Lab Report: PTC Lab

TS Biology | Winter 2022

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Abstract

The human ability to taste phenylthiocarbamide (PTC) is varied and random among a group of people, but researchers have identified patterns of higher or lower amounts of PTC tasters across certain geographic regions. In this study, the authors are comparing the phenotypes of the Transition School Class of 2021-2022 with the samples' geographic region of origin. Then, they compared these statistics to previously established phenotypic frequencies across different geographic regions to examine whether established frequencies apply for this unexplored population.

The class tasted PTC to presume their phenotypes before testing. Then, the authors gathered DNA samples from the class, amplified the samples, and then performed restriction digestion to cut only the samples that exhibited at least one allele for PTC tasting. Lastly, they used gel electrophoresis to review which samples exhibited which phenotypes.

After comparing the phenotypical results to the established frequencies, the researchers found that the majority of the data matched the established data, but there were also some outliers. Furthermore, there were many limitations to this experiment, such as the size of the TS population and the exactness of samples' geographic regions of origin. Therefore, no strong conclusions were made about this population's phenotype distribution compared to each sample's region of origin, nor the established data.

Introduction

All human populations tested to date contain some individuals who can and some who cannot taste the bitter compound phenylthiocarbamide (PTC). The reason this trait has been maintained in the population is uncertain, but this polymorphism may influence food selection, nutritional status or thyroid metabolism (Guo and Reed 2001). Furthermore, data (Wooding 2006) shows that the ability to taste PTC varies throughout different populations worldwide: native North and South Americans, Africans, and East Asians tend to have more PTC tasters, while Australian Aborigines and those of European descent are largely PTC non-tasters. This variation may be explained by environmental differences and plant toxicity rates in different territories.

The Transition School Class of 2021-2022 is a previously unexplored, albeit small, population when it comes to PTC tasting. We conducted this experiment in order to discover the genotypes and phenotypes of the individuals in this population and to compare these phenotypes to previously established geographic phenotype frequencies. Our hypothesis is that the phenotypic frequencies of our class will be similar to those already established. We want to compare these phenotypes to previously established geographic frequencies because the study we are citing is from 2001 (21 years ago at the time of writing) and may not hold true today, so examining this population may yield interesting and new results that can be further pursued.

Materials & Methods

PTC Tasting

Materials

- PTC paper

Methods

1. Taste the paper by placing it on one's tongue.
2. Record the strength of the flavor.

DNA Collection

Materials

- Permanent marker
- Paper cup
- Micropipettes and tips (10-1000 μL)
- 1.5-mL microcentrifuge tubes
- Microcentrifuge tube racks
- Microcentrifuge adapters
- Microcentrifuge
- Water bath
- Container with crushed/cracked pieces of ice
- Vortexer

Methods

1. Using a permanent marker, label a 1.5mL tube and 50mL tube with an assigned number.
2. Pour saline solution in one's mouth and rinse one's cheek pockets for 30 seconds.
3. Expel the saline solution into the 50mL tube.
4. Gently swirl the cup to mix cells that may have settled to the bottom. Using a micropipette with a fresh tip, transfer 1000 μL of the solution into the labeled 1.5mL microcentrifuge tube.

5. Place the sample tube, along with other samples, in a balanced configuration in a microcentrifuge, and spin for 2 minutes at full speed.
6. Carefully extract 900 µL of supernatant with a 1 mL micropipette, and then extract 60 µL of supernatant with a 200 µL micropipette.
7. When there is approximately 30 µL of the supernatant and cell pellet left, resuspend cells in the remaining saline by pipetting in and out while minimizing bubbles.
8. Place the PCR tube, along with other samples, in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4C hold program. If a 1.5mL tube is used, use a heat block or boiling water bath.
    a. Boiling: 99°C, 10 minutes
9. After boiling, shake the PCR tube vigorously for 5 seconds.

<u>DNA Amplification</u>

Materials

- Permanent marker
- Micropipettes and tips (1-100 µL)
- Microcentrifuge tube rack
- Thermal cycler
- Container with crushed/cracked pieces of ice

Methods

1. Label a PCR tube containing a PCR bead with the assigned number.
2. Using a micropipette with a fresh tip, add 22.5 µL of PCR primer to the tube, and let it sit for approximately one minute.
3. Using a micropipette with a fresh tip, add 2.5 µL of the cheek cell DNA into the PCR tube.
4. Place the PCR tube, along with other samples, in a thermal cycler that has been programmed for 30 cycles of the following profile.
    a. Denaturing: 94°C, 30 seconds
    b. Annealing: 64°C, 45 seconds
    c. Extending: 72°C, 45 seconds
5. Store the amplified DNA on ice.

<u>Digestion</u>

Materials

- Permanent marker
- 1.5-mL microcentrifuge tubes
- Microcentrifuge tube racks
- Micropipettes and tips (1-20 µL)
- Thermal cycler
- Incubator
- Container with crushed/cracked pieces of ice

Methods

1. Label a 1.5mL tube with the assigned number and with a "U" (undigested).
2. Transfer 10 μL of the PCR product to the "U" tube using a micropipette.
3. Add 1 μL of the restriction enzyme *Hae*III into the remaining PCR product, and label this tube with "D" (digested).
4. Intubate for one hour.

Gel Electrophoresis

Materials

- Micropipettes and tips (1-20 μL)
- Microcentrifuge tube racks
- Gel electrophoresis chamber
- Power supply
- Staining trays
- UV transilluminator (for use with ethidium bromide)
- White light transilluminator
- Water bath (60 °C)
- Container with crushed/cracked pieces of ice
- Incubator
- Agarose gel
- Buffer solution
- SybrSafe DNA gel stain

Methods

1. Seal the ends of the gel-casting tray with masking tape or other appropriate method, and insert a well-forming comb.
2. Add 2 grams of agarose to 100 mL of the buffer solution.
3. Stir using a stirring rod until the agarose powder dissolves.
4. Boil in a microwave until the mixture is boiling. Stop every 20 seconds to take out the beaker and gently shake it.
5. Pour 2% agarose solution to a depth that covers about one-half the height of the open teeth of the comb.
6. Allow the gel to solidify completely.
7. Place the gel into the electrophoresis chamber, and add enough 1× TBE buffer to cover the surface of the gel.
8. Carefully remove the comb, and add enough additional 1× TBE buffer to cover and fill in wells, creating a smooth buffer surface.
9. Use a micropipette with a fresh tip to load 20 μL of pBR322/BstNI size markers into the far left lane of the gel.
10. Use a micropipette with a fresh tip to add 10 μL of the undigested (U) and 16 μL of the digested (D) sample/loading dye mixture into different wells of a 2% agarose gel.
11. Run the gel at 130 V for approximately 30 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

12. Stain the gel using SybrSafe DNA gel stain in place of ethidium bromide.
13. View the gel using transillumination and photograph it using a camera of one's choice.

Results

Qualitative Results

Test	Strong Phenotype	Weak Phenotype	No Taste (None Phenotype)
Taste Test	9	6	3
DNA Test	5	10	3

Table 1. Results for presumed phenotypes based on taste testing, compared with results for actual phenotypes based on DNA.

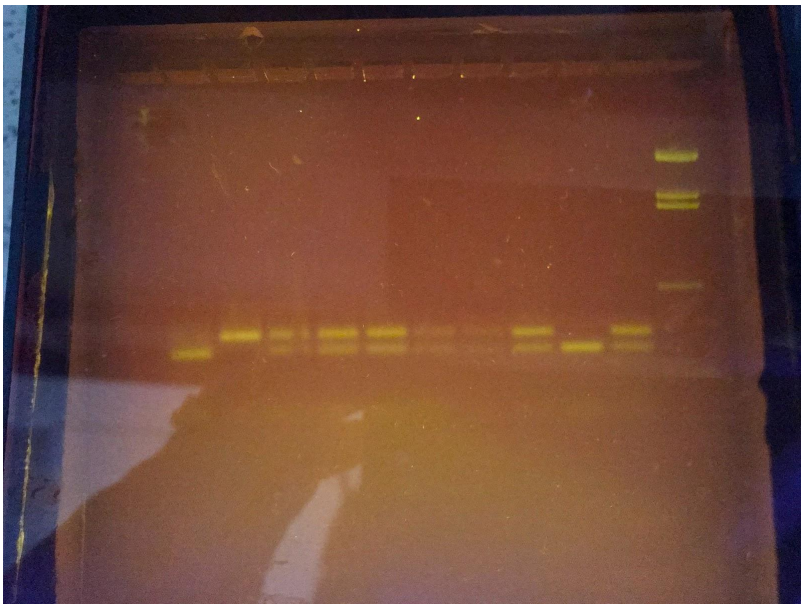

Figure 1. Gel electrophoresis results for samples 1-6 and 13-15. The leftmost well is the ladder, which is barely visible, and the second well from the left is sample 1. The seventh well from the left is sample 6, and the eighth well from the left is sample 13. The second well from the right is Lauren Witty's sample, and the rightmost well is the marker.

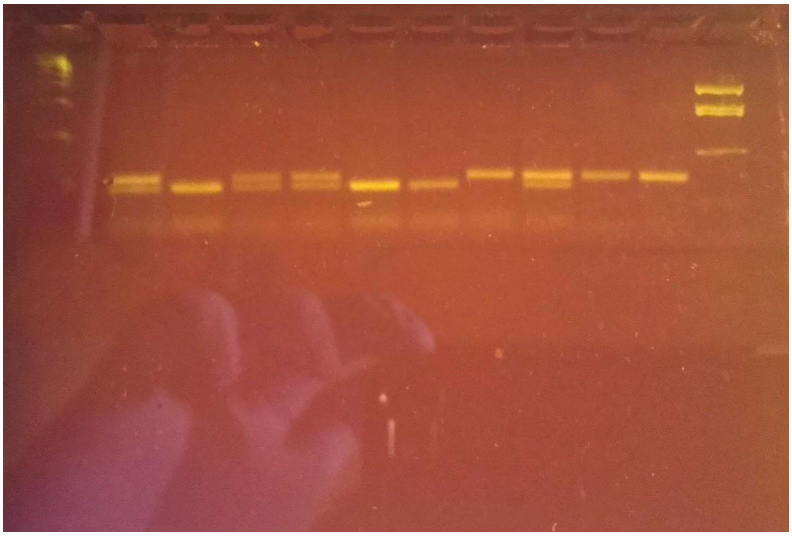

Figure 2. Gel electrophoresis results for samples 7-8, 10-12, 17, 19a, and 20. The leftmost well is the ladder. The second and third wells from the right are Cristina Valensisi's samples (as well as sample 19a), and the rightmost well is the marker.

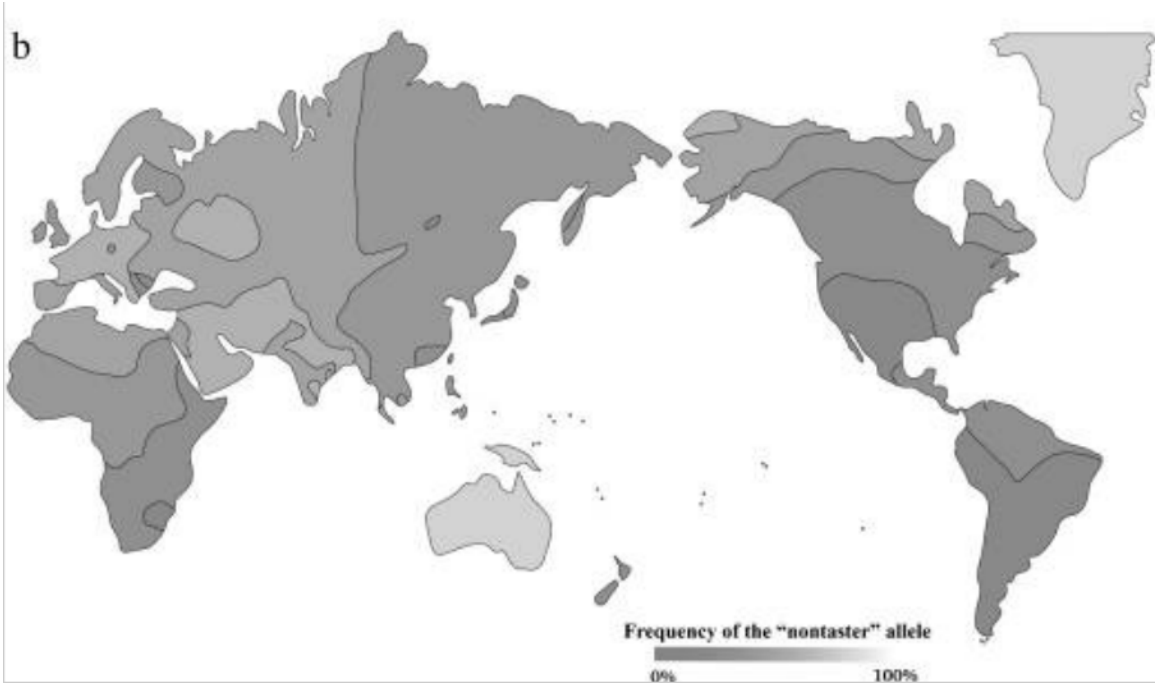

Figure 3. Frequency of the nontaster allele worldwide as reported by Wooding (2006).

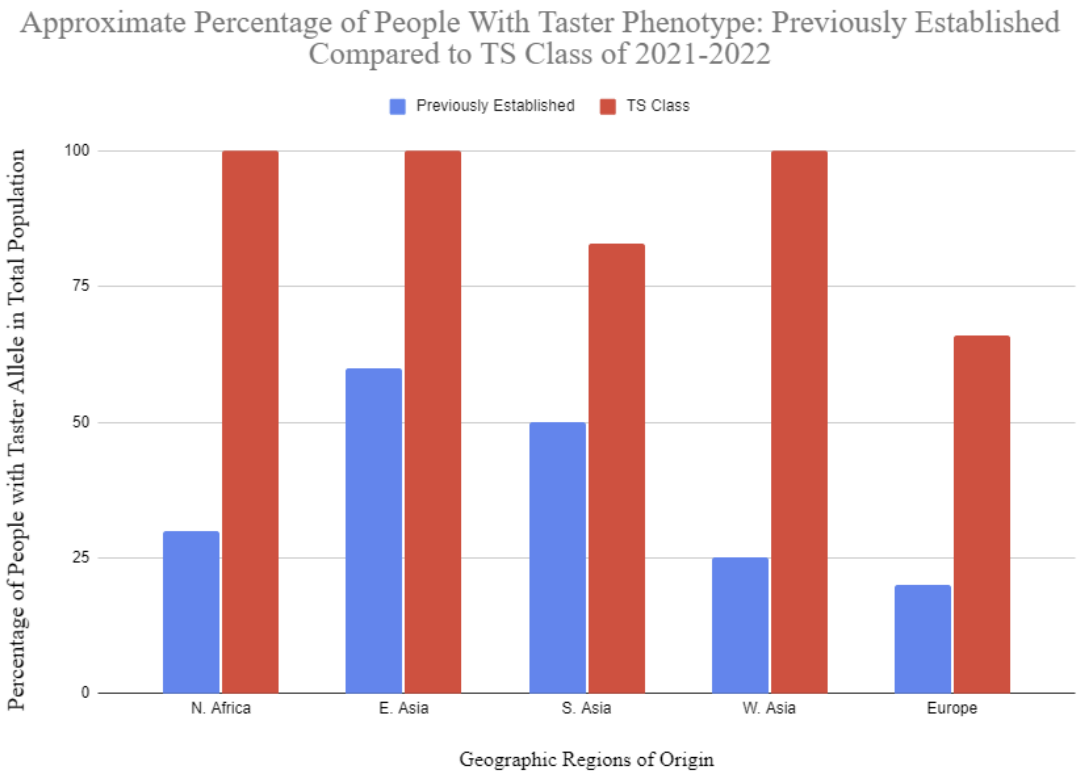

Figure 4. Frequencies of the taster phenotype in the TS Class of 2021-2022 population compared to established frequencies of the taster phenotype with samples from different geographic regions of origin.

Before we began collecting DNA samples and testing the samples' genotypes, we began by testing the sample's presumed phenotypes by allowing them to taste PTC and record what they thought their genotype was. If they recorded strong, they presumed that they had the *TT* genotype; if they recorded weak, they presumed that they had the *Tt* genotype; and if they recorded none, they presumed that they had the *tt* genotype. We found that 9 out of 18 samples or 50% of the class population presumed that they had the strong phenotype or the *TT* genotype; 6 out of 18 samples or about 33% presumed that they had the weak phenotype or the *Tt* genotype; and 3 out of 18 samples or about 17% presumed that they had the none phenotype or the *tt* genotype.

Then, we tested their DNA to confirm their genotypes and phenotypes. We found that 5 out of 18 samples or about 28% of the class population actually had the strong phenotype or the *TT* genotype; 10 out of 18 samples or about 56% had the weak phenotype or the *Tt* genotype; and 3 out of 18 samples or about 17% had the none phenotype or the *tt* genotype (see Table 1 and Figures 1 and 2).

After we collected the sample information, we compared them to the frequency of non-taster alleles and taster alleles around the world in a previously established study (Guo and Reed 2001) (see Figures 3 and 4).

Quantitative Results

Samples 1, 8, 12, 15, and 17 have the *TT* genotype, meaning they are strong tasters. Samples 2 and 19a have the *tt* genotype, meaning they cannot taste PTC at all. Finally, samples 3, 4, 5, 6, 7, 10, 11, 13, 14, 20 and LW have the *Tt* genotype, which means that they are weak tasters.

Out of this population, 61% have the *Tt* genotype, 28% have the *TT* genotype, and 11% have the *tt* genotype. Thus, 89% can taste PTC, while 11% cannot.

Furthermore, 33% of the class is East Asian. 50% of them have the strong phenotype, and the other half have the weak phenotype. 28% of the class is South Asian. Around 21% of them have the strong phenotype, around 58% of them have the weak phenotype, and around 21% of them cannot taste PTC. 11% of the class is West Asian and 100% of them have the weak phenotype. 17% of the class is European. Around 65% of them have the weak phenotype, and around 35% of them cannot taste PTC. 6% of the class is West African and 100% of them have the strong phenotype.

#	**Genotype**	**Phenotype**	**Geographic Region of Origin**	**Country of Origin**
1	*TT*	Strong	East Asia	China
2	*tt*	None	South Asia	India
3	*Tt*	Weak	East Asia	Korea, China
4	*Tt*	Weak	East Asia	Korea
5	*Tt*	Weak	South Asia	India
6	*Tt*	Weak	West Asia	Russia
7	*Tt*	Weak	East Asia	China
8	*TT*	Strong	Western Africa & Europe	-
10	*Tt*	Weak	South Asia	India
11	*Tt*	Weak	West Asia	Russia, Azerbaijan
12	*TT*	Strong	South Asia	India
13	*Tt*	Weak	South Asia	India
14	*Tt*	Weak	Europe	Poland, Croatia
15	*TT*	Strong	East Asia	China

17	*TT*	Strong	East Asia	China
19a	*tt*	None	Europe	Italy
20	*Tt*	Weak	Europe	Australia

Table 2. The genotypes and phenotypes of each sample compared to their geographic region of origin and country of origin (if available).

Region of Origin	**Strong Phenotype**	**Weak Phenotype**	**None Phenotype**
East Asia	3	3	0
South Asia	1	4	1
West Asia	0	2	0
Europe	0	2	1
Northern Africa	1	0	0

Table 3. The number of samples in each geographic region group categorized by the type of phenotype they possess.

Discussion

In this experiment, we investigated the genotypic frequencies of different geographical areas by examining the PTC receptor gene (TAS2R38) alleles of the Transition School (TS) Class of 2021-2022 population. We compared the genotypes of individuals in the TS population to general, previously established data for population distributions across different geographic areas. We found that most of the population's data corresponded to the general data for geographic regions. For example, all six individuals of East Asian origin were PTC tasters, matching previous data (Wooding 2006) showing high numbers of PTC tasters in the South Asia region (see Table 3).

Also, although our data mostly did match with previous known data, there were several individuals in the population whose genotypic data did not correspond with the general genotypic frequencies for their geographic regions of origin. For example, two-thirds of the European population in this experiment had the *Tt* genotype, meaning that they had a taster phenotype (see Tables 1 and 3). However, in the previously established data, most Europeans had the non-taster phenotype (see Figure 3).

In addition, there were multiple limitations in this experiment, the largest and most obvious of which is the size of the population being tested. Since we are using such a small population (only 20 individuals), it is hard to compare it with general geographic

data without skewing the statistics. For example, in Figure 4, the recorded percentage of North Africans in the TS population who had a taster phenotype was 100%. However, there is only one North African in the population, so the results cannot be considered as accurate as the mass-scale study we cited.

Furthermore, some individuals in the test population have vague geographical origin data and their categorization may not be entirely accurate. The experiment had some issues as well; the gel electrophoresis ladder was barely visible in some of the samples and the differentiation between strong and weak tastes when tasting the PTC is entirely subjective. These various limitations mean that we cannot definitively draw conclusions from our results, but the general trend in our data is aligned with the data for geographic PTC gene distributions.

Conclusion

Due to the limitations of this experiment (the small sample size and unconfirmed geographical data for our subjects), we were not able to conclusively determine whether the genotypic frequencies of the Transition School (TS) Class of 2021-2022 completely align with or refute previously established data. Even so, our results largely match the results of previous studies.

Future studies with larger population sizes will need to be performed in order to confirm or refute previously established data. The studies we referenced were created in the 2000's, so large scale globalization and immigration may have changed the allele frequencies found around the world. Future studies may also align with the results found in the class population. Overall, this study has intriguing implications for the future of PTC studies.

References

Guo SW, Reed DR. 2001. The genetics of phenylthiocarbamide perception. Ann Hum Biol. 28(2):2.

Wooding S. 2006. Phenylthiocarbamide: A 75-Year Adventure in Genetics and Natural Selection. Genetics. 172(4):3.