© 2023. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2023) 226, jeb246008. doi:10.1242/jeb.246008

INSIDE JEB

Crucial DNA at crux of insect wing size evolution

Drosophila melanogaster feeding on banana. Photo credit: Sanjay Acharya, CC BY-SA 4.0, via Wikimedia Commons.

Closely related species are usually remarkably similar. But take a closer look, and subtle differences begin to emerge. Some may have longer limbs or wider bodies, and some fruit fly species have dinky wings while the wings of relatives are relatively large. 'The diversity of wing size relative to whole body size is remarkable in insects, but it is currently unclear how wing-to-body proportions have changed through evolution', says Marcos Nahmad from the Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico. The proteins that control the development of wings are essentially the same across almost all fruit fly species, regardless of their physical differences, so how do these differences come about?

Regions of DNA that control how the regulatory proteins are produced could tweak the way in which a limb develops. One member of the fruit fly family, *Drosophila virilis*, has petite wings compared with *D. melanogaster* and other species, which all have relatively large wings compared with their bodies. Knowing this, Nahmad and Timothy Evans (University of Arkansas, USA) wondered if a region of regulatory DNA found in the gene for a protein known as 'vestigial' – which controls wing growth – could be responsible for *D. virilis*'s smaller wings. If so, could *D. melanogaster* grow *D. virilis*-sized wings if Keity Farfán-Pira and Teresa Martinez-Cuevas (from Cinvestav-IPN) replaced the key section of *D. melanogaster* DNA with regulatory DNA from *D. virilis*?

First, Farfán-Pira confirmed that *D. virilis* fruit flies develop smaller wings for their body size, and then she began the painstaking task of transferring a 1200 bp segment of *D. virilis* DNA from the section of DNA preceding the fly's *vestigial* gene into the correct place in the DNA of *D. melanogaster* eggs. In addition, the team added a gene that would turn the modified flies' eyes fluorescent red, to be sure that the offspring produced by the *D. melanogaster* eggs carried the crucial *D. virilis* DNA.

Eventually, after months of patience in the laboratory creating the genetically modified *D. melanogaster* eggs and then waiting for them to develop into flies, Farfán-Pira measured the wing and leg lengths of the insects and the team was astonished that the wings of the *D. melanogaster* carrying *D. virilis* DNA were far smaller for their body size than those of regular *D. melanogaster*. 'We were shocked', Nahmad admits. Inserting the *D. virilis vestigial* regulatory DNA sequence into the *D. melanogaster vestigial* gene was sufficient to make the flies' wings smaller, like the daintier wings of *D. virilis*.

But what does this discovery mean for how members of the insect family, ranging from flamboyant butterflies to stubby-winged bees, evolved such extensive wing differences given that they all use the same cellular machinery to coordinate wing development? The team suspects that the crucial section of *vestigial* gene DNA fine-tunes the distribution of the cell signalling proteins that regulate wing growth through the larvae's imaginal disk structure – which develops into the wings – to regulate wing size and allow the insect to develop either small or large wings, depending on their family history. The team concludes that some significant change in the key *vestigial* DNA segment occurred after the split between the *D. melanogaster* and *D. virilis* families, resulting in one branch of the family with relatively large wings while the other evolved their comparatively small wings, which persist to this day.

10.1242/jeb.246008

Farfán-Pira, K. J., Martínez-Cuevas, T. I., Evans, T. A. and Nahmad, M. (2023). A *cis*-regulatory sequence of the selector gene *vestigial* drives the evolution of wing scaling in *Drosophila* species. *J. Exp. Biol.* **226**, jeb244692. doi:10.1242/jeb.244692

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