Winter Lab Report: PTC Lab

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Abstract

This experiment is exploring ideas of individual genotypes and phenotypes, population genetics, and evolution. The purpose of this experiment is to find the genotypes of the PTC tasting gene in individuals, and use this information to determine whether the frequency of female strong tasters is higher than male strong tasters, as determined in a previous study (Bartoshuk et al. 1). The methods that have been used to do this experiment includes isolating and extracting DNA, PCR, digestion, and gel electrophoresis. The results of this experiment are that 1 male and 4 females have homozygous dominant alleles (strong tasters), 5 males and 6 females have heterozygous alleles (weak tasters), and 2 females have homozygous recessive alleles (non-tasters). The conclusion drawn from these results is that the claim that female strong tasters are more common holds true for this small group of individuals.

Introduction

Investigations on individuals' abilities to taste the compound PTC, or Phenylthiocarbamide, have been conducted since the early 1930s. (Wooding 1). Many analyses have been done on characteristics of people relating to their ability to taste the compound. These analyses have been performed both subjectively, by simply allowing individuals to taste PTC, and objectively, with genotype analysis. Specifically, one study investigated correlation between birth sex and ability to taste PTC. This study did not do a genotype-based analysis, only performing a rough, subjective strip-tasting test. They found that there was a larger amount of female "supertasters" (those with a strong ability to taste the compound) than male "supertasters". (Bartoshuk et al. 5) The study aims to first determine the genotypes for PTC tasting for the test group, as well as compare the frequency of female supertasters to the frequency in the aforementioned previous study. As the study relies on analyzing genotype, results can be expected to more accurately confirm the aforementioned study's conclusions on the relationship between sex and ability to taste PTC. The method in this study focuses on three SNPs in a human gene. This gene then has two alleles (and connected variants for the SNP sequences): tasting and non-tasting. They have an inheritance pattern of incomplete dominance, meaning a heterozygous individual would be a weak taster. So, individuals may have one out of three phenotypes: Strong tasting (homozygous for tasting, and also called strong taster), weak tasting (heterozygous), and lack of tasting (homozygous for non-tasting). The hypothesis is that a taste-test of a PTC-impregnated strip will give a relatively accurate prediction of one's objective ability to taste. Furthermore, female strong tasters are expected to be more common than male strong tasters.

Materials

- Saline solution
- 50 mL conical tube
- 1.5 mL tube
- Centrifuge
- Supernatant
- 1 mL pipette
- 200uL pipette
- PTC paper
- PCR primer
- PCR bead reagent
- Ice
- Thermal cycling
- 20uL pipette
- Restriction enzyme
- Thermal cycler
- Freezer
- TBE
- Agarose
- Tape
- Comb
- Tray
- Syber

Methods

Isolate & Extract DNA

1. Mouthwash with 10mL saline solution and spit into 50mL conic tube
2. Let rest for ~5 min
3. Transfer 1mL from the conic tube bottom to a 1.5mL tube
4. Centrifuge at max speed (13.3) for 2 mins
5. Remove 900uL supernatant with 1mL pipette
6. Remove 60uL with 200uL pipette
7. Resuspend mixture with 30uL on a 200uL pipette
8. Boil all tubes for 10 mins

PCR

1. Test control and flavored PTC paper by placing on tongue
2. Record taste strength prediction
3. Add 22.5uL of PCR primer to a tube (one per person) with PCR bead reagent
4. Add 2.5uL of each person's DNA to their corresponding PCR primer/reagent tube
5. Centrifuge quickly
6. Store the tubes in ice until ready for thermal cycling
7. Perform thermal cycling

Digestion

1. Use 20uL pipette to transfer 10uL of the PCR mixture to a new 1.5mL tube
2. Transfer 10uL of PCR product to the new tube ("U" or undigested), store this tube in ice
3. Add 1uL of restriction enzyme to the remaining PCR tube ("D" or digested)
4. Quickly mix the "D" tube
5. Perform thermal cycling on both tubes
6. Store samples in ice or in the freezer until Gel Electrophoresis

Gel Electrophoresis

1. Create a 2% agarose solution by boiling and mixing 2 grams of agarose and 100mL of TBE
2. Seal holes in the tray with tape and place a comb inside to form the wells
3. Pour the agarose solution up to around ½ of the gel tray and allow to solidify
4. Add TBE buffer to cover the surface of the gel
5. Remove the comb and add more TBE buffer
6. Load 20uL of markers, 10uL of the "U" sample, and 16uL of the "D" sample mixture into different wells
7. Run the gel at 130V for 30 minutes
8. Stain the gel using Syber
9. View the gel using transillumination and photograph results.

Results

The qualitative results are the predicted phenotypes that people expressed of the PTC gene by tasting a paper induced with PTC. Our results are that there are 8 strong tasters, 5 weak tasters, and 3 non-tasters as shown in Table 1.

The quantitative results are the number of females and males with the homozygous recessive alleles, homozygous dominant alleles, or heterozygous alleles. In the gel, if there are DNA bands

only at the 44 and 177 base pair marks, that means that the person's genotype for the PTC gene is homozygous tasting. If there are bands at the 44, 177, and 221 base pair marks, that means that the person's genotype for the gene is heterozygous. If there is a band only at the 221 base pair mark, that means that the person's genotype for the gene is homozygous non-tasting. In Images 1 and 2, numbers 1, 3, 5, 7, 13, and 14 are males. The rest are females. Looking at Images 1 and 2, it can be seen that 5 males and 6 females have heterozygous alleles, 1 male and 4 females have homozygous dominant alleles, and 2 females have homozygous recessive alleles. Graph 1 visually represents these results.

A control group, undigested samples, was also used in this experiment. This is to make sure that the results from the gel for the digested samples above are accurate and could be held at a benchmark. The results of the gel should show that undigested samples have only one DNA band at a 221 base pair marker. This is what happens, as shown in Image 3.

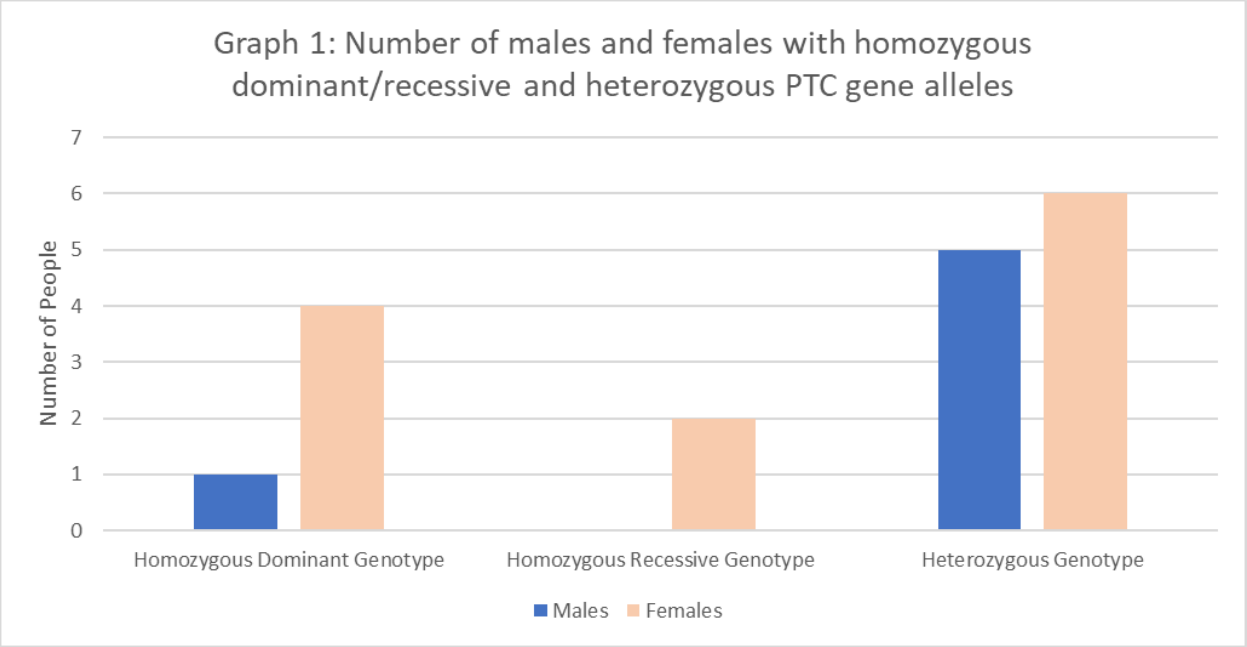
Table 1: Strong tasters, versus weak tasters, versus non-tasters			
Type of taster	**Strong taster**	**Weak taster**	**Non-taster**
Number of people	8	5	3
This table records the number of people who reported having strong, weak, or no taste from a PTC-impregnated paper strip.			

Image 1: Digested samples, Part 1

This is an image of gel electrophoresis performed on the first batch of digested samples. From this gel, we can deduce our test group's genotype for ability to taste PTC.

Image 2: Digested samples, Part 2

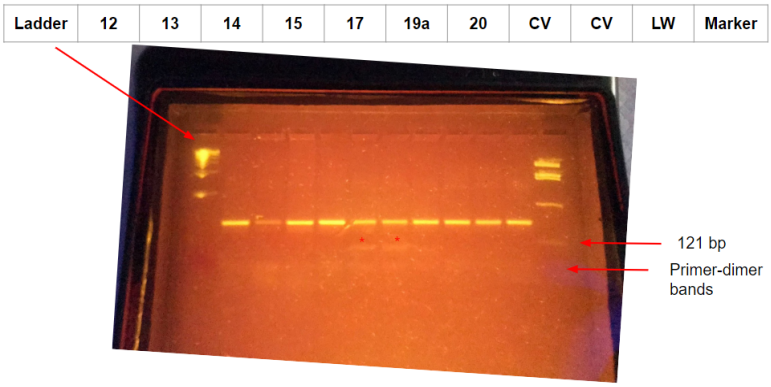
This is an image of gel electrophoresis performed on the first batch of digested samples. From this gel, we can deduce our test group's genotype for ability to taste PTC.



This is a graph that shows how many homozygous dominant, homozygous recessive, and heterozygous genotypes males and females have.

Image 3: Undigested samples

Gel #3 (undigested samples)



This is an image of gel electrophoresis performed on the first batch of undigested samples. As this is a control, we can use this gel to make sure that the electrophoresis process went smoothly.

Discussion

In this experiment, the aim is to find the genotypes of the test group as well as determine whether female or male supertasters are more frequent.

A person is a strong taster if they have homozygous dominant alleles, a weak taster if they have heterozygous alleles, and a non-taster if they have homozygous recessive alleles for the PTC gene. From the strip-tasting test, 8 people classified themselves as strong tasters, 5 as weak, and 3 as non-tasters. After the genotype analysis, though, 5 were found to be strong tasters, 11 weak, and 2 lacking taste. It can be seen that in all tasting categories, numbers of people changed. This indicates that a taste test may be too subjective to produce accurate results for an individual's ability to taste PTC.

Furthermore, it can be understood that 5 males and 6 females are weak tasters, 1 male and 4 females are strong tasters, and 2 females are non-tasters. As there was a higher proportion of female strong tasters (supertasters) than male strong tasters, this means that the article considered as a lens for this study was fairly accurate in finding that women are more likely to be supertasters.

Alleles can become more prominent in a group or a population due to their provision of fitness to the allele holder. In the context of natural selection, the PTC-taster allele may have become more common in women because it provided a natural advantage. The ability to taste PTC was found to be related to the ability to taste bitter, and often toxic, plants (Drewnowski et al. 1). As women were gatherers of plants in prehistoric hunter-gatherer civilizations, sensing toxic plants could perhaps be an advantage to their survival and ability to produce offspring, and therefore their fitness. So, the prominence of strong PTC sensitivity in women can be explained by natural selection.

Limitations of this experiment are presented in the beginning, where participants are required to taste a strip of PTC and determine whether they are a strong, weak, or non-taster of the PTC gene. Solely determining the phenotype is not entirely accurate or effective in determining the type of PTC taster that each person is because they have no point of reference, so “strong” and “weak” are entirely subjective. In order to address these limitations, PCR and Gel Electrophoresis were performed to determine the genotype and get a more determined sense of which genotype and phenotype each person had. Another limitation is that the sample size was very small and did not have the same number of males and females. To make the numbers have a better representation of the general population, a larger sample size with equal numbers of male and females would be more accurate. Some people within the testing group also opted out of doing the paper test, which means that the genotype frequencies and predicted phenotype frequencies do not match up. The last limitation was that one could not match the sex of the person to their phenotype when the PTC tasting test was done because those were kept confidential; there was only access to the total males and females and the total strong/weak/nontaster so it was not possible to tell the sex of the strong/weak/non tasters. This is again why there was a follow up experiment to determine the genotype, and for that the sex of each of the strong/weak/non tasters was known.

Conclusion

In conclusion, from the results attained through Gel Electrophoresis to establish the genotype of the members in the test group, it was found that 4 females were strong tasters, 6 were weak tasters, and 2 were non tasters, and 1 male was a strong taster, 5 were weak tasters, and none were non tasters. This supports the hypothesis that female supertasters are more common than men. This also showed a linkage more generally between assigned sex and tasting genes.

Works Cited

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