The Interplay of Amylase and Texture Perception

Presented by:

John Hayes
Associate Professor of Food Science and Director of Sensory Evaluation Center

PennState College of Agricultural Sciences
Department of Food Science

John Smythe
Head of Sensory

TATE & LYLE

Ann Colonna
Sensory Program Manager
Food Innovation Center

Oregon State University

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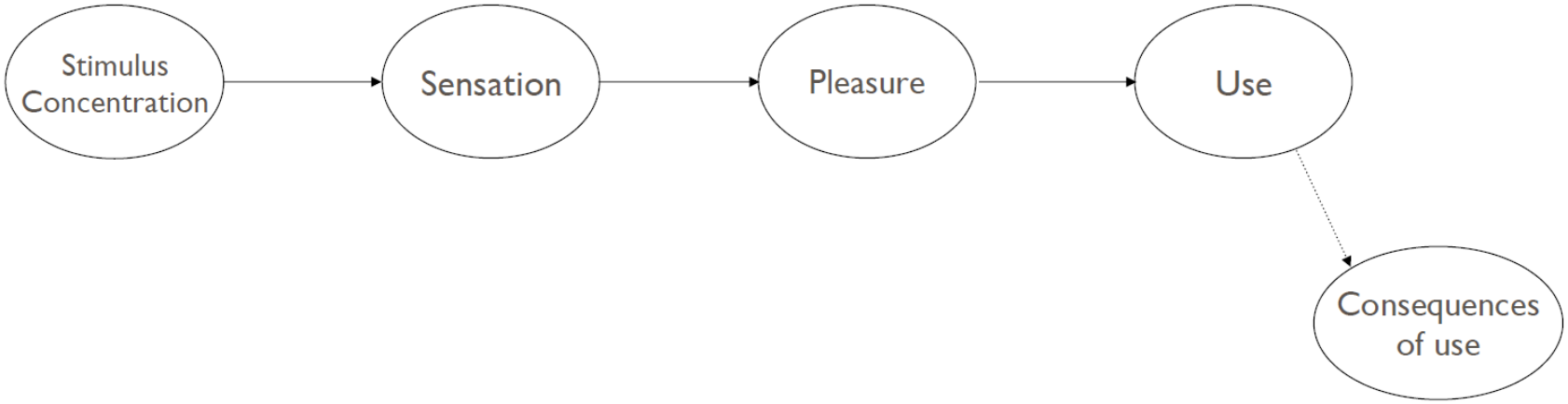
Agenda

Understanding genetic differences in amylase activity and texture perception

Demonstrating of amylase activity in sensory practice

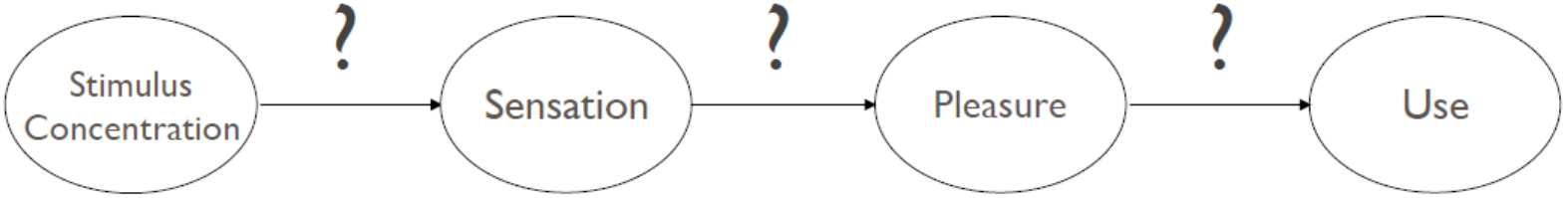
Practical culinary considerations

The implicit causal chain in most sensory research

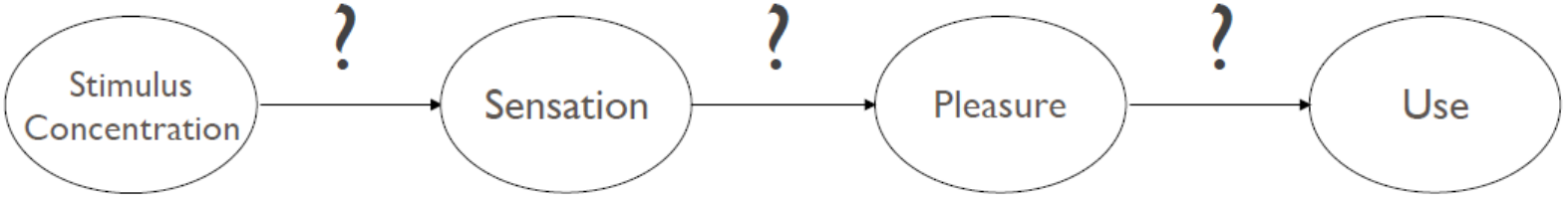



The number of civilisations in our galaxy in which communication might be possible.
The fraction of stars with planets
The fraction that can go on to support intelligent life.
Length of time such civilisations release detectable signs into space.
$N = R^* \times f_p \times n_e \times f_l \times f_i \times f_c \times L$
The average rate of star formation per year in our galaxy
The average number of planets that can potentially support life (per star with planets.)
The fraction that can go on to support life.
The fraction of civilisations that develop a technology detectable from space.
The Drake Equation.

Testing our assumed causal chain with some estimated effect sizes



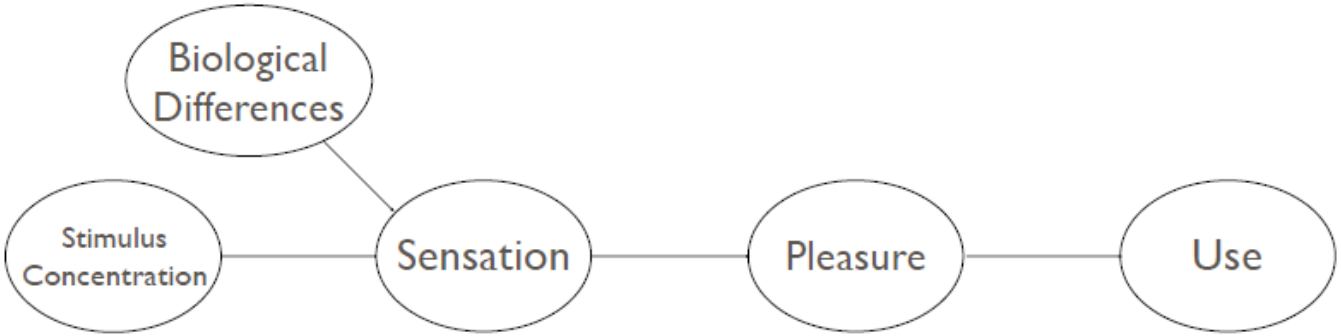
Testing our assumed causal chain with some estimated effect sizes



.97	x	.8	x	.6	=	$r_{sc,u}$~0.47
.90	x	.6	x	.4	=	$r_{sc,u}$~0.22
.80	x	.5	x	.3	=	$r_{sc,u}$~0.12

…or, formulation potentially explains ~2% to 22% of the variance in use

But formulation is *not* the only *systematic* source of variation



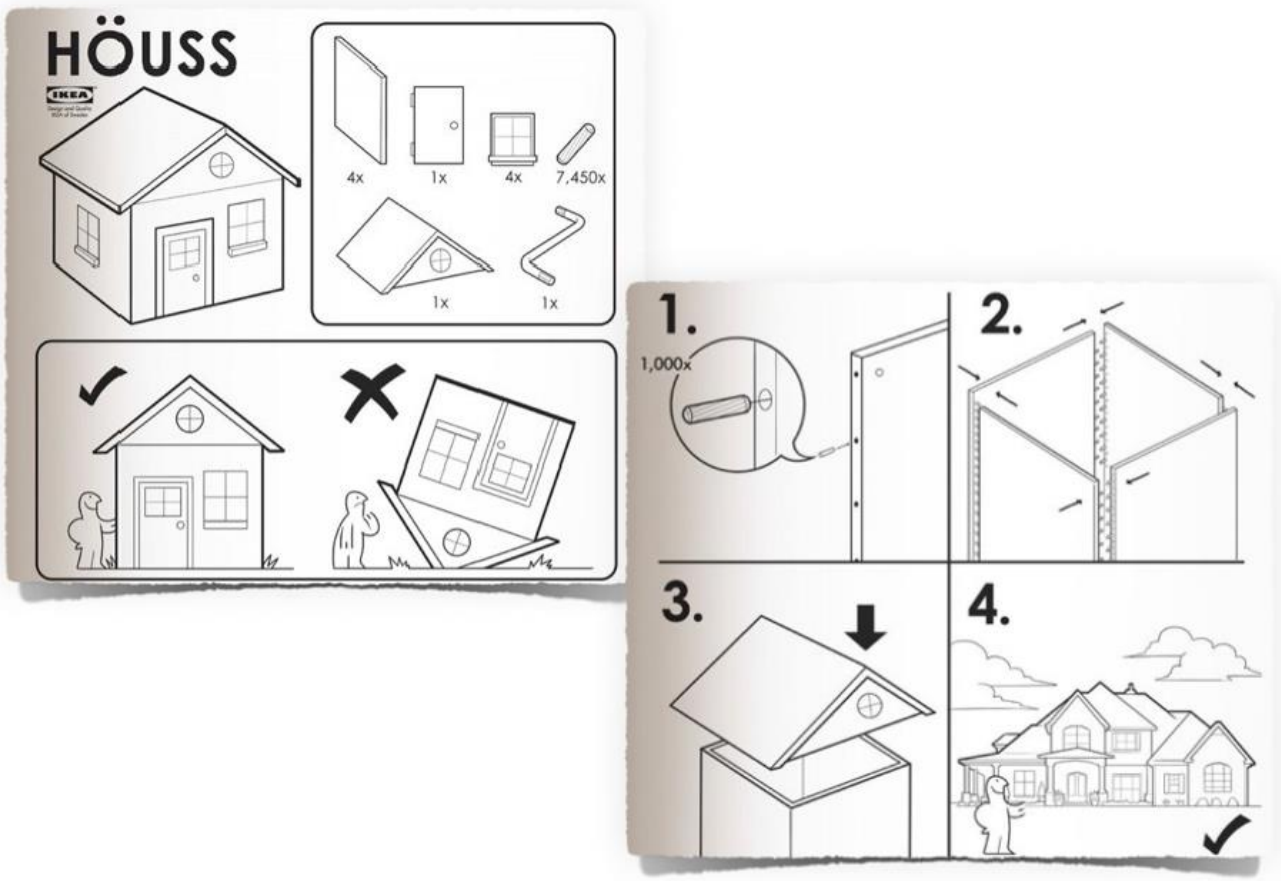
…or, why you should care about genetics

Paradox of the modern product development process

1. Sensory and market research studies often use mean liking and acceptability to predict liking in the *population*.

2. Deciding what do eat is a decision typically made by the *individual*, and tastes vary across individuals.

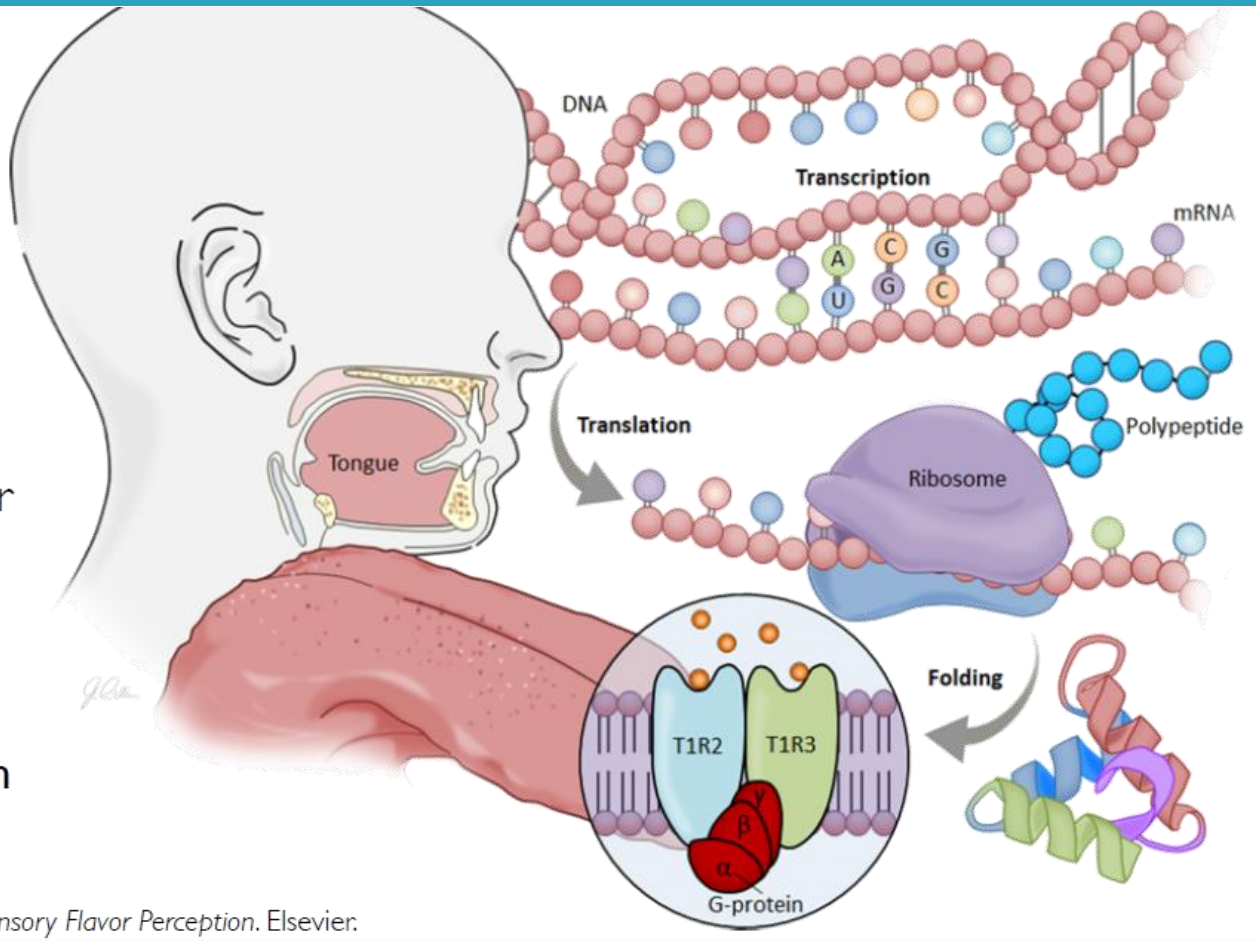
DNA = instructions for building something



DNA is transcribed to RNA, which is translated to proteins. These proteins are then folded and translocated to specific locations.

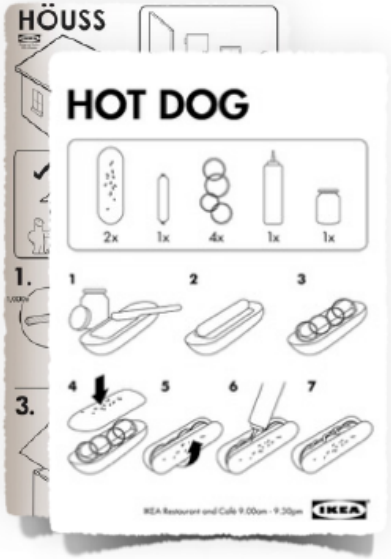
For the chemoreceptors, these locations typically occur at cell surfaces that interact with the environment.

Same process for other proteins as well, like those in saliva.



From Running & Hayes. 2016. Ch. 10 In *Multisensory Flavor Perception*. Elsevier.

Different types of variability



Genome contains many genes!
1 pg = one gene

Sources of variability

Single Nucleotide Polymorphisms (SNPs)

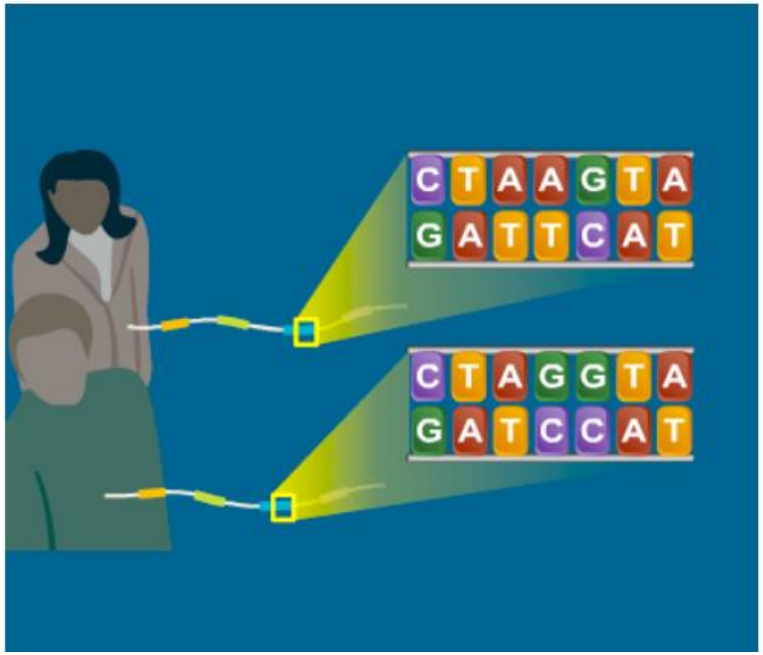


Hot Dog



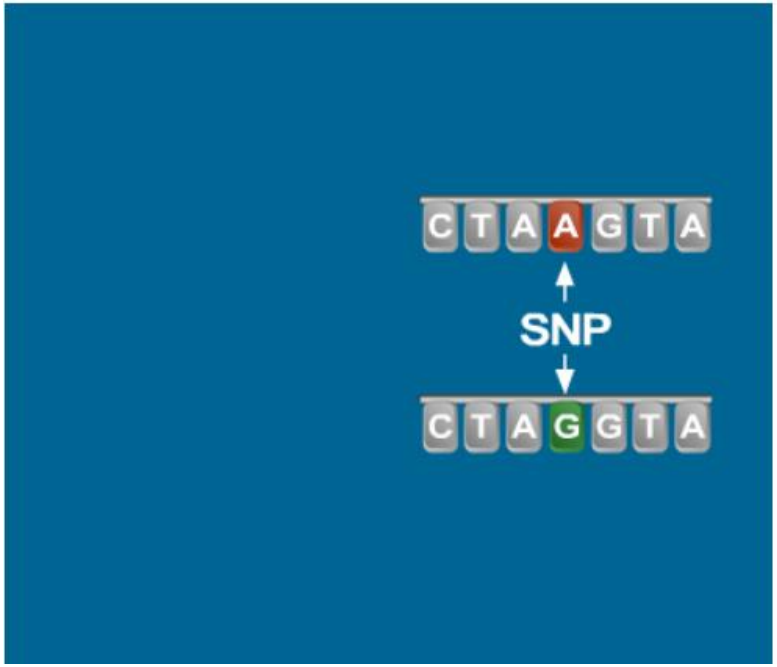
Pot Dog

SNPs



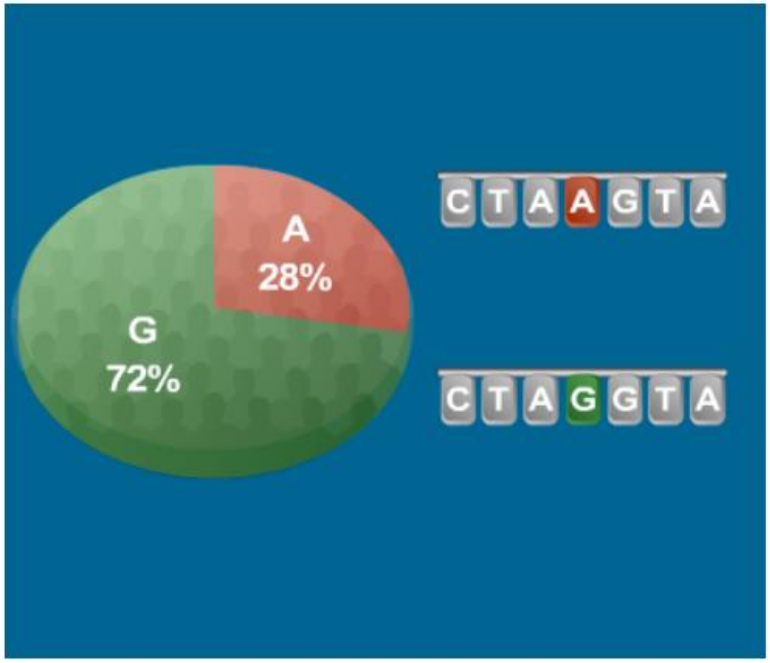
- Here are two short DNA sequences from the same genomic region of two different people

SNPs



- Here are two short DNA sequences from the same genomic region of two different people
- Notice that they are almost identical, except for the A/G SNP.

SNPs



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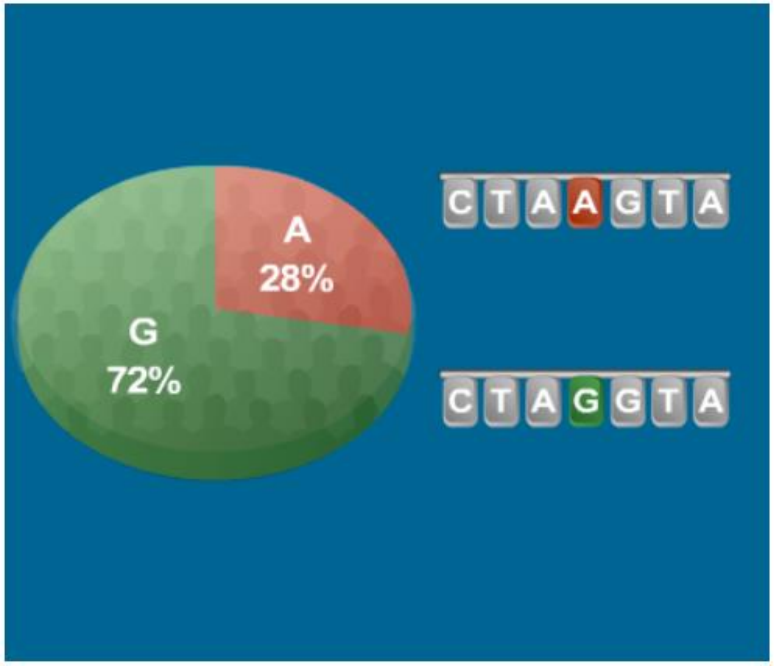
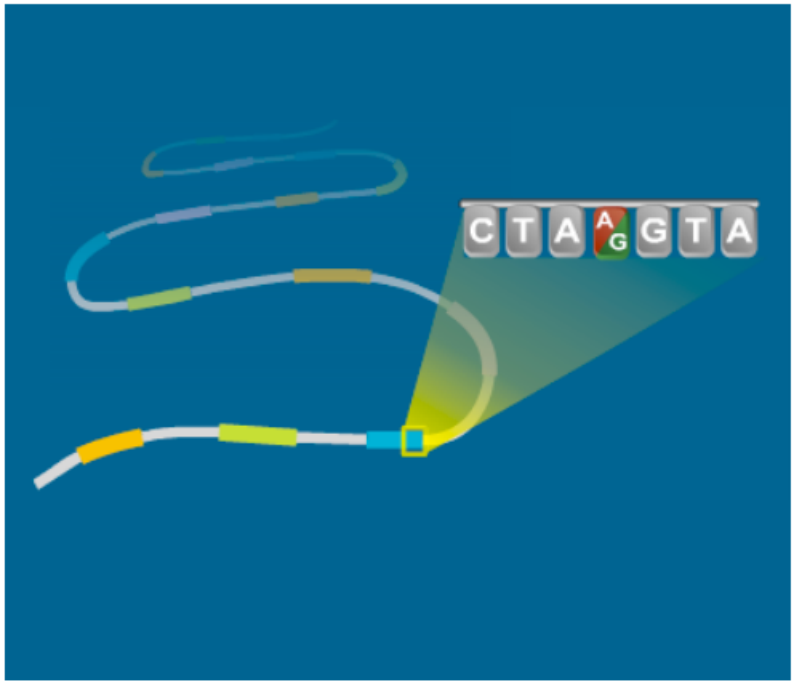
- If we look at 1000 people, we find G in this spot 72% of the time

SNPs



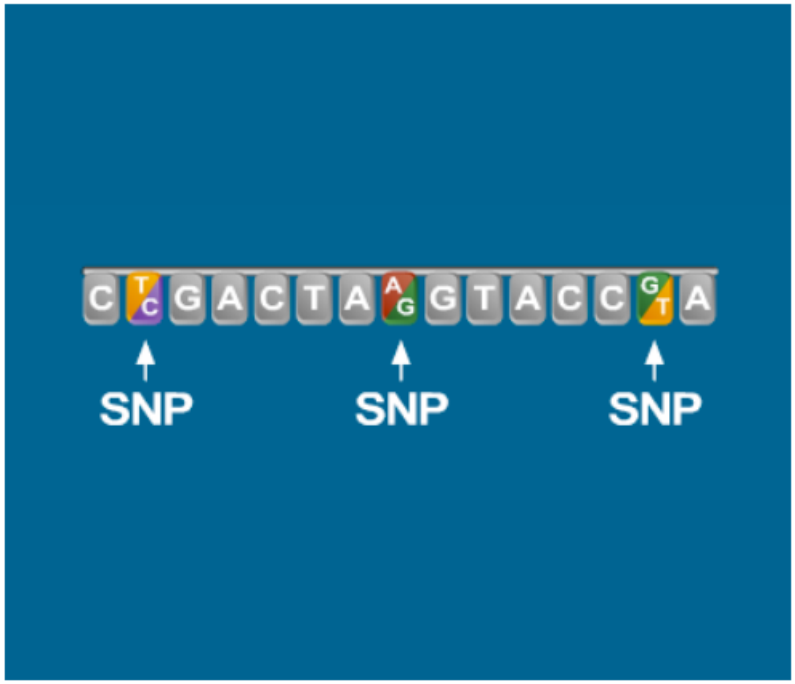
- Here are two short DNA sequences from the same genomic region of two different people
- Notice that they are almost identical, except for the A/G SNP.
- If we look at 1000 people, we find G in this spot 72% of the time
- A is called the minor allele

Haplotypes



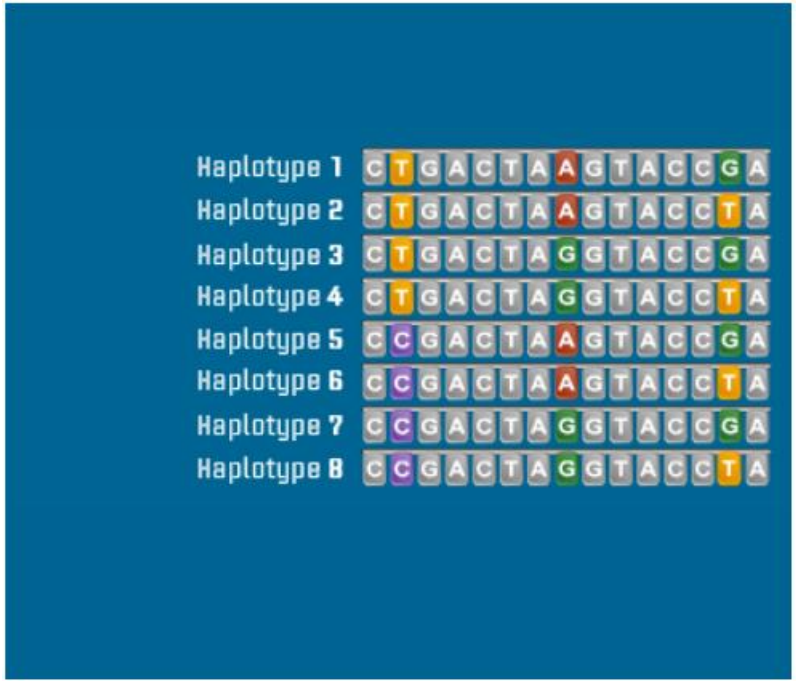
- We have ~10 million of these SNPs in our DNA

Haplotypes



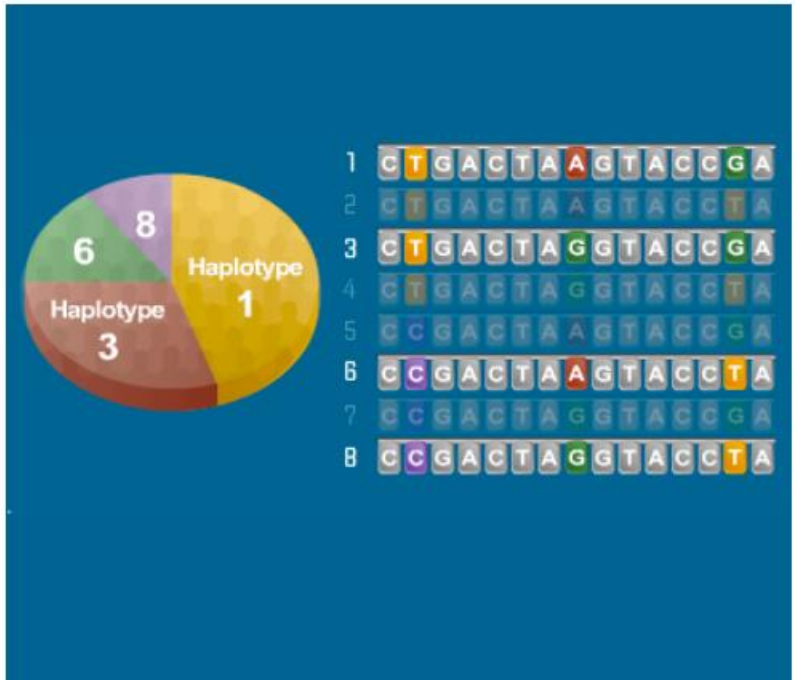
- We have ~10 million of these SNPs in our DNA
- A region of interest may have more than 1 SNP

Haplotypes



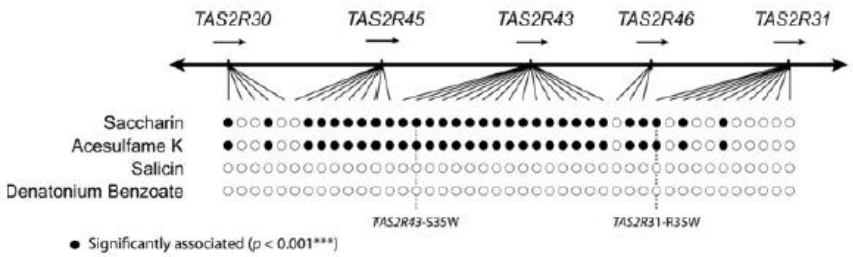
- We have ~10 million of these SNPs in our DNA
- A region of interest may have more than 1 SNP
- Just 3 SNPs result in 23 possible combinations – these are haplotypes

Haplotypes

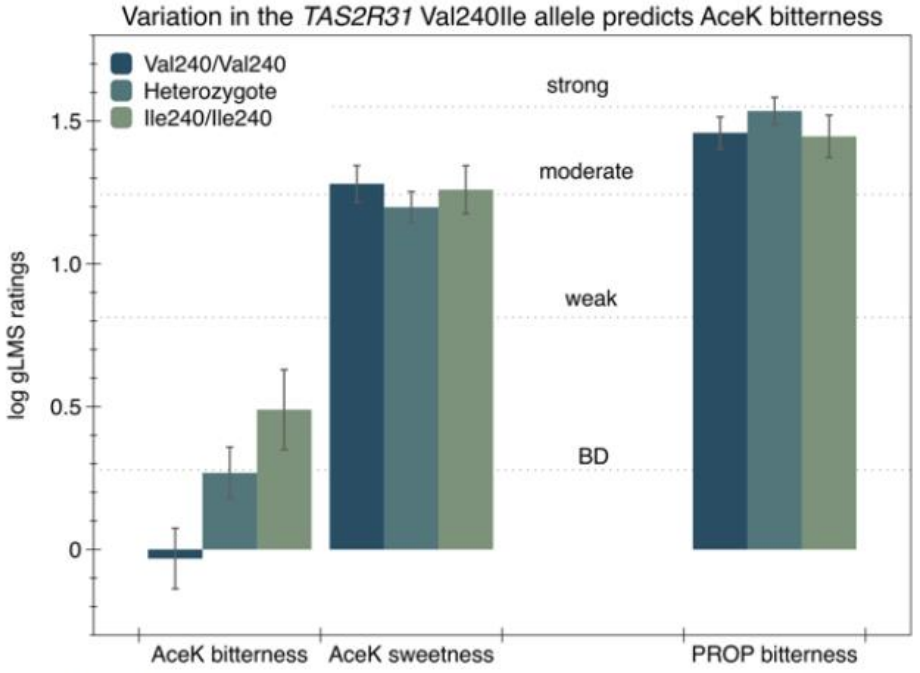


- We have ~10 million of these SNPs in our DNA
- A region of interest may have more than 1 SNP
- Just 3 SNPs result in 23 possible combinations – these are haplotypes
- all possible haplotypes are NOT seen in nature

TAS2R31 SNPs explain differences in the bitterness of AceK and Saccharin (but not PROP)



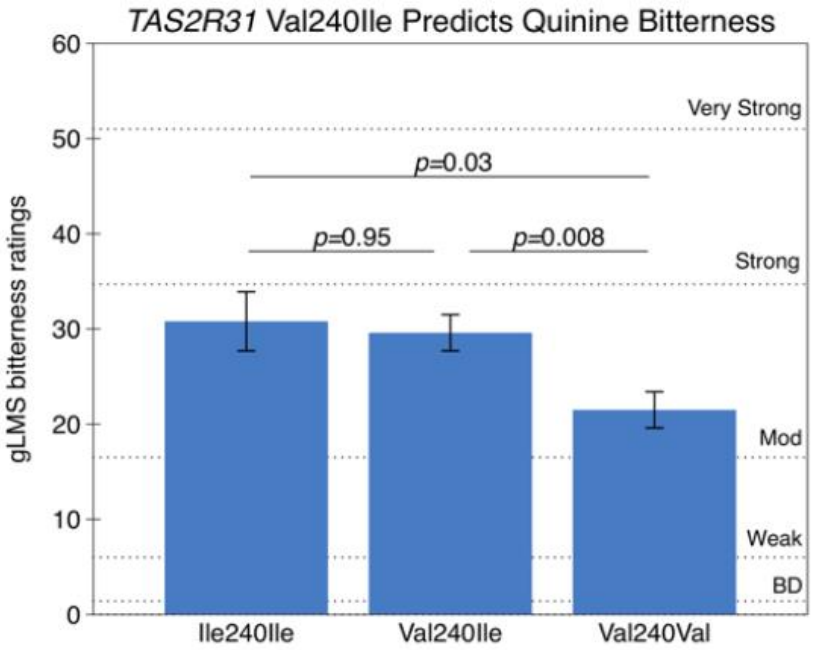
Gene		Acesulfame K			Saccharin		
		Non-Carrier	Carrier	*p*	Non-Carrier	Carrier	*p*
TAS2R31	H1	-1.65	-1.69	0.816	-1.83	-1.85	0.941
	H2	-1.33	-2.01	<0.001**	-1.46	-2.22	<0.001***
	H3	-1.60	-1.74	0.445	-1.77	-1.91	0.467
	H4	-1.61	-1.73	0.554	-1.72	-1.96	0.275
	H5	-1.58	-1.75	0.514	-1.74	-1.94	0.508
Overall				<0.001***			<0.001***

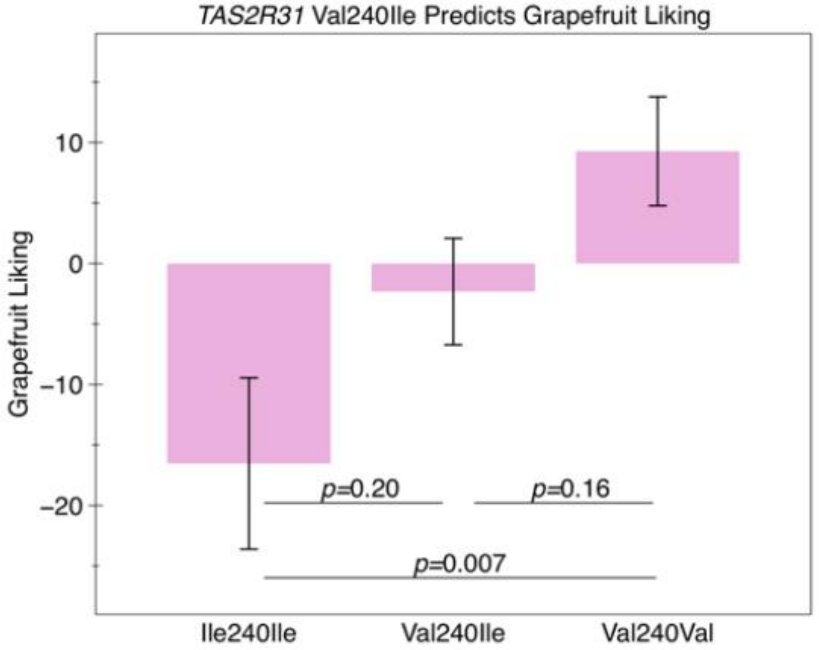


Roudnitzky, et al. 2011. Hum Mol Gen 20(17): 3437-3449

Allen, McGeary, Knopik, Hayes. 2013. Chem Senses 38(5):379-89

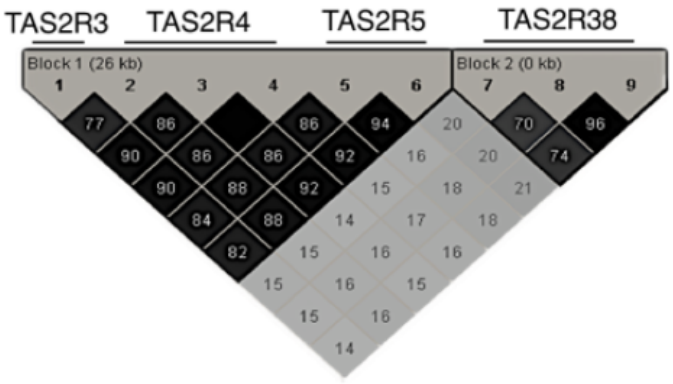
… as well as differences in quinine bitterness, and grapefruit liking

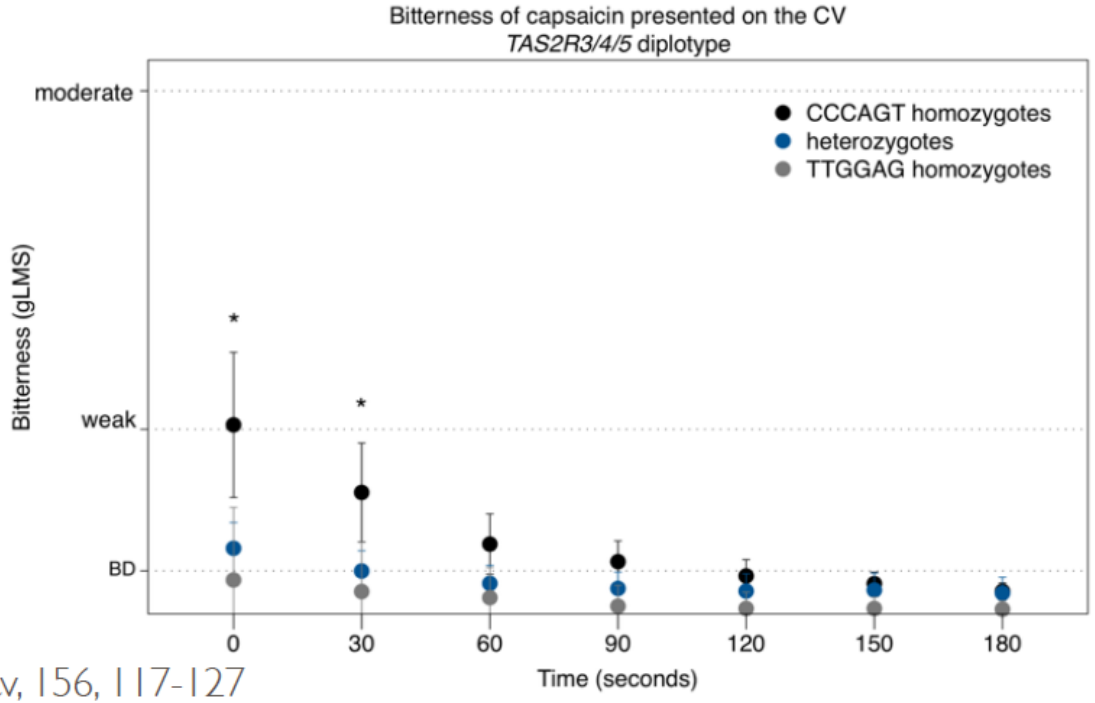




Hayes, Feeney, Nolden & McGeary. 2015. Chem Senses 40(6):437-43

SNPs across multiple *TAS2Rs* form haplotypes, and these associate with differential bitterness





Nolden, McGeary, & Hayes. 2016. Physiol Behav, 156, 117-127

Different types of variability



Genome contains many genes!
1 pg = one gene

Sources of variability

Single Nucleotide Polymorphisms (SNPs)

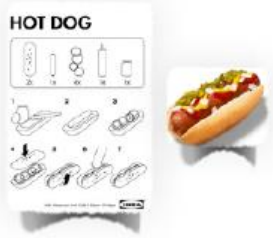


Hot Dog



Pot Dog

Copy Number Variants (CNVs)



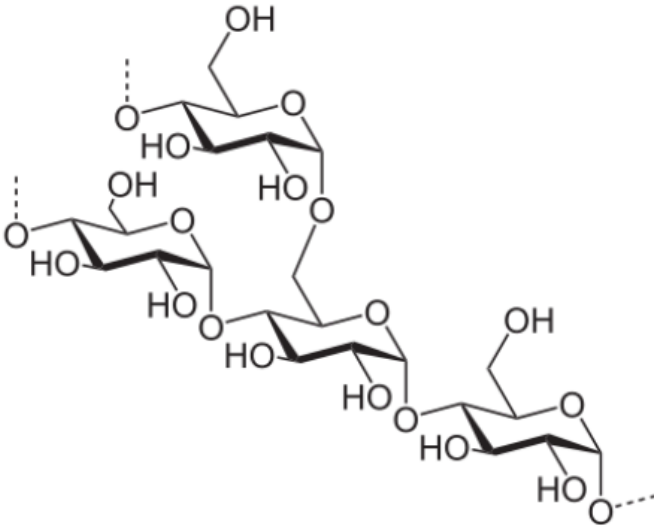
1 copy

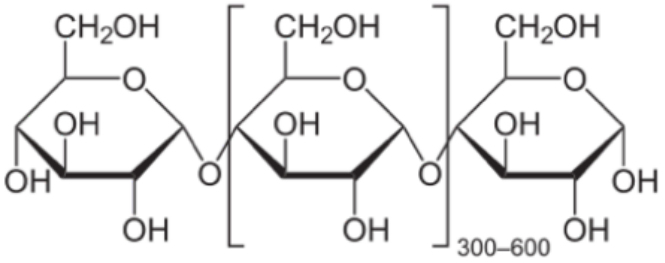


6 copies

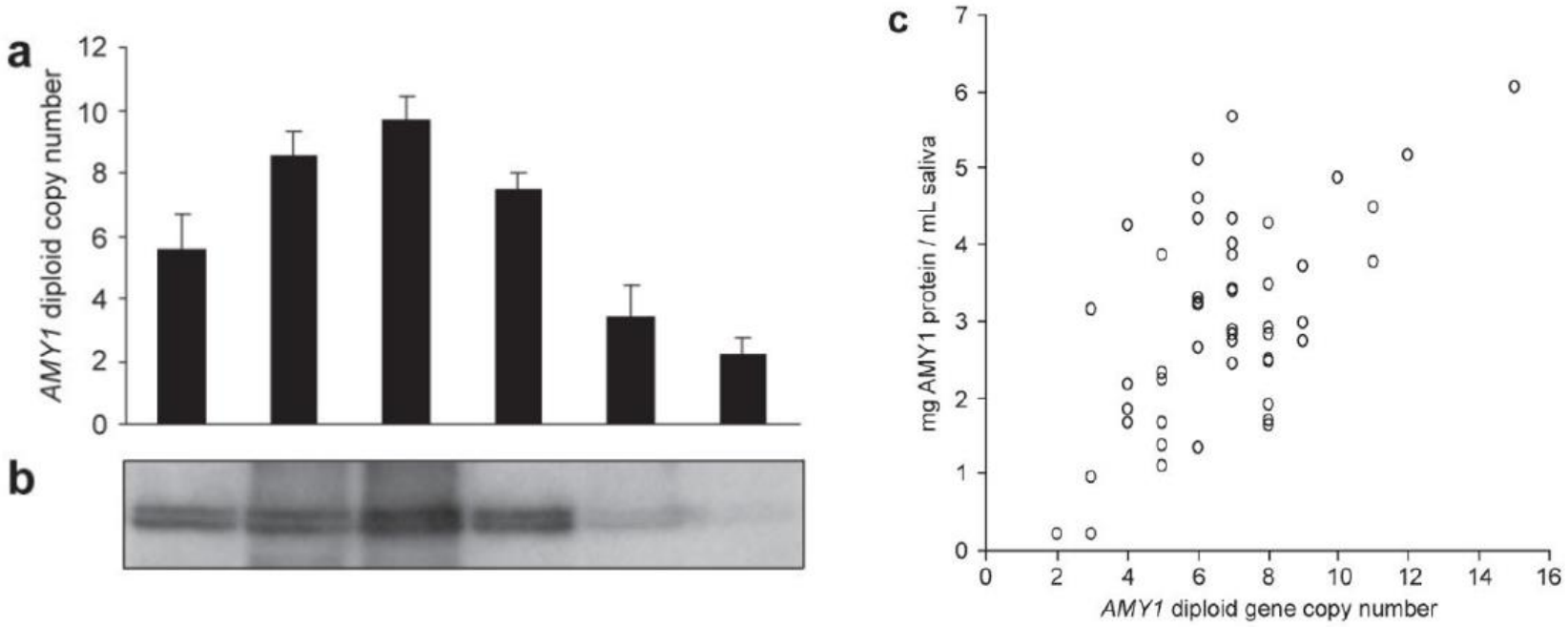
Starch, Amylase, and AMY1

- Starch consists of amylose and amylopectin.
- These are polymers, made of individual glucose units.
- They require enzymatic digestion.
- Almost half of the protein in human saliva is amylase.
- *AMY1* - the gene that encodes this enzyme shows large CNV in humans.



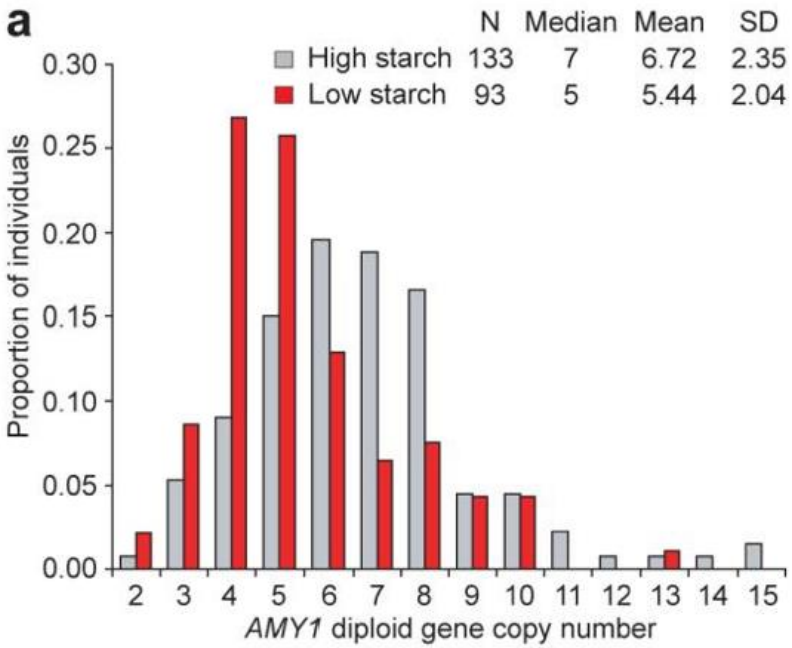


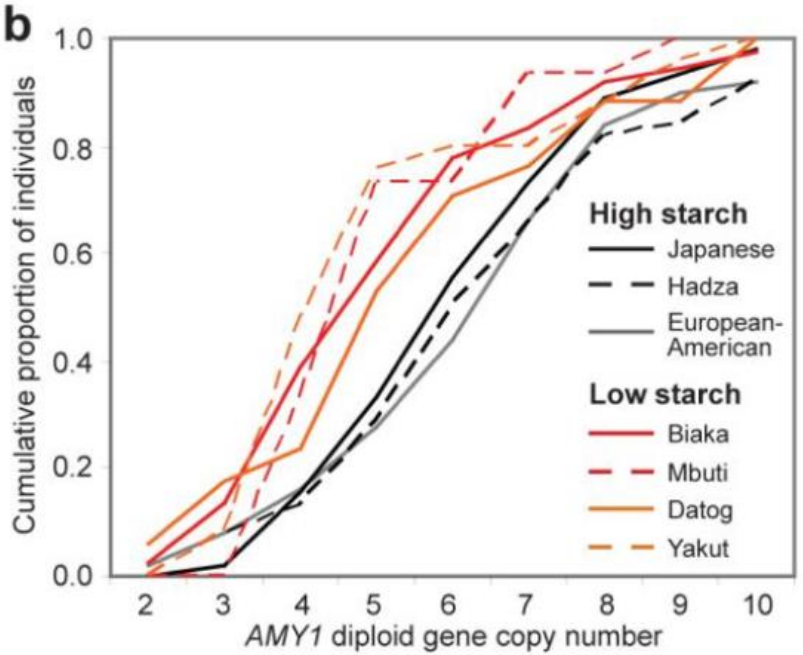
AMY1 CNVs and amylase content in 50 EAs



Perry et al. 2007. Nature Genet. 39(10):1256-1260

AMY1 CNVs and traditional diets



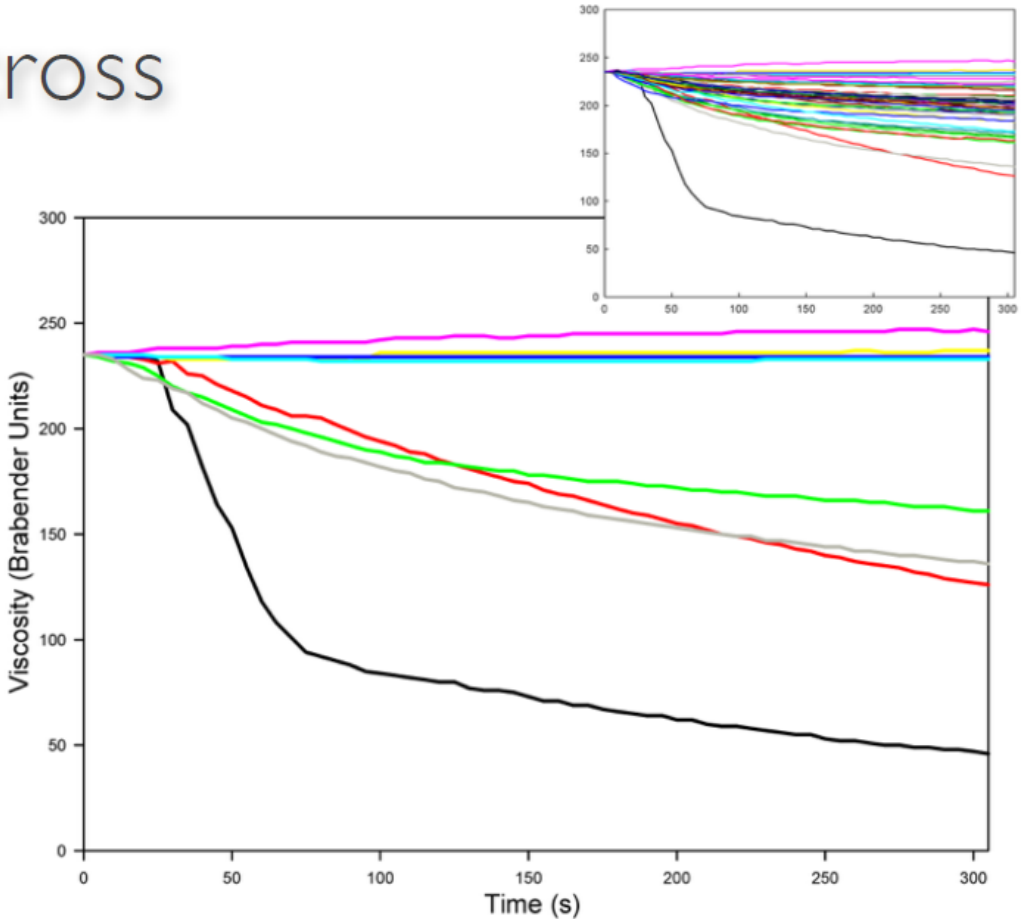


Perry et al. 2007. Nature Genet. 39(10):1256-1260

You are what your ancestors ate?

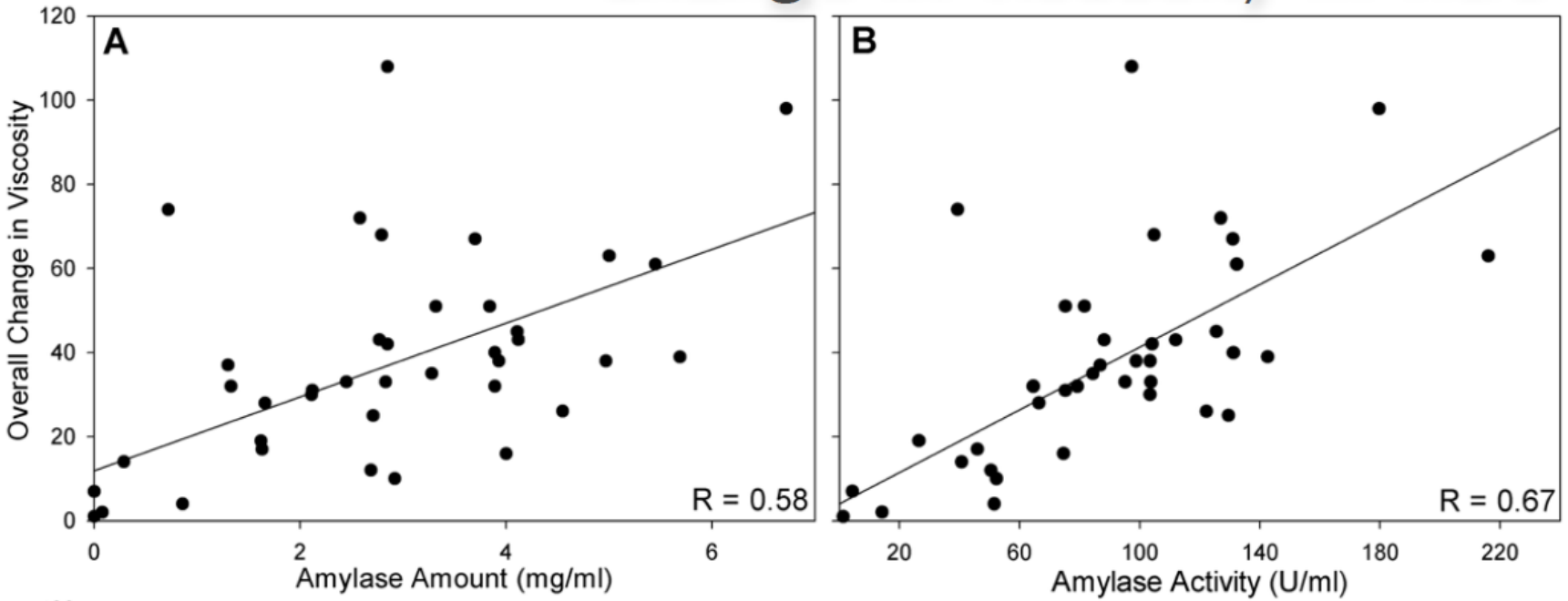
Large differences across people *in vitro*

- 100 ul of fresh saliva added to 100 g of gelled starch and tested in vitro using microviscoamylography.
- Graph shows 8 most different curves.
- All participants show lag.



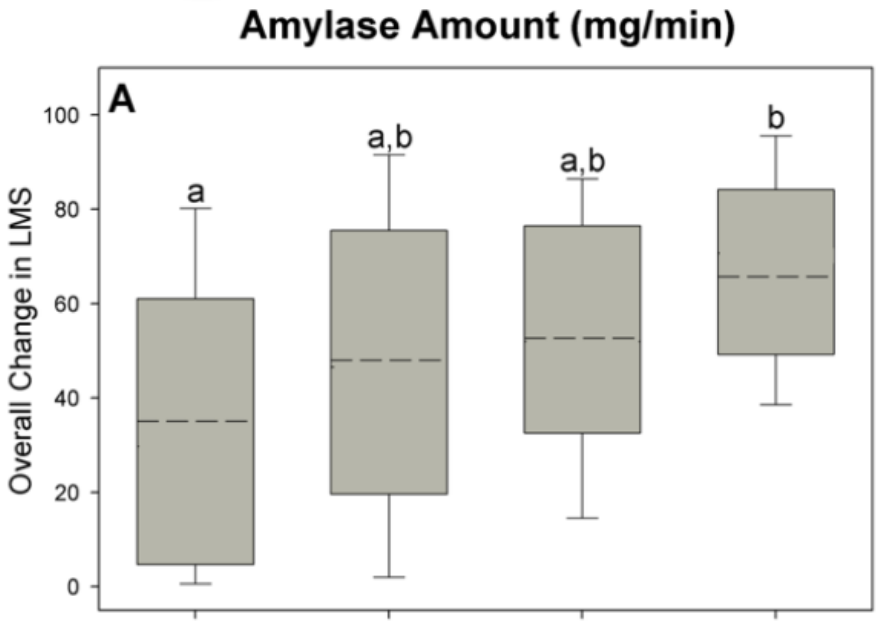
Mandel, des Gachons, et al. 2010. PLOS One.

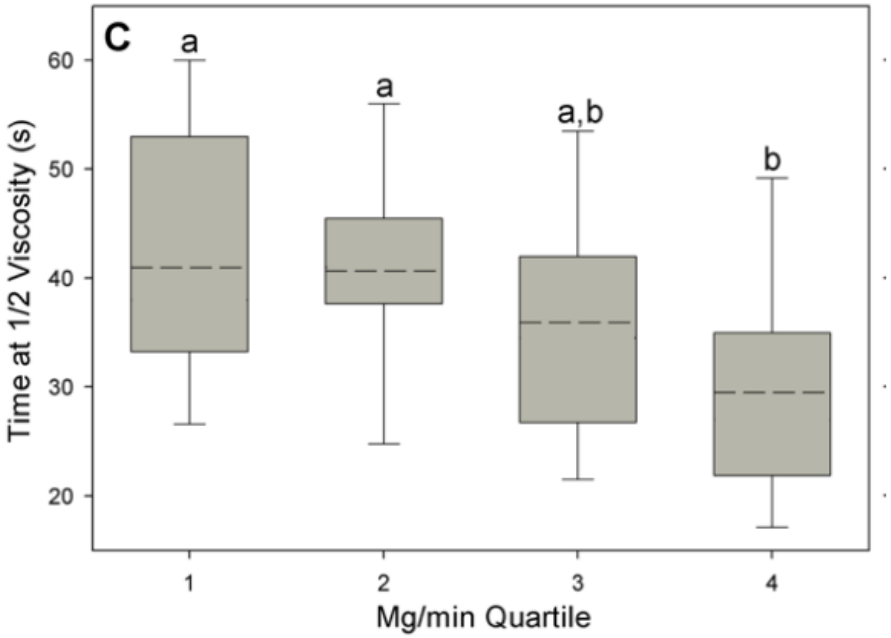
Amylase amount and activity both predict change in viscosity *in vitro*



Mandel, des Gachons, et al. 2010. PLOS One.

In vivo, more amylase predicts more overall change, and shorter time to half max

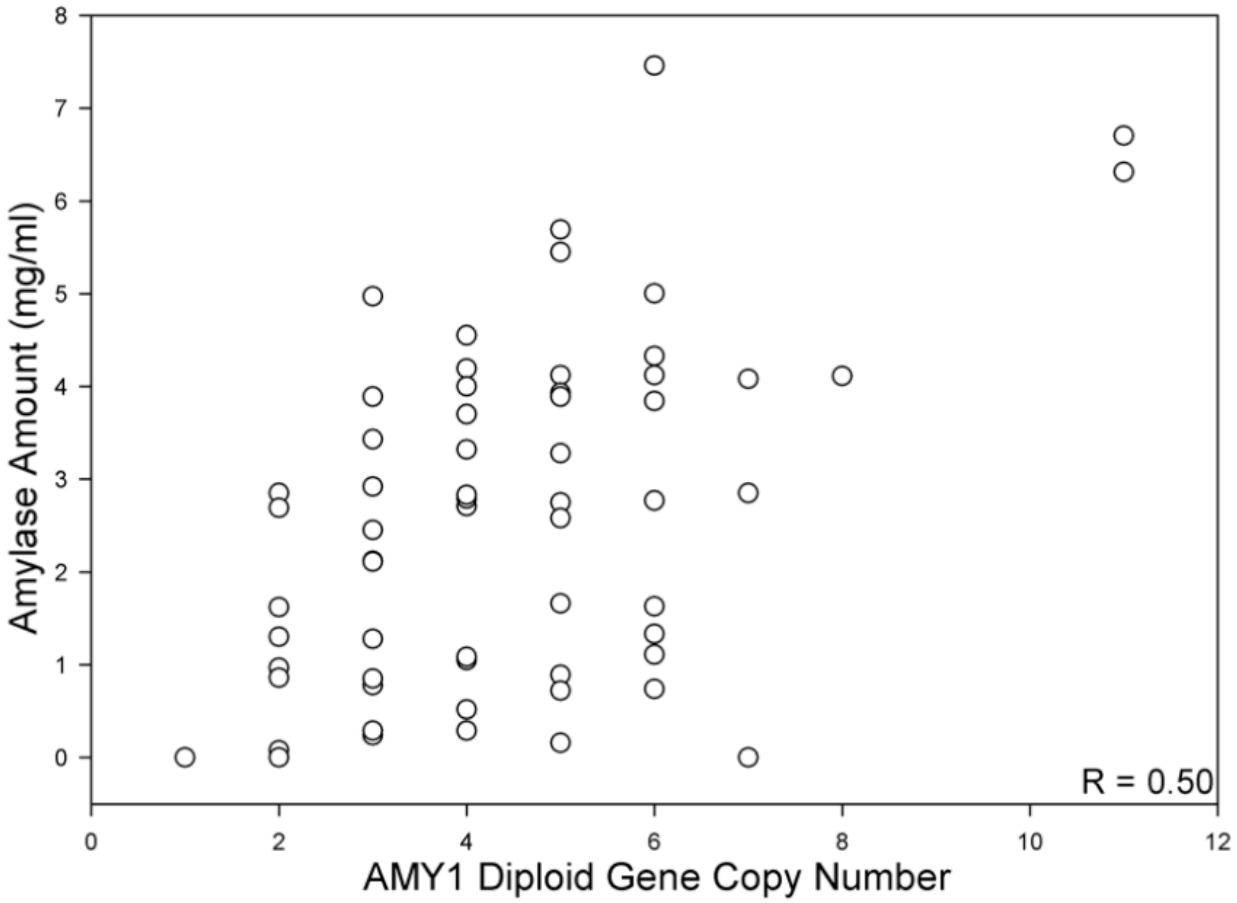




Mandel, des Gachons, et al. 2010. PLOS One.

AMY1 CNVs predict amylase amount

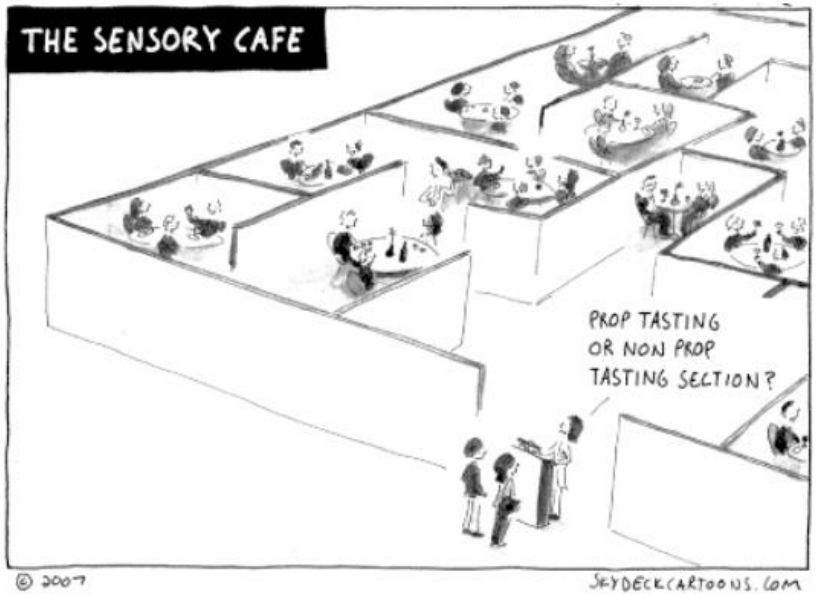
- replication = good
- but *AMY1* CNV did not predict viscosity *in vivo*



Mandel, des Gachons, et al. 2010. PLOS

Personalized genetics informs sensory science

Individual differences likely drive market segmentation



Variability across people is not a source of noise to be minimized, it is what is interesting.

Take home messages

1) There is a causal chain linking sensation to use, via liking.

2) Biological differences between people may influence this chain.

3) Bitterness is not a monolithic high or low trait.

4) Get used to caring about spit. It matters. Probably a lot.

4) Panelist training/alignment cannot make up for fundamental biological differences across people.

The Key Indicators of Descriptive Panelist Performance

Individual panelists are able to duplicate their findings

Panelists are calibrated with one another

Panel findings are reproducible over time

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Common Textural Descriptive Terms

Cohesiveness of mass

Moisture absorption

Dryness

Tooth packing

Mouth clearing

Stickiness

Amount of residue

Viscosity

Adhesiveness

Breakdown rate

Gumminess

- Many of these terms are not easily aligned among panelists
- **Foods with starch may be especially vulnerable to amylase activity**

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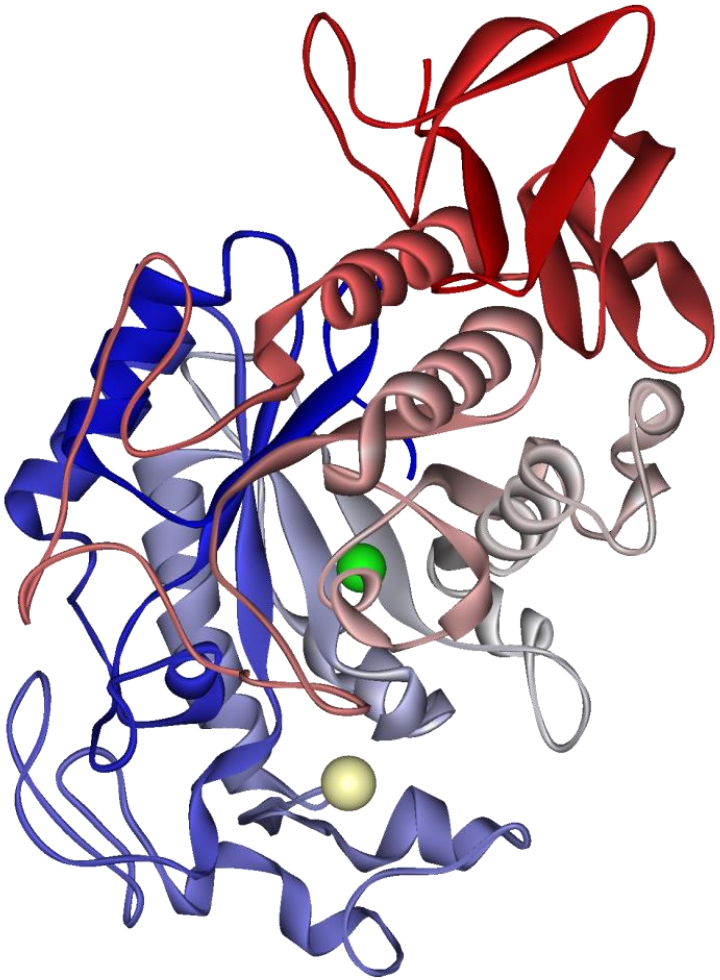
But Is Amylase Really Responsible?



Couldn't it also be affected by:

- Saliva dilution
- Thermal effects
- Thixotropic effects
- Matrix effects
- Flavor and acceptances

Ramasubbu, N.; Paloth, V.; Luo, Y.; Brayer, G. D.; Levine, M. J. (1996). "Structure of Human Salivary α-Amylase at 1.6 Å Resolution: Implications for its Role in the Oral Cavity". *Acta Crystallographica Section D*.**52** (3): 435–446.

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Remove the Confounds in Four Easy Steps

1. **Isolate saliva**
2. **Mix with model systems at fixed concentration**
3. **Measure viscosity over time with a Rapid Visco Analyzer (RVA)**
4. **Compare with appropriate controls and negative controls**
   a. Water
   b. Non-starch systems
   c. Alternate saliva sources

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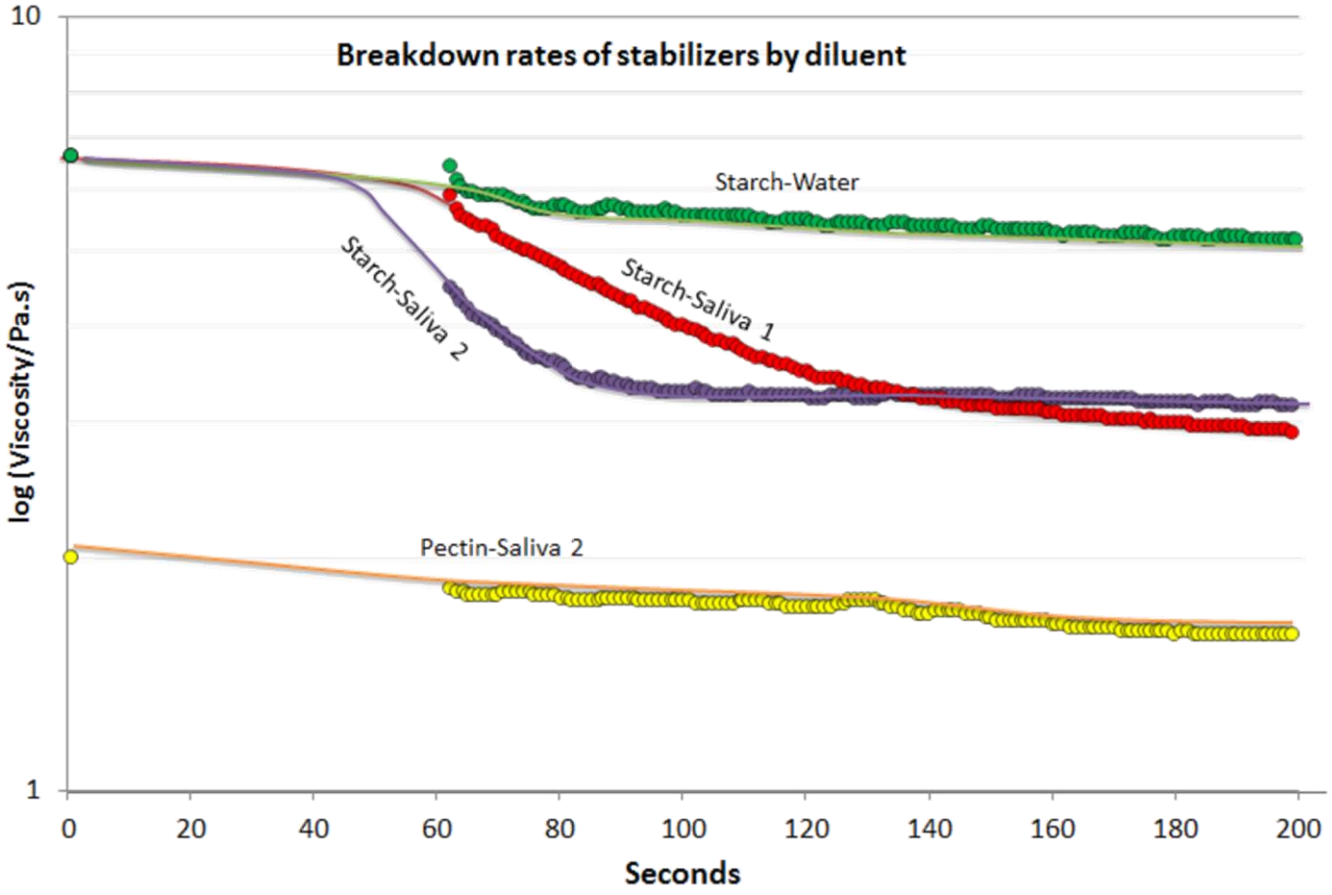
What Is Observed Instrumentally



These findings are revealed in sensory panels

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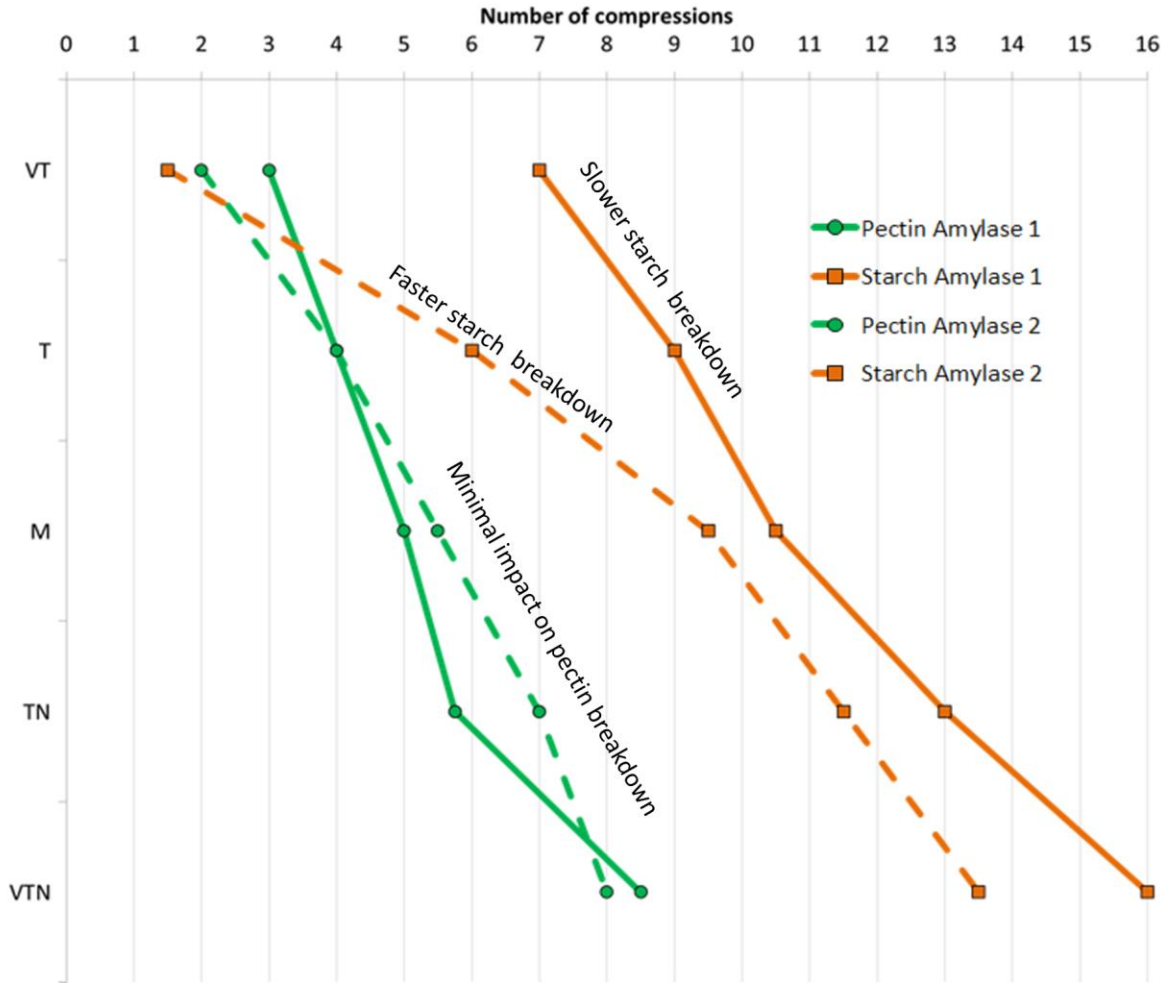
Investigating Breakdown During Oral Processing



No effect on pectin rates

Different rates of breakdown on starch

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Demonstration: Puddings

1. **Take a spoonful of pudding**

2. **Hold it in your mouth, masticating about once per second**

3. **Count the number of mastications for the pudding to**

   a) Make the first noticeable change in texture

   b) Become almost water like

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What is happening when 1g of saliva is added







Prior to saliva	~15 seconds	~45 seconds
• Firm peaks • Viscous	• Smooth peaks • Semi-viscous	• No peaks • Flowable

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Gone in 60 seconds



~60 seconds

- Material is completely liquefied

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Confounds of Amylase Activity

1. Individuals have different salivary rates
2. The number of copies of the AMY1 gene can range from 2-6, based on ethnography
3. Salivary flow rates are impacted by hunger, thirst, and liking
4. pH impacts optimal amylase activity
5. Amylase activity increases based on duration of sleep deprivation*
6. Age can impact amylase activity and salivary flow

* "First Biomarker for Human Sleepiness Identified", Record of Washington University in St. Louis, January 25, 2007

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Importance on Bolus Formation: Pretzels

1. Take 2 whole pretzels

2. Chew them in your mouth, masticating about once per second

3. Count the number of mastications for the pretzel to form a bolus

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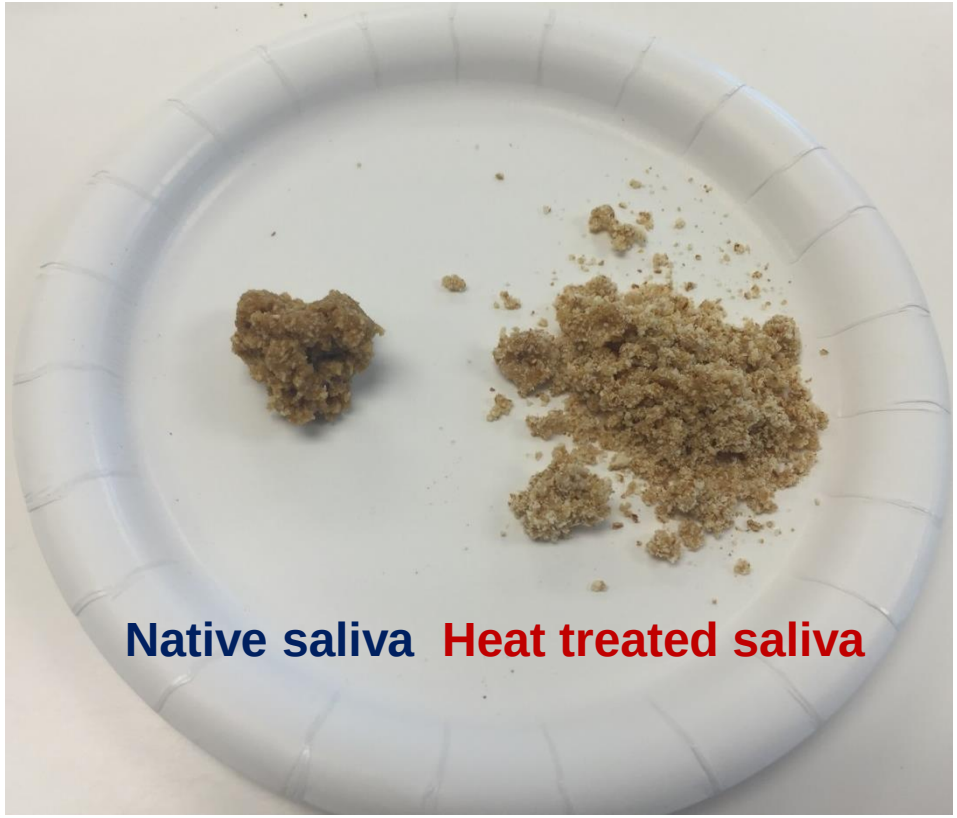
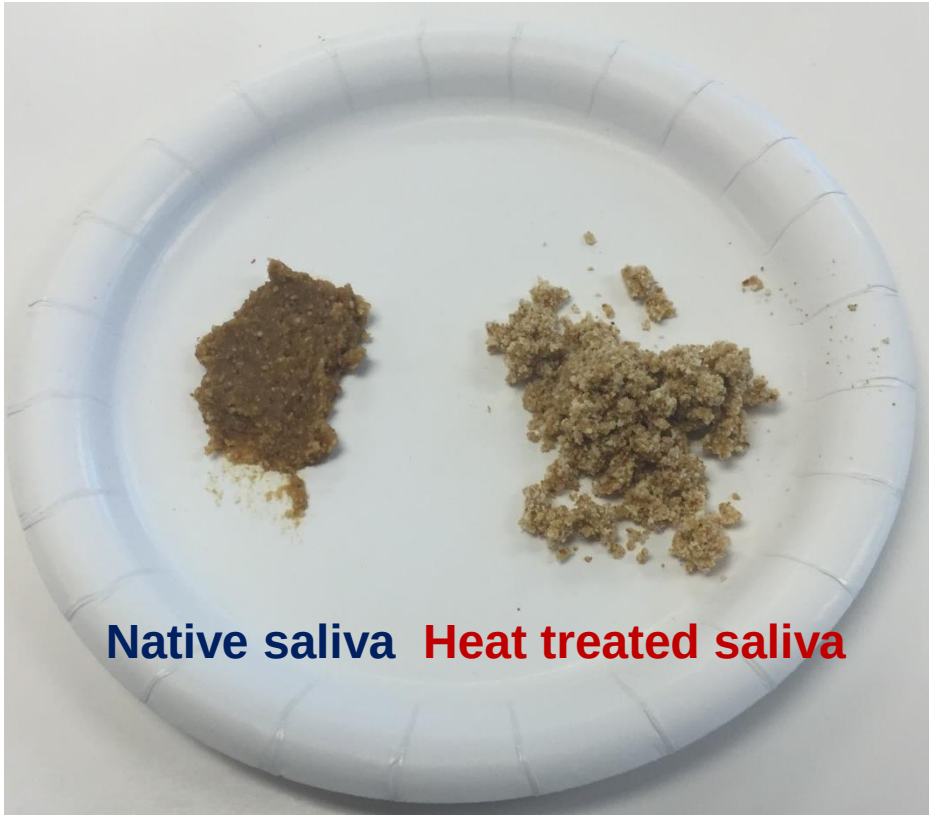
The Impact of Amylase Relative to Denatured Amylase

Compare 3g of pretzel mixed with 3g of native and heat treated saliva:



After 30 seconds of mixing



After 60 seconds of mixing

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Key Considerations Regarding Amylase

- One must consider how amylase activity impacts the perception of foods
- The longer the oral processing time of foods containing starch, the greater the impact of amylase on perception
- Amylase aids in bolus development and maintenance, creating oral processing conditions beyond what is possible with water alone
- As populations age, they will have increased incidence of dysphagia, making this phenomenon increasingly important for Baby Boomers and eventually Millennials
- Understanding how foods process can allow a product developer to create unique textural experience to meet emerging market needs

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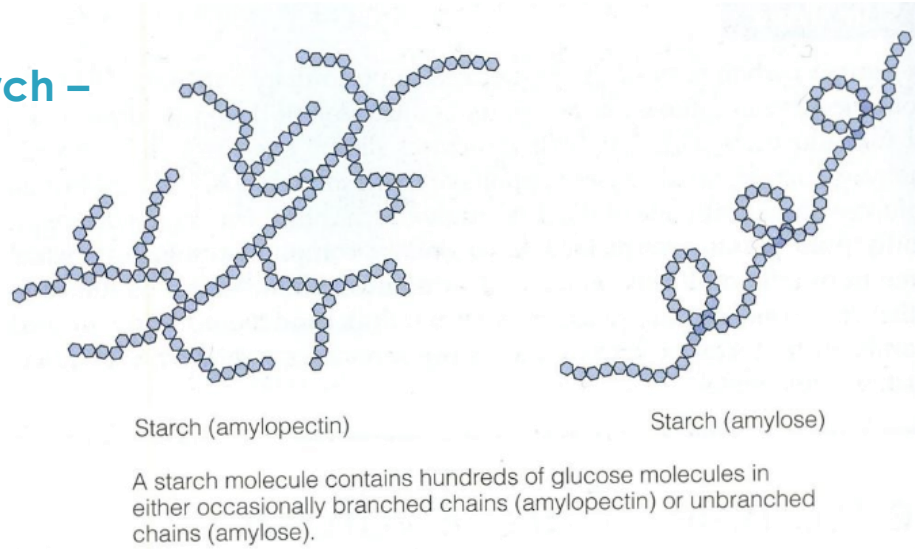
Culinary and Sensory applications with starches

Functional properties of Starch –



A starch molecule contains hundreds of glucose molecules in either occasionally branched chains (amylopectin) or unbranched chains (amylose).

Branched chain	Straight chain
Low temp (167° F)	Hi temp (212° F)
Derived from roots, tubers	Derived from grains
Glossy, thinner	Opaque, firm, sliceable
On freezing - holds	On freezing – breaks
On reheat - thins	On reheat - holds

Sources of Starch

The main commercially available starches:

Cornstarch, Tapioca, Wheat, Potato starch

To a lesser extent:

Rice, sweet potato, sago and mung bean, arrowroot

Available to the home user:	Modified Starch Blends:
Cornstarch	Base treated
Tapioca	Bleached
Wheat	Acetylated
Potato	Hydroxypropylated
Arrowroot	Pregelatinized

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Culinary Applications take advantage of known differences

Cherry pie

- Glossy sheen = amylopectin found in arrowroot or tapioca
- Prevent thinning on reheating = amylose found in cornstarch





Coconut Cream Pie

- Firm enough to cut = cornstarch, Opaqueness does not matter

Starch as a Hydrocolloid

What was once a "food science" view of starch (useful for water sequestration - safety) evolved into exploration for interesting culinary applications (molecular gastronomy)









Agar Agar	Calcium Salts	Carrageenan
Egg White Powder	Gelatin	Gellan Gum
Glycerin Flakes	Guar Gum	Isomalt
Kuzu Root Starch	Lecithin	Locust Bean Gum
Methylcellulose	Pectin	Pure-Cote B790
Sodium Alginate	Sodium Citrate	Sucrose Ester
Tapioca Maltodextrin	Transglutaminase	Ultra-Sperse
Ultra-Tex	Versawhip	

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Chefs solving Culinary Issues

The culinary applications of starch are focused on harnessing its functional properties to solve problems and/or to arrive at a desired texture-and-flavor experiences.





"For first time, we really have been able to take seriously that amazing connection between science and the food we eat. Before, we were in the dark. Today, I see light on the horizon. Today, we are far away more prepared." Jose Andres

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Thank you for joining us!

Questions or Comments?

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