Where Flowers Feed Flight

Imagine that you drink 70 liters of Coke every day, for breakfast, lunch, and dinner. It is very likely you would develop type 2 diabetes in no time. Nevertheless, there are birds that have adapted to survive and thrive on a very similar diet. These are hummingbirds, the smallest birds on the planet, with the body weight of the smallest barely exceeding 2 grams. Some of them can consume up to three times their body mass of flower nectar per day, which in human equivalent would be around 200 liters of sugar water. Now, why hummingbirds need to drink so much flower nectar is no mystery. They are the only birds that have evolved true hovering flight, allowing them to move in any direction. To do so, their wings need to flap at a rate of 80 times per second, requiring an immense amount of energy extracted from just-consumed nectar [1,2].

However, while *why* hummingbirds drink so much flower nectar is no mystery, *how* they convert it to energy literally on the fly and avoid any health consequences from their high sugar consumption is. In my PhD, I tried to solve these mysteries by studying the genomes of hummingbirds and comparing them with the genomes of other birds.

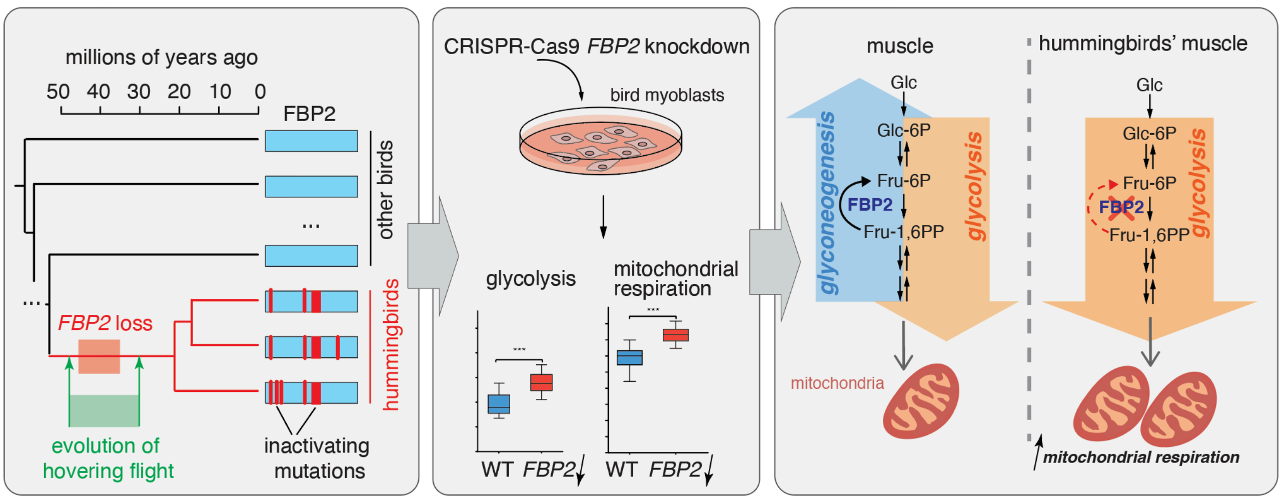
Millions of years ago, hummingbirds split from their closest insectivorous relatives, swifts, made their way from Europe to South America, and underwent a speciation burst. Now, there are over 300 species of hummingbirds [3], making them one of the most species-rich bird families. Sampling a few genomes from this vast diversity would likely reveal significant genomic variation as different species adapted to different niches. However, we wanted to identify changes that made all hummingbirds hummingbirds, the ancestral changes. In order to do so, we needed to sequence a new genome from a hummingbird belonging to a group that branched out from other hummingbirds very early: the long-tailed hermit.

While systematically searching for genes that were lost in the hummingbird ancestor, I discovered that all hummingbirds lost a gene, *FBP2*. This gene encodes fructose-1,6-bisphosphatase 2 (FBPase-2), a key enzyme in muscle glyconeogenesis, which opposes glycolysis and in serves as a pathway to build glycogen from non-glucose sources. The gene was lost due to the accumulation of inactivating mutations, like premature stop codons and frameshifts, that prevented the production of the functional protein. Using a molecular dating approach, I also found that *FBP2* was lost approximately 46-34 million years ago. Hummingbird fossils are scarce, but the two key available fossils suggest that hovering flight co-evolved with nectar-feeding and happened between 48 and 30 million years ago [4,5]. Intriguingly, this indicates hummingbirds lost *FBP2* around the time when they evolved to hover and feed on nectar, raising the question whether there is a connection between *FBP2* loss and evolution of these traits.

This seemed like a pretty compelling coincidence, but at this point, it was just that: a coincidence, a “just-so” story. In evolution, gene loss rarely leads to trait gain. More often, gene loss is part of a “use it or lose it” scenario, like subterranean mammals and cavefish losing eye-related genes [6,7] or aquatic mammals losing the gene to grow hair [8]. But, occasionally, gene loss follows a “less is more” scenario, like the loss of the *DARC* gene in some human populations giving those humans a gain in malaria resistance [9]. Could hummingbirds’ loss of *FBP2* be one of those rare “less is more” scenarios?

In order to find out, I needed to move beyond comparative genomics into the wet lab. Using CRISPR-Cas9, I knocked down *FBP2* in a bird muscle cell line. This gave me a simplified model, a simulator of the evolutionary process of the gene loss in bird cells. I found that these cells processed glucose faster than wildtype cells. This was because these modified cells had *accelerated* glycolysis. As it turns out, removing the inhibition antagonizing glycolysis actually served to shift the balance towards increased glucose breakdown. It is like releasing the brakes in a car and flooring the gas pedal.

Surprisingly, these cells also had increased respiration capacity. At first, we were puzzled by this, as there wasn't an obvious connection between glyconeogenesis and mitochondria. A recent study helped to shed light on our discovery, as it showed that *FBP2* has also a non-catalytic function and suppresses mitochondrial biogenesis via transcription regulation [10]. Therefore, when *FBP2* is downregulated, the number of mitochondria in cells increases. Given that glycolysis and mitochondrial respiration are the two main energy-generating pathways in the presence of oxygen, the loss of *FBP2* could therefore make sugar processing and energy turnover more efficient. Thus, this gene loss would have been a key change contributing to the evolution of the unique adaptations of hummingbirds.



The loss of the FBP2 gene in the ancestor of all hummingbirds coincided with the evolution of hovering flight and nectar-feeding. The knockdown of FBP2 in a model system showed enhanced glycolytic capacity and mitochondrial respiration, suggesting that the loss of the gene could be a key genomic change contributing to the evolution of hummingbirds' extreme metabolic traits.

Interestingly, cancer cells seem to use a very similar strategy to ensure they get enough energy for rapid growth: it was shown that FBPases are downregulated in tumors, and restoring their expression suppresses tumor growth [11,12]. Other studies show that inhibition of FBPases is effective for lowering blood glucose levels [13,14]. This makes *FBP2* a promising target for treating human conditions like cancer and type 2 diabetes. So, while we may never be able to eat as much sugar as hummingbirds, it's possible that their genomes might still hold a key to improving human health. At the very least, it provides excellent, albeit sweet, food for thought.

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