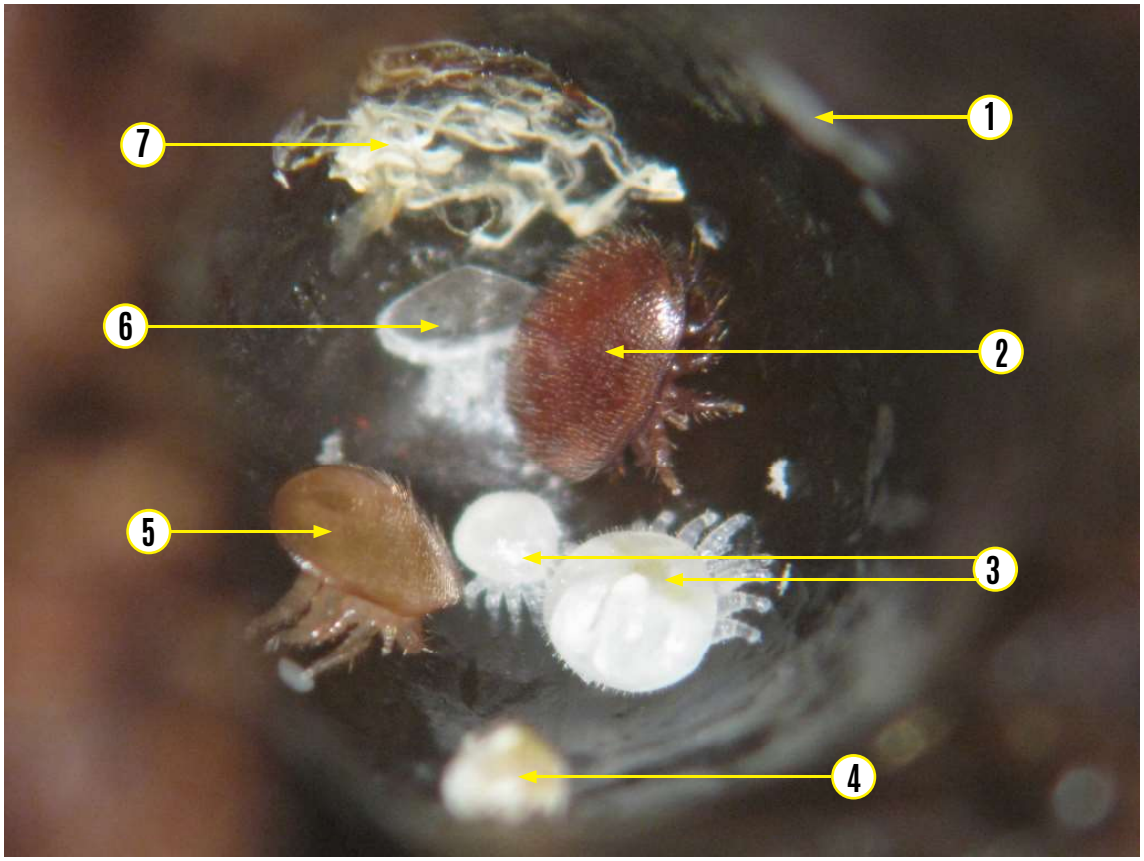
These stages are usually found in honey bee brood cells 5 to 8 days post capping. Two distinct features are helpful, as illustrated by the red arrows:

- Legs: Young males have longer and thinner legs than female protonymphs.
- Body shape: the posterior part of the body is round in female protonymphs (apple shape), whereas it is more sharp-featured (pear shape) in males.

An adult male, featuring a light-orange body colour, is presented for comparison. The scale bar indicates 200 µm.

For further details on developmental stages of bees and mites see also the Bee Book chapters by Human et al., 2013 (Miscellaneous methods) and Dietemann et al, 2013 (Varroa research).

In order to try to understand better what happens if the cell is recapped, it would be useful during the brood examination to note the **presence/absence of Varroa feces** which clearly indicates that a mite was present which might have gone out of cell while it was uncapped. The Varroa "faecal accumulation site" is usually located on the side wall of the cell. Another unusual (and very uncommon) situation is the **absence of Varroa mother** while the progeny is present in the cell. This could be difficult to notice, in particular when brood with purple eyes pupae are examined and young light stages of mite offspring are in the cell. If such a case is noticed, it would mean that mother mite left the cell while it was uncapped.



Inside of the cell with reprodction of the mites. 1 - faecal accumulation site is usually located on the side wall of the cell (faecal deposit is out of focus), 2 - mother mite, 3 - protonimph and deutonimph stages of mite development, 4 - male, 5 - new emerged adult female, 6 - exuvia, 7 - Honey bee larva faecal deposit.
(Photo by C. Costa)

Preliminary statistical simulations by Sreten Andonov based upon brood samples from Kirchhain show that at least 335 brood cells need to be checked in samples with an average infestation of 10 % and level of non-reproduction rates of 30 % with a confidence level equal or greater 95%. We therefore recommend to check at least 35 single infested cells per brood sample. These numbers can be reduced for higher levels of non-reproduction, but a minimum number of 10 single infested cells will be demanded is necessary to include the sample in further evaluations.

References

Kirrane, M. J., De Guzman, L. I., Rinderer, T. E., Frake, A. M., Wagnitz, J., Whelan, P. M., 2011. Asynchronous development of honey bee host and Varroa destructor (Mesostigmata: Varroidae) influences reproductive potential of mites. J. Econ. Entomol. 104, 1146–1152. doi:10.1603/EC11035

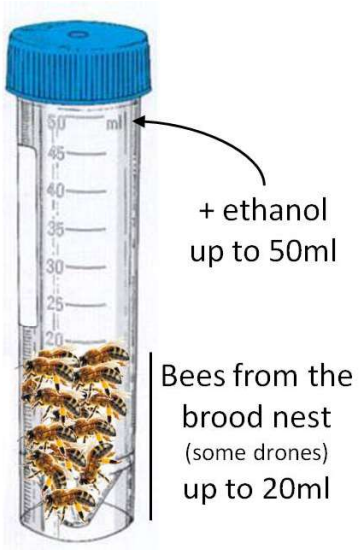
Locke, B., Conte, Y. Le, Crauser, D., Fries, I., 2012. Host adaptations reduce the reproductive success of Varroa destructor in two distinct European honey bee populations. Ecol. Evol. 2, 1144–50. doi:10.1002/ece3.248

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of Varroa destructor. J. Invertebr. Pathol. 103. doi:10.1016/j.jip.2009.07.016

Attachment:

Sampling protocol for molecular genetic analysis of colonies tested for SMR - Collect about 50 young workers and 10 drones per colony

HOW TO COLLECT THE SAMPLES



1. Fill the tube up to approximately **30 ml with bees taken from the brood nest** (≈ 50 bees) **and up to 50 ml with absolute ethanol** (96 %). If possible, add up to 10 drones.
2. **Label the samples** (see below in "HOW TO LABEL THE SAMPLES")
3. Store the samples **overnight at 4 °C** (in the fridge).
4. **Change the absolute ethanol (96 %) the day after sampling** to ensure optimal preservation
5. **Keep the samples at 4 °C until shipping**
6. **Report the sampling to the coordinators of the experiment and await instructions for shipping**

HOW TO LABEL THE SAMPLES

Please prepare labels with **your name and the colony number and affix them to the outside of the sampling tubes**.

The ID code of each sample must be copied to a piece of paper with a pencil (not pen!) **and put inside the tube** that it can be read from the outside.

MATERIALS NEEDED FOR SAMPLING

Product	**Catalog Code**
50ml plastic tube (skirted)	e.g. 525-0243 (VWR)
Pre-prepared labels	-
Absolute Ethanol 96%	e.g. ALCH0110F5_T (VWR)
Pieces of paper	-
Pencil	-
Gloves	e.g. 112-2756 (VWR)
Storage boxes or plastic bags	-