Caterpillars pass down food preferences to offspring through blood

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By conducting smell tests with the caterpillars of the squinting bush brown butterfly, Bicyclus anynana, NUS scientists demonstrated that caterpillars' food preferences are determined by factors, or substances, present in their blood and these preferences could be passed on to offspring through the bloodstream. Credit: Associate Professor William Piel, Yale-NUS College

Many caterpillars are known for their specific food preferences, which they bring with them when they morph into butterflies. For instance, the

monarch butterfly only feeds on milkweed plants, while the Lime butterfly feeds on lime leaves. Despite deriving from a common ancestral species, these unique diet preferences are a point of interest for researchers at the National University of Singapore (NUS).

In an earlier study by researchers at the Department of Biological Sciences under the NUS Faculty of Science, they demonstrated that when caterpillars fed on leaves outside of their usual diet, they would prefer the smell of that type of plant after a few days. Remarkably, these caterpillars also passed on the acquired smell preference to their offspring.

Such a phenomenon is also seen in nature when caterpillars find themselves on a new food plant when the female butterfly lays eggs on the wrong plant by mistake. The new plant is edible but has a new smell, the caterpillars will learn to prefer this new smell and pass this preference on to their offspring.

This type of inheritance may facilitate host switching and ultimately the formation of new species, each with their own food preferences.

"Since new food preferences develop in the brain of caterpillars, it was unclear how such preferences were inherited by their offspring," explained Professor Antónia Monteiro, who led the research team.

Dr. V. Gowri, the Ph.D. graduate who is first author of the study said, "Offspring develop from the fusion of two cells (an egg and a sperm) produced in the gonads of each parent, which are located far from the brain. It was unclear how a smell preference was communicated to these cells."

To discover the factors that influence this adaptive behavior, the research team conducted experiments and showed that the blood of the

caterpillar, called hemolymph, which bathes both the brain and the reproductive organs, contains factors that promote the inheritance of the new smell preference.

The NUS team published their findings in the scientific journal *Biology Letters* on 15 May 2024.

The caterpillar's smell test

A caterpillar's blood can mediate the transport of factors from the brain to the gonads, impacting smell preferences in the next generation. Alternatively, it could transport these factors from the food to the brain of the embryo in the next generation, if included in the sperm or egg cells that create that embryo.

To test if the blood of caterpillars contained such factors, the newly hatched caterpillars were fed either the plants containing the new smell or a control plant. Some caterpillar's blood was then collected from their body once they matured. The collected blood was injected into caterpillars that did not consume either type of food.

From this experiment, the researchers observed that the caterpillars that received the blood from the control-fed caterpillars stuck to their usual menu. By contrast, those that received blood from caterpillars fed with food containing the new smell started to lean towards this change in diet. Most interestingly, so did their offspring, born many days later.

"This was very surprising to us, as this experiment shows that learning a preference towards a smell can occur without the need for the smell to enter the caterpillar's body via the antennae, as suggested in textbooks," said Prof Monteiro.

These experiments suggest a possible mechanism that could help

caterpillars switch their food preferences over the course of evolution. The researchers hope to further explore the mechanism of smell preference inheritance and isolate the specific factors being inherited from one generation to the next.

More information: V. Gowri et al, Haemolymph transfusions transfer heritable learned novel odour preferences to naive larvae of Bicyclus anynana butterflies, *Biology Letters* (2024). DOI: 10.1098/rsbl.2023.0595

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