THE REASON WE DRINK ALCOHOL IS ROOTED IN OUR EVOLUTION

El porqué de que bebamos alcohol tiene sus raíces en nuestra evolución

*Matthew A Carrigan**

Gracias a la resurrección de varias enzimas de nuestros antepasados primates se han identificado varias mutaciones que ocurrieron hace, aproximadamente, 10 millones de años, las cuales le confirieron a nuestros antepasados una capacidad mucho mayor para metabolizar etanol. Este episodio de evolución enzimática coincidió con un cambio climático global asociado con la reducción de los bosques africanos y la transición de nuestros antepasados de un estilo de vida arbórea a un estilo de vida terrestre en el cual la fruta altamente fermentada era más común. Estos estudios sugieren que la evolución de las enzimas de nuestros antepasados pueden haberles permitido explotar una fuente alternativa de alimento cuando la comida era escasa.

Palabras clave: etanol, alcohol deshidrogenasa, ADH2, ADH4

We have resurrected ancient enzymes from our primate ancestors and identified several mutations occurring approximately 10 million years ago that endowed our ancestors with an enhanced capacity to metabolize ethanol. This episode of enzyme evolution coincided with a global climate change associated with shrinking African forests and our ancestor's transition from an arboreal lifestyle to a terrestrial lifestyle where highly fermented fruit was more common. These studies suggest that the evolution of our ancestor's enzymes may have enabled our ancestors to exploit an alternative source of nourishment during a time when food was scarce.

Keywords clave: ethanol, Alcohol dehydrogenase, ADH2, ADH4

There is a growing appreciation in medicine that many modern human diseases, from back pain to diabetes and hypertension, are rooted in our evolutionary history. More specifically, these "lifestyle" diseases are attributed to an incompatibility between our modern environment and the more ancient environment for which our genome is adapted. Could this idea/approach serve us to understand alcoholism in humans?

Ethanol - or alcohol, in common parlance - is widely consumed across cultures and throughout history, even though its toxic potential is widely known. Why would the consumption of a potentially toxic substance be so universal? Understanding this paradox may give insight into why some who consume ethanol become addicted, while others can consume healthy amounts throughout their life.

Some researchers have hypothesized that the modern human tendency to consume ethanol arises from our distant evolutionary history. In much the same way our bodies are wired to encourage consumption of sugar, salt and fat – a tendency that leads to over-consumption in today's environment – perhaps our genes adapted millions of years ago to encourage ethanol consumption when this may have provided an important dietary benefit, presumably because ethanol was present in fermenting fruit. Others have argued that ethanol in naturally fermenting fruit was insignificant[1] y and that ethanol became abundant only ~10,000 years ago when humans learned how to intentionally manipulate fermentation [2]. This recent source of ethanol would have been unique to modern humans and is less likely to have shaped

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2. McGovern, P. E.; Zhang, J.; Tang, J.; Zhang, Z.; Hall, G. R.; Moreau, R. A.; Nuñez, A.; Butrym, E. D.; Richards, M. P.; Wang, C.-s.; Cheng, G.; Zhao, Z.; Wang, C., Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (51), 17593-17598.

our genome to promote ethanol consumption[3].

Determining *when* humans began consuming ethanol would help us understand *why* humans consume ethanol…but how does one go back in time to determine this? Until recently, the matter was limited to speculation, but the new field of paleogenetics provides an opportunity to glimpse into the past and answer historical questions such as these*. That is what a group of researchers from Santa Fe College, the Foundation for Applied Molecular Evolution and the Indiana University School of Medicine (USA) decided to try.

To investigate the history of adaptation to ethanol, we first sequenced the genes involved in metabolizing ethanol from a wide variety of modern primates, from chimpanzees and gorillas to monkeys and lemurs. We then used these DNA sequences to predict the genes in our distant ancestors throughout the past 60 million years of our evolution (**Figure 1**). Finally, we re-created in the laboratory these prehistoric enzymes and tested how effectively they could metabolize ethanol.

Our first study examined a protein called ADH4 (**al**cohol **deh**ydrogenase **4**). This enzyme is produced in the mouth and stomach and is therefore one of the first enzymes in our bodies with an opportunity to metabolize ethanol.

The results were clear – the ancestor of humans and our close relatives, the chimpanzee and gorilla, acquired a mutation ~10 million years ago (Mya) that enabled them to metabolize ethanol much more efficiently than previous ancestors[4]. Prior to this mutation, our ancestors' ADH4 enzyme could effectively metabolize toxic alcohols, such as citronellol and geraniol (Figure 2) commonly found in plant leaves, but not the alcohol (ethanol) in fermented fruits.

3. Milton, K., Ferment in the Family Tree: Does a Frugivorous Dietary Heritage Influence Contemporary Patterns of Human Ethanol Use? *Integrative and Comparative Biology* **2004**, *44* (4), 304-314.

4. Carrigan, M. A.; Uryasev, O.; Frye, C. B.; Eckman, B. L.; Myers, C. R.; Hurley, T. D.; Benner, S. A., Hominids adapted to metabolize ethanol long before human-directed fermentation. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (2), 458-463.

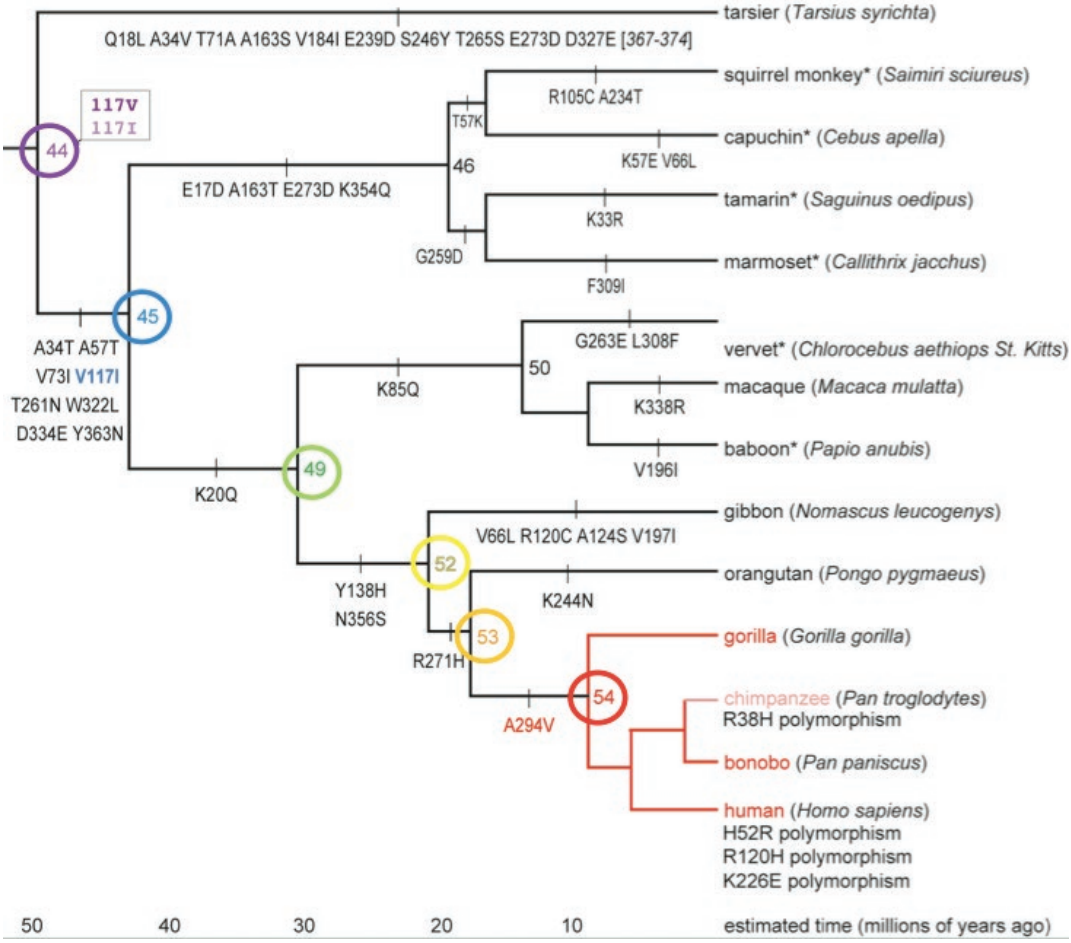

Figura 1. Phylogenetic tree shows the reconstructed the evolutionary history of the alcohol dehydrogenase 4 (ADH Class IV, ADH4) enzymes. Amino acid changes throughout the evolution of ADH4 proteins are shown along branches of the tree. Ancestral ADH4 proteins that were recreated in the lab are indicated by numbered nodes within the tree. Ambiguities in the ancestral sequences are indicated within grey boxes. Branches of the tree in red indicate enzymes active against ethanol. The branch leading to chimpanzee is pink to indicate that the R38H polymorphism is frequent among Pan troglodytes (based on the limited sequences in current genomic databases), and the impact of this polymorphism is not known.

** It is highly recommended that the reader consults the following paper to gain more insight into paleogenetics Laos, R. y Benner, S., "Linking chemistry and biology: protein sequences", Rev. Quim. PUCP, **2016**, 30(1-2). 23-28.* (🖳)

The timing of the ADH4 mutation coincides with a major climate change called the Mid-Miocene Climatic Transition (MMCT) during which the Earth cooled and dried. This climate shift caused African forests to shrink, a change that would likely increase pressure for forest animals to adapt to the changing environment. The fossil record also indicates our distant ancestors were shifting from a primarily arboreal (tree dwelling) life to an existence increasingly adapted to living on the ground. This is significant because more fermented, hence more alcoholic fruit, is more common on the ground. The increased activity of ADH4 in our ancestors during this time suggests that ethanol consumption may have helped our ancestors cope with a dwindling food supply by exploiting an otherwise toxic food.

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While people today may seek the intoxication that results when ethanol consumption is faster than its metabolism, intoxication would likely be detrimental to an animal climbing in trees high above the ground and surrounded by predators. But in this context, an animal with a highly efficient ADH4 could gain the caloric value associated with the fermented fruit without the life-threatening intoxication.

Alcohol metabolism involves many enzymes, and we suspected that if our ancestors had in fact adapted to consume ethanol, as indicated by ADH4, other enzymes that contribute to ethanol metabolism would also show a shift towards greater efficiency for metabolizing ethanol. We therefore reconstructed the evolutionary history of a second enzyme, ADH2. This enzyme is abundant in the liver, and like ADH4, metabolizes many types of alcohol. These more recent studies shows that an ancient form of ADH2 (dating to ~30 Mya) had a low level of activity towards ethanol (Kcat = 3.8 per min†), but by ~20 Mya, the enzyme in our ancestors had evolved to one with much higher activity towards ethanol (19.7 per min). Interestingly, as ADH2 continued to evolve, its activity decreased to an intermediate level (7.1 per min) at around the time ADH4 was increasing in activity (~10 Mya).

If our ancestors were adapting to exploit ethanol between 10 and 20 Mya, why would the new form of ADH2 that arose 10 Mya with an intermediate activity become common, and eventually replace the faster form that was present in our ancestors 20 Mya? One possibility is that by 10 Mya, ethanol was no longer a significant part of these ancestors' diet. The fact that ADH4 evolved to have an increased activity at nearly the same time argues against this. Alternatively, it is possible that an attenuated (moderate, but not too high) rate of ethanol metabolism is optimal.

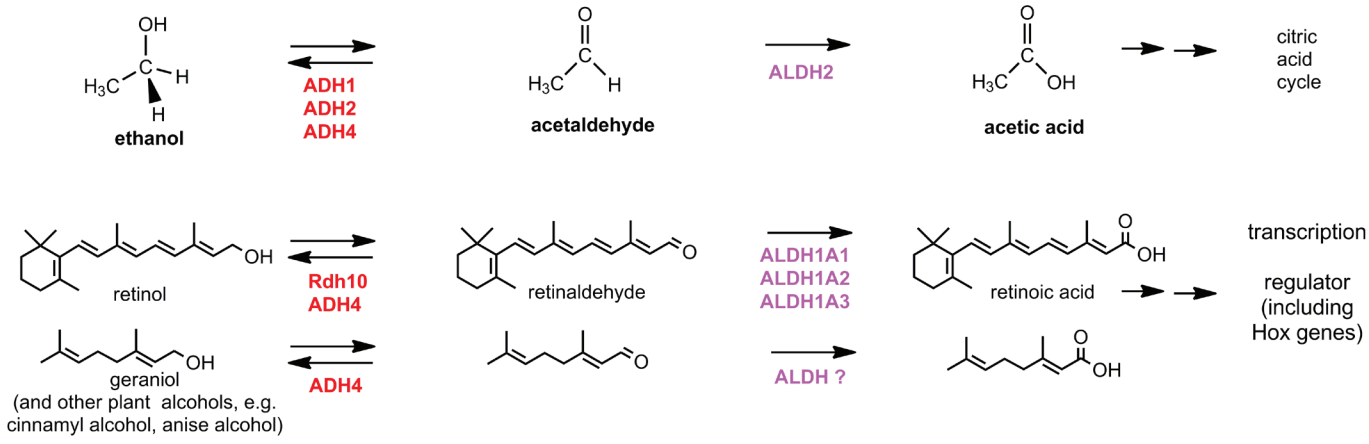

Figura 2. Pathway for the metabolism of some alcohols

†k_{cat} *is a way to indicate the number of substrate molecules per molecule of enzyme per minute, or simply "per minute", as it is called in the rest of this article.*

But if intoxication is dangerous, why would moderate ethanol metabolism be superior to extremely rapid ethanol metabolism? To understand this, one must consider the entire pathway for ethanol metabolism (Figure 2). As ethanol is metabolized by ADH2 and ADH4, the ethanol is converted into acetaldehyde. Acetaldehyde is significantly more toxic than ethanol, and it is the primary agent responsible for ethanol-induced liver damage. Normally the acetaldehyde produced from ethanol is metabolized (or removed) rapidly by another enzyme called aldehyde dehydrogenase (ALDH), limiting the damage and dysphoria that results if acetaldehyde is created faster than it is removed. An "optimally" designed ethanol metabolism would pair increased ethanol metabolism with increased acetaldehyde metabolism, but because evolution relies on random mutations to generate new properties, the evolutionary process does not necessarily arrive at the optimal solution. Further, enzymes often have more than one function, and optimization for one function is often limited by a need to remain functional for other roles. We are currently investigating these possibilities by reconstructing the evolution of the ALDH enzymes that detoxify acetaldehyde.

Taken together, the results of ADH4 and ADH2 suggest that fermented fruit was an important part of our ancestors' diet long before modern human civilization. While this evolutionary analysis suggests our ancestors adapted to ethanol in fruit, it does not mean our genomes are adapted to the much higher levels of ethanol found in modern alcoholic beverages…so much like with sugars, salt and fat, we are at risk of over-consuming something that was once scarce but important.

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The spanish version of this paper is available online here

ESSENTIAL LITERATURE

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