



**This is a work in progress. The main body comes from several papers and common open sources. The main point here is that I am merging the papers on the science and biology to the papers on genes and evolution and then putting it into simple English.**

**Well its more like a cut and paste of the information that I want to write about right now. I have been slowly cutting down 50 pages of PHD geek talk to English. When done it will be about 2 pages of simple English.**

## **What is Smell & How does it Work ?**

People respond to the sense of smell on an emotional level more strongly than any other sense. For example, a single scent can trigger a whole string of forgotten memories. The area of the brain associated with smell is connected to the same area that is associated with memory.

Some of these connections are genetic and are expressed from birth. Although smell-identification ability increases during childhood, even newborn infants are highly sensitive to some important smells, newborn babies locate their mothers' nipples by smell. Other experiments have also shown that babies are responsive to very faint differences in body odor, and can identify their mother by smell imprints from birth. Although an infant's sense of smell may be highly sensitive, it's only to specific smells, rather than a wide range of odors.

Because the olfactory nerves are a direct extension of the brain's limbic system, reaction to smell is relayed immediately. The connection between odors and memory was a necessary skill, to find food, avoid dangers, a survival skill that was naturally selected by our ancestors.

Our sense of smell is made possible by the detectors of odor molecules by our olfactory nerves. The olfactory nerves are located within the nasal cavity and respond to particular aromas. They send the information to the part of the brain where memories are stored and emotional responses are formed. This area connects with another part of the brain (hypothalamus and pituitary gland) which governs our hormonal systems. These aromas trigger a variety of chemical actions within the body, including the release of specific chemicals. Enkephalin reduces pain and creates a feeling of well-being. Endorphins also reduce pain and induce sexual feelings. Serotonin helps relax and calm.

When these inner connection in the brain malfunction or get cross wired problems can occur. One such disease called Parosmia, also known as Troposmia or Cacosmia, is one such problem. Parosmia is an olfactory

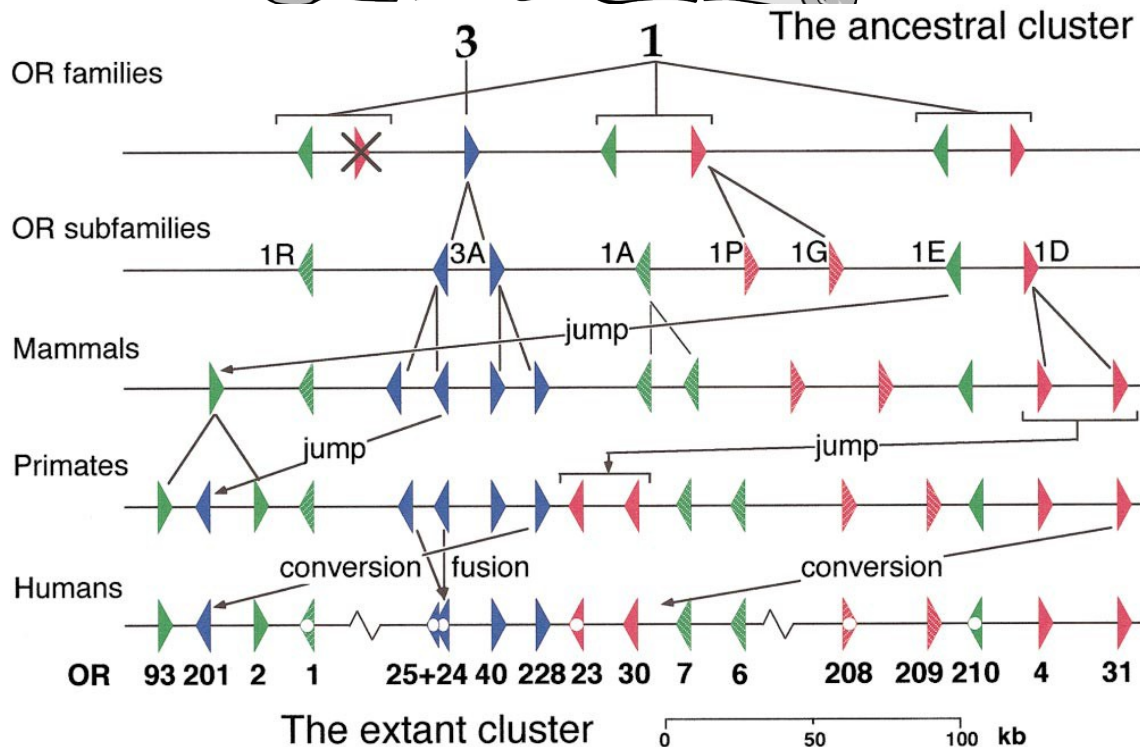


dysfunction that is characterized by the inability of the brain to properly identify an odor's "True" smell. What happens instead is that the odor is interpreted by the brain as different unpleasant aroma, typically described as burned, rotting, fecal, or chemical smell. There are instances however, of that the scent is interpreted as other pleasant odors, this is more specifically called Euosmia.

## **The Science & Biology of smell**

### **Evolution of the sense of smell in humans**

The evolution of our sense of smell can be seen in our genes. The genomic organization in human Olfactory Receptor (OR) genes may be best accounted for by a complex series of evolutionary events. The oldest of these genes is about 100 million years old and is traced back to Fish like receptors (Class I) genes. At that time there was only a limited amount of Class I genes in existence approximately 100. The Class I OR are specialized for recognizing water-soluble odorants. Even today we have approximately 38 functional ancient class 1 genes and OR, even though their exact functions are not completely understood in humans. Over time as amphibians (tetrapod specific class II OR) evolved the number of OR genes grew (to well over 1,000) and became more complex. The Class II OR became more specialized in detecting for airborne odorants. As mammals evolved our Class II genes continued to change. In our more recent past approximately 150,000 years ago changes from retrovirus again changed the genes to more closely resemble what they are today in the human genome.



The color vision priority hypothesis states that the evolution of color vision in primates may have decreased primate reliance on olfaction, which explains the relaxation of selective pressure that accounts for the accumulation of olfactory receptor pseudogenes (*DNA sequences that resemble functional genes but are generally thought to have no purpose*) in primates. This change along with the change in modern human females to be sexually receptive even when she is not in estrus reduced the need for the genes that expressed human pheromone sexual responses and thus eliminated the need for the human Vomeronasal organ functionality. When the human Vomeronasal organ functionality was decreased and eliminated some of the scent responses mechanisms were incorporated into the main Olfactory Receptors to brain coding functions.

## The sense of smell

The sense of smell gives rise to the perception of odors or scents. The perception of an odor effect is a two-step process. First, there is the physiological part; the detection of stimuli by receptors in the nose. The stimuli are then processed by the region of the human brain which is responsible for olfaction. Because of this, an objective and analytical measure of odor is



impossible. Because of this higher level function in the brain everyone perceives scents differently. Although the human sense of smell is feeble compared to that of many animals, it is still very acute. We can recognize thousands of different smells, and we are able to detect certain odors even in infinitesimal quantities.

### Typical odorants

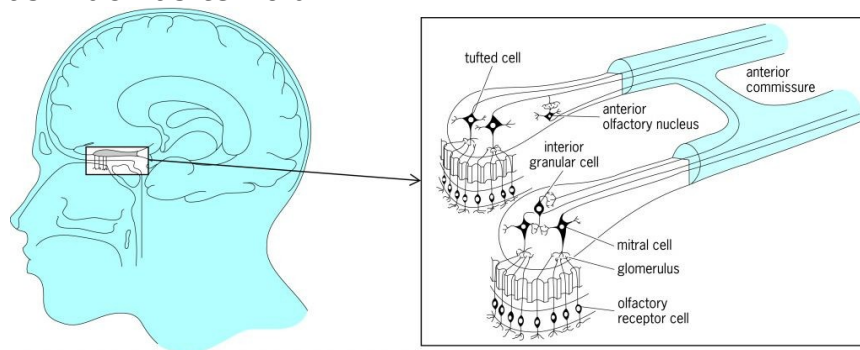
The typical Odorants are airborne volatile compounds with a low molecular weight (30-300 Dalton), typically organic, hydrophobic and polar. A few inorganic chemicals can also stimulate olfaction, notably hydrogen sulfide, ozone, ammonia, and the halogens.

### The process of how we smell (olfaction).

When odorant molecules reach the olfactory epithelium, a small patch of tissue located at the roof of each nasal cavity, they stimulate a large population of olfactory receptor neurons. This initiates a chain of biochemical and electrical signals that results in the sensation that we know as an odor. The olfactory pathway can be divided into three general subsystems:

- (1) olfactory epithelium, where primary reception takes place,
- (2) olfactory bulb, where an organized olfactory image is formed and,
- (3) olfactory cortex, where odor associations are stored. These multidimensional images are then interpreted and acted upon by the brain.

In humans the olfactory receptors are located inside the nasal passages. Humans have two airway passages for breathing. The mouth is used for intake of large amounts of air or for quiet breathing. During quiet breathing eddy currents may carry just enough of a odorant to evoke a sensation, whereupon sniffing can occur. Sniffing concentrates the amount of odorant reaching the receptors by as much as tenfold.



The central neural pathways of the olfactory system have a complexity unmatched among the sensory systems. One pathway carries information to the pyriform cortex (paleocortex of the temporal lobe), to a sensory relay in the



thalamus (dorsomedial nucleus), and to the frontal cortex (orbitofrontal region). This pathway seems rather strictly sensory.

Another pathway carries information to the pyriform cortex, the hypothalamus, and other structures of the limbic system. The latter have much to do with the control of emotions, feeding, and sex. The strong affective and motivational consequences of olfactory stimulation seem compatible with projections to the limbic system and with the role of olfaction in certain types of physiological regulation.

## Trigeminal nerve

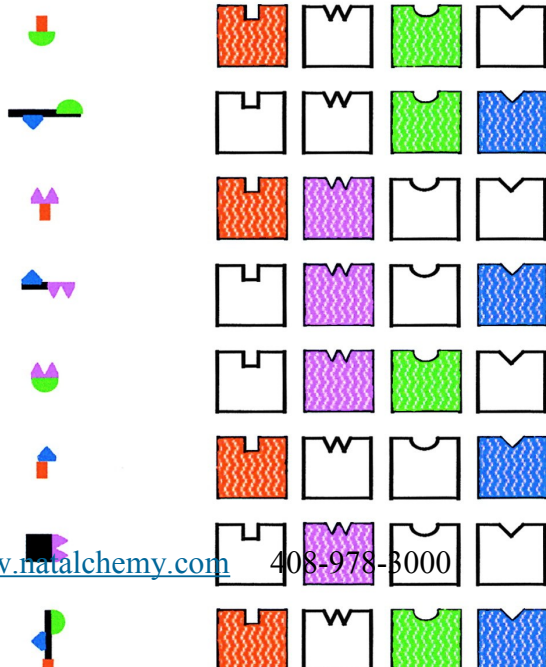
In addition to exciting olfactory receptors, many odorous substances excite the sensory endings of fibers of the trigeminal nerve, which are distributed in the mucosa of the nose and which serve as nonspecific, common chemoreceptors. The receptors of the trigeminal nerve are especially important in the complex perception of the odors of irritants, such as acetic acid and ammonia.

## Olfactory epithelium

Our smelling process starts at the olfactory epithelium. These olfactory receptors are made up of about five or six million yellowish cells. They are located high up in the nasal passages occupying the size of a small postage stamp. They are in fact the main organ of taste as well as smell. The so called taste buds on our tongues can only distinguish four qualities; sweet, sour, bitter and salty, all other tastes are detected by the olfactory receptors. These slender nerve cells are called cilia. The nerve endings are sensitive to molecules floating about in the air that get stuck on the mucous surface. The

### ODORANTS

### RECEPTORS



contact of such molecules with the cilia of the olfactory receptors is translated into neural messages. The genetic variation in people is also expressed in the olfactory receptors in different ethnic populations, i.e. not everyone has the same sense of smell.

Olfaction involves a large family of (48) receptors, each selective to a specific molecular feature of an odorant, such as carbon chain length, molecule shape, or functional groups. Each odorant is detected by multiple receptors and each receptor can detect multiple odorants,



leading to a high-multidimensional array and combinatorial code at the olfactory receptors level. For example, five ORs recognize only odorants with seven, eight, or nine carbon atoms (C7–C9 odorants), and one OR recognize only C5–C7 odorants. Similarly, only 1 OR recognize odorants of the lengths (C4–C9), and the majority (38) respond only to odorants of 2–4 consecutive carbon chain lengths. This multidimensional array and coding system gets even more complex as certain odors can be triggers to block certain OR, or to heighten the sensitivity of the signals from other OR. This system of antagonism, agonist may be directly correlated to the mixture of odors in the natural world. This system evolved to help us detect that food may be tainted over the sweet pleasant smells. The system of antagonism, agonist is well known in aromatherapy as a complete essential oil may be pleasant, but the individual parts of the oil may be irritating. The systematic coding in our OR seems to be in response to our evolution in harmony with plants and life's natural processes. Even human Sperm cells have odor receptors to help them find the egg.

### Olfactory bulb

Nerve fibers leave the olfactory receptor cells and enter the skull through the ethmoid bone, then disappear into the 'olfactory bulb' located at the anterior end of the 'olfactory tract'. The olfactory bulb is supported and protected by the cribriform plate of the ethmoid bone, which in humans separates it from the olfactory epithelium, and which is perforated by olfactory nerve axons. The olfactory bulb is a programmable preprocessor for odors. As a neural circuit, it receives direct input from the large family of olfactory nerves. By tuning in certain receptors it allows enhancing discrimination between odors, enhancing sensitivity of odor detection, the filtering out many background odors also serves to enhance the transmission of a few select odors. This allows the higher brain areas involved in arousal and attention to modify the detection or the discrimination of odors. This organ which then leads to the frontal - and eventually the temporal lobe of the cerebrum which is responsible for emotions, memory and sexual arousal.

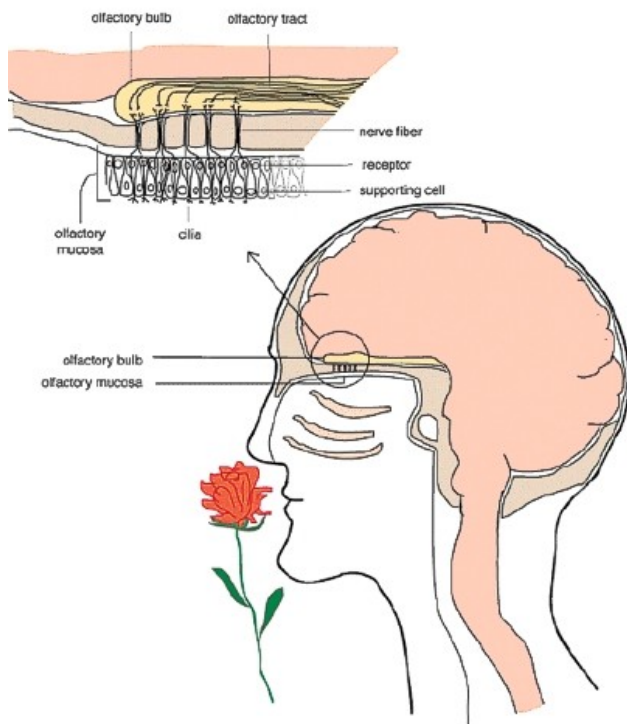
# NatAlchemy

Nature makes good scents

## Our response to odors

Odors can trigger many different behaviors in the body. Different odors can influence emotions, bring back memories, increase or decrease appetite, and even stimulate sexual arousal. Dangerous odors can even trigger a fight-or-flight response.

Compared to many other animals like dogs, and insects like mosquitoes, humans are quite scent-challenged. We have a much smaller quantity of odor receptors in the nasal cavity, rodents have 4 times more. But when scent molecules hit our odor receptors they go directly into the brain. Humans have the capacity to identify more than ten thousand different scents.



There is little understanding of how transduction occurs and how various molecules are transduced differently. The physical structure (shape) of a molecule plays a deciding role. The shape compatibility of the molecule is one of the main reasons that natural scents are more compatible with our biological systems than synthetic fragrances. Olfaction tells us how our food tastes. It is also used in identification (e.g., a mother can identify her infant by smell alone almost immediately after birth).

## Conditions that impair Olfaction

The most common ways that people lose their sense of smell are through the common cold (too much mucous), viral infection of olfactory epithelium, or a cracked cribriform plate caused by an accident. Olfactory impairment is especially marked in many disease processes, tumors or traumas of certain regions of the brain. Disturbances of olfaction include the increased sensitivity to odors, olfactory hallucinations with certain mental diseases, in which nonexistent, usually unpleasant, odors are perceived. Hyposmia is the abnormally decreased sensitivity to odors, while anosmia is the total loss of olfactory sensitivity. Disturbances in the recognition of odors can also occur. Smoking does affect the sense of smell. The tars can coat the cilia and make them less responsive and the odor of the tars decrease the sensitivity of the sense of smell since the receptors always have the constant background noise



from the scent from the chemicals that coat the hairs in the nose. This also masks many of the more sensitive scents.

### Changes in our sense of Smell throughout our lifetime

Our ability to distinguish different scents i.e. "our sense of smell" increases to reach a plateau at about the age of eight. In many people the sense of smell changes again around the age of puberty, and then again around the late 20's to early 30's. The most noticeable changes in the earlier years are the loss of the ability to determine specific smells from our genetic imprinted scents we are born with, but as these senses decrease the ability to determine a broader sense of smell is acquired through the learning process. We are able to link more scents with memories and an adult "Nose" can distinguish thousands of scent profiles.

One's smelling ability also depends on the person's state of mental and physical health, with some very healthy 80-year-olds having the same olfactory prowess as young adults. Across all age groups women consistently out-perform men on tests of smelling ability.

## Olfactory perception

Olfactory perception involves three basic tasks: *intensity estimation*, *qualitative description*, and *hedonic tone*.

### *Intensity estimation*

The relationship between odorant concentration and perceived intensity (sensitivity) is well understood, and follows a logarithmic law common to other sensory systems. *Odor detection thresholds*, defined as the lowest concentration at which a stimulus is perceived, can be as low as parts-per-trillion for some odorants, although these estimates may differ across subjects by as much as 50-fold.

Odor intensity can be expressed using an odor intensity scale, which is a verbal description of an odor sensation to which a numerical value is assigned. Odor intensity can be divided into the following categories according to intensity:

- 0 - no odor
- 1 - very weak (odor threshold)
- 2 - weak
- 3 - distinct
- 4 - strong
- 5 - very strong



6 - intolerable

This method is applied by in the laboratory and is done so by a series of suitably trained panelists/observers who have been trained to appropriately define intensity.

With prolonged or repeated contact with an odorous substance, the olfactory threshold to that substance and, to a lesser degree, to other odorous substances is increased. This attenuation of olfactory sensitivity is partially due to fatigue in the olfactory receptors and partially due to changes in the brain centers of the olfactory analyzer.

## Olfactory Sensitivity

Olfactory sensitivity decreases in most elderly persons. On the other hand, during pregnancy the degree of olfactory sensitivity may become uncomfortably acute. Olfaction decreases or completely disappears when the mucosa of the nose are congested or atrophied. Although some sense of smell is still active through the activation of the pain nerve cells pathways. Many odorous substances activate not only the olfactory system but also the 'somatosensory' system - the nerve endings in our noses which are sensitive to temperature, pain etc. This is why 'anosmics' people who have completely lost their sense of smell can still detect menthol, phenylethyl alcohol and some other substances.

### *Qualitative description*

*Qualitative description* of an odorant is a much more difficult task. It is estimated that humans have the ability to discriminate up to 10,000 different scents, though most of us only experience a fraction of these in our lifetime.

It appears that the relevant sensory code for olfactory quality is probably a pattern of excitation across different receptor groups.

Various schemes, such as Henning's odor prism (flowery, putrid, fruity, spicy, burned and resinous), have been proposed in the past in an attempt to classify odors into a smaller number of dimensions. Due to the lack of success of these efforts, current approaches employ odor profiling techniques, in which a large number of verbal descriptors are used to describe individual odors.



### *Hedonic tone*

The hedonic tone is a qualitative property related to the pleasantness of an odorant. The hedonic quality is highly subjective, and is influenced by cultural factors and emotional associations. In addition, hedonic tone is non-monotonically dependent on the exposure levels: some odorants are pleasant at low concentration but not at high (or persistent) doses. The hedonic tone of an odor is not hardwired in the brain, but shaped through experience; whether a subject likes an odor or not depends largely on the associations that are made throughout one's lifetime.

### **Conclusion:**

Nature has shaped the evolution of a complex sense of smell in humans. This sense of smell is acutely connected to plants and our natural life processes. The sense of smell is very individual from the beginning of our life due to the expression of our own unique genes to the development of scent memories during our lifetime. No two people have the exact same sense of smell.

Nature has designed our sense of smell to be compatible with the aromas of plants, and their scents do have a marked influence on our psyche and our well being.

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## Comparison of Odorant Specificity of Two Human Olfactory Receptors from Different Phylogenetic Classes and Evidence for Antagonism

Chem. Senses 30: 69–80, 2005 doi:10.1093/chemse/bji002

Guenhae¨ I Sanz, Claire Schlegel, Jean-Claude Pernollet and Lo¨c Briand

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## Evolution of olfactory receptor genes in the



## human genome

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Contributed by Masatoshi Nei, August 11, 2003

## Sequence, Structure, and Evolution of a Complete Human Olfactory Receptor Gene Cluster

Gustavo Glusman,\* Alona Sosinsky,\* Edna Ben-Asher,\* Nili Avidan,\* Dina Sonkin,\* Anita Bahar,\* Andre´ Rosenthal,† Sandra Clifton,‡ Bruce Roe,‡ Concepcio´ n Ferraz,§ Jacques Demaille,§ and Doron Lancet\*,1

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Cell, Vol. 87, 675–686, November 15, 1996, Copyright ©1996 by Cell Press

## Visualizing an Olfactory Sensory Map

Peter Mombaerts,\* Fan Wang, Catherine Dulac, Steve K. Chao, Adriana Nemes, Monica Mendelsohn, James Edmondson, and Richard Axel

(Ressler et al., 1993; Vassar et al., 1993;

Howard Hughes Medical Institute Strotmann et al., 1992, 1994. In con New York, New York 10032

Proc. Nati. Acad. Sci. USA

Vol. 89, pp. 8948–8952, October 1992

Neurobiology

## Spatial pattern of receptor expression in the olfactory epithelium

(guanine nucleotide binding protein-coupled receptors/odor coding/olfaction/olfactory neurons/bilateral symmetry)

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## Combinatorial Receptor Codes for Odors

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# A Multigene Family Encoding a Diverse Array of Putative Pheromone Receptors in Mammals

Hiroaki Matsunami and Linda B.

Howard Hughes Medical Institute Department of Neurobiology neurons (Buck and Axel, 1991; Levy et al., 1991; Nef et al., 1992; Strotmann et al., 1992; Ngai et al., 1993; Ressler et al., 1993)  
Boston, Massachusetts 02115

## REFERENCES

- Asai, H., Kasai, H., Matsuda, Y., Yamazaki, N., Nagawa, F., Sakano, H., and Tsuboi, A. (1996). Genomic structure and transcription of a murine odorant receptor gene: Differential initiation of transcription in the olfactory and testicular cells. *Biochem. Biophys. Res. Commun.* **221**: 240–247.
- Bailey, W. J., Fitch, D. H., Tagle, D. A., Czelusniak, J., Slightom, J. L., and Goodman, M. (1991). Molecular evolution of the psi eta-globin gene locus: Gibbon phylogeny and the hominoid slowdown. *Mol. Biol. Evol.* **8**: 155–184.
- Barth, A. L., Dugas, J. C., and Ngai, J. (1997). Noncoordinate expression of odorant receptor genes tightly linked in the zebrafish genome. *Neuron* **19**: 359–369.
- Baumeister, H., Gronostajski, R. M., Lyons, G. E., and Margolis, F. L. (1999). Identification of NFI-binding sites and cloning of NFI-cDNAs suggest a regulatory role for NFI transcription factors in olfactory neuron gene expression. *Brain Res. Mol. Brain Res.* **72**: 65–79.
- Ben-Arie, N., Lancet, D., Taylor, C., Khen, M., Walker, N., Ledbetter, D. H., Carrozzo, R., Patel, K., Sheer, D., Lehrach, H., and North, M. A. (1994). Olfactory receptor gene cluster on human chromosome 17: Possible duplication of an ancestral receptor repertoire. *Hum. Mol. Genet.* **3**: 229–235.
- Ben-Arie, N., North, M., Khen, M., Gross-Isserof, R., Walker, N., Horn-Saban, S., Gat, U., Natchin, M., Lehrach, H., and Lancet, D. (1993). "Olfactory Receptor: from Signal Modulation to Human Genome Mapping," Vol. 1, pp. 122–126, Springer-Verlag, Berlin/New York, Koseinenkin Kaikan, Sapporo, Japan.
- Bernardi, G. (1993). The isochore organization of the human genome and its evolutionary history—A review. *Gene* **135**: 57–66.
- Bodenteich, A., Chissoe, S., Wang, Y. F., and Roe, B. A. (1993). Shotgun cloning as the strategy of choice to generate templates for high-throughput dideoxynucleotide sequencing. In "Automated DNA Sequencing and Analysis Techniques" (J. C. Venter, Eds.), pp. 42–50, Academic Press, London.
- Brand-Arpon, V., Rouquier, S., Massa, H., de Jong, P. J., Ferraz, C., Ioannou, P. A., Demaille, J. G., Trask, B. J., and Giorgi, D. (1999). A genomic region encompassing a cluster of olfactory receptor genes and a myosin light chain kinase (MYLK) gene is duplicated on human chromosome regions 3q13–q21 and 3p13. *Genomics* **56**: 98–110.
- Brandeis, M., Frank, D., Keshet, I., Siegfried, Z., Mendelsohn, M., Nemes, A., Temper, V., Razin, A., and Cedar, H. (1994). Sp1 elements protect a CpG island from de novo methylation. *Nature* **371**: 435–438.
- Britten, R. J. (1994). Evolutionary selection against change in many Alu repeat sequences interspersed through primate genomes. *Proc. Natl. Acad. Sci. USA* **91**: 5992–5996.
- Brosius, J. (1999). Many G-protein-coupled receptors are encoded by retrogenes. *Trends Genet.* **15**: 304–305.
- Brunak, S., Engelbrecht, J., and Knudsen, S. (1991). Prediction of



- human mRNA donor and acceptor sites from the DNA sequence. *J. Mol. Biol.* **220**: 49–65.
- Bucher, P., and Yagil, G. (1991). Occurrence of oligopurine z oligopyrimidine tracts in eukaryotic and prokaryotic genes. *DNA Seq.* **1**: 157–172.
- Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* **65**: 175–187.
- Buettnner, J. A., Glusman, G., Ben-Arie, N., Ramos, P., Lancet, D., and Evans, G. A. (1998). Organization and evolution of olfactory receptor genes on human chromosome 11. *Genomics* **53**: 56–68.
- Buiakova, O. I., Krishna, N. S., Getchell, T. V., and Margolis, F. L. (1994). Human and rodent OMP genes: Conservation of structural and regulatory motifs and cellular localization. *Genomics* **20**: 452–462.
- Burge, C., and Karlin, S. (1997). Prediction of complete gene structures in human genomic DNA. *J. Mol. Biol.* **268**: 78–94.
- Carver, E. A., Issel-Tarver, L., Rine, J., Olsen, A. S., and Stubbs, L. (1998). Location of mouse and human genes corresponding to conserved canine olfactory receptor gene subfamilies. *Mamm. Genome* **9**: 349–354.
- Chalifa-Caspi, V., Rebhan, M., Prilusky, J., and Lancet, D. (1997). The Unified Database (UDB): A novel genome integration concept. *Genome Digest* **4**: 15.
- Chess, A., Simon, I., Cedar, H., and Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. *Cell* **78**: 823–834.
- Chisoe, S. L., Bodenteich, A., Wang, Y.-F., Wang, Y.-P., Burian, D., Clifton, S. W., Crabtree, J., Freeman, A., Iyer, K., Li, J. A., Ma, Y., McLaury, H.-J., Pan, H.-Q., Sarhan, O., Toth, S., Wang, Z., Zhang, G., Heisterkamp, N., Groffen, J., and Roe, B. A. (1995). Sequence and analysis of the human ABL gene BCR gene and regions involved in the Philadelphia chromosomal translocation. *Genomics* **27**: 67–82.
- Crowe, M. L., Perry, B. N., and Connerton, I. F. (1996). Olfactory receptor-encoding genes and pseudogenes are expressed in humans. *Gene* **169**: 247–249.
- Engels, W. R. (1993). Contributing software to the internet: The Amplify program. *Trends Biochem. Sci.* **18**: 448–450.
- Fritsch, E. F., Lawn, R. M., and Maniatis, T. (1980). Molecular cloning and characterization of the human beta-like globin gene cluster. *Cell* **19**: 959–972.
- Garcia-Fernandez, J., and Holland, P. W. (1994). Archetypal organization of the amphioxus Hox gene cluster. *Nature* **370**: 563–566.
- Gardiner, K. (1995). Human genome organization. *Curr. Opin. Genet. Dev.* **5**: 315–322.
- Gentles, A. J., and Karlin, S. (1999). Why are human G-proteincoupled receptors predominantly intronless? *Trends Genet.* **15**: 47–49.
- Gimelbrant, A. A., and McClintock, T. S. (1997). A nuclear matrix attachment region is highly homologous to a conserved domain of olfactory receptors. *J. Mol. Neurosci.* **9**: 61–63.
- Glusman, G., Clifton, S., Roe, R., and Lancet, D. (1996). Sequence analysis in the olfactory receptor gene cluster on human chromosome 17: Recombinatorial events affecting receptor diversity. *Genomics* **37**: 147–160.
- Glusman, G., Sharon, D., Kalush, F., Clifton, S., Roe, B., Ferraz, C., Demaille, J., Ben-Asher, E., and Lancet, D. (1998). Genome dynamics, polymorphisms and mutations in a 350 kb human olfactory receptor gene cluster. *Math. Model. Sci. Comput.* **9**: 30–44.
- Hanke, J. H., Hambor, J. E., and Kavathas, P. (1995). Repetitive *Alu* elements form a cruciform structure that regulates the function of the human CD8 alpha T cell-specific enhancer. *J. Mol. Biol.* **246**: 63–73.
- Higgins, D. G., Thompson, J. D., and Gibson, T. J. (1996). Using CLUSTAL for multiple sequence alignments. *Methods Enzymol.*



266: 383–402.

Ioannou, P. A., Amemiya, C. T., Garnes, J., Kroisel, P. M., Shizuya, H., Chen, C., Batzer, M. A., and de Jong, P. J. (1994). A new

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bacteriophage P1-derived vector for the propagation of large human DNA fragments. *Nat. Genet.* **6**: 84–89.

Issel Tarver, L., and Rine, J. (1996). Organization and expression of canine olfactory receptor genes. *Proc. Natl. Acad. Sci. USA* **93**: 10897–10902.

Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B., and Smale, S. T. (1994). DNA sequence requirements for transcriptional initiator activity in mammalian cells. *Mol. Cell. Biol.* **14**: 116–127.

Jukes, T. H., and Cantor, C. R. (1969). Evolution of protein molecules. In "Mammalian Protein Metabolism" (H. N. Munro, Eds.), pp. 21–123, Academic Press, New York.

Jurka, J. (1997). Sequence patterns indicate an enzymatic involvement in integration of mammalian retroposons. *Proc. Natl. Acad. Sci. USA* **94**: 1872–1877.

Karlin, S., Campbell, A. M., and Mrazek, J. (1998). Comparative DNA analysis across diverse genomes. *Annu. Rev. Genet.* **32**: 185–225.

Kulp, D., Haussler, D., Reese, M. G., and Eeckman, F. H. (1996). A generalized hidden Markov model for the recognition of human genes in DNA. *Ismb* **4**: 134–142.

Lancet, D. (1986). Vertebrate olfactory reception. *Annu. Rev. Neurosci.* **9**: 329–355.

Lancet, D. (1991). Olfaction. The strong scent of success. *Nature* **351**: 275–276. [News]

Lancet, D., Sadvovsky, E., and Seidemann, E. (1993). Probability model for molecular recognition in biological receptor repertoires: Significance to the olfactory system. *Proc. Natl. Acad. Sci. USA* **90**: 3715–3719.

Laurent, G. (1997). Olfactory processing: Maps, time and codes. *Curr. Opin. Neurobiol.* **7**: 547–553.

Malnic, B., Hirono, J., Sato, T., and Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell* **96**: 713–723.

Merino, E., Balbas, P., Puente, J. L., and Bolivar, F. (1994). Antisense overlapping open reading frames in genes from bacteria to humans. *Nucleic Acids Res.* **22**: 1903–1908.

Mombaerts, P. (1999). Molecular biology of odorant receptors in vertebrates. *Annu. Rev. Neurosci.* **22**: 487–509.

Nassif, N., Penney, J., Pal, S., Engels, W. R., and Gloor, G. B. (1994). Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Mol. Cell. Biol.* **14**: 1613–1625.

Nikolaev, L. G., Tsevegiyn, T., Akopov, S. B., Ashworth, L. K., and Sverdlov, E. D. (1996). Construction of a chromosome specific library of human MARs and mapping of matrix attachment regions on human chromosome 19. *Nucleic Acids Res.* **24**: 1330–1336.

Nizetic, D., Zehetner, G., Monaco, A. P., Gellen, L., Young, B. D., and Lehrach, H. (1991). Construction, arraying, and high-density screening of large insert libraries of human chromosomes X and 21: Their potential use as reference libraries. *Proc. Natl. Acad. Sci. USA* **88**: 3233–3237.

Page, R. D. (1996). TreeView: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **12**: 357–358.

Pearson, W. R., Wood, T., Zhang, Z., and Miller, W. (1997). Comparison of DNA sequences with protein sequences. *Genomics* **46**: 24–36.

Pilpel, Y., Sosinsky, A., and Lancet, D. (1998). Molecular biology of olfactory receptors. *Essays Biochem.* **33**: 93–104.

Qasba, P., and Reed, R. R. (1998). Tissue and zonal-specific expression of an olfactory receptor transgene. *J. Neurosci.* **18**: 227–236.

Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. (1995). MatInd and MatInspector: New fast and versatile tools for



- detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res.* **23**: 4878–4884.
- Raja, M., Zevin-Sonkin, D., Shwartzburd, J., Rozovskaya, T. A., Sobolev, I. A., Chertkov, O., Ramanathan, V., Lvovsky, L., and Ulanovsky, L. E. (1997). DNA sequencing using differential extension with nucleotide subsets (DENS). *Nucleic Acids Res.* **25**: 800–805.
- Reese, M. G., Harris, N. L., and Eeckman, F. H. (1996). “Large Scale Sequencing Specific Neural Networks for Promoter and Splice Site Recognition,” World Scientific, Singapore.
- Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1993). A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* **73**: 597–609.
- Rouquier, S., Taviaux, S., Trask, B. J., Brand-Arpon, V., van den Engh, G., Demaille, J., and Giorgi, D. (1998). Distribution of olfactory receptor genes in the human genome. *Nat. Genet.* **18**: 243–250.
- Rowen, L., and Koop, B. F. (1994). Zen and the art of large-scale genomic sequencing. In “Automated DNA Sequencing and Analysis” (M. D. Adams, C., Fields, and J. C. Venter, Eds.), pp. 167–174, Academic Press, New York.
- Rychlik, W. (1995). Selection of primers for polymerase chain reaction. *Mol. Biotechnol.* **3**: 129–134.
- Sakano, H., Kurosawa, Y., Weigert, M., and Tonegawa, S. (1981). Identification and nucleotide sequence of a diversity DNA segment (D) of immunoglobulin heavy-chain genes. *Nature* **290**: 562–565.
- Schuler, G. D., Altschul, S. F., and Lipman, D. J. (1991). A workbench for multiple alignment construction and analysis. *Proteins* **9**: 180–190.
- Selbie, L. A., Townsend Nicholson, A., Iismaa, T. P., and Shine, J. (1992). Novel G protein-coupled receptors: A gene family of putative human olfactory receptor sequences. *Brain Res. Mol. Brain Res.* **13**: 159–163.
- Sharon, D., Glusman, G., Pilpel, Y., Khen, M., Gruetzner, F., Haaf, T., and Lancet, D. (1999). Primate evolution of an olfactory receptor cluster: Diversification by gene conversion and recent emergence of pseudogenes. *Genomics* **61**: 24–36.
- Shen, L., Wu, L. C., Sanlioglu, S., Chen, R., Mendoza, A. R., Dangel, A. W., Carroll, M. C., Zipf, W. B., and Yu, C. Y. (1994). Structure and genetics of the partially duplicated gene RP located immediately upstream of the complement C4A and the C4B genes in the HLA class III region. Molecular cloning, exon–intron structure, composite retroposon, and breakpoint of gene duplication. *J. Biol. Chem.* **269**: 8466–8476.
- Smit, A. F. A., and Green, P. (1997). RepeatMasker at [http://repeatmasker.genome.washington.edu/cgi-bin/RM2\\_req.pl](http://repeatmasker.genome.washington.edu/cgi-bin/RM2_req.pl).
- Solovyev, V., and Salamov, A. (1997). The Gene-Finder computer tools for analysis of human and model organisms genome sequences. *Ismb* **5**: 294–302.
- Solovyev, V. V., Salamov, A. A., and Lawrence, C. B. (1995). Identification of human gene structure using linear discriminant functions and dynamic programming. *Ismb* **3**: 367–375.
- Sullivan, S. L., Adamson, M. C., Ressler, K. J., Kozak, C. A., and Buck, L. B. (1996). The chromosomal distribution of mouse odorant receptor genes. *Proc. Natl. Acad. Sci. USA* **93**: 884–888.
- Trask, B. J., Friedman, C., Martin-Gallardo, A., Rowen, L., Akinbami, C., Blankenship, J., Collins, C., Giorgi, D., Iadonato, S., Johnson, F., Kuo, W. L., Massa, H., Morrish, T., Naylor, S., Nguyen, O. T., Rouquier, S., Smith, T., Wong, D. J., Youngblom, J., and van den Engh, G. (1998). Members of the olfactory receptor gene family are contained in large blocks of DNA duplicated polymorphically near the ends of human chromosomes. *Hum. Mol. Genet.* **7**: 13–26.
- Uberbacher, E. C., Xu, Y., and Mural, R. J. (1996). Discovering and understanding genes in human DNA sequence using GRAIL. *Methods Enzymol.* **266**: 259–281.



Valcarcel, J., and Gebauer, F. (1997). Post-transcriptional regulation: The dawn of PTB. *Curr. Biol.* **7**: R705–R708.

Vanderhaeghen, P., Schurmans, S., Vassart, G., and Parmentier, M. (1997). Molecular cloning and chromosomal mapping of olfactory receptor genes expressed in the male germ line: Evidence for their  
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wide distribution in the human genome. *Biochem. Biophys. Res. Commun.* **237**: 283–287.

van Helden, J., Andre', B., and Collado-Vides, J. (1998). Extracting regulatory sites from the upstream region of yeast genes by computational analysis of oligonucleotide frequencies. *J. Mol. Biol.* **281**: 827–842.

van Helden, J., Andre', B., and Collado-Vides, J. A Web site for the analysis of regulatory sequences in the yeast *Saccharomyces cerevisiae*. In preparation.

Vassar, R., Ngai, J., and Axel, R. (1993). Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* **74**: 309–318.

von Sternberg, R. M., Novick, G. E., Gao, G. P., and Herrera, R. J. (1992). Genome canalization: The coevolution of transposable and interspersed repetitive elements with single copy DNA. *Genetica* **86**: 215–246.

Walensky, L. D., Ruat, M., Bakin, R. E., Blackshaw, S., Ronnett, G. V., and Snyder, S. H. (1998). Two novel odorant receptor families expressed in spermatids undergo 59-splicing. *J. Biol. Chem.* **273**: 9378–9387.

Wang, M. M., Tsai, R. Y., Schrader, K. A., and Reed, R. R. (1993). Genes encoding components of the olfactory signal transduction cascade contain a DNA binding site that may direct neuronal expression. *Mol. Cell. Biol.* **13**: 5805–5813.

Wang, S. S., Tsai, R. Y. L., and Reed, R. R. (1997). The characterization of the Olf-1/EBF-like HLH transcription factor family: Implications in olfactory gene regulation and neuronal development. *J. Neurosci.* **17**: 4149–4158.

Xu, Y., Mural, R., Shah, M., and Uberbacher, E. (1994). Recognizing exons in genomic sequence using GRAIL II. *Genet. Eng.* **16**: 241–253.

## References

Abdulaev, N.G., Popp, M.P., Smith, W.C. and Ridge, K.D. (1997) Functional expression of bovine opsin in the methylotrophic yeast *Pichia pastoris*. *Protein Expr. Purif.*, **10**, 61–69.

Araneda, R.C., Kini, D. and Firestein, S. (2000) The molecular receptive range of an odorant receptor. *Nat. Neurosci.*, **3**, 1248–1255.

Araneda, R.C., Peterlin, Z., Zhang, X., Chesler, A. and Firestein, S. (2004) A pharmacological profile of the aldehyde receptor repertoire in rat olfactory epithelium. *J. Physiol.*, **555**, 743–756.

Buettner, J.A., Glusman, G., Ben-Arie, N., Ramos, P., Lancet, D. and Evans, G.A. (1998) Organization and evolution of olfactory receptor genes on human chromosome 11. *Genomics*, **53**, 56–68.

Bulger, M., van Doorninck, J.H., Saitoh, N., Telling, A., Farrell, C., Bender, M.A., Felsenfeld, G., Axel, R., Groudine, M. and van Doorninck, J.H. (1999) Conservation of sequence and structure flanking the mouse and human beta-globin loci: the beta-globin genes are embedded within an array of odorant receptor genes. *Proc. Natl Acad. Sci. USA*, **96**, 5129–5134.



- Cometto-Muniz, J.E., Cain, W.S., Abraham, M.H. and Gola, J.M. (1999) Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. *Physiol. Behav.*, 67, 269–276.
- Duchamp-Viret, P., Chaput, M.A. and Duchamp, A. (1999) Odor response properties of rat olfactory receptor neuron. *Science*, 284, 2171–2174.
- Duchamp-Viret, P., Duchamp, A. and Chaput, M.A. (2003) Single olfactory sensory neurons simultaneously integrate the components of an odour mixture. *Eur. J. Neurosci.*, 18, 2690–2696.
- Freitag, J., Krieger, J., Strotmann, J. and Breer, H. (1995) Two classes of olfactory receptors in *Xenopus laevis*. *Neuron*, 15, 1383–1392.
- Gaillard, I., Rouquier, S., Pin, J.P., Mollard, P., Richard, S., Barnabeu, C., Demaille, J. and Giorgi, D. (2002) A single olfactory receptor specifically binds a set of odorant molecules. *Eur. J. Neurosci.*, 15, 409–418.
- Gaillard, I., Rouquier, S., Chavanieu, A., Mollard, P. and Giorgi, D. (2004) Amino-acid changes acquired during evolution by olfactory receptor 912–93 modify the specificity of odorant recognition. *Hum. Mol. Genet.*, 13, 771–780.
- Gimelbrant, A.A., Haley, S.L. and McClintock, T.S. (2001) Olfactory receptor trafficking involves conserved regulatory steps. *J. Biol. Chem.*, 276, 7285–7290.
- Glusman, G., Yanai, I., Rubin, I. and Lancet, D. (2001) The complete human olfactory subgenome. *Genome Res.*, 11, 685–702.
- Hague, C., Uberti, M., Chen, Z., Bush, C.F., Jones, S.V., Ressler, K.J., Hall, R.A. and Minneman, K.P. (2004) Olfactory receptor surface expression is driven by association with the *b2*-adrenergic receptor. *Proc. Natl Acad. Sci. USA*, 101, 13672–13676.
- Johnson, B.A., Ho, S.L., Xu, Z., Yihon, J.S., Yip, S., Hingco, E.E. and Leon, M. (2002) Functional mapping of the rat olfactory bulb using diverse odorants reveals modular responses to functional groups and hydrocarbon structural features. *J. Comp. Neurol.*, 449, 180–194.
- Kajiyama, K., Inaki, K., Tanaka, M., Haga, T., Kataoka, H. and Touhara, K. (2001) Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants. *J. Neurosci.*, 21, 6018–6025.
- Kaluza, J.F. and Breer, H. (2000) Responsiveness of olfactory neurons to distinct aliphatic aldehydes. *J. Exp. Biol.*, 203, 927–33.
- Katada, S., Nakagawa, T., Kataoka, H. and Touhara, K. (2003) Odorant response assays for a heterologously expressed receptor. *Biochem. Biophys. Res. Commun.*, 305, 964–969.
- Kostenis, E. (2001) Is Galphai6 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends Pharmacol. Sci.*, 22, 560–564.
- Krautwurst, D., Yau, K.W. and Reed, R.R. (1998) Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell*, 95, 917–926.



Laing, D.G. and Francis, G.W. (1989) The capacity of humans to identify odors in mixtures. *Physiol. Behav.*, 46, 809–814.