Honeybee evolution: royal jelly proteins help queen larvae to stay on top

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Summary: A new study shows that, in honey bees, the main role of certain proteins in royal jelly is to ensure that the larva stays in its cell, thereby allowing it to develop into a queen.

A honey bee (Apis spp.) colony is made up of thousands of individuals that are nearly all female. Most of these females develop into workers, that are normally sterile, and only a small number will become reproductive queens [1]. As there is no strict genetic basis for determining which individual becomes a queen or a worker [2] and since each female larva has the potential to become a queen, other factors must play a role in determining the caste of a female larva.

Honey bee larvae are progressively fed by workers with a protein rich jelly of which the two main components, proteins and fatty acids are produced in two glands of the nurse bees: the hypopharyngeal gland and the mandibular gland. One third of royal jelly is made up of proteins and sugars in a ration of 1:1, while the balance consists of water and a fair proportion of fatty acids [3]. Sugar is added by the nurse bees and originates from the honey stores, whereas the proteins and fatty acids are synthesised in the hypopharyngeal gland and the mandibular gland respectively. Larvae which receives royal jelly exclusively during her larval development will develop into a queen, whereas larvae that receive less and jelly with sugar diluted will become a worker.

Four proteins, the so called major royal jelly proteins (MRJP), make up the majority of the proteins in royal jelly, two of which not only provide nitrogen to the larva but also antimicrobial protection. Proteins were for decades considered to be prime candidates as potential “queen maker factors” but quests to identify the “royal substance” were all unsuccessful. Recently, it was shown that one of the four protein does not have any determining function [3]. The long history of unsuccessful attempts to identify a queen determining substance has resulted in the conclusion that the amount of food provided to larvae is the determining factor [3-6] rather than a royal substance.

Nevertheless, the function of the proteins, besides being an important nitrogen source for the larvae was still unknown. Despite countless evidence to the contrary [3-6] and the focus always came back to the point of caste determination. Here the results of Buttstedt et al [7] are instructive, as they are able to show the function of two proteins found in royal jelly and thereby shift the paradigm from t a queen determining substance to an alternative even-more important function. They thought out of the box by investigating how honey bees overcome another problem when it comes to raising queens.

Honeybee workers are raised in combs of horizontally orientated "bunk beds " (Fig 1a) which are slightly sloped so that the larvae slide down into cell but not out [8]. Queens on the other hand are develop in vertical cells which are open at the bottom with the larva placed at the top, which is the cell bottom (Fig 1b-e) [8]. So, to become a queen you have to defy gravity.

Buttstedt and colleagues [7] show that two proteins in royal jelly create a pH-dependent fibril network allowing the larva to hold on to the royal jelly surface. They show that during the secretion of the jelly the interaction between the two glandular products results in a decrease of the pH of the jelly from around 7 down to 4. This is achieved by adding fatty acids from the mandibular glands which result in an increase in viscosity to the levels required to keep the larva at top of the cell. It would be the equivalent of having dinner while being attached to the ceiling by our dinner. The simplicity of this mechanism can be seen in what happens to larvae adhering to royal jelly when the authors increase the pH. The network of proteins collapses and the larva falls out of the cup (supplementary video).

By changing the pH the bees either allow the protein network to form or to dissolve. The more remarkable aspect is the adhesive properties of the royal jelly. A queen larva during her development in the cells while defying gravity can reach a weight of more than 300 mg [9]. She only will receive around 50 µg of food per day [5, 6] and this amount of food is for her to eat, grow and to glue herself to the cell. This would be very roughly comparable to using 12.5 grams of glue to attach a 75 kg human to the ceiling.

The article by Buttstedt and colleagues [7] is significant on three levels: Firstly, at the functional level they identify the underlying additional function of the two main proteins in royal jelly. Secondly, this provides convincing evidence that will undermine the idea that the proteins in royal jelly have to have any caste deterministic function and assist in overcoming an outdated paradigm thereby allowing us to re-focus our research efforts. Thirdly, the adhesive properties and the fact that the fibril network is pH dependent could also open up new avenues in other more distantly related fields. Understanding the adhesive properties of royal jelly in concert with the properties of the larva itself might yield results applicable in other fields such as use of bioadhesive with their applications in medical research.

As the authors of the study state themselves a highly adaptive solution to two aspects of raising a queen: Feeding the larva excellent food and providing it with a two-component adhesive ensuring that future queens stay on top. And indirectly they answer the third question: What does it take to make a queen? Just stay on top!

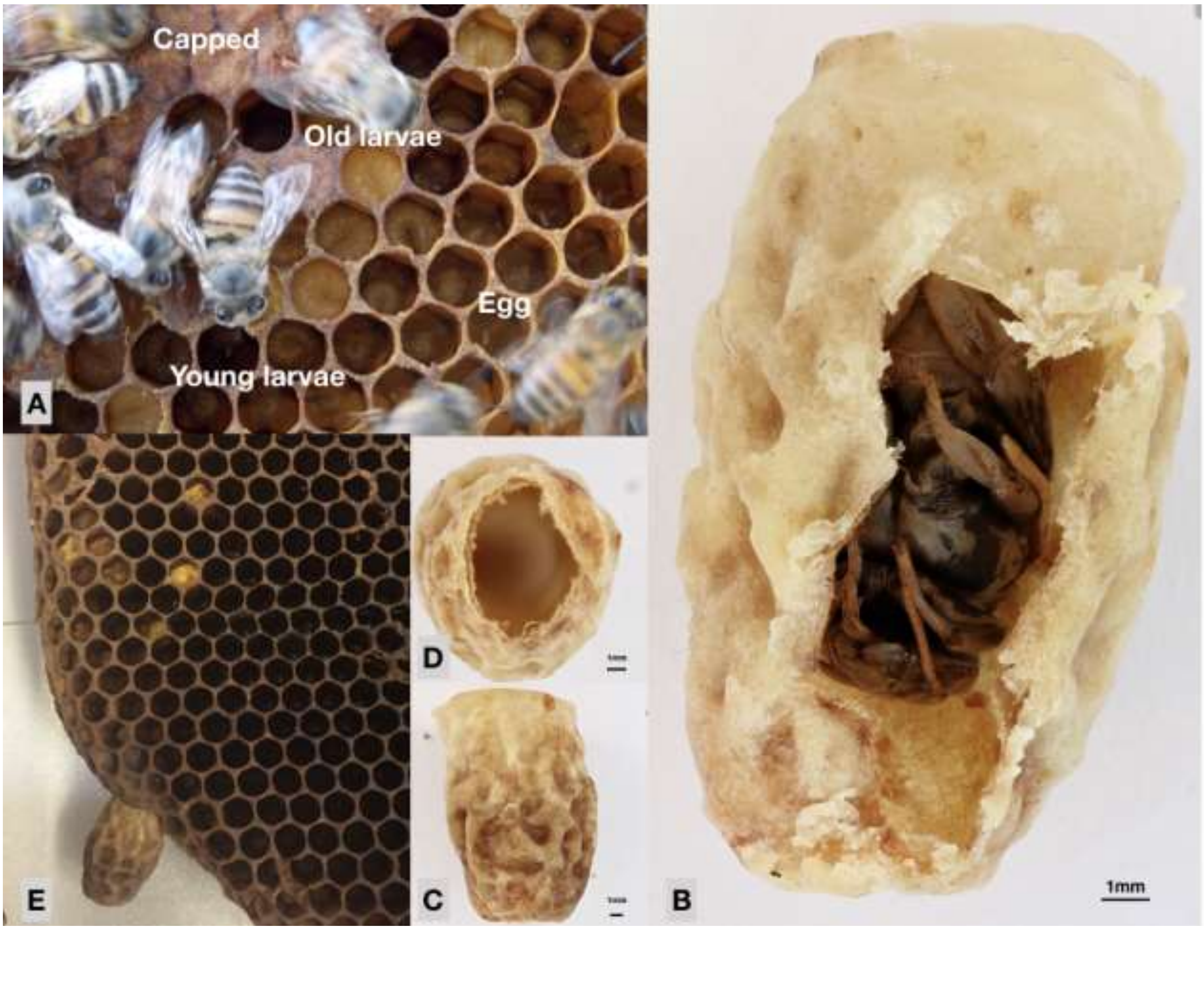

Figure 1: A) comb with larvae of different ages in the cells as well as eggs and sealed/ capped brood (Photo by C.L. Laing). B) queen pupae in a queen cell head facing down. C) queen cell: side view. D) queen cell: view from the bottom with larva sitting on top. E) Queen cell on comb.

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