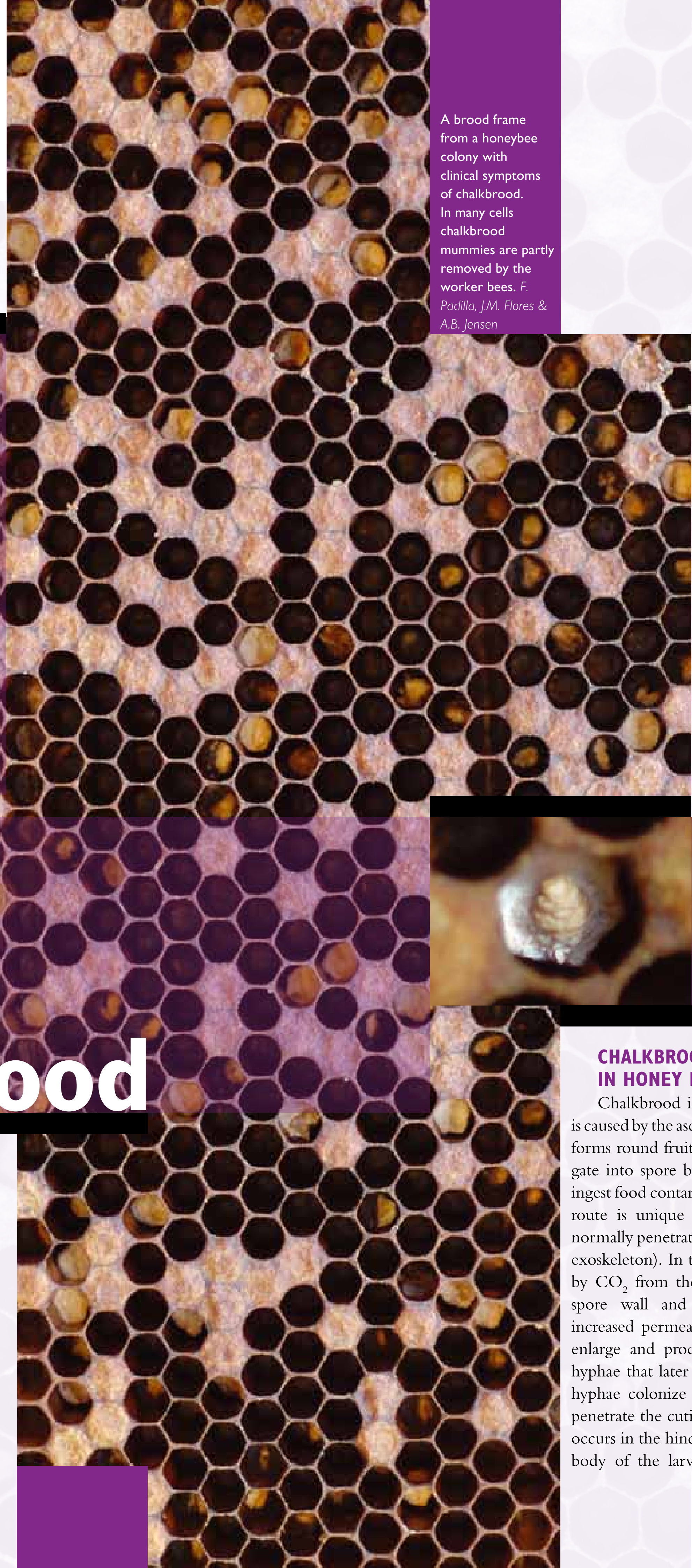
Fungal infections in honeybee brood

ANNETTE BRUUN JENSEN & JOSÉ MANUEL FLORES

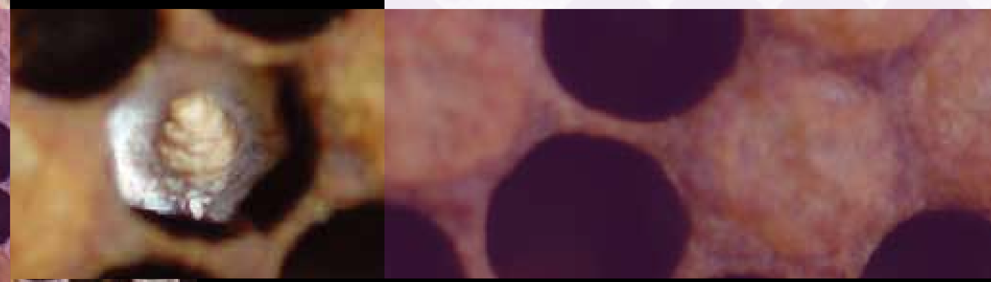
HONEYBEES ARE SOCIAL INSECTS that live in big societies and can become ill. Each society consists of one queen, hundreds of drones and thousands of workers. Honeybees build their home in a confined space within a hive or in a hollow tree furnished with wax combs made up of hexagonally shaped cells. These cells are not only used for storing food, such as honey and pollen, but also for housing the brood. The queen lays her eggs in the cells and when they hatch the larvae are fed and tended by workers. Shortly before pupation, the workers seal the cells with a wax lid and the pupae contained inside develop and mature into new bees.

Contact between individuals (adult–adult / adult–larva) in the hive is high and such contact naturally increases disease transmission. When a pathogenic microbe enters a colony it can quickly spread throughout the hive, just as in a school classroom or in the confined space of an aeroplane. The bees within a single hive are closely related; the workers and drones are all either sisters or brothers. This close relatedness means that individuals within a hive are similarly susceptible to microbial attack.



A brood frame from a honeybee colony with clinical symptoms of chalkbrood. In many cells chalkbrood mummies are partly removed by the worker bees. *F. Padilla, J.M. Flores & A.B. Jensen*

Honeybees, the most important pollinators on the planet, are just like other living creatures; they are vulnerable to microbial attack and sometimes fall victim to a variety of viruses, bacteria and fungi. One of the commonest fungal infections is chalkbrood.



CHALKBROOD – FUNGAL INFECTION IN HONEY BEE LARVAE

Chalkbrood is a fungal disease of honeybee larvae. It is caused by the ascomycetous fungus *Ascosphaera apis*, which forms round fruiting bodies containing spores that aggre-gate into spore balls. Larvae become infected when they ingest food contaminated with fungal spores. This infection route is unique for the insect-pathogenic fungi which normally penetrate their insect host through the cuticle (the exoskeleton). In the larval gut, *A. apis* spores are activated by CO_2 from the host tissue. In an activated spore, the spore wall and membrane become permeable. This increased permeability allows the spore to absorb water, enlarge and produce a germ tube which gives rise to hyphae that later penetrate the gut wall of the larva. The hyphae colonize the body cavity and, after several days, penetrate the cuticle from the inside out – this most often occurs in the hind end of the larva's body. Soon, the entire body of the larva, except for the head which remains unaffected, is covered by a white mycelium. This mycelium may or may not go on to produce dark fruiting bodies.

The larvae killed by the fungus appear dark or white, depending on whether fruiting bodies form or not. When they dry up, those lacking fruiting bodies look like small Egyptian mummies or a piece of chalk, hence the name chalkbrood. Fruiting bodies only develop when hyphae of the opposite sex (designated + and –) come together. The presence of both sexes in infected larvae and relative humidity determine fruiting body development and the concomitant production of millions of infective spores.

CHALKBROOD IS A STRESS-RELATED DISEASE

Clinical symptoms of chalkbrood are typically seen when honeybees are stressed and when workers are not able to keep the brood temperature around the optimal 34 °C. Larvae are more susceptible to chalkbrood when chilled, just as we are more prone to catch a cold if we are stressed. A chilled brood can be caused by an imbalance between the number of brood and the number of workers, due to high mortality of workers (e.g. other diseases or pests), to cold weather during hive build-up in early spring or to hive management on a cold day.

Chalkbrood of honeybees has been recognized since the early 1900s in Europe and is among the first known honeybee diseases. The disease is documented almost anywhere honeybees are kept by man. Chalkbrood is easy to recognize. A typical sign of a hive with a high infection is the piling up of mummies by workers on the bottom board or outside the hive entrance. As the colony weakens, the workers cannot keep up with the removal of so many of their dead sisters. For this reason, both white and dark mummies can be found still sitting in the brood frame next to cells containing healthy bees. Such colonies typically do not survive the winter. But since most colonies are able to regain their strength after a mild chalkbrood outbreak, curing themselves, many beekeepers don't see chalkbrood as a major threat; however, chalkbrood may actually have a greater impact on colony performance than anticipated.

INDIVIDUAL AND SOCIAL DEFENCE AGAINST FUNGAL INFECTIONS

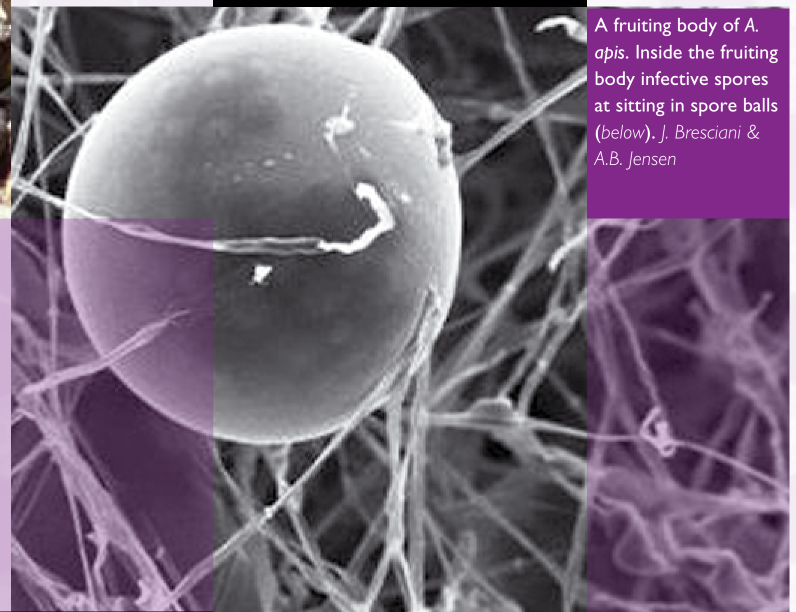
Worldwide, there is no effective chemical treatment against chalkbrood disease. Fortunately, the main defence against chalkbrood comes from the bees themselves, both individually and as a collective response by the colony. Bees have an innate immune system and can express various antimicrobial substances towards pathogens. They even have phagocytes, cells that can detect and ingest pathogens. But unlike us, they lack an acquired immune system and cannot produce specific antibodies.

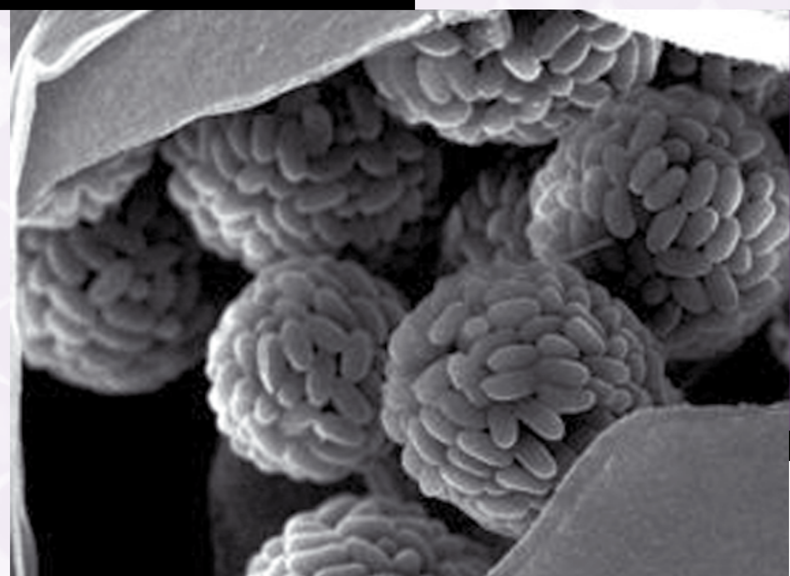
Recent genome sequencing suggests that honeybees have fewer genes involved in immune defences compared to other studied insects, such as the mosquito *Anopheles gambiae* (malaria vector). A possible explanation for this surprising fact is that honeybees have reduced the cost of maintaining an expensive immune system by developing collective behavioural defence mechanisms.

Chalkbrood mummies that have been pulled out of the frame and dropped by the worker bees into the bottom board of a hive. *A.B. Jensen*

Dark and white chalkbrood mummies. The black mummies contain millions of new infective spores. *A.B. Jensen*

"Chalkbrood fungi potentially play a major role in the pollinator decrease observed worldwide in the past few decades."





A fruiting body of *A. apis*. Inside the fruiting body infective spores at sitting in spore balls (*below*). *J. Bresciani & A.B. Jensen*

One very important collective defence is hygienic behaviour. This behaviour includes the detection and removal of sick or dead brood. Because chalkbrood most often kills the brood after the cells are capped, hygienic behaviour involves a complex multistep performance in which the honeybees must first find the cells with infected brood inside, open the caps and finally remove the mummies. Some colonies have bees that are better at performing these hygiene tasks than others. The bees in these colonies are able to quickly find and remove sick brood and thus reduce the likelihood of the disease spreading. In colonies where removal of diseased brood is delayed, the fungus remains inside the hive long enough to produce and release a large number of mature spores which can infect other larvae. Hygienic behaviour is genetically controlled, thus breeding for more hygienic colonies is a way to help honeybees combat chalkbrood.

Honeybees show other sophisticated behaviours. They collect propolis, a resinous mixture from the leaf buds of trees, which contains antimicrobial substances. Propolis is used by honeybees for disinfecting combs and the inner surfaces of the hive, and to close holes and crevices. Honeybees are also able to increase the colony temperature in an effort to eliminate or reduce the pathogen number; this strategy is analogous to when we have a fever.

CHALKBROOD FUNGI AND WILD POLLINATORS

Other pollinators also struggle with fungal diseases, in particular solitary bees. Leaf cutter bees, which are used commercially for alfalfa pollination services in the USA, have many problems with chalkbrood. Chalkbrood in solitary bees is caused by closely related but different fungal species from that which causes chalkbrood in honeybees. It is intriguing that all species within the genus *Ascosphaera* only grow and proliferate within bee environments. Some kill the larvae, whereas others have the ability to grow on the pollen provisions, faecal matter and other nest debris.

The impact of non-pathogenic species on bee fitness is currently unknown – but all these 'chalkbrood' fungi potentially play a major role in the pollinator decrease observed worldwide in the past few decades.

ANNETTE BRUUN JENSEN is Associate Professor in the Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsenvej 40, 1871 Frederiksberg C, Denmark (tel. +45 33 26 62; email abj@life.ku.dk)

JOSÉ MANUEL FLORES is Associate Professor in the Departament of Zoology, University of Córdoba, Campus de Rabanales 14071, Córdoba, Spain (tel. +34 957 21 86 98; email ba1flsej@uco.as)

FURTHER READING

Aronstein, K.A. & Murray, K.D. (2009). Chalkbrood disease in honey bees. *J Invertebr Pathol*, 103 Suppl. 1, 20–29.

Flores, J.M., Ruiz, J.A., Ruz, J.M., Puerta, F., Bustos, M. Padilla, F. & Campano, F. (1996). Effect of temperature and humidity of sealed brood on chalkbrood development under controlled conditions. *Apidologie* 27, 185–192.

Jensen, A.B., Pedersen, B.V. & Eilenberg, J. (2009). Differential susceptibility across honey bee colonies in larval chalkbrood resistance. *Apidologie* 40, 524–534.