Introduction to olfaction + Human olfactory genetics, genomics and Transcriptomics

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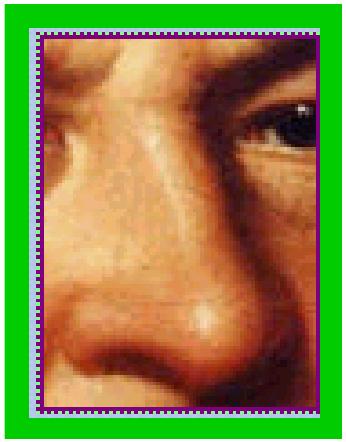
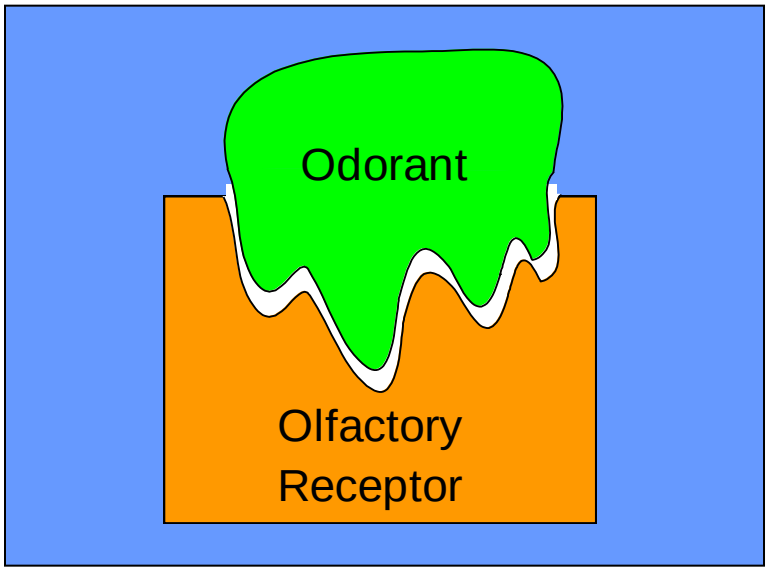
Smell (olfaction): a universal molecular recognition device





Olfaction:

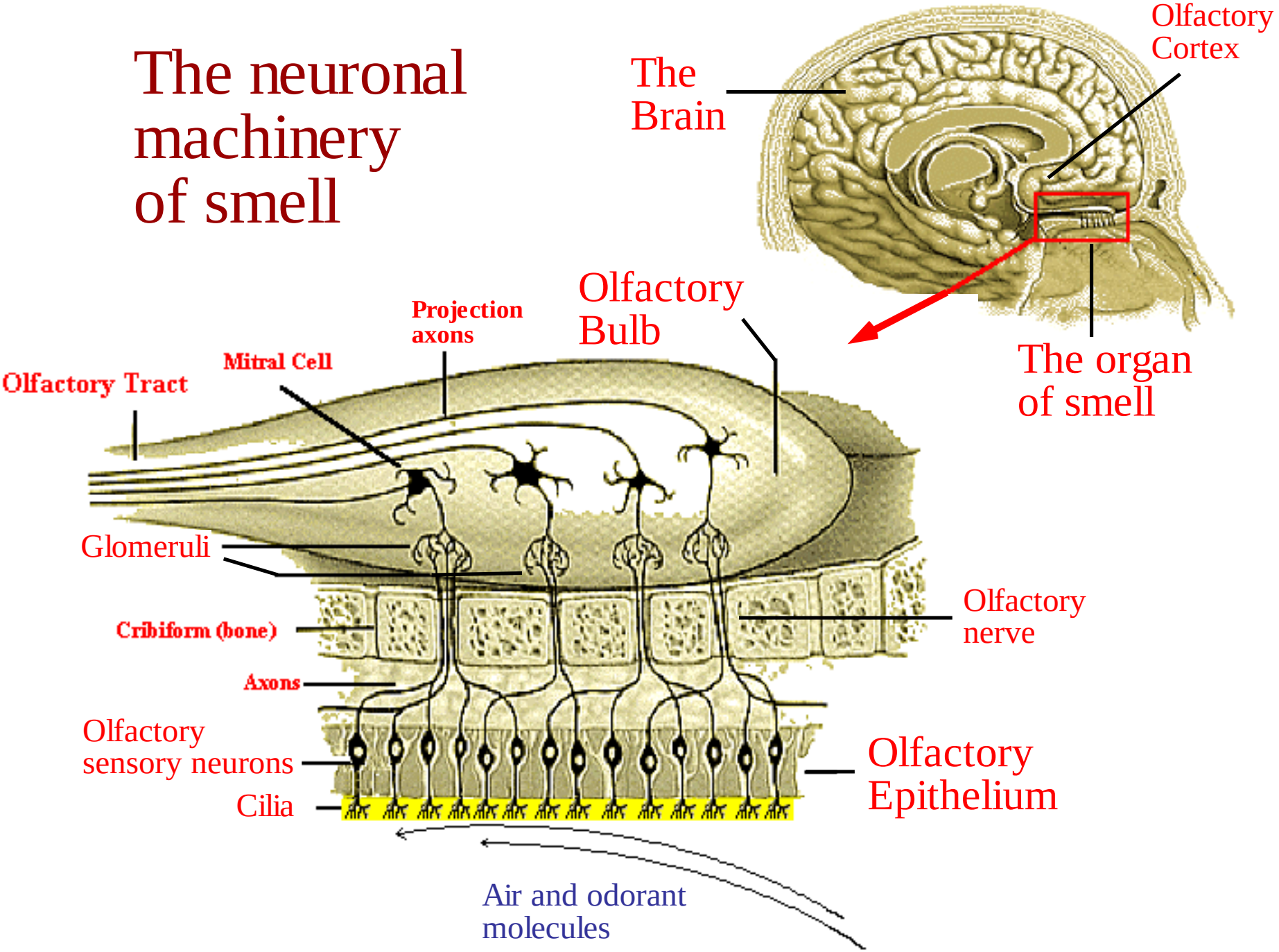
Sensitivity

Selectivity

Speed

Credit: Wysocki and Gilbert
National Geographic Magazine, 1986

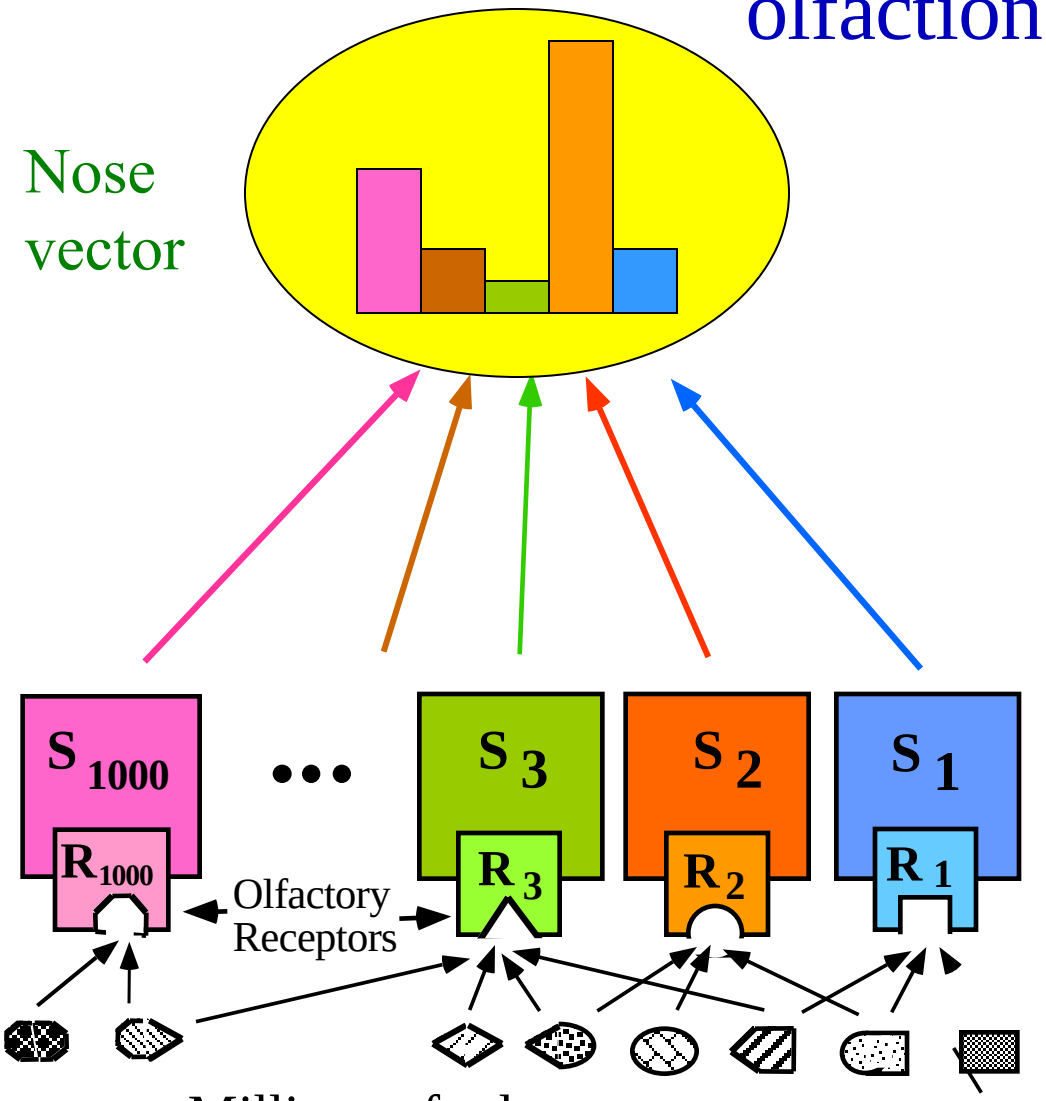
The neuronal machinery of smell



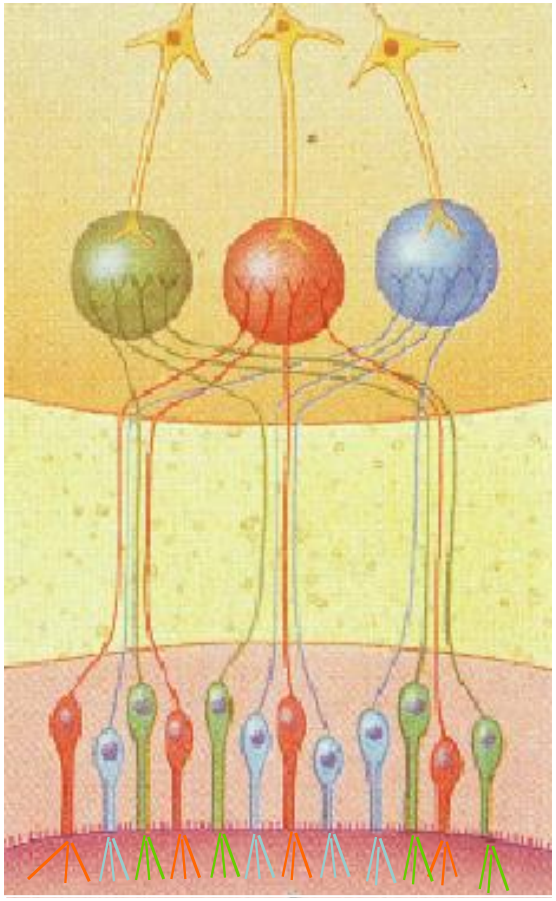
The logic of olfaction

Brain processing

Nose vector



Olfactory Bulb



Secondary Neurons

Glomeruli

Olfactory Nerve

Sensory Neurons

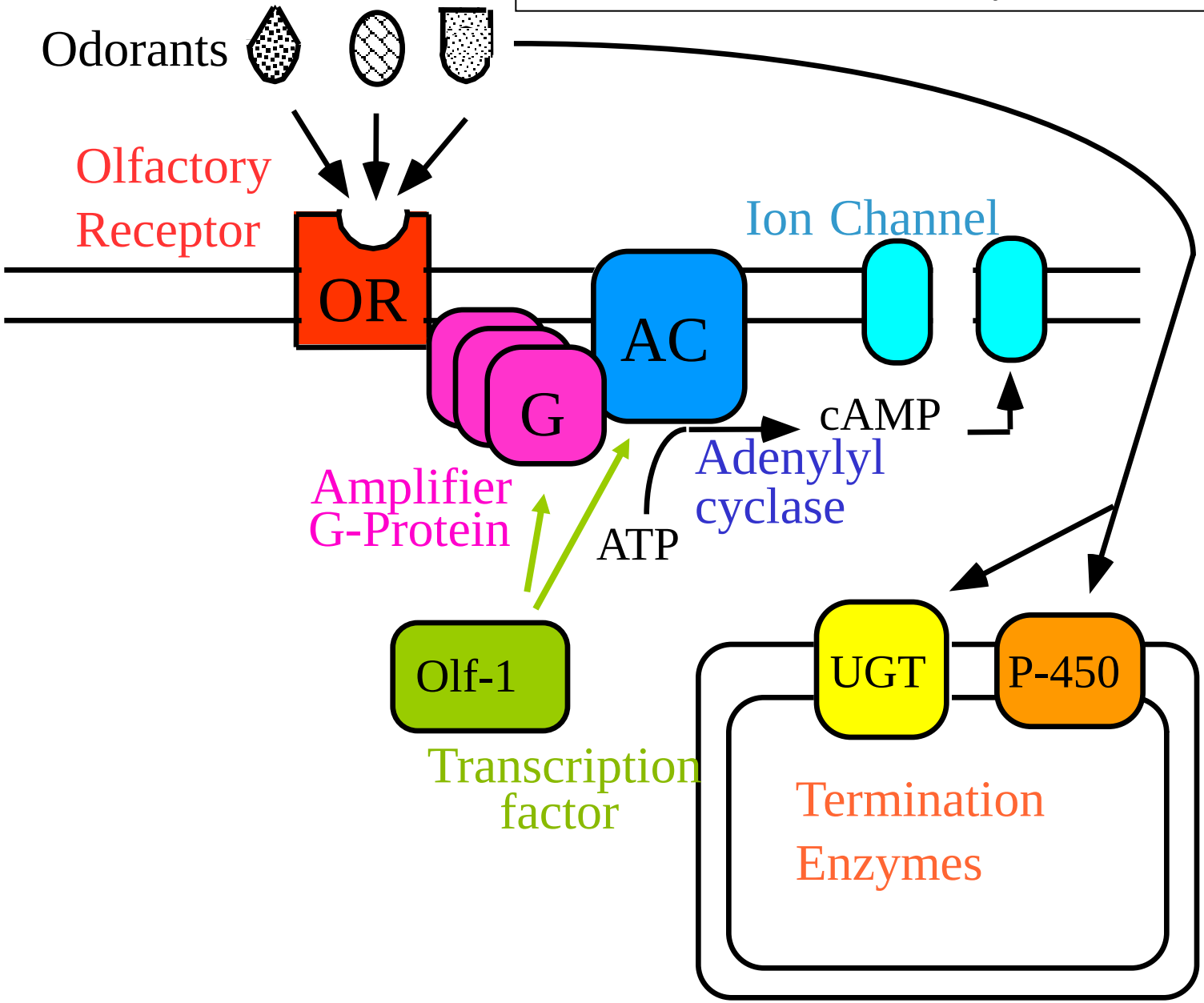
Cilia

Olfactory Epithelium

Early olfactory receptor (OR) hypotheses 1985-1990
(Prior to OR gene cloning, Buck and Axel 1991)

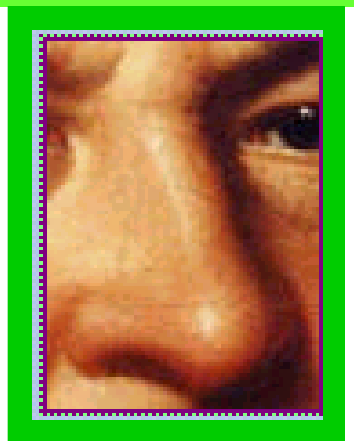
- Olfaction utilizes “run of the mill” protein receptors
- The receptors should be encoded by a large gene family
- Odorants are encoded as across-receptor patterns
- The receptors should be G-Protein-coupled, seven-helix
- Clonal exclusion (each sensory cell expresses only one OR type)
- A glomerulus is the target for same-OR axons

The Biochemistry of olfaction

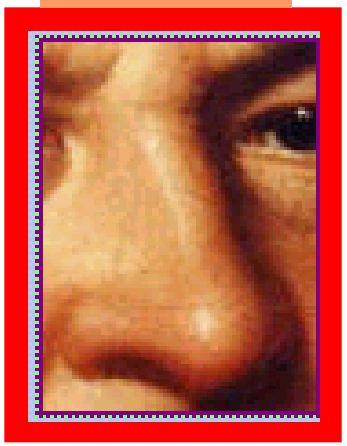


Olfactory genetic disfunction (smell blindness)

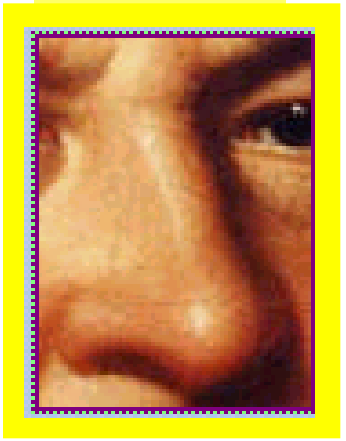
Normosmia
Does not exist!

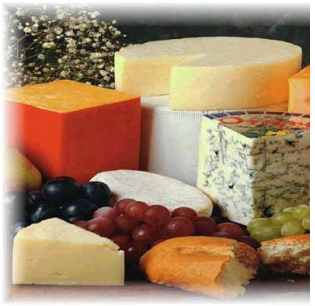


General
anosmia



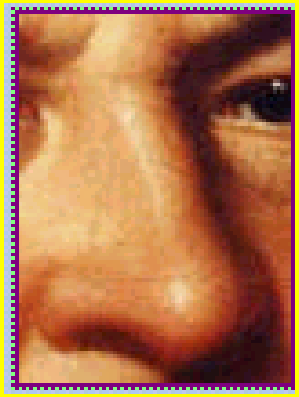
Specific
anosmia





Odorant-specific olfactory disfunction (smell blindness)

Specific anosmia



Everey person in this room is affected!

John Amoore: Specific anosmia is prevalent!

Eugenol

β-Ionone

Tri-methyl amine

Hydrogen cyanide

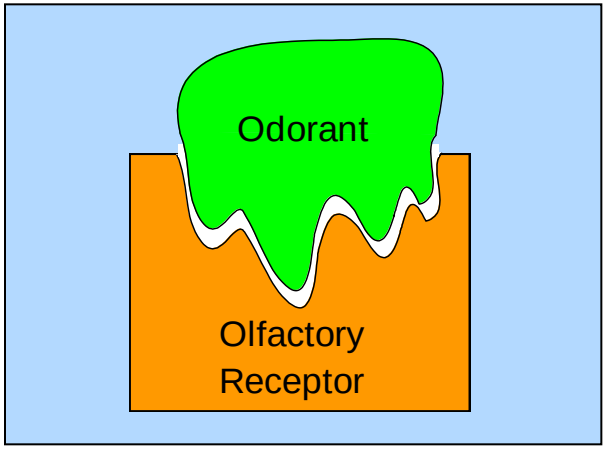
Dihydromyrcenol

Many more…

Typical prevalence:
1-10%

Typical threshold decrement:
1-2 decimal log units

Early evidence for the existence of stereo-specific receptors in olfaction

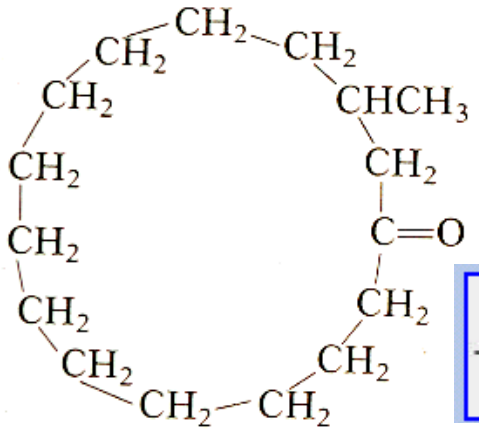


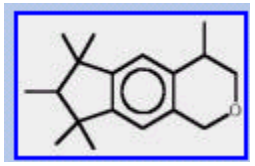
Animal pheromone

Human Anosmia

Musk deer

Musk



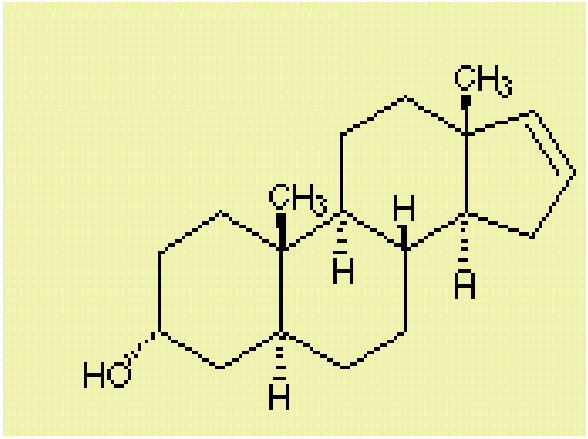


~10% no detection

~90% pleasant

Boar (wild male pig)

Androstenone

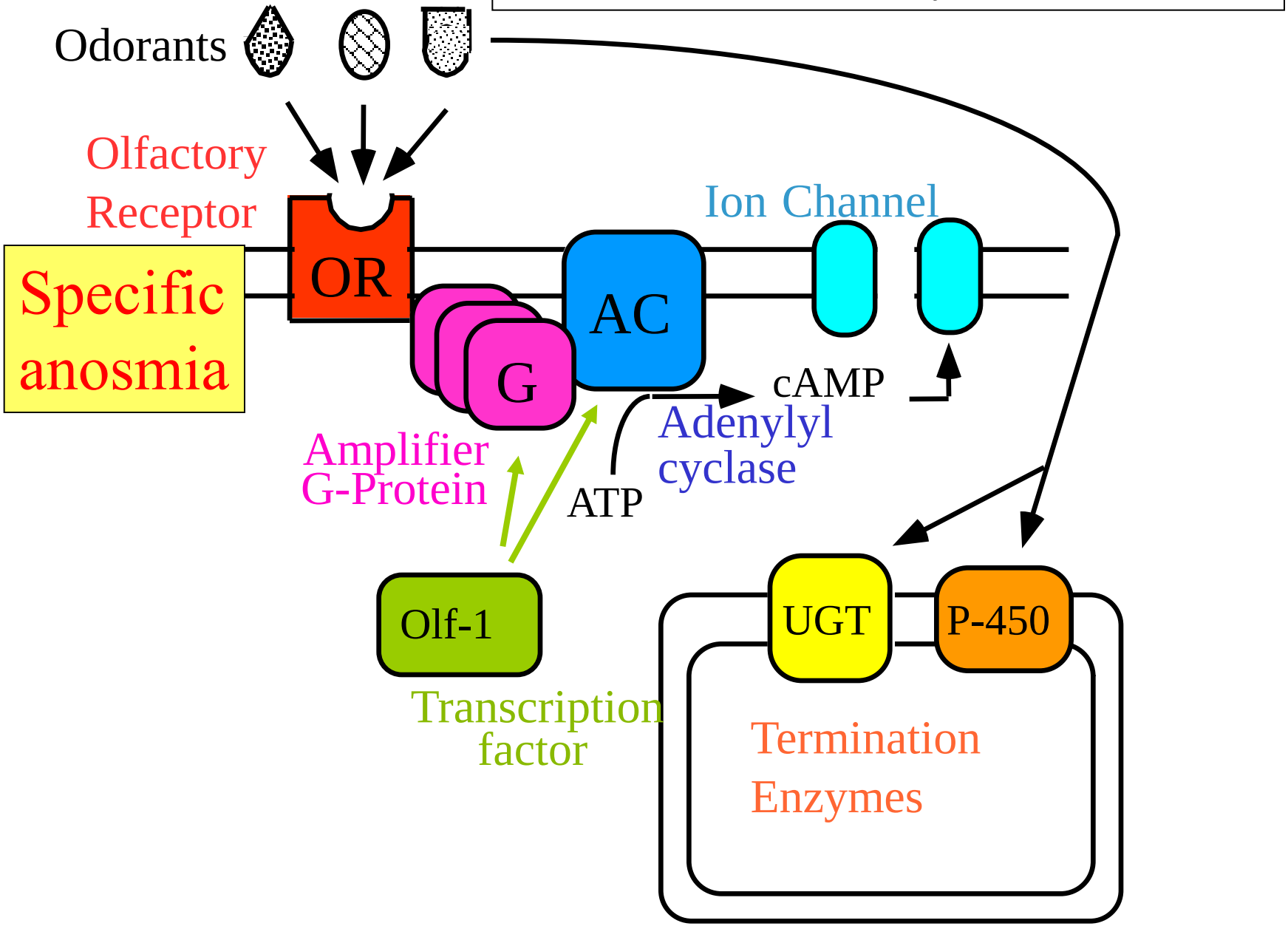


~30% no detection

~40% weak & pleasant

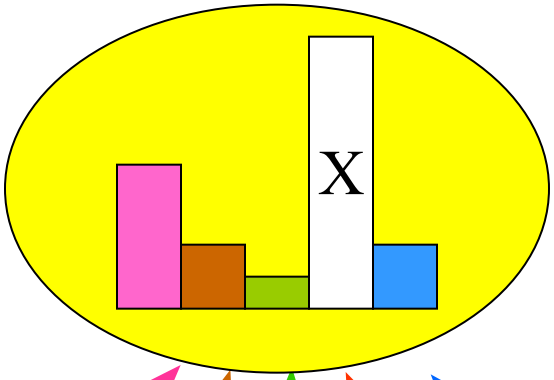
~30% strong & foul

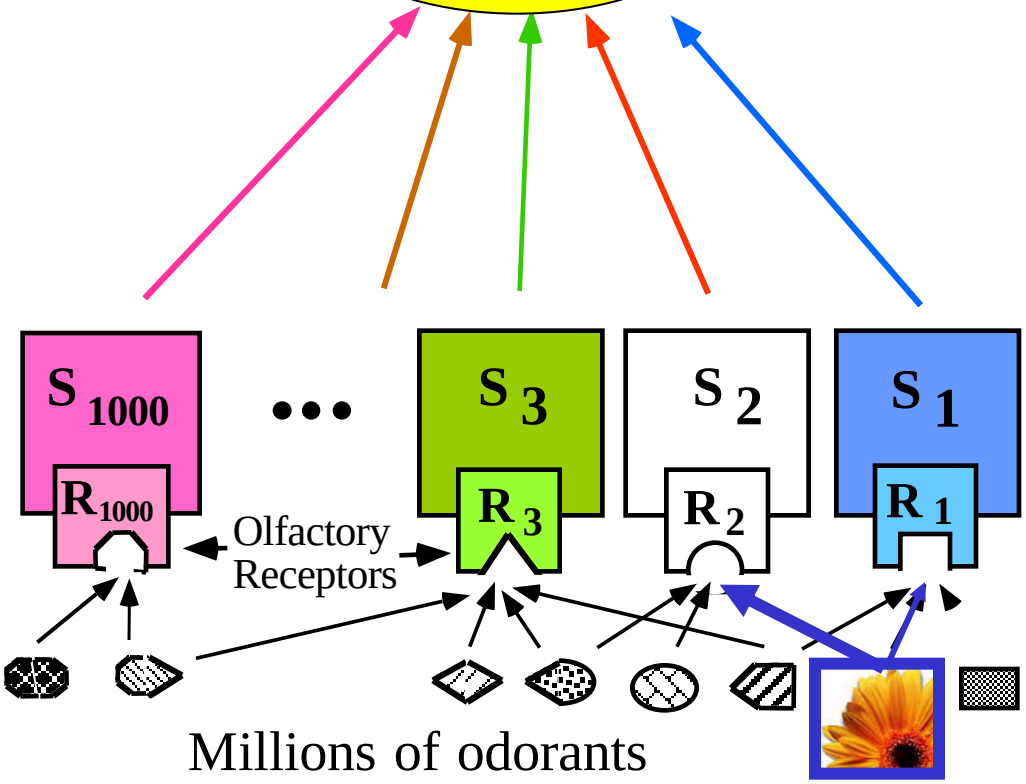
The Biochemistry of olfaction



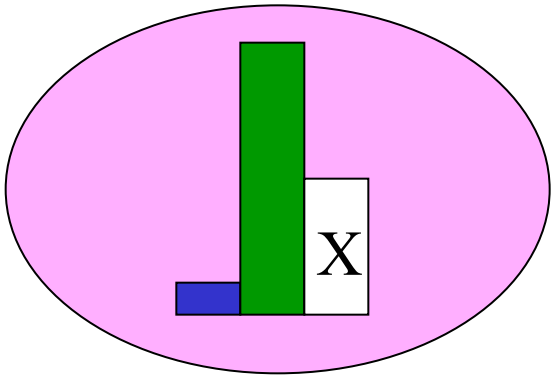
Smell blindness
- Specific anosmia

Nose Vector = Odor quality

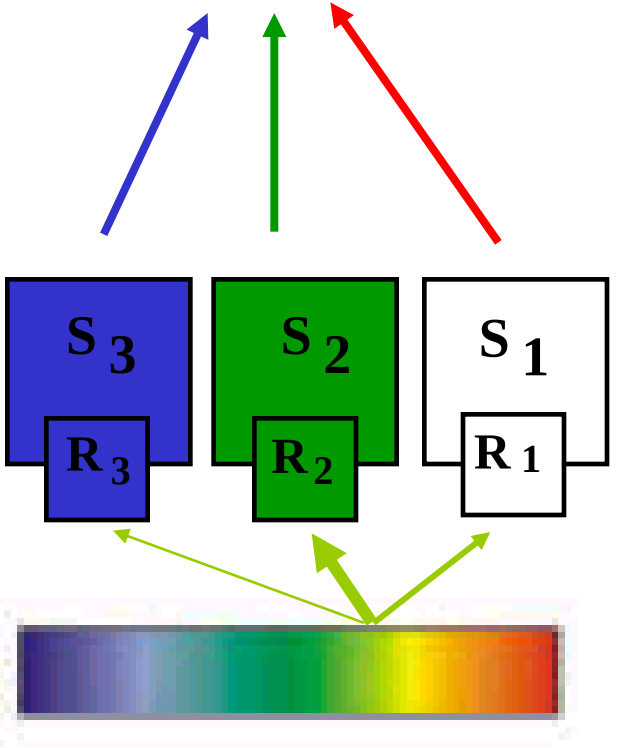




Color blindness

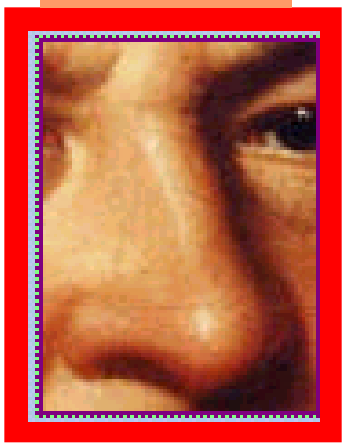


Eye Vector = Hue



Congenital General Anosmia (innate smell blindness)

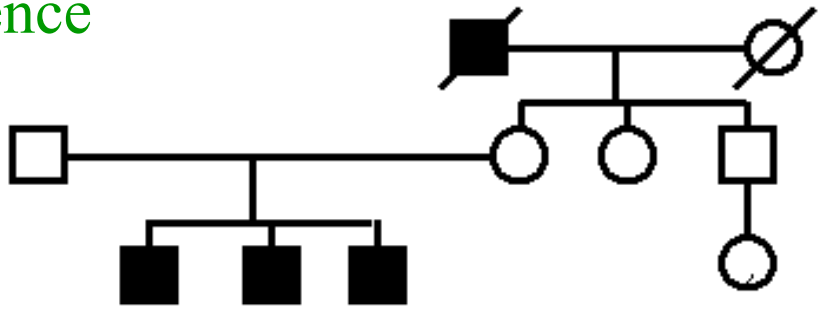
General anosmia



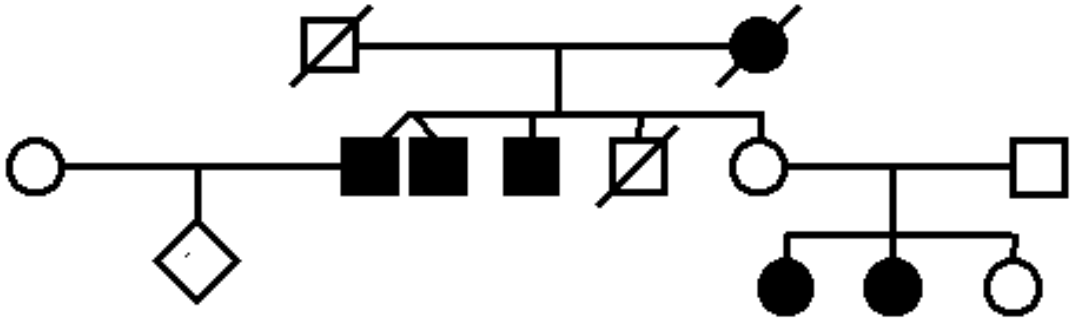
Congenital General Anosmia (CGA)

~1/10,000 estimated incidence

	Male	Female
Normal	□	○
Affected	■	●



Family A001 - Ashkenazi origin

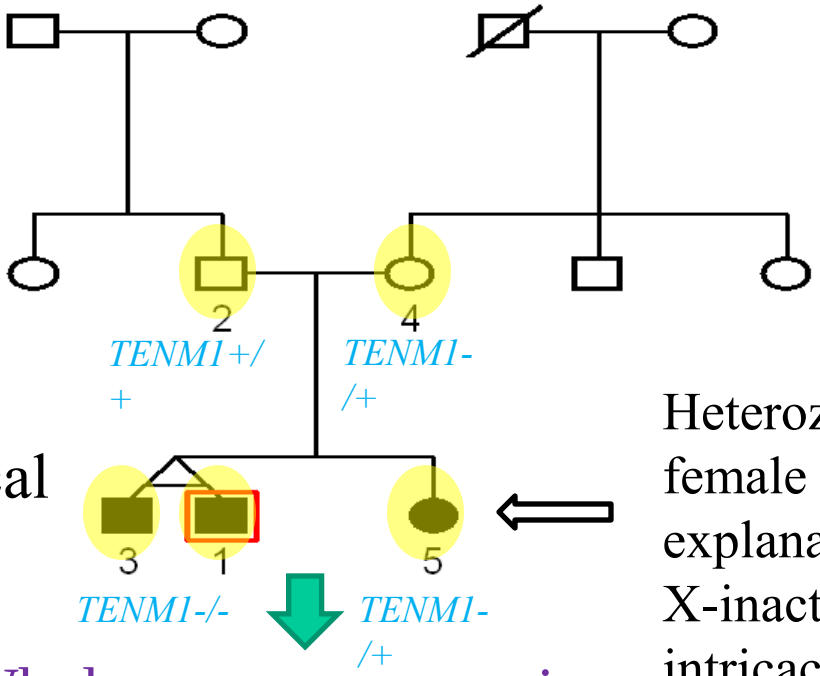


Family A002 - Greek origin

Total: 162 CGA subjects

62 familial, in 24 families

Family A-230 presumed recessive inheritance
Ashkenazi/Yemenite



Identical twins

Heterozygous affected female requires explanation by X-inactivation intricacies

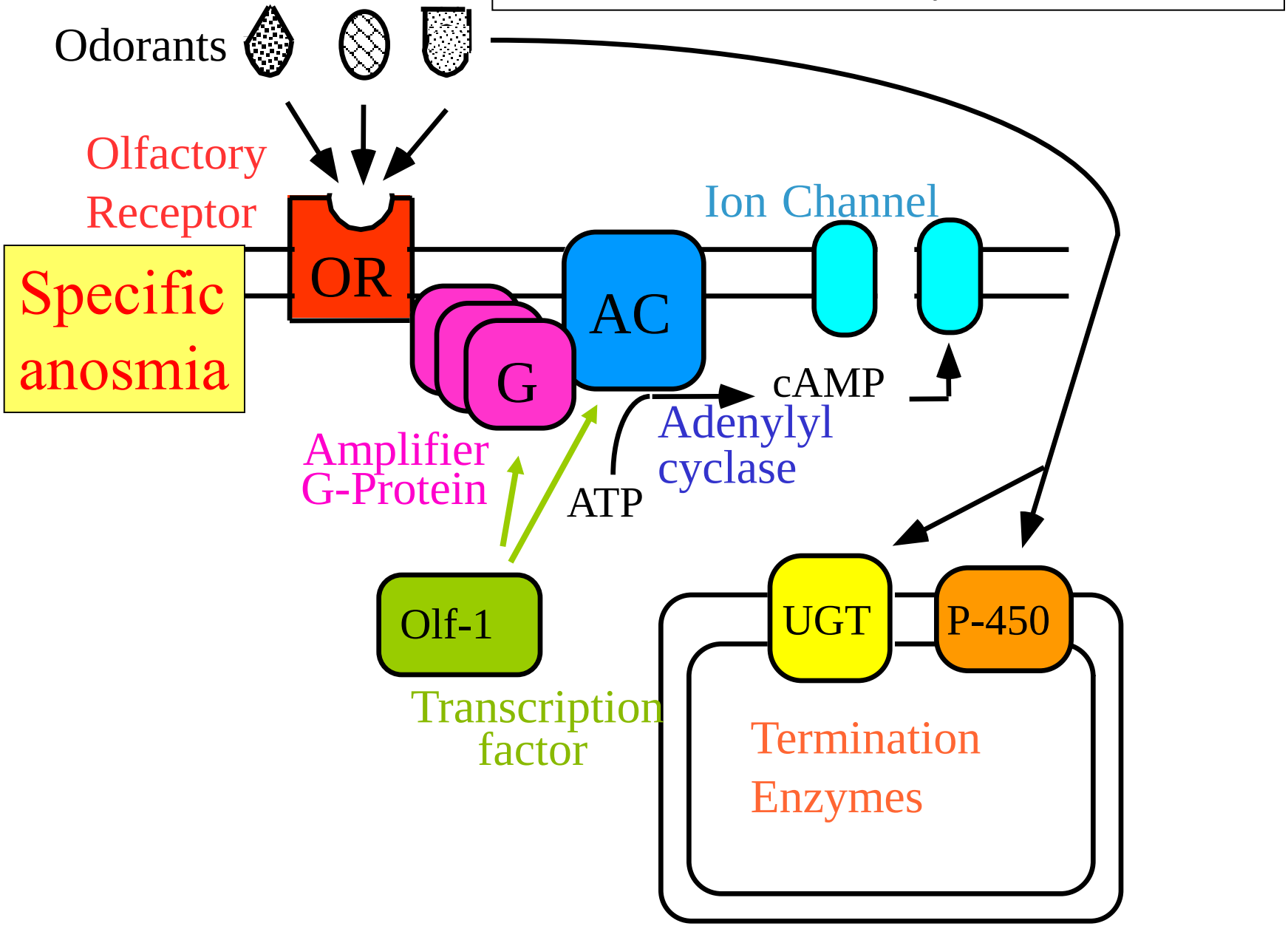
Whole exome sequencing

nonsynonymous deleterious variant in *TENM1*

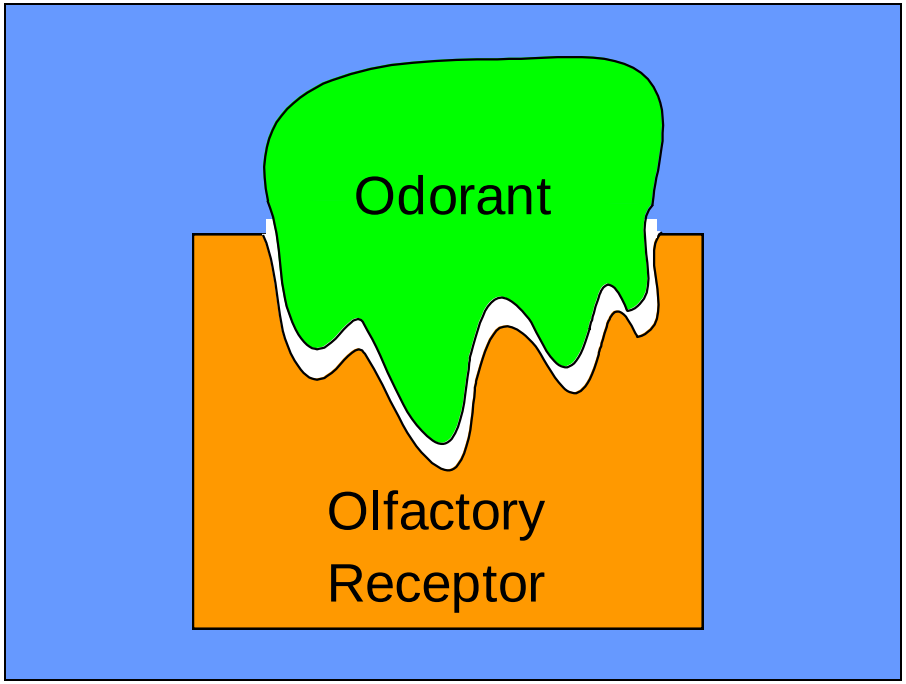
gene on chrX

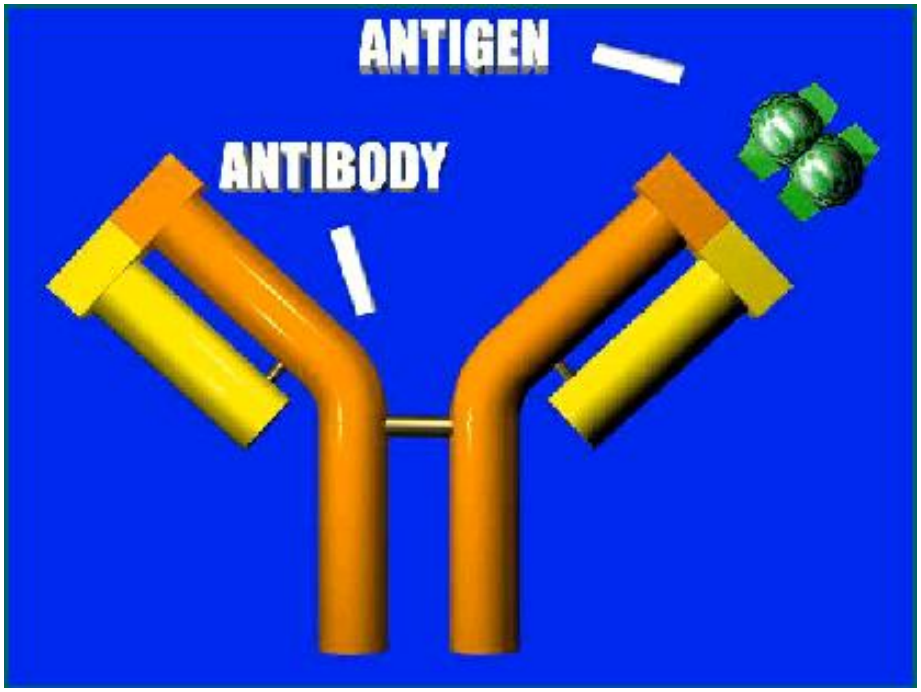
Known to play a role in neuronal connectivity

The Biochemistry of olfaction

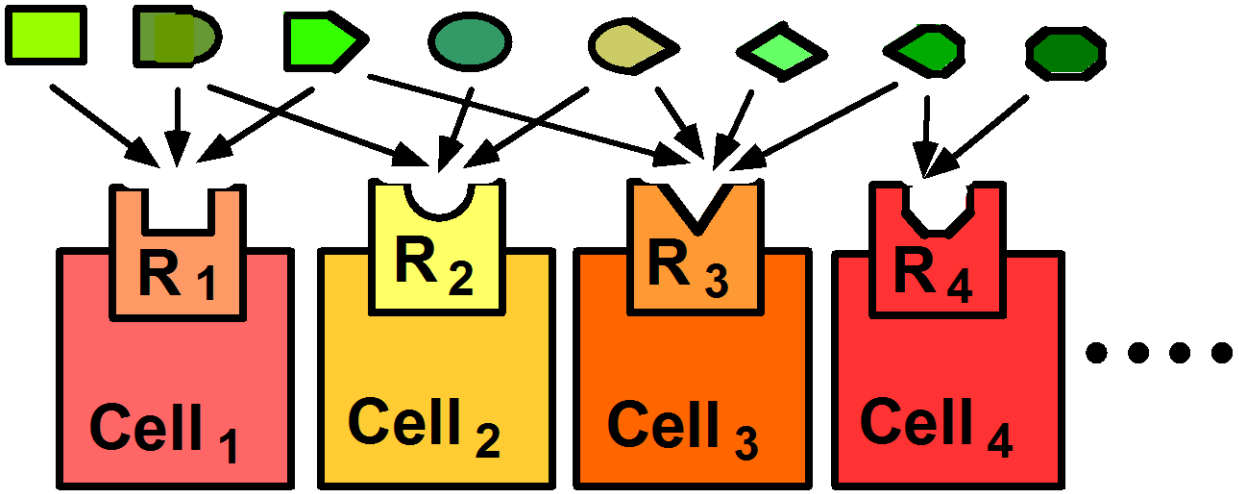


A hypothesis: olfaction is like immunity





Chance governs the binding



Probabalistic receptor repertoires

Immunoglobulins

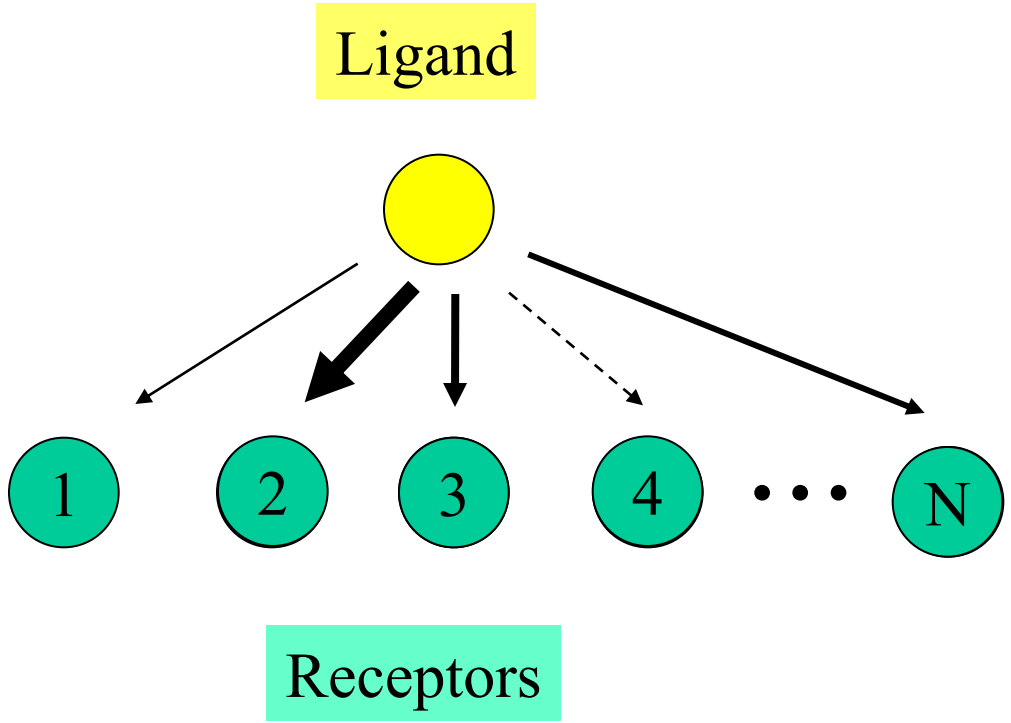
T-Cell receptors

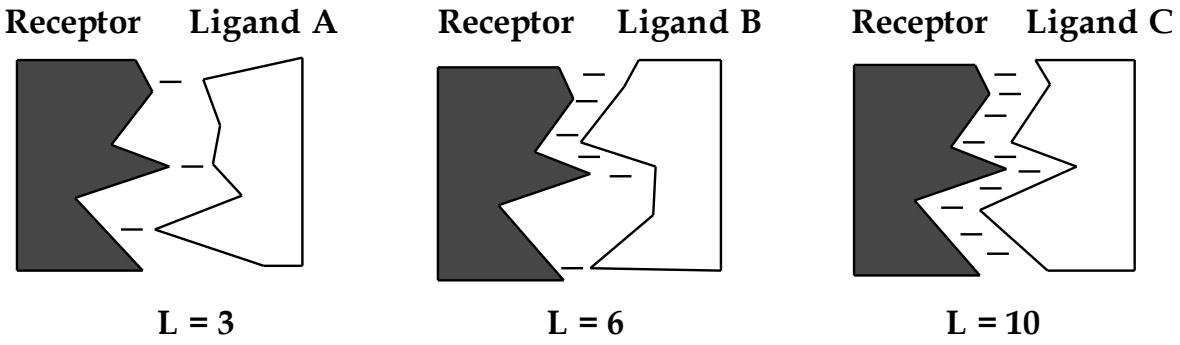
MHC proteins

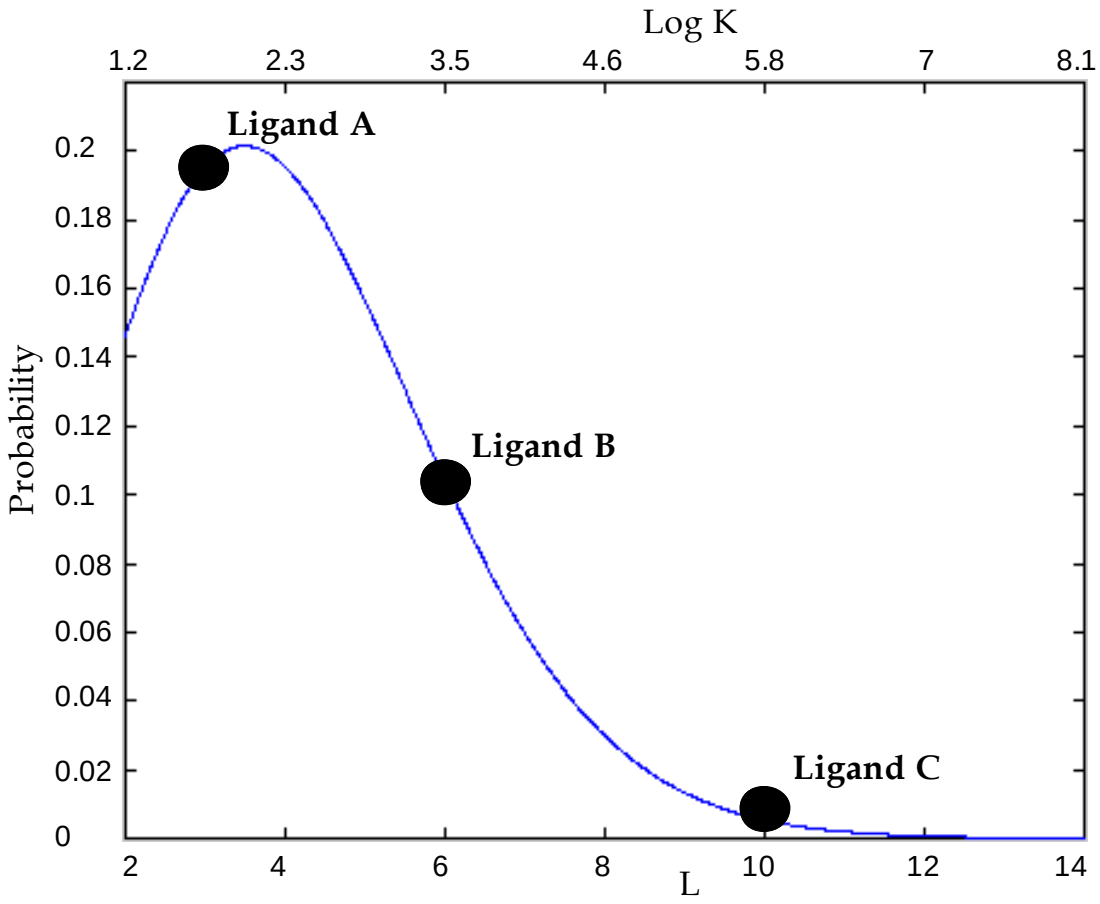
Cytochromes P-450

Olfactory receptors

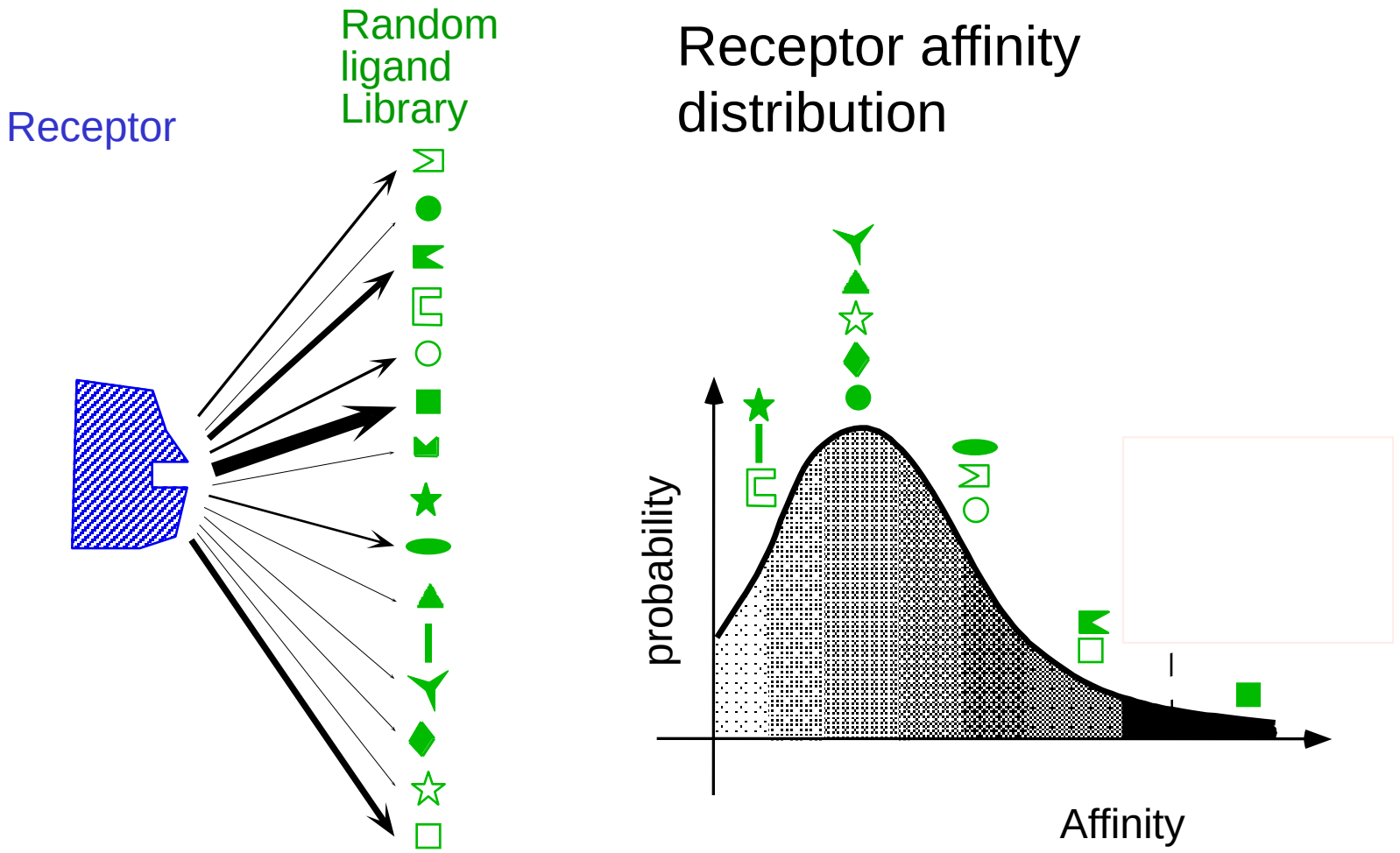
Taste receptors







Probability of different degrees of stereospecific fit


Receptor
Random ligand Library
Receptor affinity distribution
probability
Affinity

Receptor Affinity Distribution (RAD) model:

The binomial distribution

Receptor

4	**5**	**1**
3	1	2
2	2	**3**

$\bar{r}$

B=9

S=5

L=4

Ligand (Hapten)

3	**5**	**1**
4	2	3
2	1	**3**

$\bar{h}$

B = number of subsites

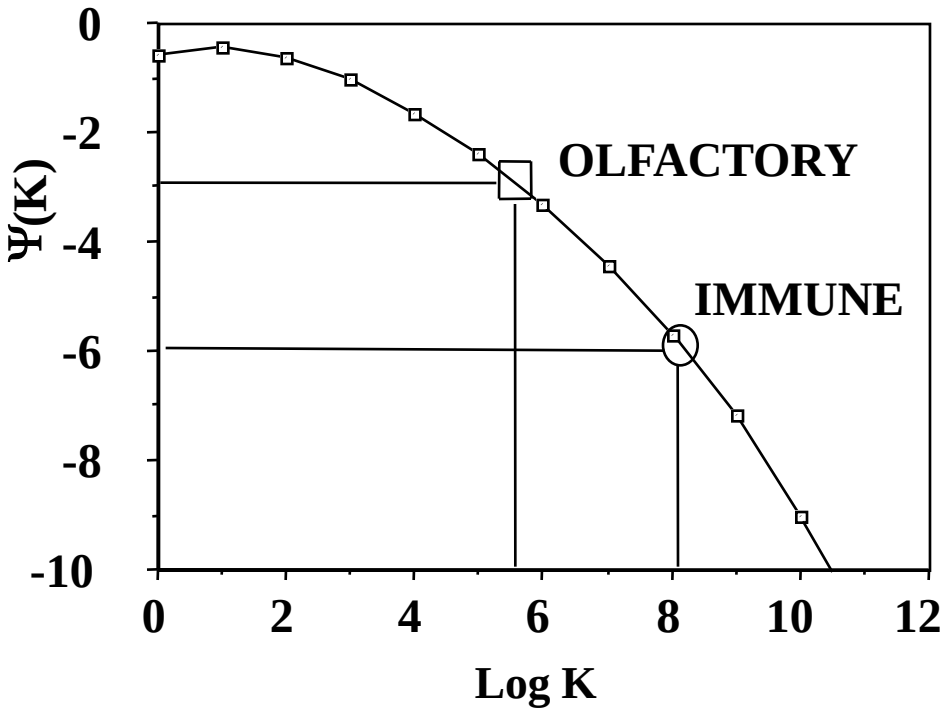
S = Subsite diversity ("alphabet" size)

$$P(L) = \frac{B!}{L!(B-L)!}\left(\frac{1}{S}\right)^{L}\left(1-\frac{1}{S}\right)^{B-L}$$

$$\text{Binom}(1/S, B, L); \quad L=0,\ldots,B$$

Consequences of the RAD model:

Relationship between affinity and repertoire size N



In general, $N \cong 1 / \Psi (K_{max})$

$K_{max}^{olf} \cong 10^5 \leftrightarrow N_{olf} \cong 1000$

$K_{max}^{imm} \cong 10^8 \leftrightarrow N_{imm} \cong 10^6$

Seidemann…Lancet PNAS 1993

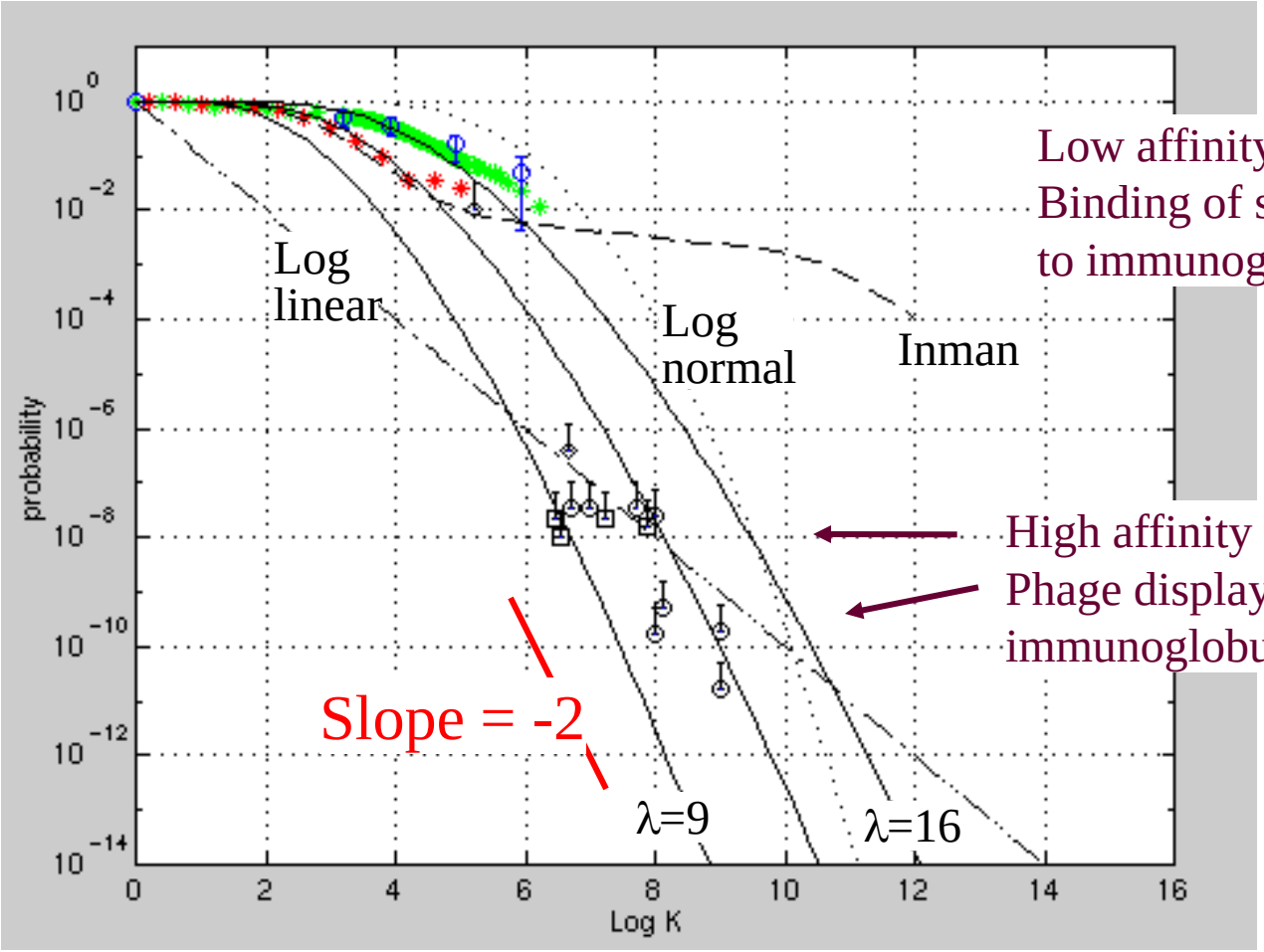
Test of the Poisson approximation of RAD statistics for recognition in biological repertoires

$$\Psi(L) = \frac{\lambda^{L}}{L!} \cdot e^{-\lambda}$$

Inman (1988)

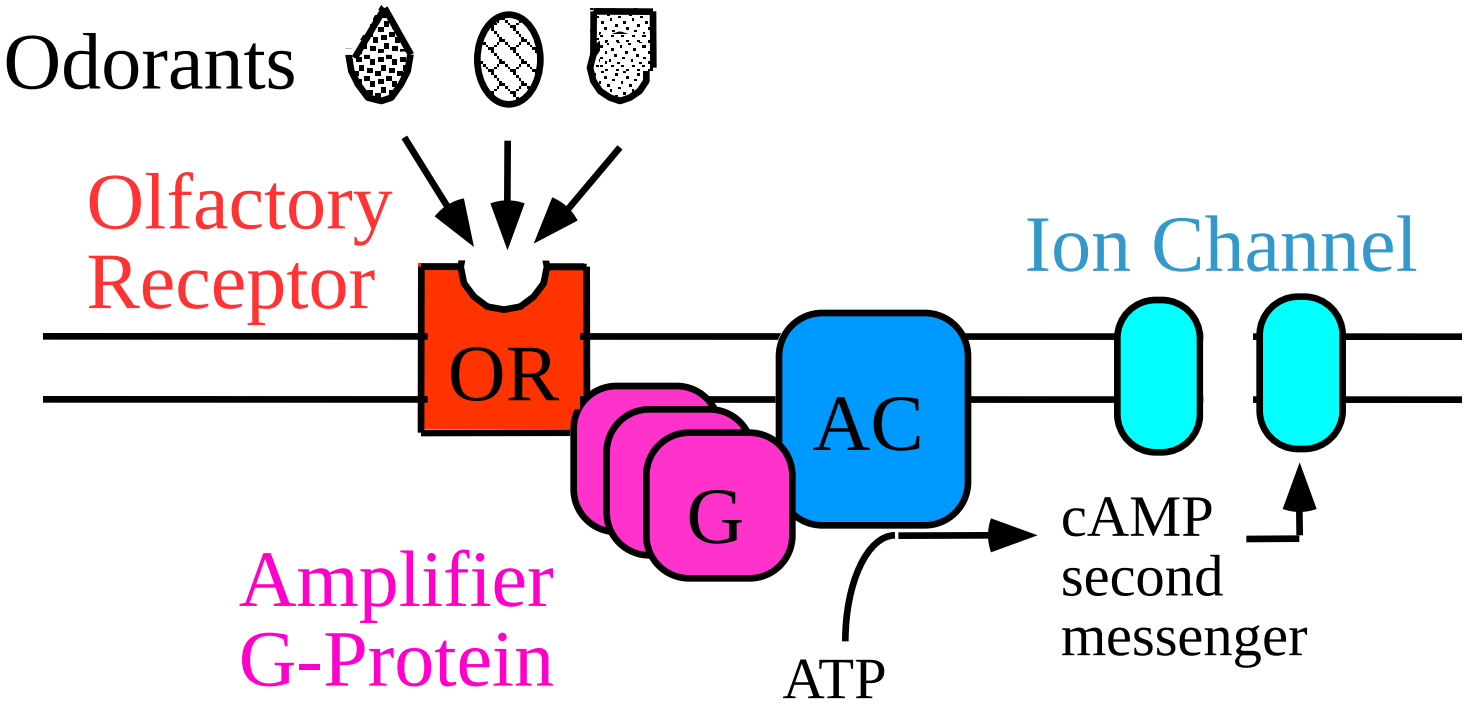
Varga (1991)

Kauvar (1995)



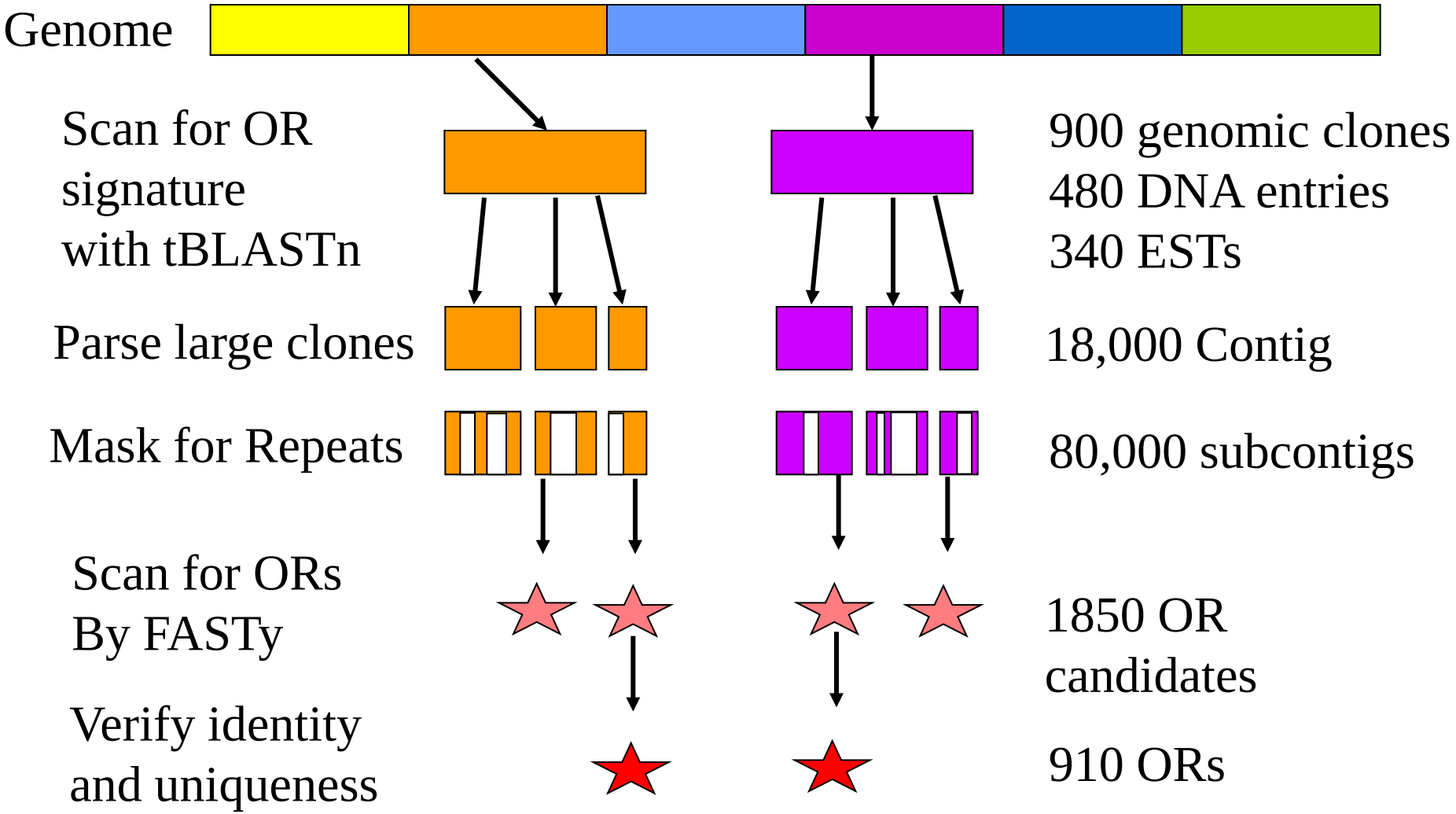
Low affinity domain:
Binding of small molecules to immunoglobulin

High affinity domain:
Phage display peptides immunoglobulin libraries



Olfactory receptor (OR) genomics

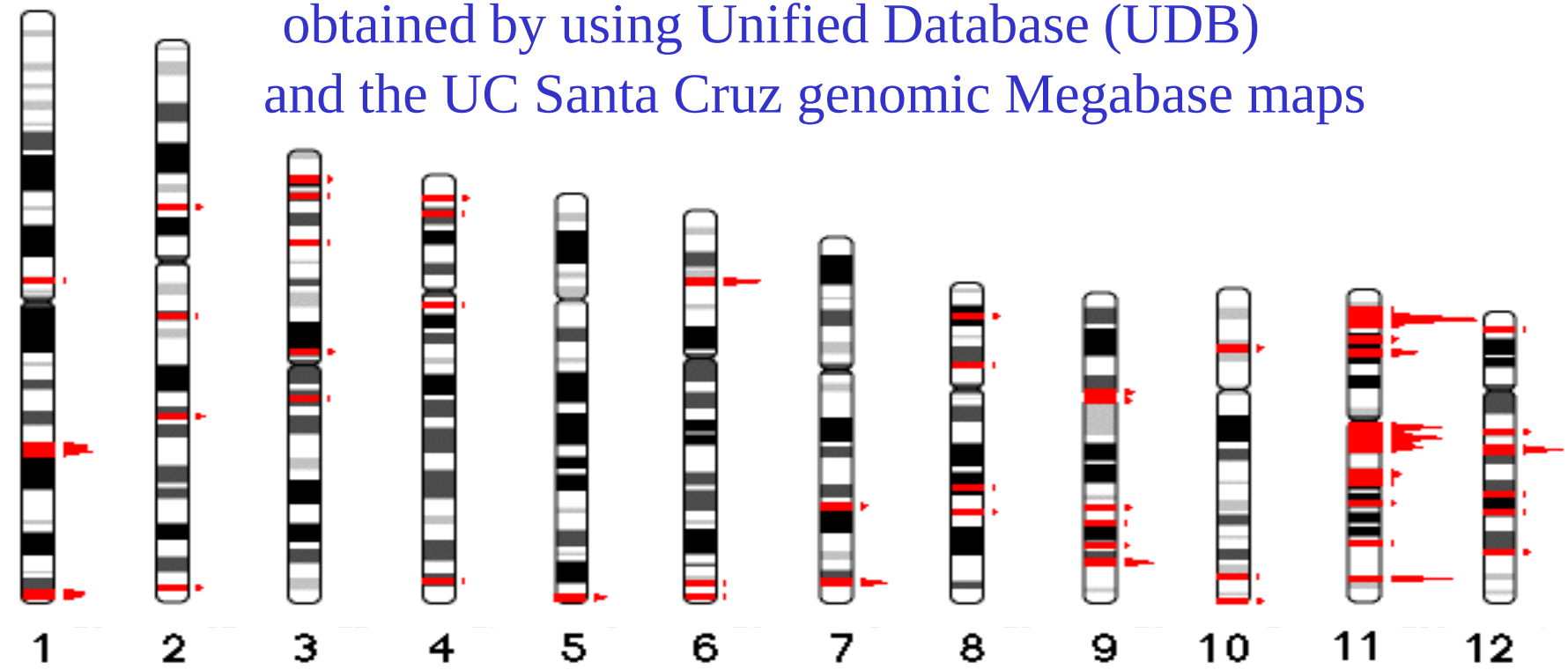
Olfactory Receptor Gene Mining Pipeline



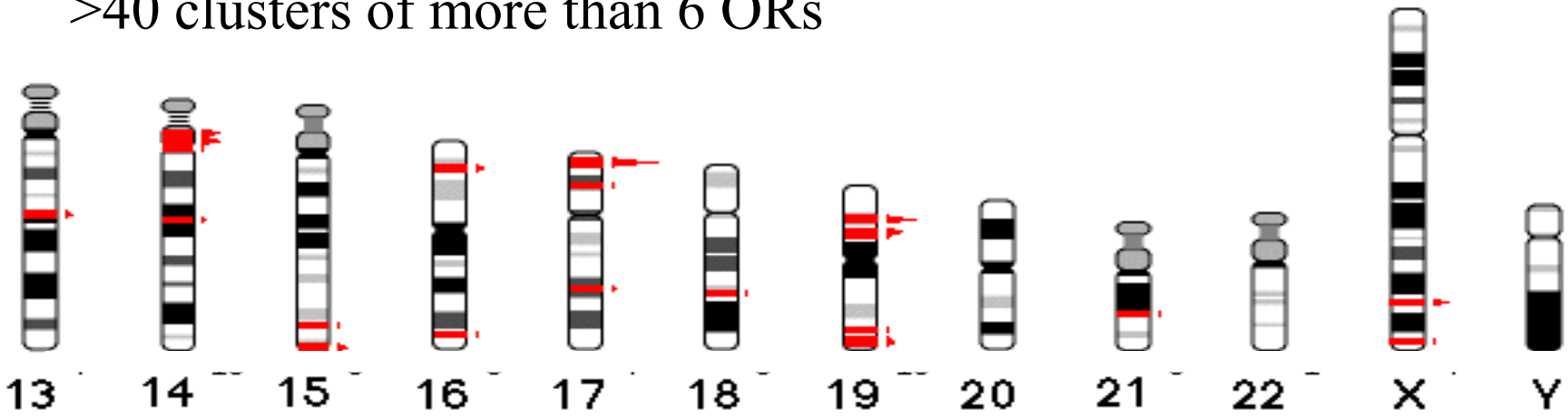
Glusman, Yanai, Rubin & Lancet, Genome Research, May 2001

Map of the human Olfactory Receptor (OR) sub-genome

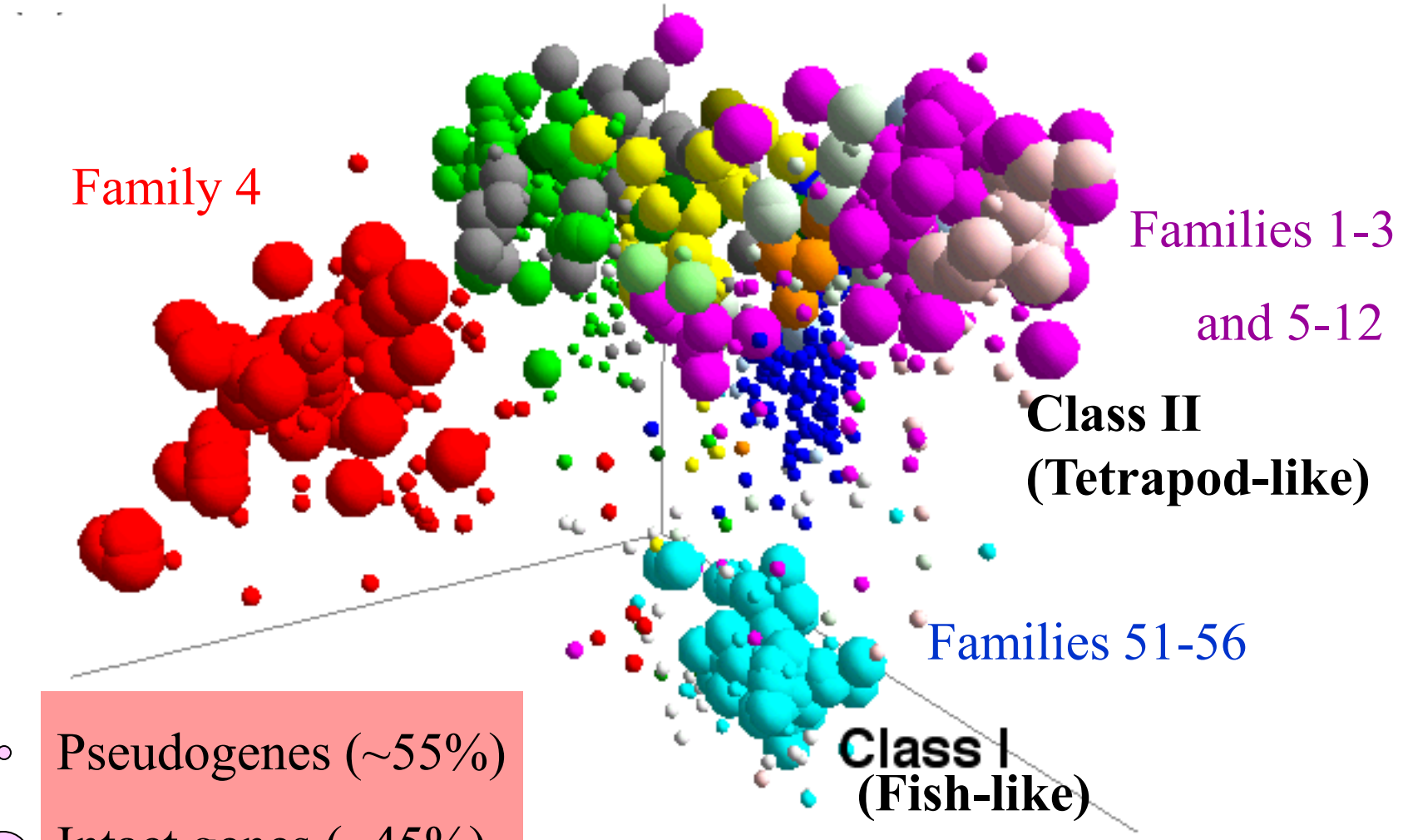
obtained by using Unified Database (UDB)
and the UC Santa Cruz genomic Megabase maps



>40 clusters of more than 6 ORs

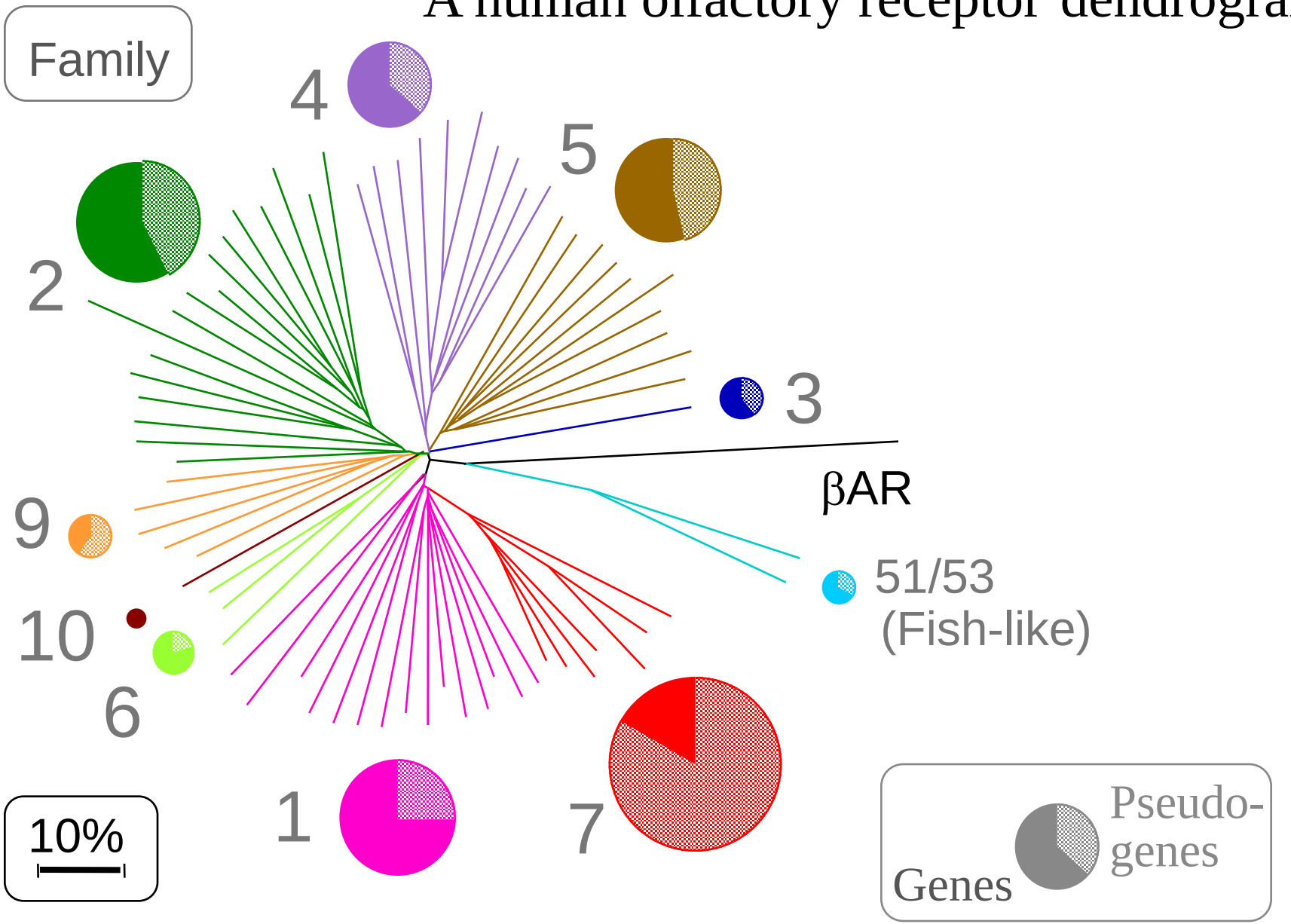


Human OR gene repertoire: 850 genomic loci

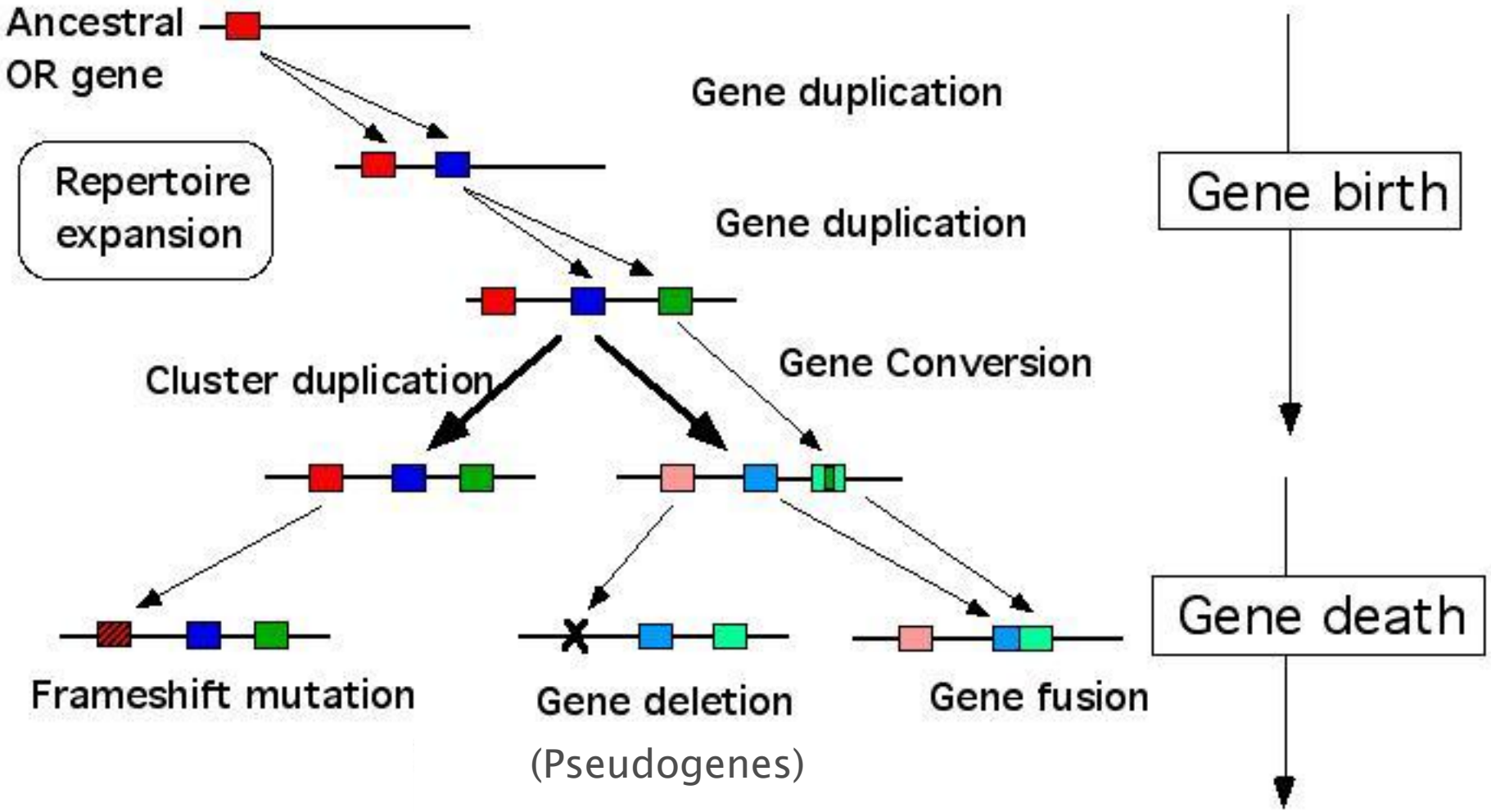


Glusman, Yanai, Rubin, Lancet Genome Res 2000

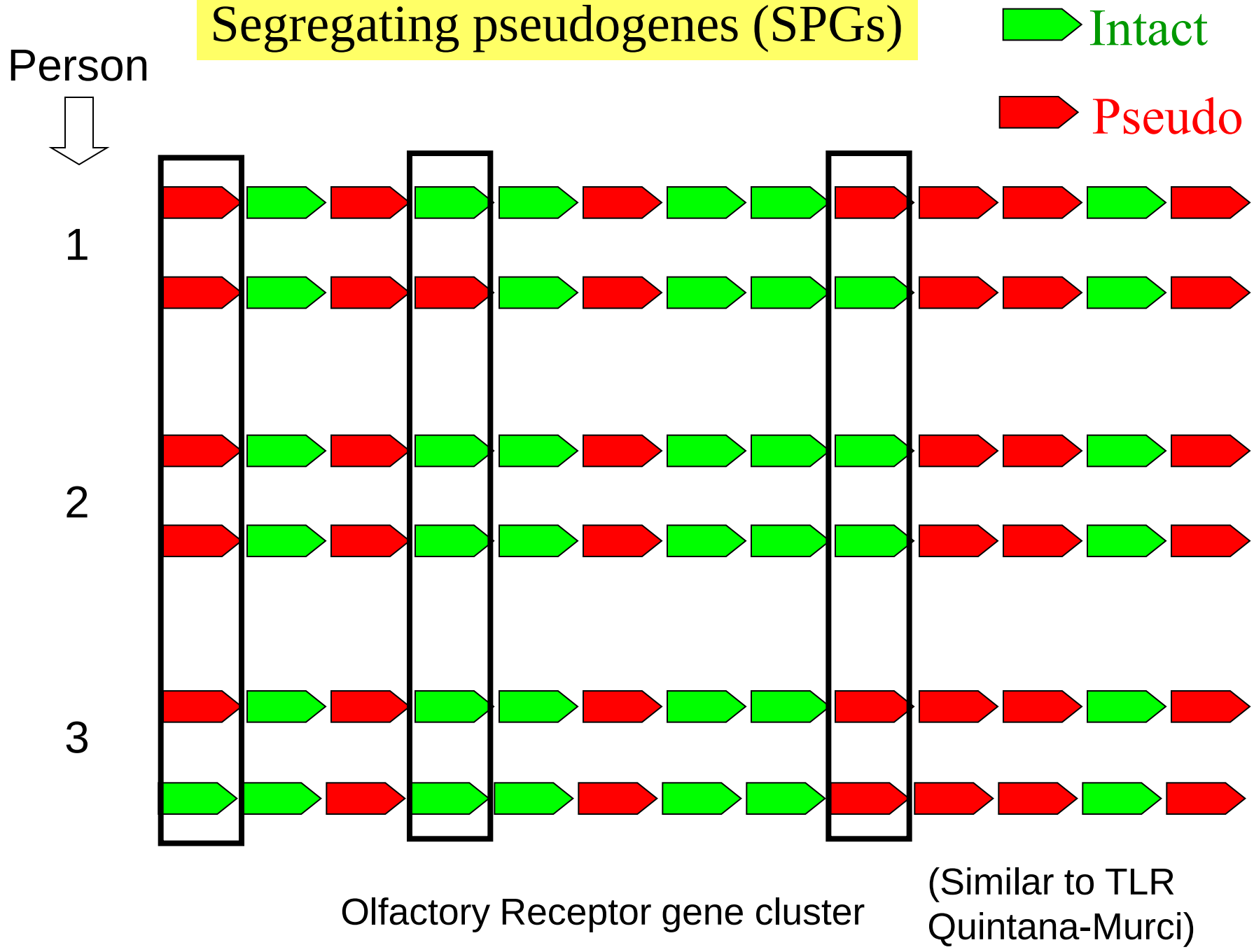
A human olfactory receptor dendrogram



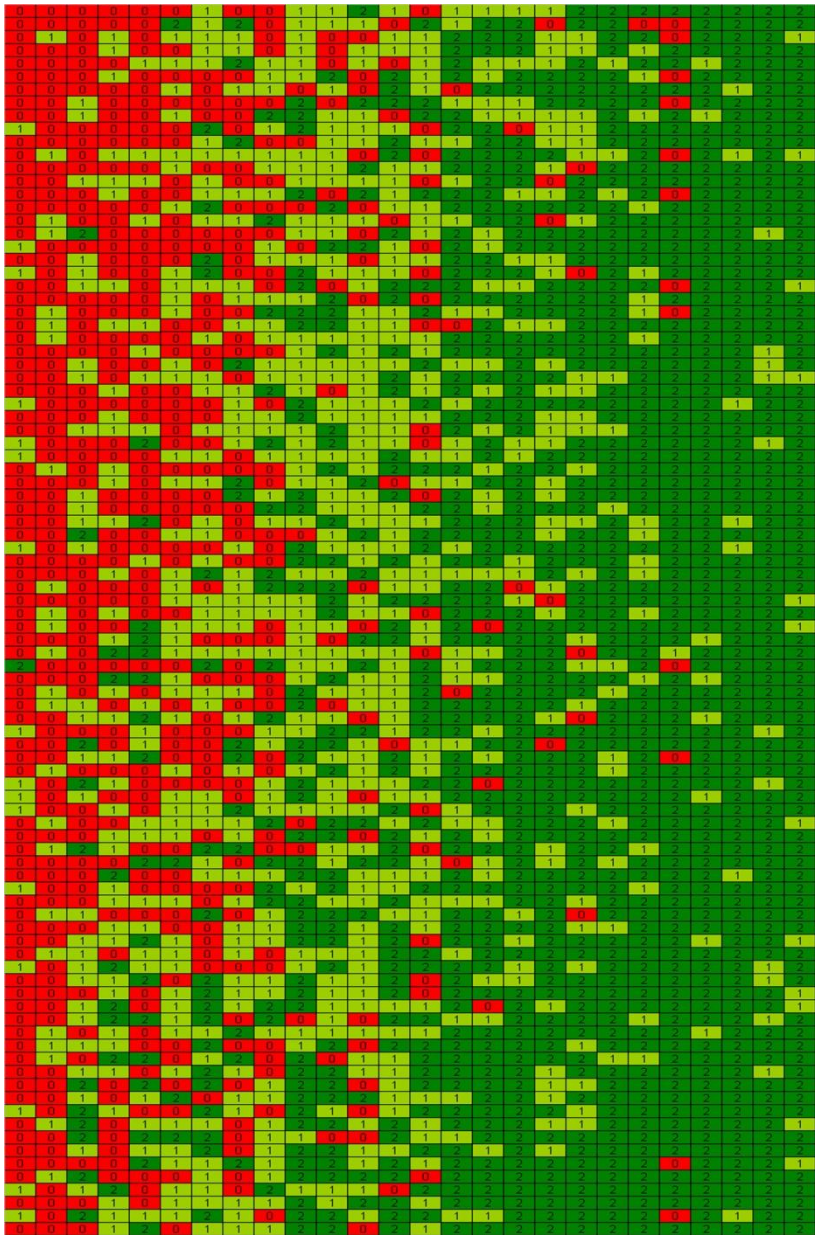
Olfactory sub-genome dynamics



Segregating pseudogenes (SPGs)



Nasodiversity



26 OR gene Loci

100 Persons

Almost every person has a different OR genotype!

Homozygote disrupted

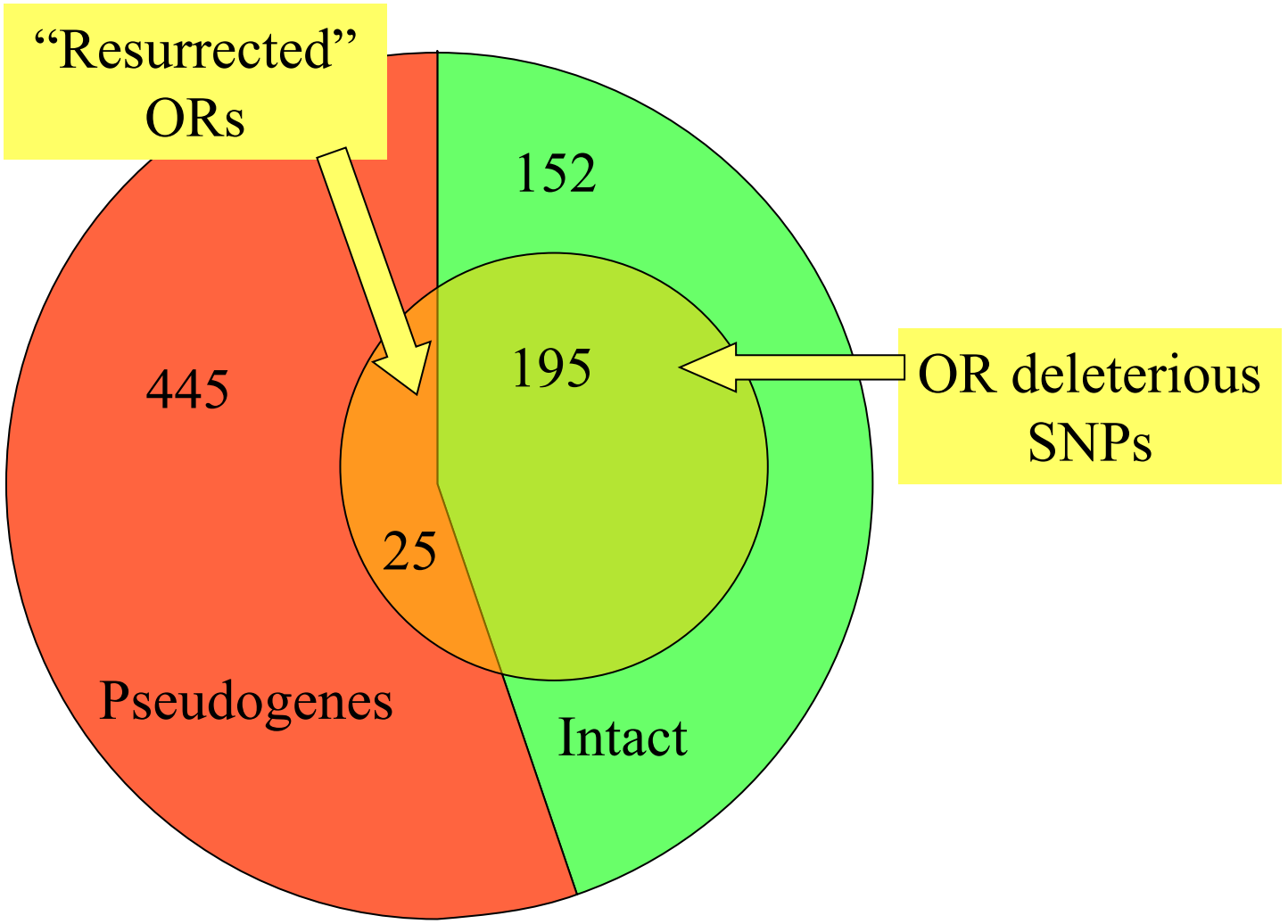
One of the most pronounced cases of genetic diversity

Heterozygote

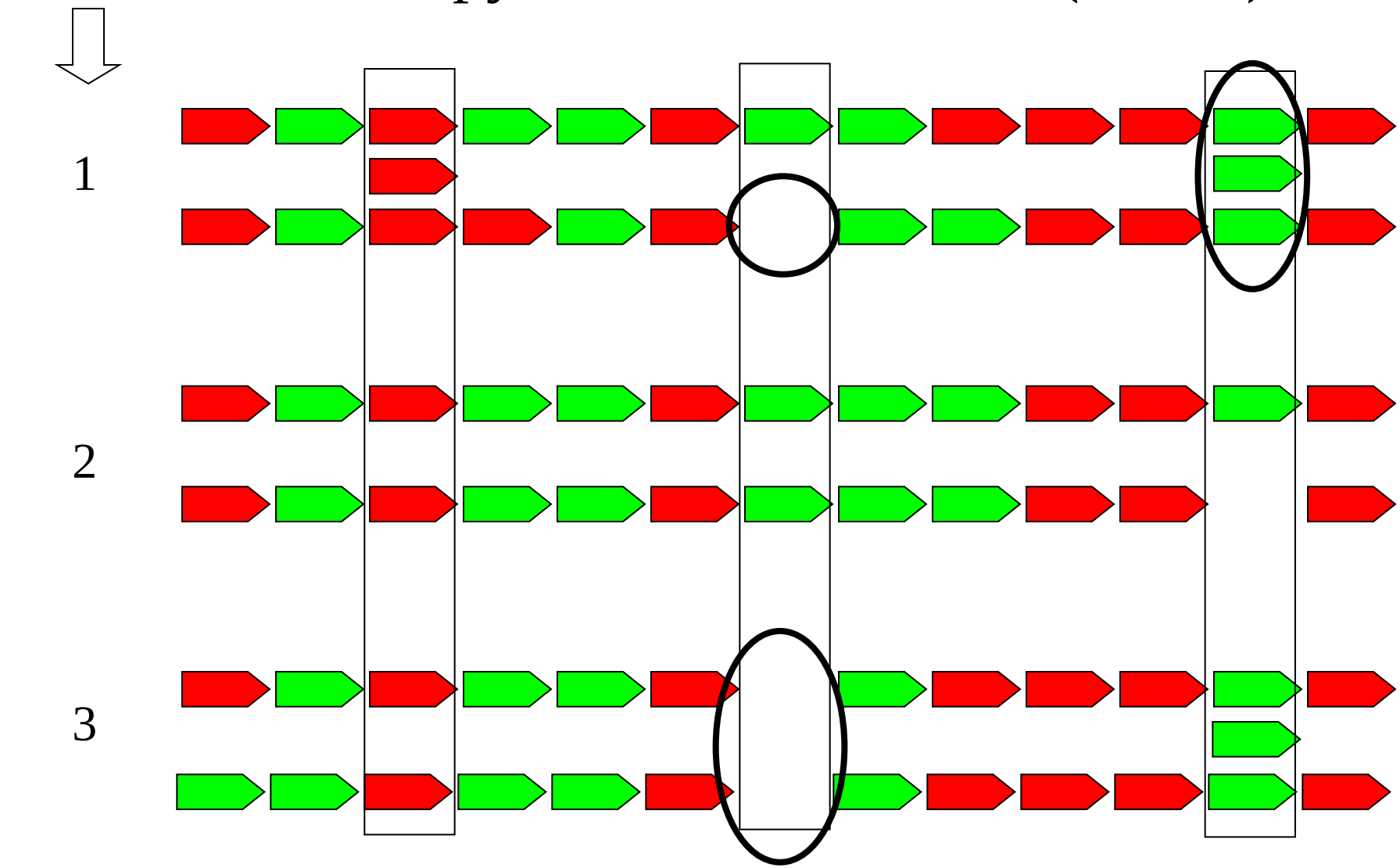
Homozygote intact

Menashe…Gilad Nature Genetics 2003

240 of 400 (60%) of intact ORs are affected by loss-of function variations



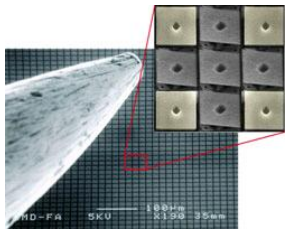
OR Copy Number Variations (CNVs)



Special interest in OR homozygous deletions

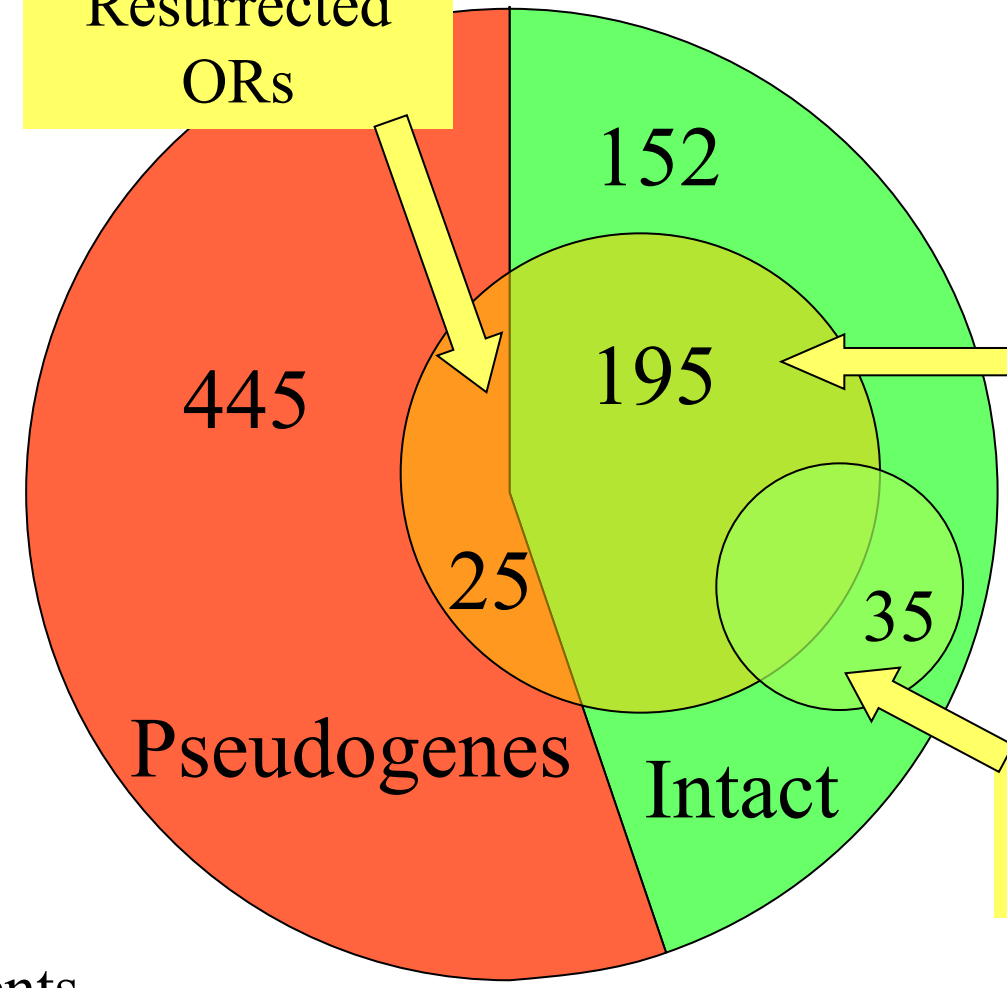
240 of 400 (60%) of intact ORs are affected by loss-of function variations

CNVs



Array experiments

"Resurrected" ORs



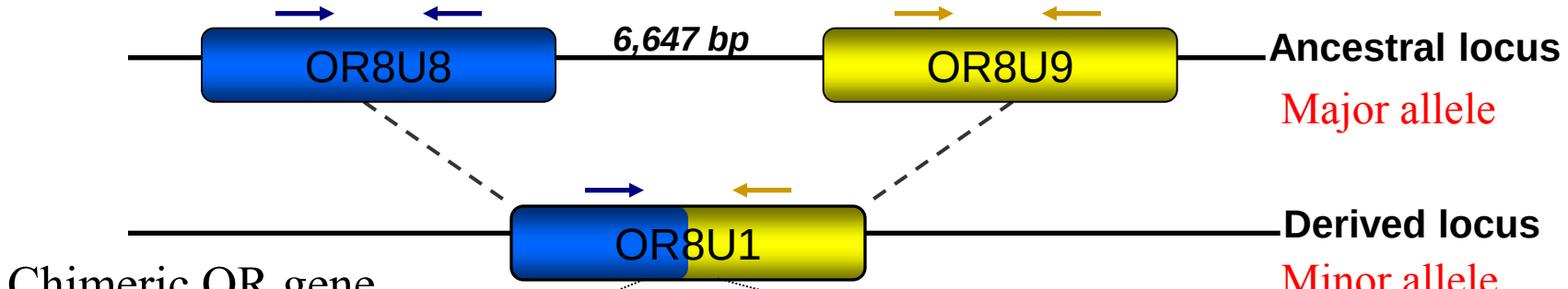
Unpublished

OR deleterious SNPs

OR deletion CNVs

Hasin et al PLoS Genet 2008

CNV breakpoints within OR coding regions



Evidence that OR genes may promote
CNV formation through NAHR

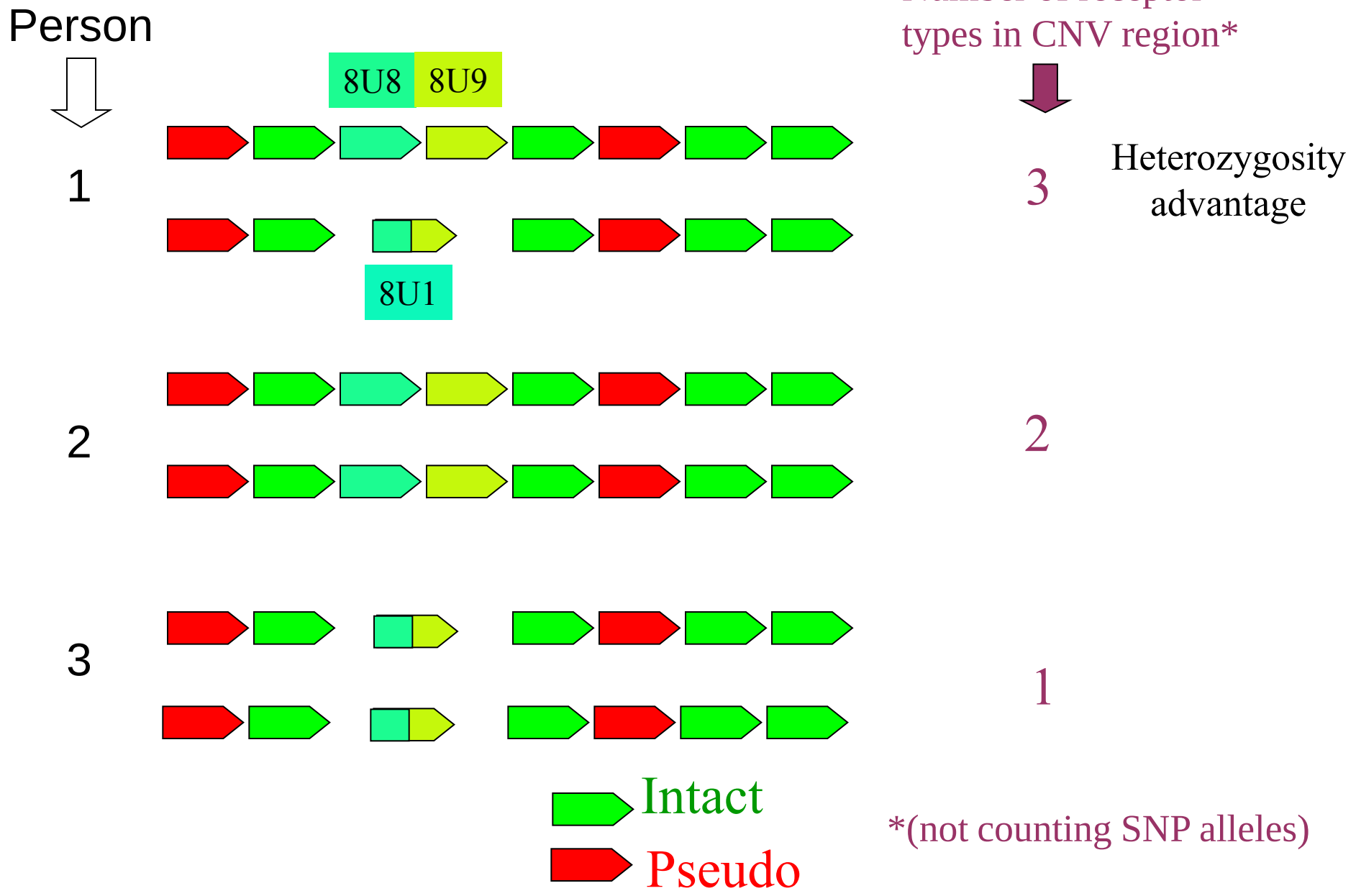
```
OR8U8 CCCTCTATTGTATATGGTTGTAATGACTCCAGGAATCTGCATTCAACTTGTAGCAGTTCCTTATAGCTATAGCTTCCTAATGGCACTATTTCACACCATC 483
OR8U1 CCCTCTATTGTATATGGTTGTAATGACTCCAGGAATCTGCATTCAACTTGTAGCAGTTCCTTATAGCTATAGCTTCCTAATGGCACTATTTCACACCATC 483
OR8U9 CCCTCTGATGTATATGGTGGTAATGTCCCCAGGAATCTGCATTCAGCTTGTGGCTGCTCCCCATAGCTATAGCATCTTGGTTGCACTGTTTCACACCATC 483
      ******  ********** ****** * ********************* ***** ** * ***  ********** ** *  * ***** ***********

OR8U8 CTCACCTTCCGCCTCTCCTATTGCCACTCCAACATTGTCAACCATTTCTATTGTGATGACATGCCTCTCCTCAGGCTAACTTGCTCAGACACTCGCTTCA 583
OR8U1 CTCACCTTCCGCCTCTCCTATTGCCACTCCAACATTGTCAACCATTTCTATTGTGATGACATGCCTCTCCTCAGGCTAACTTGCTCAGACACTCGCTTCA 583
OR8U9 CTCACCTTCCGCCTCTCCTATTGCCACTCCAACATTGTCAACCATTTCTATTGTGATGACATGCCTCTCCTCAGGCTAACTTGCTCAGACACTCGCTTCA 583
      ************************************************************************************************

OR8U8 AACAGCTATGGATTTTGGCCTGTGCTGGTATCACATTCATCTGCTCTGTTCTGATTGTCTTTGTCTCCTACATGTTCATTATTTTTGCCATCCTGAGGAT 683
OR8U1 AACAGCTCTGGATCTTTGCCTGTGCTGGTATCATGTTCATTTCCTCCCTTCTGATTGTCTTTGTCTCCTACATGTTCATCATTTCTGCCATCCTGAGGAT 683
OR8U9 AACAGCTCTGGATCTTTGCCTGTGCTGGTATCATGTTCATTTCCTCCCTTCTGATTGTCTTTGTCTCCTACATGTTCATCATTTCTGCCATCCTGAGGAT 683
      ******* ***** ** ***************  ***** * ***  ******************************** **** ***************
```

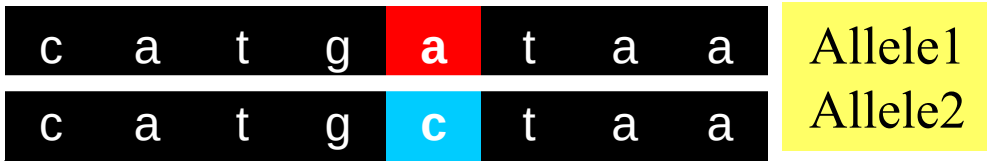
Exact breakpoint obtained by Celera data mining

Gene diversity enhancement by CNV



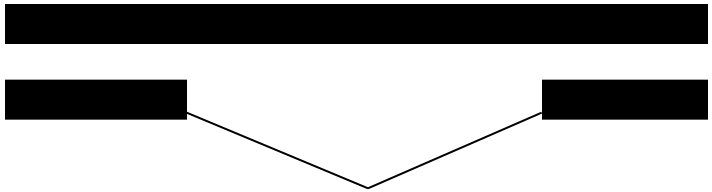
Types of genetic variations

Single nucleotide polymorphism (SNP)



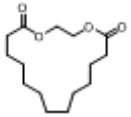
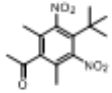
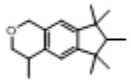
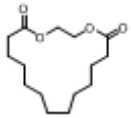
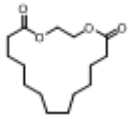
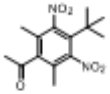
Single base change
Insertions/deletions (indels)
Deleterious (stops)
Silent or protein-changing
Exon-intron junction

Copy number variation (CNV)



Large segment (>1kb) of DNA deleted, inserted or inverted

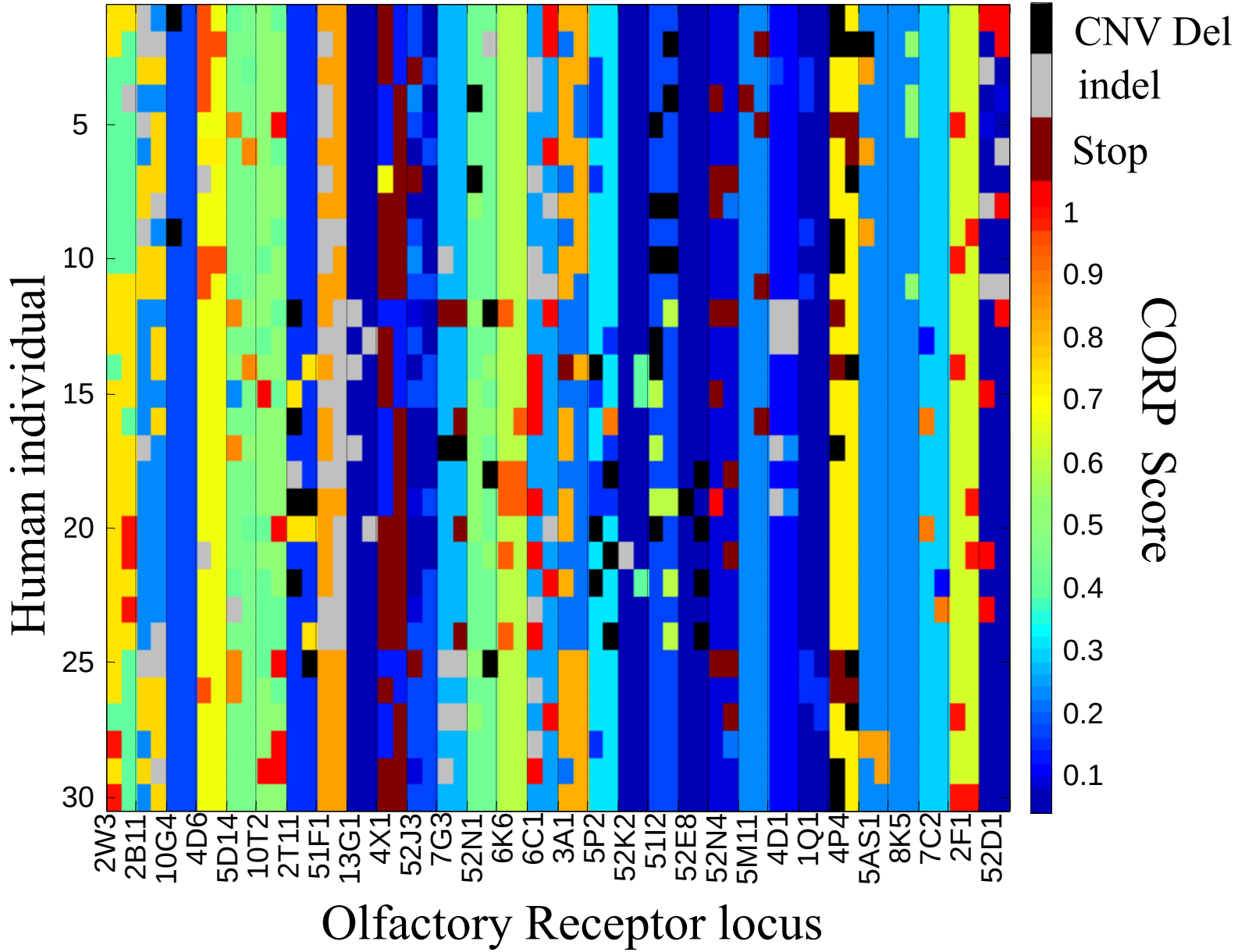
Different OR alleles may bind odorants differently

SNPs →	W/S	G/F	R/C	Q/E
	W	G	R	Q
	W	F	R	Q
	S	F	R	E
	W	G	C	Q
	S	G	C	E
	W	G	C	E
	S	G	R	E

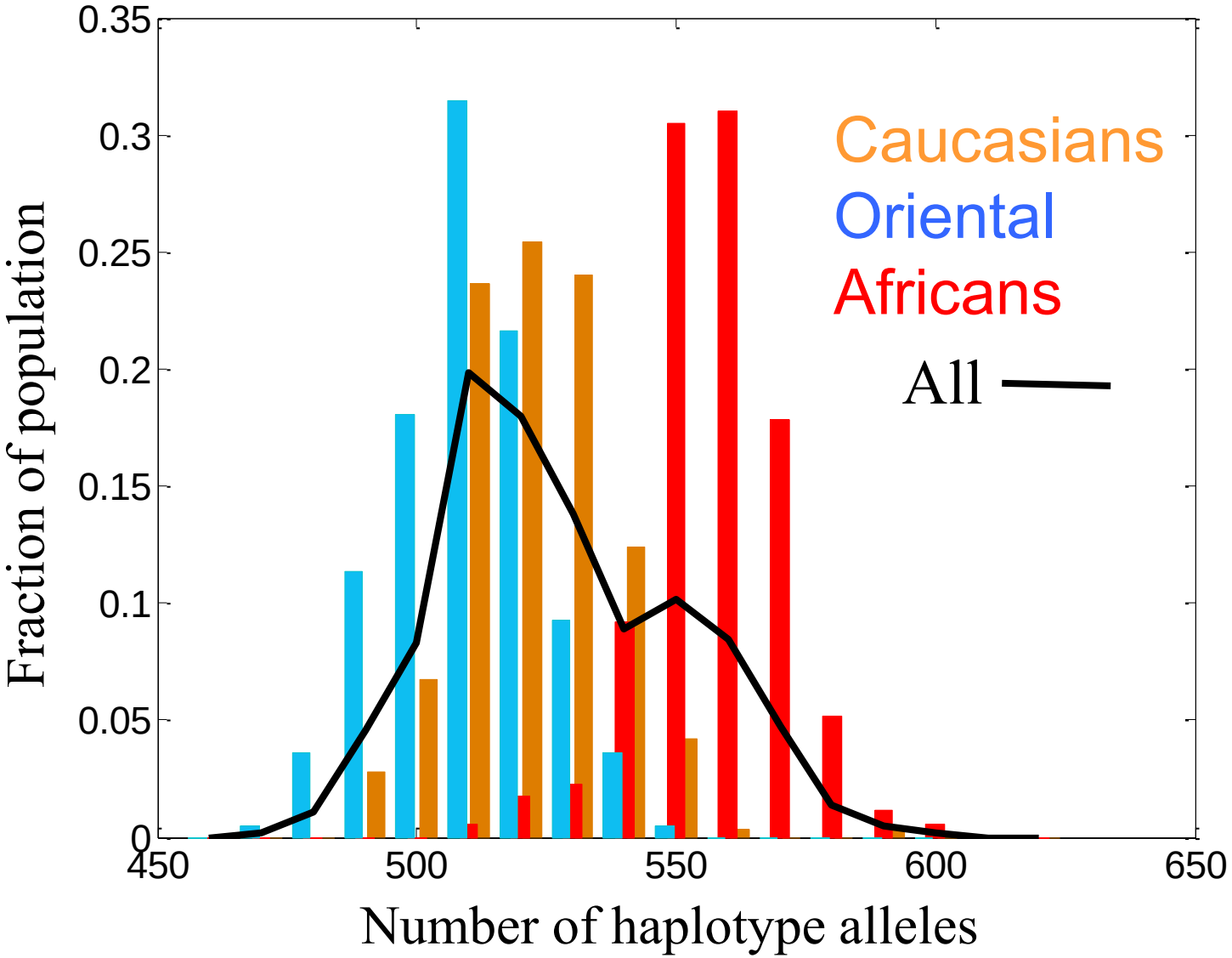
Odorant

OR allelic haplotypes

Haplotype diversity for 30 ORs in 30 individuals

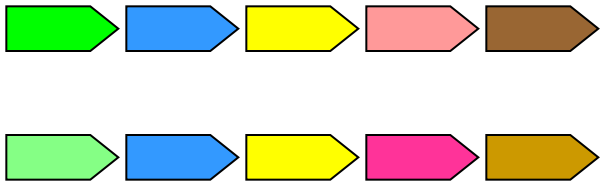


Number of OR Haplotypic variants per 405 OR loci

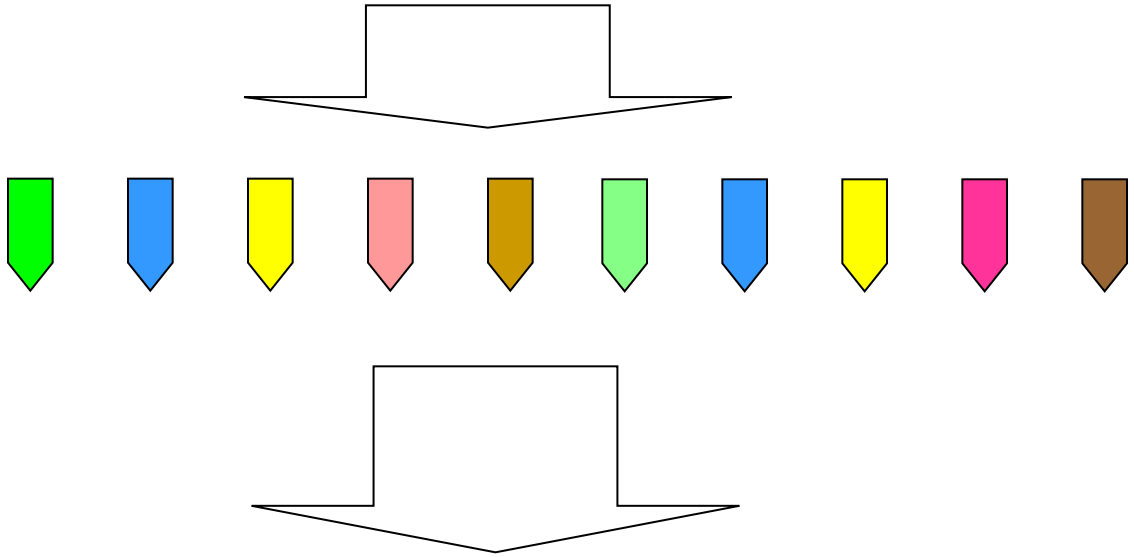


OR allelic input to the brain

OR loci



Allele1
Allele2



Allelically excluded sensory neuron expression