A queen bee (labelled with blue paint) surrounded by workers.

A QUEEN IS MADE, NOT BORN

Ryszard Maleszka explains how the bees took an ancient gene and, via multiple duplications, created a specialised family of proteins that serve their most important social need: raising a queen.

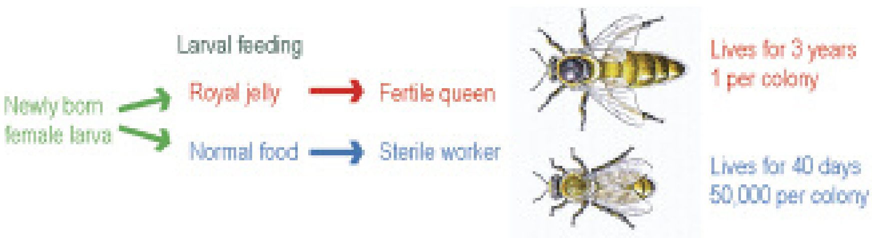
Honey bees are a highly social species. They live in dense colonies, often reaching as many as 60,000 individuals occupying a small space equivalent to roughly 15 adult humans in a one-bedroom apartment. The hive environment is well-protected, food is constantly shared among individuals, and temperature and humidity are virtually constant. But their societies are different from ours.

In both honey bees and humans, the dependence of the individual upon society is a fact of nature that cannot be eliminated. However, humans are social beings and solitary beings, and always attempt to protect their existence. Bees, on the other hand, have no self-value and work as a whole, with most aspects of their life controlled by fixed, inherited processes.

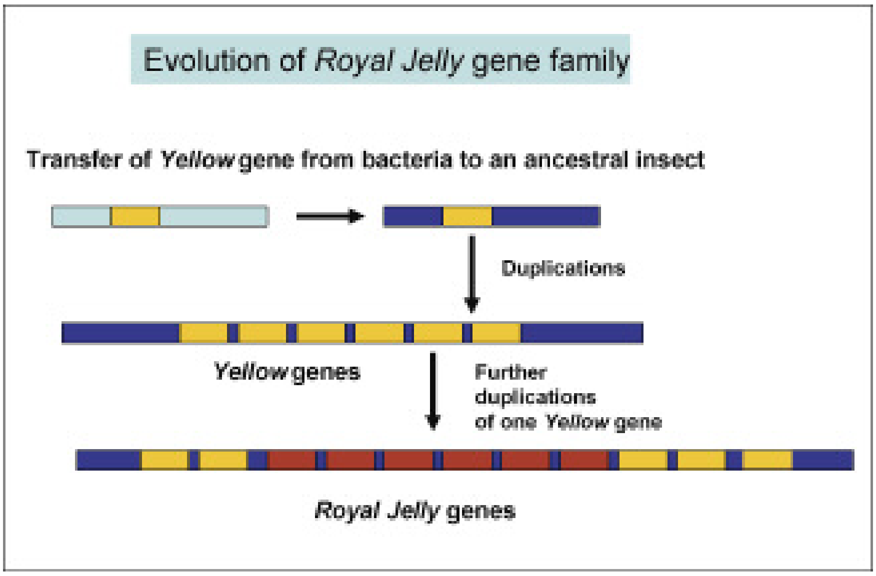
One of the prerequisites of insect sociality is a striking biological phenomenon known as reproductive division of labour. Only one individual in the colony, a queen, is permitted to produce new offspring, whereas her daughters are reduced to mere hive employees who selflessly work around the clock to ensure the existence of an elaborate society built around a single reproductive individual.

A queen bee is made, not born. She is selected to become queen when she is either an egg or a very young larva.

When worker bees decide to make a new queen, either because the old one is weakening or was killed, they choose several eggs or small larvae and begin feeding them with a nutritious substance called royal jelly. Raising a new queen is a race against time because a queen-



Honey bees make a queen by feeding a selected female larva with large quantities of royal jelly.



Recent evidence suggests that the royal jelly proteins evolved from an insect gene family known as "yellow" that has its origins in bacteria.

less colony cannot survive.

Royal jelly is a thick, creamy and highly concentrated source of proteins, essential amino acids, unusual fats, vitamins and other nutrients, and is produced by worker bee head glands. A huge amount of royal jelly, in which a developing larva virtually swims, sets her on a journey to become a very lucky creature. Not only will she get full attention and an unlimited amount of food during development, but when born she will continue to be spoiled with good food, constant grooming, and protection by her assistants: the royal court. Moreover, her life span will be at least 20 times longer than that of a regular worker bee.

But nothing is free in the honey bee society. In return for all these privileges and rewards she will lay up to 2000 eggs per day, equal to almost three times her bodyweight, for the next 2–3 years. This may sound unfair from a human point of view, but such an arrangement within the colony is essential to its survival because the workers must fully concentrate on fulfilling another prerequisite of insect sociality: parental brood care.

The molecular mechanism underlying the action of royal jelly is not yet known. The queen larva has exactly the same genetic composition as a regular worker larva, but these two virtually different organisms follow two very different developmental paths.

These paths must be controlled by subtle modifications of the genome that don't alter its DNA sequence. These are known as epigenetic or "above the genome" changes.

Genetically identical female larvae develop into queens or workers on the basis of larval nutrition. Developmental switches render workers almost entirely sterile while queens develop into one of the most fecund animals known. It is widely believed that this remarkable biological potency of royal jelly is largely controlled by a group of proteins known as royal jelly proteins.

Until recently, little was known about the function and evolution of royal jelly proteins. Now, thanks to the published sequence of the honey bee genome, the mysteries behind the royal jelly protein family are slowly being unveiled.

Using the genomic data our team at the Australian National University discovered that the royal jelly proteins are encoded by an array of nine genes arranged in a small cluster lying within a chromosomal region occupied by another gene family named "yellow". The name comes from the "originator" of this family, which has a role in pigmentation.

Each insect genome sequenced so far encodes 10–12 copies of yellow genes, which share a high level of sequence similarity consistent with the common origin. The yellow genes perform diverse functions related to pigmentation, development and sexual maturation.

The genomic architecture of the yellow/royal jelly gene family in the honey bee suggests that, in the ancestor of social bees, repeated duplications of one member of the yellow cluster produced a new gene family – royal jelly – that over a period of millions of years evolved new "social" functions.

Furthermore, comparative genomic analyses revealed that genes belonging to the yellow family exist only in insects and in bacteria. This suggests that during the early stages of insect evolution a bacterial yellow gene was "captured" by an insect, and over a period of time found a new role in an entirely different context: that of a complex multicellular organism.

Such movement of genetic material across species boundaries is called horizontal transfer, and can occur when two species are closely associated with each other. In the case of insects the most likely scenario would be a gene transfer from symbiotic bacteria living within the insect's body that have been associated with their insect host for millions of years.

Thus, in evolutionary terms, the royal jelly gene family is relatively young, but has evolved from a very ancient bacterial gene via multiple duplications.

What is the molecular function of royal jelly proteins? It is widely accepted that a high jelly intake by a feeding larva is sensed by the fat body (the insect equivalent of a liver), which activates the insulin pathway. This, in turn, increases the level of a hormone controlling the metabolic genes. The activation of these genes increases the larval queen's metabolism, and subsequently leads to an increased demand for even more food.

Thus, the system operates via a feed-forward loop that stimulates larval growth at an astonishing rate. Interestingly, the same hormone that activates the metabolic genes has an inhibitory effect on the development of certain organs that would be useless for a queen, such as the royal jelly-producing glands and pollen-collecting baskets.

By inventing the royal jelly proteins, honey bees solved a difficult problem: how to provide the hungry larva, which cannot forage outside the little cell in which she develops, with essential but limiting nutrients.

Recent evidence suggests that some of these proteins function as deposits of biologically accessible nitrogen. Although nitrogen is an essential component of nucleic acids and proteins, it is often considered a limiting dietary component because animals cannot produce it. Instead they must obtain it from their diet. Consequently, high levels of nitrogen stored in royal jelly proteins may be critical for rapidly growing young larvae and for the well-being of fertile queens.

In addition, royal jelly may provide another limiting nutrient, sulfur. Royal jelly proteins have a high content of sulfur-containing amino acids.

Our data also suggest that royal jellies retain some of the ancestral roles associated with the yellow proteins, and may serve as developmental regulators or activators of biochemical pathways.

Although we know very little about the roles of these proteins in bacteria, the yellow protein family in insects mainly appears to be required for sex-specific reproductive maturation and development. Even the classic yellow, discovered in a fruit fly 100 years ago, is required for pigmentation in a sex-specific manner, and is important for male sexual behaviour. Other members of this family evolved diverse functions, acting either as enzymes in biosynthetic pathways or as molecules interacting with regulatory proteins during development. Not surprisingly, yellow genes in insects are under hormonal control.

The genes essential for complex social behaviours are largely a mystery. Royal jelly genes have thus far only been found in honey bees and closely related insects belonging to the same order. We hypothesise that these genes may have specifically evolved to modulate sociality via nutrition.

Since their mode of action is nutritionally based, an understanding of the genetic architecture behind this queen/worker determination can help answer fundamental questions related to reproduction, nutrition and growth rates, and ageing, and provide insight into the interaction of genes and the environment.

Because reproductive division of labour is one of the prerequisites for insect sociality, a rare but highly successful evolutionary transition, this work is of particular value to sociogenomics. It will generate novel data on how an entire genome responds to diet and environment, and will advance our understanding of the genetic basis of whole-organism phenotypes that in this case encompass the genetic regulation of an entire society.

Finally, because molecular processes are evolutionarily conserved, the findings of this project have the potential to be applicable to explaining diet-induced regulatory changes underlying human gene expression.

The honey bee genome is a crucial link to understanding the co-evolutionary interactions between genes and the environment.

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