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An Evolutionary Review on the Substrate Specificity of the Gustatory Receptors

Noor Srivastava[1], Arianna Broad[2]

[1]Vasant Valley School, New Delhi, Delhi, India

[2]Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA

Abstract: ***Gustatory receptors are vital for discerning tastes, such as sweet, salty, bitter, sour, and umami, playing a fundamental role in determining the nutritional value or potential harm of substances. In humans, taste receptor cells (TRCs) are predominantly located on the tongue and upper digestive tract, while in adult fruit flies (Drosophila), taste sensors are distributed across appendages like the proboscis, legs, wings, and ovipositor, with varying receptor types. For humans, T1R2+3 receptors respond to various sugars due to their importance as a calorie source. In contrast, fruit flies employ multiple receptors like Gr61a, Gr64a, Gr64a-f, and Gr43a to detect sugars, necessitating specific receptor combinations for substrate specificity. Bitter taste receptors (T2R) in humans not only identify bitter compounds but also trigger protective mechanisms. In fruit flies, receptors like Gr66a and Gr93a work collectively to detect bitter compounds. Fruit flies rely on gustatory receptors on their appendages to sample potential food, compensating for their limited visual discrimination abilities, necessitating numerous receptors for effective communication with their brains. Conversely, humans rely on advanced sensory cues and higher intelligence to assess food safety, requiring fewer receptors. This underlines the contrast in gustatory receptor strategies between these species. Understanding the evolutionary relationships and implications of gustatory receptors is crucial. Analyzing the divergent and convergent evolution of these receptors provides insights into the adaptive mechanisms that have shaped the complex interplay between taste perception and survival strategies across different species. By studying these relationships, we can unravel the underlying genetic, functional, and ecological factors that have influenced the development of gustatory receptors over time. Such research is imperative for comprehending the fundamental principles of sensory evolution and its broader implications for ecological adaptation and species survival.***

Keywords: Gustatory receptors, taste receptors, humans, fruit flies, evolution

1. Introduction

Gustatory receptors are proteins that are abundant in sensory organs that are the catalysis of initiation for the signaling cascade that occurs to the neurons, which transmit to the brain that perceives and distinguishes between different tastes of food an organism consumes. Gustatory sensory organs are composed of gustatory receptors or taste receptor cells (TRCs) that contain proteins on their surfaces that detect the presence of specific compounds consumed by the taster.[1] These chemical compounds are known as substrates. The five primary tastes most organisms can detect are sweet, salty, bitter, sour and umami. Sweet and umami are registered as “good” tastes that signify nutritionally caloric-rich foods (those that contain macromolecules that can be utilized in energy harnessing and protein synthesis).[1] Bitter and sour are typically considered “bad” tastes and are recognized as harmful substances, alerting the organisms of the presence of toxins or extreme pH; for example, bleach has a high pH and is bitter while coffee has a low pH and is also bitter – both contain compounds (ammonia or caffeine) that are toxic at different concentrations dependent on the organism.[1] Salt can be a “good” or a “bad” taste depending on the concentration of sodium and the physiological needs of the organism.[1] This is likely due to different concentrations of salt, high concentrations taste “bad” as the body doesn't want to overload the cells with salt for risk of them shrinking and dying due to osmosis. Hence, lower concentrations of salt taste “good”. Apart from taste, texture also plays a role in determining if something is safe to consume. Sandy or sharp sensations signal that the food may damage the intestine and is not safe to consume while a creamy consistency signals physical safety as well as the presence of desirable fats.[2] The gustatory system is the most important regarding the regulation and driving of the feeding process. Receptors detect nutritionally beneficial or harmful compounds and then trigger responses that accept or reject the substance. This is likely due to the prior knowledge stored in our brains. The brain knows what nutrients the body needs, so it communicates with the gustatory receptors to “accept” the substrates the body requires nutritionally while demonstrating a rejecting behavior (such as not eating it) to those substrates if the body does not need this substrate or if the substrate is harmful to the organism. Different gustatory sensory organs comprise different gustatory receptor proteins, which detect specific substrates. This phenomenon of gustatory proteins recognizing and signaling the proximal neurons of the presence of this specific compound is known as substrate specificity.

As evolution progresses, organisms adapt to the changing environment. Hence, vast differences are expected in the anatomy, functioning and substrate specificity of human beings and lower life forms. One such example of the evolution of characteristics over time is the niche construction of lactase persistence. Niche construction is a process by which organisms modify their own and others' evolutionary niches.[18] The lactase enzyme is responsible for the digestion of lactose and its production decreases after the weaning phase in most mammals. Some humans, however, continue to produce lactase throughout adulthood, this is known as lactase persistence. The mutation (−13910*T) explains the distribution of the phenotype. Lactase persistence occurs due to the cultural practice of consuming dairy products.[19] This process showcases how

genes mutate/diverge due to environmental pressure such as the consumption of lactose by humans once dairy became the primary food.[19]

This review will focus on examining the substrate specificity of gustatory receptors between lower- and higher-level organisms. The lower-level organism examined was the common fruit fly (*Drosophila melanogaster*) whereas the higher-level organism examined was humans (*Homo sapiens*) I will compare, contrast, and hypothesize why there is such a wide variation between substrate specificity of gustatory receptors between lower and higher-level organisms.

Anatomy of Gustatory Receptor Sensory Organs

In humans, TRCs are located in gustatory papillae. Most papillae are of three types- fungiform, foliate and vallate- and are located on the tongue. A substantial number of papillae are also located in the palate, pharynx, larynx, epiglottis, and upper esophagus concluding that the tongue and upper digestive tract are the only places where sensory organs are found in humans. (Figure 1A).[4] This is in contrast to *Drosophila*, where there are many sensory organs located throughout the body of the fly to sense viable and nutritionally valuable substrates. The closest appendage to the mammalian tongue is the proboscis which extends from the head. It is composed of an external taste organ with two labella and internal gustatory structures that line the pharynx and are the final mechanism that helps the fly decide whether to expel or consume the food. Taste sensors are also found on the legs, anterior wing margins and even on the ovipositor.[3]

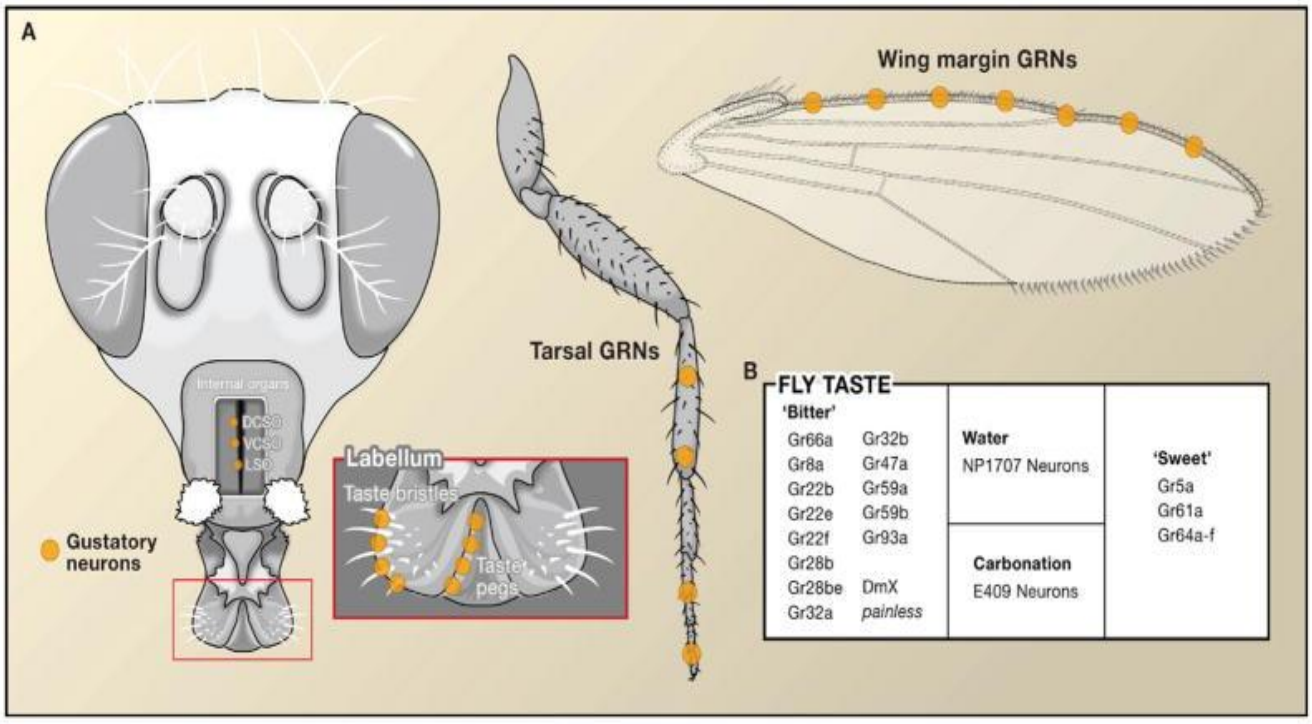

Figure 1: Location of Gustatory neurons in *Drosophila melanogaster* [1]

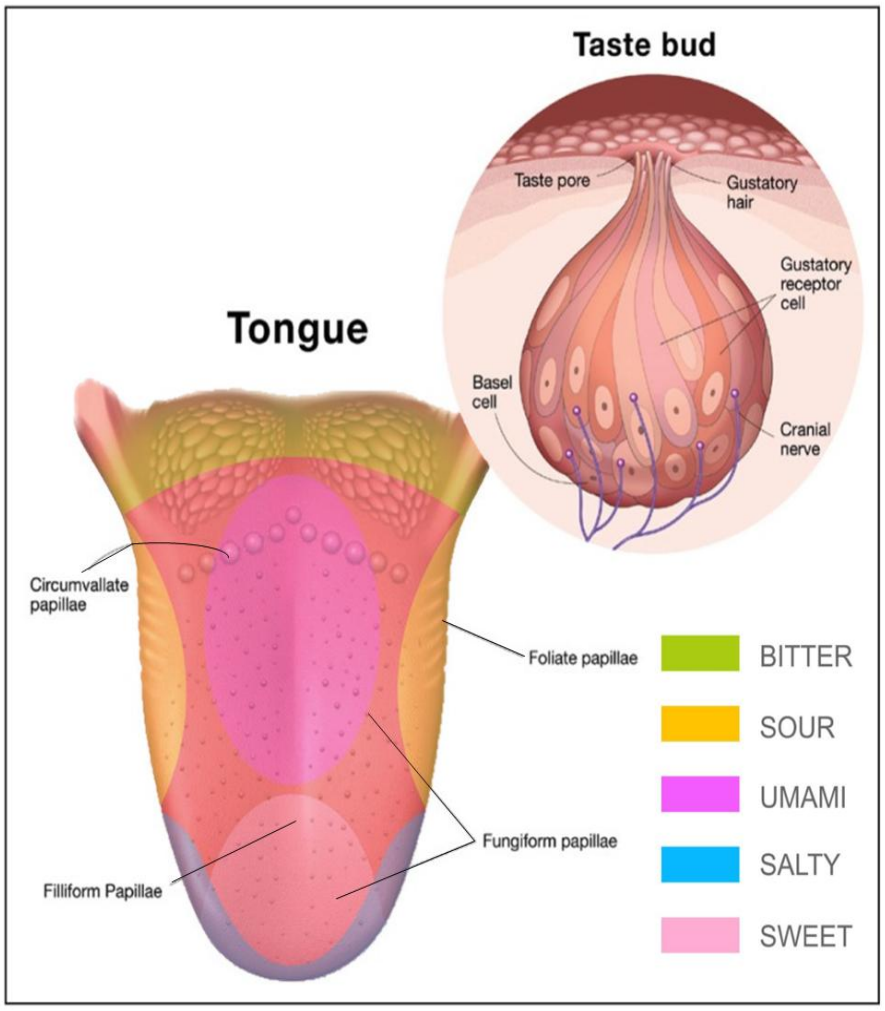

Figure 2: Anatomy of tongue and taste buds and location of different taste receptors in *Homo sapiens*[2]

Within the human TRCs, there are many divergent protein family which includes T1R1, T1R2 and T1R3 (taste receptor, type 1, members 1, 2, and 3, respectively, with gene symbols TAS1R1, TAS1R2, and TAS1R3).[4] Whereas, in *Drosophila*, only the gustatory receptor (Gr) gene family mediates most taste qualities. However, there are many more Gr proteins in flies than in humans. Furthermore, the gustatory sensation is unique to lower-level organisms than humans because one receptor in humans can detect multiple substrates, whereas in flies' gustatory sensation is dependent on a specific combination of gustatory receptors coming together in a complex to sense a single substrate. Different combinations of gustatory receptor proteins sense different and discrete substrates.[12] Lastly, most fly Gr proteins are receptors for sweet, bitter and pheromone signals, whereas typically humans do not utilize TRCs for hormone reception yet use hormone receptors which are a different class of proteins.[12]

"Sweet" Gustatory Receptor Substrate Specificity

The T1R2+3 mammalian sweet taste receptors respond to the sugars sucrose, fructose, galactose, glucose, lactose, and maltose. This is because sugars are a primary source of calories and are used for energy production.[5][6] Sucrose is essential for memory, reaction time, attention, ability to solve mathematical problems and reduction of fatigue.[7] Fructose also plays a role in insulin secretion.[8] In *Drosophila*, Gr61a, Gr64a, Gr64a-f and Gr43a are the sweet receptors that respond to fructose, sucrose and glucose.

A study conducted to determine the organization and number of neurons expressing eight different Gr genes resulted in the Gr5a-expressing neuron being the most abundant and well-distributed.[12] Gr5a is required by the fly for behavioral and sensory response to a disaccharide known as trehalose. Trehalose is abundant in yeast and fungi which are present in fermented fruit, a food source vital for *Drosophila*. It can be used as an energy source for flight as well as an osmoregulator for hemolymph- a fluid found in invertebrates analogous to blood in vertebrates.[13]

Looking at the substrate specificity for different receptors of the two organisms we can say that flies seem to require certain combinations of receptors to obtain specificity while humans do not have this, and they have what can be called one catch-all receptor which means a single receptor can have the specificity for a larger group of substrates. For example, in *Drosophila* Gr64f functions in combination with Gr5a for trehalose detection and with Gr64a for sensing sucrose, maltose, and glucose. However, these pairings are not sufficient to bring about responses to the sugars therefore detection of a single sugar may require more than two receptors.[17] In humans, the T1R2+3 mammalian sweet taste receptors respond to a much larger variety of sugars (sucrose, fructose, galactose, glucose, lactose, and maltose)

without needing to be in complex with other gustatory receptors.[5]

"Bitter" Gustatory Receptor Substrate Specificity

In humans, the bitter T2R taste receptors when detecting bitter compounds, have the additional responsibility to trigger mechanisms that reject them. They regulate behavioral responses such as vomiting, coughing, and sneezing. All these protect the body from toxins and irritants associated with bitter tastes.[9]

Flies have significantly more bitter gustatory receptors than sweet receptors.[1] This is likely because bitter receptors detect toxins and other harmful substances whereas sweet receptors taste sugars. Since there is a larger number of toxins that exist compared to sugars, there is a higher number of bitter receptors present; however, for this study we will be focusing on the most abundant bitter receptors in *D. melanogaster*, which is not an exhaustive review of all of their bitter gustatory receptors. Following Gr5a, Gr66a expression in sensory neurons was found to be expressed in the largest number. The cells for this receptor respond to bitter compounds like caffeine, theophylline, valine, or threonine. It also senses N, N- Diethyl-meta-toluamide (DEET), the most used insect repellent worldwide, and L-canavanine, a plant-based insecticide.[21] Bitter substances are perceived as toxic and harmful and are therefore avoided by flies. For example, caffeine reduces and fragments sleep in *Drosophila* and lengthens the circadian period, which is a 24-hour cycle of alertness and sleepiness that responds to the changing of light in our environment.[14] Theophylline reduces their visual learning performance.[15] The Gr93a receptor is co-expressed with the Gr66a receptor and together they respond to caffeine.[17] Once again, we see the requirement for fly receptors to work together while humans have a single receptor.

"Umami" Gustatory Receptor Substrate Specificity in Humans

The umami taste receptor TAS1R1/TAS1R3 responds to all 20 amino acids that constitute the proteins in our body but has the highest affinity for glutamate.[2] Glutamate is a neurotransmitter that is crucial for the maintenance of ideal energy levels, necessary for most Central Nervous System (CNS) functions, and neuroplasticity, which is important for adaptation to changes in the environment.[10] Amino acids are the building blocks of proteins in our bodies, without which almost all life processes in our bodies would not be able to take place.[11] It is still unclear if there are gustatory receptors that are umami sensors in flies, this may just be a higher-level organism trait.

Other Gustatory Receptors

A receptor specific to males in flies is the Gr68a receptor which is required for a tapping step during courtship, it is likely to encode for a long-chain hydrocarbon.[12] The tapping step is crucial to the completion of the entire courtship sequence and mating. It is also required for detecting the CH503 male sex pheromone which is transferred from males to females and inhibits courtship by other males.[21]

On the other hand, humans use vomeronasal receptors to sense pheromones instead of gustatory receptors.(Pantages, E, and C Dulac.) Detection of pheromones through gustatory receptors is a feature unique to lower-level organisms emphasizing their importance in dictating behavioral responses in flies.

2. Discussion

Gustatory receptor organs are present on the wings and legs, and this enables the organism to sample potential food without consuming it. They can taste favorable or toxic chemicals before deciding whether or not to extend the proboscis and consume the food.[20] This feature is vital in *Drosophila* because their brains are not developed enough to distinguish between harmful and favorable substances visually. They solely rely on the gustatory senses for survival. For the same reason, flies require many different combinations of gustatory receptors to detect a single substrate – to effectively communicate the correct signals to the brain as they cannot rely on other senses to intake this information. However, humans possess a higher level of intelligence than flies, being able to look at an object and instantly know if it is safe to eat or not based on smell, texture, color, and more. Therefore, humans do not need as many receptors as flies to help discern different substances.

Upon concluding this literature review, the authors decided to construct a genetic phylogeny of the gustatory receptors we reviewed in this literature curation to aid in our understanding of the relationship between gustatory receptors within and across species. Contrary to initial expectations, TAS1R2 and TAS1R3, both serving as wide array sugar receptors, are not closely related genetically, suggesting that their similar function has evolved through convergent evolution. TAS1R3 and Gr64f are identified as orthologs, indicating a common ancestral gene shared between them. Meanwhile, TAS1R3, a wide array sugar receptor, and T2R3, associated with detecting bitter compounds, are divergent paralogs, reflecting their genetic similarity despite their differential functions, with TAS1R3 having a closer genetic affinity to Gr64f. Additionally, observations suggest that Gr64e likely arose from the duplication of Gr64d, suggesting a series of gene duplication events among Gr64c, Gr64d, and Gr64e. Gr64f, Gr64b, and Gr5a are identified as convergent paralogs, indicating similar functions that evolved independently. In contrast, Gr61a and Gr93a are divergent paralogs, showing shared genetic ancestry but significant functional divergence. Similarly, TAS1R1 and TAS1R2 are described as divergent paralogs, with a common genetic origin but different roles in taste perception. Likewise, Gr64a and Gr66a are noted as divergent paralogs. Gr43a and TAS1R2 are identified as orthologs, suggesting they originated from a common ancestral gene. Overall, these genetic relationships underscore the dynamic nature of taste receptor evolution, shedding light on the molecular basis of taste perception and the diverse adaptations of different species to their dietary and ecological niches.

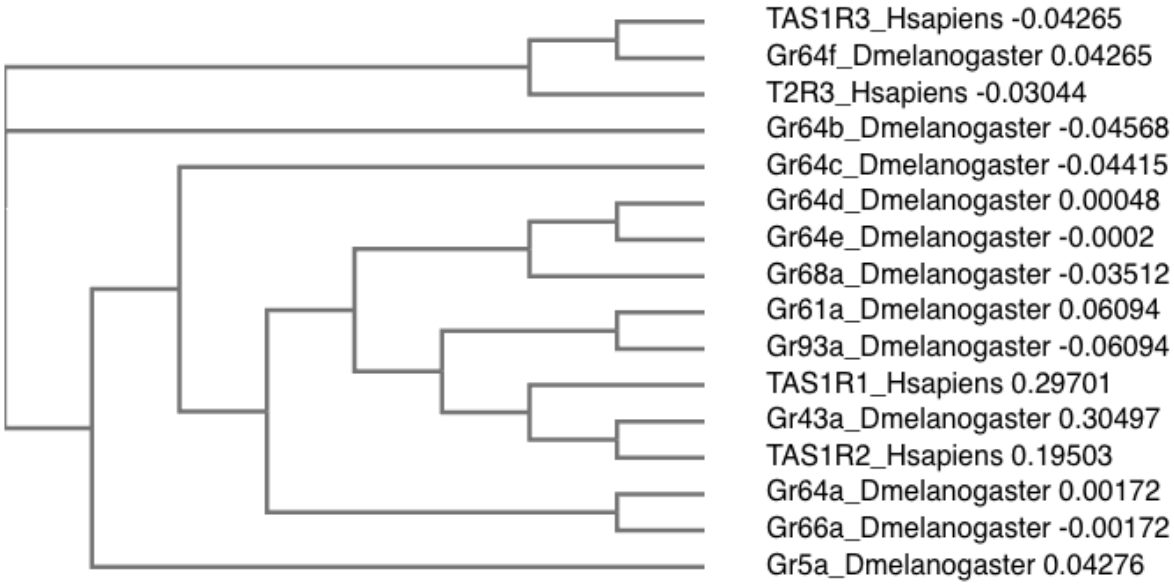

Figure 3: Phylogenetic analysis of gustatory receptors reviewed in the study

The study of gustatory receptors offers valuable insights into the evolutionary progression of these sensory systems across a wide range of organisms, underscoring their importance in an organism's survival throughout the course of evolution. However, as evolution has advanced, there has been an overall loss of substrate specificity in gustatory receptors. This trend has culminated in the case of *Homo sapiens*, where we possess fewer overall gustatory receptors, but these receptors have adapted to detect a wide array of chemically similar substrates. Interestingly, lower-level organisms such as *Drosophila melanogaster* exhibit a higher number of gustatory receptors, notably an abundance of bitter receptors compared to sweet receptors. This disparity may be attributed to the need for these organisms to avoid a multitude of poisons and toxins in their environment, outweighing the importance of detecting sugars for nourishment.

Our constructed phylogeny has yielded several key observations that shed light on the evolutionary relationships of these receptors and their implications for organism survival. Notably, TAS1R2 and TAS1R3, originally expected to be closely related due to their similar function as wide-array sugar receptors, are genetically distinct, suggesting convergent evolution. TAS1R3 and Gr64f emerge as orthologs, indicating shared ancestral origins despite their distinct roles. TAS1R3's genetic similarity to T2R3, a receptor for bitter compounds, points to divergent paralogy, raising questions about the underlying evolutionary mechanisms. The presence of gene duplication events, as evident in Gr64c, Gr64d, and Gr64e, further underscores the dynamic nature of gustatory receptor evolution. The convergent paralogy of Gr64f, Gr64b, and Gr5a, in contrast to the divergent paralogy of Gr61a, Gr93a, TAS1R1, and TAS1R2, highlights the interplay between genetic relationships and functional divergence in these receptors.

The evolution of gustatory receptors presents an excellent model for studying the loss of substrate specificity in receptors responding to external stimuli, known as exteroceptors. This loss of specificity is likely influenced by the acquisition of additional sensory traits such as advanced touch, sight, and hearing in evolving organisms. These sensory adaptations have enabled organisms to distinguish between harmful and beneficial substrates, reducing the environmental pressure to maintain broad substrate detection in gustatory receptors. Continued research on gustatory receptor evolution is imperative, as numerous questions remain unanswered, such as the combinatorial receptor approach used by flies for specificity. These inquiries can be addressed through a series of structural biology experiments aimed at deciphering the specific changes occurring within receptor complexes containing different proteins. In conclusion, the evolutionary journey of gustatory receptors offers a captivating window into the dynamic interplay of genetics, functionality, and sensory adaptation in the world of taste perception.

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