

Post -16 bitter taste perception workshop

Background information

Summary

This workshop forms part of a programme of educational projects to celebrate the bicentenary of Darwin's birth, and 150 years since the publication of his most famous work, *On the Origin of Species by Means of Natural Selection*. It uses molecular biology techniques to investigate the process by which humans and chimpanzees have both evolved to lose their ability to taste a bitter chemical. For decades scientists believed that some humans and chimpanzees share this trait as they had inherited it from common ancestors before the two species diverged in evolution. However, recent genetic evidence has shown that both humans and chimpanzees have undergone independent evolutionary processes that have resulted in this shared trait. This new evidence exemplifies Darwin's theory; selection pressures have acted on both species, and each has adapted accordingly. In this case, the adaptation (an inability to taste) has occurred via independent routes through a process known as convergent evolution.

The workshop will be delivered by trained scientific and exhibition staff to young people, aged 16-19, in three science discovery centres across the UK. The purpose of this document is to provide workshop deliverers with necessary background information, and has been written for a general scientific audience who may have little previous experience of genetics or evolution.

Overview

The taste test workshop is based on the recent findings of Stephen Wooding *et al*, published in Nature in 2006¹. Before this paper was published it was known that both humans and chimpanzees can be either 'tasters' or 'non-tasters' of a bitter tasting chemical, phenylthiocarbamide (PTC). More recently, the (in)ability of humans to taste PTC has been attributed to differences in the *TAS2R38* gene, which are now well understood². The *TAS2R38* gene encodes a taste receptor, which is located on the tongue and is responsible for bitter taste perception in both humans and chimpanzees.

As humans and chimpanzees have evolved from common ancestors, and they share the same traits (being unable or able to taste PTC), for decades scientists assumed

that they had inherited these characteristics from a common ancestor before the divergence of the two species. As a logical conclusion, this went untested until 2006 when Wooding *et al* set out to identify the differences in the DNA sequence of *TAS2R38* in chimpanzees that cause the (in)ability to taste PTC. The group found that the genetic differences resulting in an inability to taste PTC in the chimpanzee *TAS2R38* gene were in a different location to those resulting in an inability to taste PTC in the human *TAS2R38* gene. Therefore, this research shows that the previously held hypothesis was wrong; humans and chimpanzees did **not** share the inability to taste PTC when the two species diverged in evolution. Rather, the two species had diverged from a common ancestor and, a later time, independently evolved the inability to taste PTC through unrelated changes to the *TAS2R38* taste receptor gene. This is one of the first papers to provide genetic evidence for a form of convergent evolution; independent evolutionary events which converge towards a common function.

This workshop sets out to allow students to investigate their own *TAS2R38* gene and see if the results correlate with their ability to taste PTC. This practical work can then be extended, through bioinformatics, to understand evolutionary processes through analysis of the genetic similarities and differences between humans and our closest relative, the chimpanzee.

What causes differences in bitter taste perception?

The *TAS2R38* gene encodes a taste receptor, which is found on the tongue. Differences in this gene affect whether people can taste a chemical called phenylthiocarbamide (PTC). These differences in genetic make-up can be described as genotypes. The ability to taste or not taste PTC is described as a phenotype, an observable effect of the genotype. To people who can taste it, PTC is very bitter.

These genetic differences can be described as single nucleotide polymorphisms (SNPs) – that is – only one nucleotide, or letter, within the DNA sequence of the *TAS2R38* gene is changed. SNPs can be utilised to genotype individuals using a variety of methods involving PCR, a technique which allows DNA to be copied millions of times so that it can be visualised in the laboratory. It is important to remember that each individual has two copies of every gene, one inherited maternally and one paternally. Therefore at each nucleotide position, people can be homozygous, i.e. have two copies of one letter, or heterozygous, have one copy of

each letter. If we consider the single nucleotide polymorphism at nucleotide 145 in the human *TAS2R38* gene:

CC – homozygous ‘taster’

CG – heterozygous ‘taster’

GG – homozygous ‘non-taster’

Heterozygotes can taste PTC, but with less sensitivity than homozygous ‘tasters’.

The *TAS2R38* gene accounts for up to 85% of variation in the ability to taste PTC, and is therefore a good predictor of an individual’s (in)ability to taste it. However, ability to taste PTC can be affected by other factors, such as other genes involved in taste, age and smoking habits, amongst others.

Another important factor contributing to an individual’s (in)ability to taste is the number of taste buds that are present on the tongue. Some taste buds are contained within fungiform papillae, the visible red dots found mainly on the tip of the tongue. Individuals who are very sensitive to a variety of tastes have a greater density of fungiform papillae than others. These individuals are often termed ‘supertasters’. It is important to stress that the number and density of fungiform papillae can affect an individual’s response to PTC. As the density of fungiform papillae increases, the ability to taste PTC increases for all ‘non-taster’ and ‘taster’ genotypes³. This effect would only be observable in the taste test; there is no genetic link between an individual’s *TAS2R38* genotype and their fungiform papillae density.

What is convergent evolution?

Convergent evolution describes the acquisition of the same biological trait in species that have evolved through divergent evolutionary pathways. In this example, the trait is the inability to taste the chemical PTC. This trait has arisen from small changes in the DNA sequence of the taste receptor gene, *TAS2R38*. As humans, chimpanzees and gorillas have evolved from common ancestors the DNA sequence of the gorilla’s *TAS2R38* gene is used as a known starting point before the evolutionary changes occurred. This information suggests that the common ancestor of human and chimpanzees was a “taster” of PTC at the point of evolutionary divergence, and that the two species have since evolved so that some individuals cannot taste PTC. As the changes to the gene are distinct between humans and chimpanzees, but they cause the same ‘non-taster’ function, the evolutionary pathway is convergent.

What is the evidence for convergent evolution between humans and chimps?

There are several SNPs that occur within the DNA sequence of *TAS2R38* that can cause differences in ability to taste PTC. The information below has been simplified to reflect DNA changes that correlate closely with tasting or non-tasting in the *TAS2R38* gene. For example, nearly all human 'non-tasters' have a guanine (G) at position 145 and nearly all human 'tasters' have a cytosine (C) at this position. The gorilla DNA sequence is identical to the most common 'taster' DNA sequence at these two positions, suggesting that some humans and chimpanzees have evolved from 'tasters' to 'non-tasters'.

Table 1: Substitution of a guanine (G) nucleotide at position 145 results in non-tasting in humans, but is unchanged in non-tasting chimpanzees.

Species	NUCLEOTIDE 145	
	C	
	TASTER Genotype	NON-TASTER Genotype
Chimpanzee	C	C
Human	C	G

The DNA change at position 145 is investigated with the Carolina Biological Supplies kit.

This SNP changes the DNA sequence so that it encodes a different amino acid. A cytosine (C) at nucleotide 145 encodes a proline, whereas a guanine (G) at this position encodes an alanine. This changes the taste receptor protein by reducing its affinity for the PTC chemical.

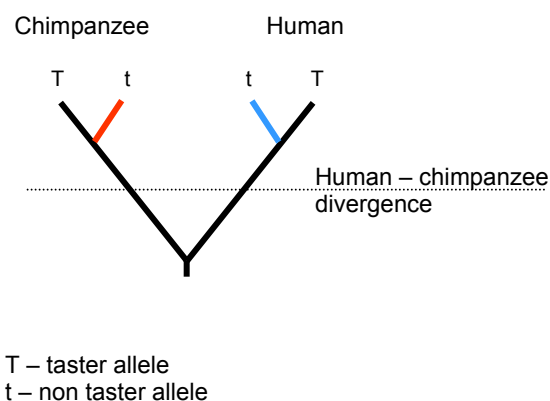
Table 2: Substitution of a guanine (G) nucleotide at position 2 results in non-tasting in chimpanzees, but is unchanged in non-tasting humans.

Species	NUCLEOTIDE 2	
	T	
	TASTER Genotype	NON-TASTER Genotype
Chimpanzee	T	G
Human	T	T

This SNP is within the codon at which protein synthesis is initiated in the *TAS2R38* gene. Therefore, chimpanzees with this mutation do not make a full length functional *TAS2R38* protein.

Observational work has shown that humans and chimpanzees can be either 'tasters' or 'non-tasters' of a bitter tasting chemical, PTC. Specific changes to the DNA sequence of *TAS2R38* result in an inability to taste PTC in both humans and chimpanzees. From the information above, it is evident that chimpanzees and humans differ in the SNPs that they have, which result in their (in)ability to taste PTC. Therefore, humans and chimpanzees have adapted to become 'non-tasters' via independent evolutionary processes, but share the same phenotype. This is an example of convergent evolution.

Figure 1: A diagram to show the mechanism of convergent (or independent) evolution for the PTC tasting phenotype (adapted from Wooding *et al*, 2006)



Why might bitter taste perception be evolutionarily important?

These findings have led to a discussion about why some humans and chimps have evolved not to taste PTC. It is unclear what selection pressure has led to, and maintained, this evolutionary change. Some scientists have proposed that differences in ability to taste may be advantageous in different geographical locations. *Brassica* vegetables such as broccoli and brussel sprouts contain isothiocyanates which act as a ligand for the *TAS2R38* receptor. These vegetables have been shown to have potent anti-cancer effects, and are therefore beneficial to eat. However, in geographical regions of low iodine, over-ingestion of isothiocyanates has been associated with thyroid disease and goitre. Therefore, differences in the

TAS2R38 gene may be beneficial in preventing, or allowing the ingestion of isothiocyanates without experiencing an unpleasant taste ^{reviewed in 4}.

An assumption to date has been that changes to the *TAS2R38* gene that result in an inability to taste PTC mean that the taste receptor is non-functional or broken. However, it may be that the receptor has changed to become responsive to a different ligand. Two studies have shown that fruits of the plant *Antidesma bunius* taste bitter to PTC 'non-tasters', but sweet to PTC 'tasters', which might suggest that this plant contains a chemical which may act upon the *TAS2R38* receptor. If the *TAS2R38* gene which results in an inability to taste PTC, also results in an *ability* to taste a different chemical, this suggests a mechanism by which both alleles are advantageous. This is known as heterozygote advantage.

Both examples above describe advantages to maintaining both 'taster' and 'non-taster' SNPs within the *TAS2R38* gene of humans and chimpanzees. These advantages prevent one trait being 'selected out' over generations by means of natural selection and rather, the traits are maintained, or steady, within the populations. This process is called balancing selection. Heterozygote advantage is a mechanism through which balancing selection occurs.

How does this build upon Darwin's work?

Darwin proposed that all species have evolved from common ancestors through a process termed natural selection. In his most famous work *On the Origin of Species by Means of Natural Selection* the only diagram is a tree of life, which shows the relationships between species that have evolved, in response to selection pressures, from common descent. This workshop builds on the principle that humans and chimpanzees have evolved from common ancestors, and investigates the process by which this evolution has occurred.

Darwin's work has been supported by fossil evidence and comparative studies in anatomy, physiology and biochemistry. Genetic information provides further supporting evidence for his theories. As DNA sequencing techniques and computing capability continue to advance, the scientific community has access to an increasing body of DNA sequence data with which to study evolution. Projects are underway to collect genetic information from all of the world's species which can then be compared using internet-based bioinformatics software. Experiments like these have

shown humans and chimpanzees to be equally evolutionarily divergent from the gorilla, with a DNA sequence similarity of over 95%.

A practical bridging evolutionary research and molecular biological techniques

The work of Wooding and others provides an opportunity to extend the scope of PCR-based workshops that are traditionally offered to post-16 students in centres throughout the UK. The practical reflects current scientific research, and therefore students' results could be used, through a mass participation experiment, to generate further data for research groups working in this area. Nowgen has corresponded with Stephen Wooding who is supportive of the project.

The practical component of the workshop allows students to perform DNA extraction, PCR and gel electrophoresis and is therefore attractive to teachers in that it enables their students to cover these aspects of the curriculum. The practical will also focus on the analysis of results from a restriction digestion, which is an objective in most A level biology curricula, and currently omitted from the majority of existing PCR-based workshops aimed at this audience. A further marked difference is that students will identify differences using their own DNA which we anticipate they will find very exciting. The protocol has been considered by a leading UK geneticist who has stated that it is highly unlikely to reveal any health-related genetic information to the students, nor does it raise any other ethical issues.

Finally, and perhaps most importantly, the practical workshop demonstrates how a molecular technique is applied to a major scientific question. Students will learn the theory of techniques, and apply them practically to investigate their own DNA, and to compare differences to their classmates, other participating students, and their evolutionary ancestors. This work brings together the history of evolution, and the cutting edge of genetics research. Surely there is no better way to bring evolution to life than to look inside your cells and understand the evolutionary history of part of your own DNA?

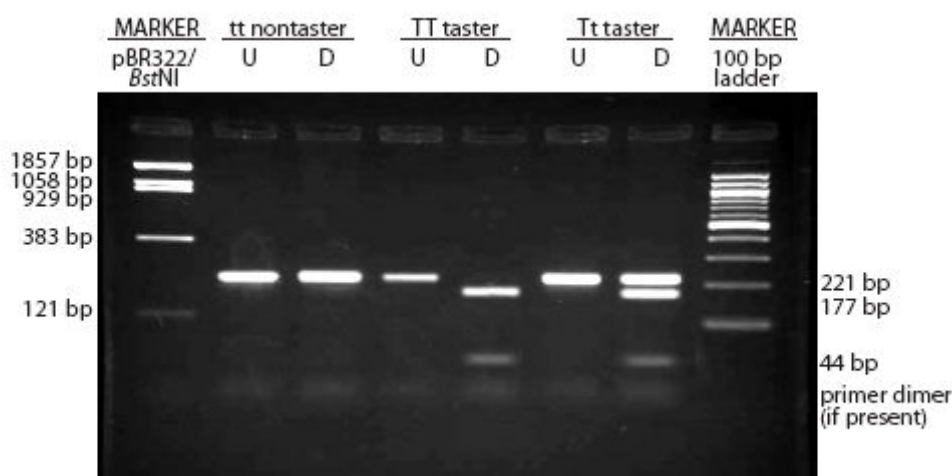
How will the practical work?

Students will use a range of techniques to investigate their genotype and phenotype for ability to taste PTC.

Phenotypic investigation – students will taste PTC (probably using impregnated paper strips) in comparison to a control. Students will assess their own ability to taste PTC.

Genotypic investigation – students will extract their DNA and amplify the *TAS2R38* gene using polymerase chain reaction (PCR). The students will perform a restriction digest which will cut the DNA if the students have a cytosine (C) at nucleotide position 145. They will run their products on an agarose gel, and interpret the results in order to understand their genotype for PTC tasting.

Figure 2: Expected gel results from the practical using the Carolina Biological Supplies kit



Gel picture taken from the DNA Dolan Learning Center/ Carolina Biological Supplies teacher resources.

Results - Generally, there is a high correlation between genotype and phenotype in this experiment, but it is important to stress that this correlation is not absolute. The study that first discovered that *TAS2R38* affects PTC sensitivity found that 50-85% of the (in)ability to taste PTC is due to polymorphisms in the gene. This is an important learning point within the practical that ensures students understand that a combination of several genes and environmental conditions are often determinants of a single trait. Other factors that may contribute are differences in other genes, (including taste receptors), the density of fungiform papillae, and the students' own perception of taste.

References

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4. Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol*. 2005;15(4):322-7.