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Why Are Olfactory Systems of Different Animals So Similar?

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Key Words

Convergence · Adaptation · Constraint · Odorant binding protein · G Protein-coupled receptor · Sensory transduction · Glomerulus

Abstract

As we learn more about the neurobiology of olfaction, it is becoming increasingly clear that olfactory systems of animals in disparate phyla possess many striking features in common. Why? Do these features provide clues about the ways the nervous system processes olfactory information? This might be the case if these commonalities are convergent adaptations that serve similar functions, but similar features can be present in disparate animals for other reasons. For example, similar features may be present because of inheritance from a common ancestor (homology), may represent responses to similar constraints, or may be superficial or reflect strategies used by researchers studying the system. In this paper, I examine four examples of features of olfactory systems in members of different phyla: the presence of odorant binding proteins in the fluid overlying olfactory receptor neurons; the use of G protein-coupled receptors as odorant receptors; the use of a two-step pathway in the transduction of odorant signals; and the presence of glomerular neuropils in the first central target of the axons of olfactory receptor cells. I analyze data from nematodes, arthropods, molluscs, and vertebrates to investigate the phylogenetic distribution of these features, and to try to explain the presence of these features in disparate animals. Phylogenetic analyses indicate that these features are not homologous across phyla. Although these features are often interpreted as convergent adaptations, I find that alternative explanations are difficult to dismiss. In many cases, it seems that olfactory system features that are similar across phyla may reflect both responses to similar constraints and adaptations for similar tasks.

Introduction

Striking similarities in the organization of vertebrate and insect olfactory systems have long been noted by biologists. As researchers have broadened the scope of inquiry to include nematodes, molluscs, and crustaceans, the impression that some features are consistently present in olfactory systems has only been strengthened. What is the underlying cause of these similarities? Do they represent elements that are essential for olfaction, or are there other explanations for their appearance in distantly-related phyla? In this paper, I will describe features that are

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present in the olfactory system of members of several phyla, and will analyze possible explanations for the existence of these similarities.

Some researchers who take a 'model system' approach pay little attention to evolutionary issues, and apparently assume that similarities in the development, anatomy, physiology, or behavior of different animals reflect basic principles of biology. Others assume that the features of interest arose once, perhaps in a distant ancestor of the animals being compared; this similarity due to shared inheritance is called *homology*. Similar features might also have evolved independently, through *convergence*. (In this paper, I will consider *parallelism*, in which similar features evolve independently from the same precursor, to be a special case of convergence, as the two are conceptually related and are difficult to distinguish in practice [Eldredge and Cracraft, 1980].) The possibility that similarities in the organization of olfactory systems of diverse animals are due to convergence, and therefore might provide clues about mechanisms of processing of odorant information, has received increased attention in recent years [Ache, 1994; Hildebrand and Shepherd, 1997; Strausfeld and Hildebrand, 1999; Ache and Restrepo, 2000].

Convergent features can arise through different processes, and we should not assume that features that have arisen independently represent adaptations for processing odorant information. In some cases, similar features could arise independently due to *constraints* rather than as a result of adaptation [Wake, 1991]. Constraints can be attributed to many causes, and the problem of constraint has been the subject of many excellent reviews [Maynard Smith et al., 1985; Schwenk, 1994/95]. Developmental and genetic processes constitute an important source of constraint, as these factors can limit the possible phenotypes that can be produced. For example, Finlay and Darlington [1995] have argued that, in general, the cellular events in early neural development constrain size changes such that selection for an increase in size of a particular brain region in mammals will result in an increase in overall brain size, rather than an increase in the size of just that particular region. Constraints are also imposed by physical laws, such as those governing the transmission of sensory stimuli or the biomechanical properties of animals' bodies. Physical laws similarly constrain the architecture of the nervous system, as illustrated by Ringo's [1991] analysis of the relationships among brain size, cell number, connectivity, and regional specialization. If cell number increases with brain size, the amount of fiber needed to maintain the same connectivity increases disproportionately. The result is that larger brains will tend to consist of a larger number of specialized regions than will smaller brains, where broad interconnectivity can more easily be achieved.

It is important to note that adaptation and constraints may both play a role in the evolution of a feature, and these hypotheses are not mutually exclusive. For example, selection might favor an increase in brain size; because of constraints on connectivity, the result may be a larger brain (adaptation) with an increased number of discrete subdivisions (constraint). Many features that have arisen through convergent evolution might represent a combination of adaptation and constraints.

Organization of Olfactory Systems

In general, the term *olfaction* is applied to chemosensory systems that detect chemicals emanating from a distant source. Other chemosensory systems generally require physical contact with the source for detection, and in invertebrates such systems are called 'gustatory'. Vertebrates have anatomically distinct olfactory and gustatory systems, as well as other chemoreceptors, such as the common chemical sense and solitary chemoreceptors. Because analogous systems in other phyla are difficult to identify, I will not consider these chemosensory systems here. In this paper, I will also not consider examples presented by the vomeronasal system, a vertebrate olfactory subsystem frequently but erroneously assumed to be convergent with specialized pheromone-sensing systems of insects [Eisthen, 1997].

The olfactory systems of vertebrates, molluscs, arthropods, and nematodes share many features that are intriguingly similar, and markedly different from the features of other sensory systems. In general, olfactory receptor cells are bipolar neurons with a dendrite that protrudes into a fluid medium. The dendrite is capped with cilia and/or microvilli, which are probably the site of odorant transduction, and where the membrane-bound odorant receptors are presumably localized. The odorant receptor genes are members of a large superfamily that produces molecules with seven membrane-spanning regions; most of these receptors interact with G proteins that activate intracellular signaling pathways. At the opposite pole of the cell, the axon projects directly into the central nervous system, usually without branching, and often forms synapses with many other cells in tangles of fibers known as *glomeruli*.

Table 1. Model organisms commonly used in neurobiological olfactory research

Phylum	Class	Genus and species	Common name(s)
Nematodes	Secernenteans	***Caenorhabditis elegans***	
Arthropods	Malacostracans	*Homarus americanus*	American lobster
		Panulirus argus	Caribbean spiny lobster
	Insects*	*Periplaneta americana*	American cockroach
		Apis mellifera	honeybee
		Manduca sexta	tobacco hornworm, hawk moth, sphinx moth
		Heliothis virescens	tobacco budworm
		Schistocerca americana	American grasshopper
		Drosophila melanogaster	fruit fly
Molluscs	Gastropods	*Achatina fulica*	giant African snail
		Helix aspersa	brown garden snail
		Limax (Lehmannia) marginatus	tree slug
		Limax maximus	spotted garden slug, great gray garden slug, European giant garden slug
Vertebrates	Actinopterygians	***Brachydanio rerio***	zebrafish
		Carassius auratus	goldfish
		Ictalurus punctatus	channel catfish
		Oncorhynchus mykiss	rainbow trout
	Amphibians	***Ambystoma tigrinum***	tiger salamander
		Necturus maculosus	mudpuppy
		Rana spp.**	true frogs
		Xenopus laevis	African clawed frog
	Mammals	*Mesocricetus auratus*	Syrian hamster, golden hamster
		Mus musculus domesticus	house mouse
		Rattus norvegicus	Norway rat
		Cavia porcellus	guinea pig
		Sus scrofa	pig
		Bos taurus	cow
		Ovis aries	sheep
		Homo sapiens	human

Animals that have proved to be particularly valuable models, for which much data are available, are indicated in ***boldface.***

* Many insects, particularly those of agricultural importance, have been studied; most such research focuses more on chemical ecology and behavior than on neurobiology.

** Various species of ranid frogs are used in olfactory research, including *Rana catesbeiana, R. pipiens, R. ridibunda, R. temporaria,* and *R. 'esculenta'* [a naturally-occurring hybrid of *R. ridibunda* and *R. lessonae*; Berger, 1967, 1968].

The olfactory systems of vertebrates possess all these features, and those of animals in other phyla generally conform to this pattern, with a few exceptions. Vertebrate olfactory receptor neurons are found in a pseudostratified epithelium inside the nasal cavity, and the axons project to glomerular structures in the olfactory bulb at the rostral end of the telencephalon. In insects, the olfactory receptor neurons are found in clusters inside cuticle-covered sensillae along the antennae, and sometimes on other appendages such as the maxillary palp in *Drosophila* [Singh and Nayak, 1985; Ayer and Carlson, 1992]. Odorants gain access to olfactory receptor neurons via pores in the sensillar cuticle. The axons of the olfactory receptor neurons project to the antennal lobe in the brain. In lobsters, olfactory receptor neurons are found in clusters inside specialized sensillae along the antennules, and the axons of these cells project to the olfactory lobe in the deutocerebrum. The olfactory receptor neurons of snails are clustered in

groups, and their dendrites project to a dense olfactory epithelium at the tip of the tentacle. The axons of most of these cells project a short distance into regions associated with the tentacle ganglion [Chase and Tolloczko, 1993]. In *Caenorhabditis elegans*, olfactory receptor neurons are found in the paired amphid organs near the mouth, which are sensitive to mechanosensory stimuli as well as chemicals in water (gustation) and volatile chemicals in air (olfaction). Of the 12 neurons in each amphid organ, 3 respond exclusively to volatile compounds and may be considered purely olfactory neurons, and another 2 respond to volatile chemicals as well as other stimuli [Bargmann et al., 1993; Mori and Ohshima, 1997]. The cell bodies of these neurons are located in the lateral ganglia, and make synapses with each other and with interneurons in the lateral and ventral ganglia [White et al., 1986].

Our ability to recognize cases of similarity and analyze underlying causes is necessarily limited by the available data. During the past 40 years, the most popular model animals for laboratory studies of the anatomy and physiology of the olfactory system have been a few selected species of insects, lobsters, snails, fishes, salamanders, and rodents. More recently, researchers have initiated studies of chemoreception in the nematode *C. elegans*, and have made such progress that we can reasonably compare the organization of the olfactory system of *C. elegans* with those in members of other phyla. Table 1 contains a list of animals for which a substantial body of data is available concerning the organization and function of the peripheral olfactory system and/or the first central target. In analyzing the features of olfactory systems in diverse animals, we must bear in mind the problem of incomplete data sets, as the information available for a given species reflects the strengths of that animal as a laboratory model. For example, the sphinx moth *Manduca sexta* has proved to be an excellent model for anatomical and electrophysiological studies because of its large brain and large, accessible antennae; in contrast, the fruit fly *Drosophila melanogaster* is more suitable for molecular and genetic studies, but because of its small size fewer anatomical and electrophysiological experiments have been carried out.

In this paper, I will discuss four features that are found in olfactory systems in more than one phylum. These features are: (1) the presence of odorant binding proteins in the fluid overlying the receptor cell dendrite; (2) the use of G protein-coupled receptors as odorant receptors; (3) the use of a two-step signaling cascade in odorant transduction; and (4) the presence of glomerular structures at the first central target in the olfactory pathway. These features may represent adaptations that have evolved independently, and therefore might provide us with valuable information about the way the nervous system processes odorant stimuli. Alternatively, these similar features may instead reflect underlying homology, or could have arisen independently due to similar constraints. A further possibility should be considered the null hypothesis: the perceived similarity may be superficial, and may not reflect any of these processes. For each olfactory system feature, I will consider the data bearing on each of these hypotheses to try to determine whether or not the feature has arisen as a result of convergence; if so, I will assess the evidence that the feature arose as an adaptation or as a result of constraints. I will then discuss the relevance of this analysis for our understanding of olfactory system function, and for the use of evolutionary convergence as a tool in neurobiology. In the following analysis, I will generally assume that features present in several members of a large group are homologous within that group, although there is clear evidence that characters can evolve independently several times even within narrow taxonomic groups [for examples, see Bell, 2002; Nishikawa, 2002; Wray, 2002].

Odorant Binding Proteins

Before an odorant molecule can bind to a receptor, it must pass through the fluid overlying the olfactory receptor neurons, above the sensory epithelium or within the sensillum or amphid organ. In some insects and mammals, this fluid has been found to contain specialized molecules that bind odorants, and which are called odorant binding proteins (OBPs) [Vogt and Riddiford, 1981; Pelosi et al., 1982; Pevsner et al., 1985]. OBPs are soluble proteins dissolved in the fluid overlying the receptor neurons, and are not membrane-bound.

The first OBP was discovered in the pheromone-specific sensillae of the silk moth *Antheraea polyphemus* [Vogt and Riddiford, 1981]. OBPs have since been found in at least 17 species of endopterygote insects and 1 species of hemipteran insect [Vogt et al., 1999]. Within species, multiple OBPs have been identified. For example, 7 different OBPs have been isolated from *Manduca*, and 17 have been found in *Drosophila* [Robertson et al., 1999]. The molecules that function as OBPs in insects are of similar size and are characterized by six cysteines that are found in particular portions of the sequence, but these molecules also have diverse structures, even within a species, and do not appear to belong to a single class of proteins [Vogt et al., 1999]. Each type of OBP appears to have a fairly narrow odorant binding affinity and is found in

the lymph of particular subsets of sensillae, rather than being broadly distributed across the antenna [Vogt et al., 1991]. Within a single sensillum, more than one OBP may be expressed [Hekmat-Scafe et al., 1997].

In mammals, all OBPs discovered to date are lipocalins, and are therefore structurally less diverse than those found in insects. The lipocalin family comprises a group of carrier proteins with diverse functions, including retinol binding proteins, α_2 microglobulins, and the major urinary proteins (MUPs) [Tegoni et al., 2000]. Interestingly, MUPs have been shown to bind with pheromones in urine [Singer, 1991; Bacchini et al., 1992; Böcskei et al., 1992; Robertson et al., 1993], and may also serve as pheromones themselves [Singer and Macrides, 1993; Mucignat-Caretta et al., 1995]. As in insects, the olfactory mucus contains several different OBPs. The largest number found to date is in porcupines *(Hystrix cristata),* which have at least 8 different OBPs [Felicioli et al., 1993]. Mice *(Mus musculus domesticus)* have 6 different OBPs that are not closely related within the lipocalin family [Utsumi et al., 1999]. Multiple OBPs have also been found in rabbits [probably *Oryctolagus cuniculus;* Garibotti et al., 1997], rats [*Rattus norvegicus;* Löbel et al., 2001], and humans [Lacazette et al., 2000]. Mammalian OBPs, like those of insects, bind ligands selectively [Löbel et al., 2002]; however, unlike those of insects, mammalian OBPs are not restricted to the fluid near particular olfactory receptor cells, and are distributed throughout the mucus overlying the olfactory epithelium, as well as adjacent regions of nonsensory epithelium.

An olfactory-specific lipocalin that might function as an OBP has been described in the clawed frog *Xenopus laevis* [Lee et al., 1987], but Baldaccini et al. [1986] were unable to measure OBP-like binding in another amphibian, the common frog *(Rana temporaria)*. Indeed, these authors examined OBP-like activity in a variety of vertebrates, and were able to demonstrate binding in several mammalian species but not in birds (rock doves, *Columba livia,* and Muscovy ducks, *Cairina moschata*), Hermann's tortoises *(Testudo hermanni),* teleost fishes (American eels, *Anguilla rostrata,* rainbow trout, *Salmo gairdneri,* and black bullhead catfish, *Ictalurus melas*), or thornback rays *(Raja clavata)*. OBPs have not been reported in other non-mammalian vertebrates, in lobsters, or in *C. elegans*. OBPs have not been identified in molluscs, but Chase and Tolloczko speculate that a protein they found in the mucus overlying the olfactory epithelium in snails *(Achatina fulica)* may function as an OBP [Chase and Tolloczko, 1993]. The phylogenetic distribution of known OBPs is illustrated in figure 1.

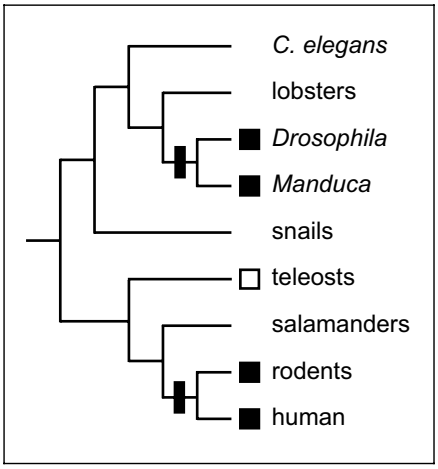

Fig. 1. Phylogenetic distribution of odorant binding proteins (OBPs) among common model animals used in olfactory research. Taxa in which OBPs have been identified are indicated with solid boxes, and animals for which OBPs may not be present are indicated with an empty box. Based on these data, a hypothesis concerning the evolutionary history of OBPs is illustrated, with separate origins indicated by bars; the hypothesis illustrated here postulates that OBPs evolved once in terrestrial insects and once separately in mammals.

Why do we find OBPs in insects and mammals? Is the presence of OBPs in these two groups an example of evolutionary convergence that informs us about the workings of the olfactory system, or are alternative hypotheses also tenable?

Homology

The presence of OBPs in insects and mammals could be due to homology; that is, OBPs could have arisen once, in the common ancestor of insects and mammals. Given the complete lack of similarity in the structure of mammalian and insect OBPs, we can reject this hypothesis, and conclude that OBPs have arisen independently at least twice.

Convergence

Given that OBPs have arisen independently in insects and vertebrates, they may be an example of convergent evolution. If so, are they adaptations that serve a particular function, or did they arise as the result of constraints?

Adaptation. Several hypotheses about the adaptive function of OBPs have been proposed, and fall into three categories. All draw support from studies demonstrating that OBPs in insects and mammals bind odorants selectively, and in some insects are associated with functional-

ly specialized sensillae [Vogt et al., 1991; Du and Prestwich, 1995; Steinbrecht, 1996; Plettner et al., 2000; Löbel et al., 2002]. (1) OBPs may serve as filters before odorant stimuli arrive at the receptors [Vogt et al., 1991]. (2) OBPs form a complex with odorants, and the complex may interact with the odorant receptor [Prestwich et al., 1995]. (3) OBPs might inactivate odorants, perhaps to prevent desensitization and/or to allow detection of new stimuli. Different mechanisms of inactivation have been proposed, including the possibility that OBPs directly inactivate odorants by binding them [Vogt and Riddiford, 1981], that the OBP-odorant complex serves as a substrate for enzymes that degrade odorants [Vogt et al., 1985], or that OBPs remove excess odorant from the lymph or mucus surrounding the receptor neurons [Kaissling, 1998].

Constraint. Similar features can arise independently because of constraints. Although it is difficult to imagine how the existence of OBPs could result from developmental or genetic constraints, OBPs might have arisen in terrestrial insects and mammals because of physical constraints imposed by the problem of detecting odorants in air. Specifically, the observation that OBPs have been identified in terrestrial animals but not in any aquatic animal has led to the popular hypothesis that OBPs function in insects and mammals to transport hydrophobic molecules through lymph or mucus to the odorant receptors [Bignetti et al., 1987; Vogt, 1987]. If so, it is difficult to understand why OBPs have not been found in non-mammalian terrestrial vertebrates [Baldaccini et al., 1986], but perhaps broader assays would detect different lipocalins or other classes of odorant-binding molecules in these animals. In any case, this hypothesis could be falsified independently for insects and for mammals by the discovery of homologous proteins in the olfactory sensillae or epithelia of aquatic relatives. Conversely, this hypothesis would gain support if researchers could demonstrate that OBPs bind to a membrane-bound docking protein, releasing odorant very close to the receptor, as has been suggested by Rogers and colleagues [Rogers et al., 1997]. Indeed, a membrane-bound OBP receptor has been discovered in cows [Boudjelal et al., 1996], although its relationship with odorant receptors is still unclear.

The Null Hypothesis

In considering a feature that has arisen independently several times, the null hypothesis is that the observed similarities are superficial and of no functional or evolutionary significance. Perhaps the presence of proteins that can bind odorants in sensillar lymph and olfactory epithelial mucus is coincidental, and OBPs do not serve the same function in insects and mammals. A variant null hypothesis suggests that OBPs may serve a general role not specific to olfaction, such as detoxification of external chemicals [Boudjelal et al., 1996]. Support for the latter hypothesis comes from the observation that, in mammals, OBPs and other lipocalins are distributed throughout the mucus overlying the nasal epithelia, including nonsensory regions, and that OBP-like lipocalins are found in non-nasal tissues [Lacazette et al., 2000]. Similarly, in insects, homologues of OBPs have been found in non-olfactory tissues [Vogt et al., 1999]. Because the function of OBPs has not yet been demonstrated for any species, we cannot discount either of these null hypotheses.

Although we cannot at present dismiss the null hypotheses, OBPs may represent an example of convergent evolution. If so, the evolution of OBPs may have been shaped by both adaptation and constraints. Indeed, features that arise to serve one function are not infrequently co-opted for another function later [Gould and Vrba, 1982]. For example, OBPs might have initially arisen to transport odorants across the air/liquid interface surrounding receptor neurons (i.e., as a response to a constraint), and each organism originally had only one form of OBP; the OBP genes may then have duplicated and diverged, and different OBPs began to function in filtering odorant stimuli before they arrive at the receptors. Conversely, OBPs may have arisen to function in odorant processing (i.e., as an adaptation), and their *absence* in aquatic organisms may reflect a constraint. OBPs are present in mucus or lymph at concentrations up to 10 mM and are water-soluble [Vogt and Riddiford, 1981]. Perhaps OBPs serve a valuable function in odorant processing in insects and mammals, and aquatic animals such as fishes are constrained from using OBPs because of the high energetic cost of producing proteins that could diffuse away into the environment.

These different scenarios can be supported or refuted by new data. If further research reveals that OBPs serve the same function in insects and mammals and are not present in aquatic animals, we may conclude that OBPs are an adaptation and are constrained from being used by aquatic animals. On the other hand, if we learn that OBPs are only present in terrestrial vertebrates and insects but serve different functions in these groups, we might conclude that OBPs arose to mitigate the constraints involved in detecting hydrophobic odorants in air, and that they were secondarily co-opted for different uses in odorant processing. Finally, if the distribution of OBPs is not confined to terrestrial animals and OBPs serve different func-

tions in different groups, or serve a general function not specific to olfaction, we may conclude that any perceived similarities were superficial, and that the null hypothesis is supported.

Structure of Odorant Receptors

Odorant receptors are part of the large, diverse superfamily of G protein-coupled receptors (GPCRs) with seven membrane-spanning domains. The family of GPCRs includes opsins and muscarinic acetylcholine receptors as well as receptors for sweet taste, serotonin, dopamine, prostaglandin, and gonadotropin releasing hormone. Odorant receptors were first described in rats *(R. norvegicus)* by Buck and Axel [1991], who developed probes for GPCRs based in part on physiological and biochemical evidence indicating that odorant binding activates G proteins. Homologous odorant receptor genes have now been found in more than 20 mammalian species, as well as in birds, amphibians, coelacanths, teleosts, and lampreys [Ngai et al., 1993a; Freitag et al., 1995; Nef et al., 1996; Freitag et al., 1998, 1999]. The vertebrate odorant receptor genes constitute large families: catfish *(I. punctatus)* and zebrafish *(Brachydanio rerio)* are estimated to have about 100, and mice *(M. musculus domesticus)* have at least 100 and perhaps as many as 1000 odorant receptor genes [Buck and Axel, 1991; Levy et al., 1991; Ngai et al., 1993a; Barth et al., 1996].

The identification of odorant receptors in non-vertebrates has been difficult. Nevertheless, odorant receptor genes were recently sequenced from *Drosophila* and *C. elegans* using a bioinformatics approach, in which researchers examined data derived from genome projects for sequences encoding proteins that would be predicted to have seven transmembrane domains [Troemel et al., 1995; Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999]. Although it is conceivable that insects, nematodes, and vertebrates could use fundamentally different receptors for odorant detection, the large numbers of GPCR-type odorant receptor genes found seem to rule this out: *Drosophila* has about 60 odorant receptor genes and *C. elegans* may have 100 – 500 [Troemel et al., 1995; Bargmann, 1998; Vosshall et al., 2000]. Nevertheless, the sequences of the odorant receptor genes in *C. elegans* and *Drosophila* are quite different from those of vertebrates, indicating that the odorant receptor genes in these three phyla were coopted independently from the larger family of GPCR genes. The phylogenetic distribution of known G protein-coupled odorant receptors is illustrated in figure 2.

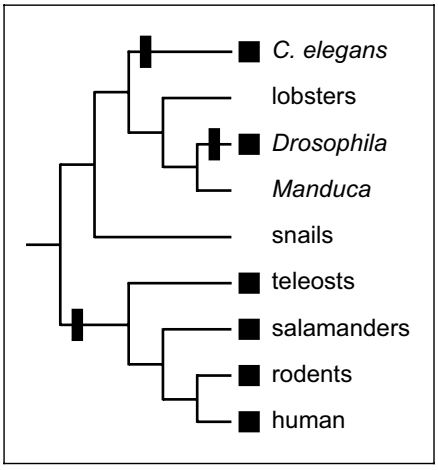

Fig. 2. Phylogenetic distribution of G protein-coupled odorant receptors among common model animals used in olfactory research. Taxa in which these receptors have been identified are indicated with solid boxes. A hypothesis concerning the evolutionary history of these receptors is illustrated. Given that the receptor genes are quite different in *C. elegans, Drosophila,* and vertebrates, the hypothesis illustrated here postulates that members of the large family of GPCR genes were co-opted independently at least three times to serve as odorant receptors.

Some features of the odorant receptor genes are similar across phyla, and some differ. The number of receptor genes expressed in each olfactory neuron remains controversial, because direct measures are extremely difficult to obtain. Based on the proportion of olfactory neurons hybridizing with individual odorant receptor gene probes, evidence that individual olfactory receptor neurons express a single allele of an odorant receptor gene, and the observation that neurons expressing the same receptor project to the same glomerulus, several groups have proposed that each vertebrate olfactory receptor neuron expresses only one odorant receptor gene [Nef et al., 1992; Ngai et al., 1993b; Chess et al., 1994; Ressler et al., 1994; Vassar et al., 1994]. Contrary evidence has been obtained from goldfish *(Carassius auratus)*, in which two odorant receptor genes are each expressed in a large proportion of the olfactory receptor neurons, suggesting that each neuron expresses at least 2 or 3 receptor genes [Speca et al., 1999]. In *Drosophila,* each olfactory receptor neuron expresses the OR83b receptor gene and at least one other, but probably not more than a small number of receptor genes [Vosshall et al., 1999, 2000]. In contrast, *C. elegans* olfactory receptor neurons may express many odorant receptor genes. Troemel et al. conservatively estimate the

total number of *C. elegans* odorant receptor genes at 100, but a more recent estimate based on the full genome suggests that the actual number could be closer to 500 [Troemel et al., 1995; Bargmann, 1998]. Given that *C. elegans* has 10 neurons that respond to volatile odorants [Bargmann et al., 1993; Mori and Ohshima, 1997], each olfactory neuron may express 10–50 odorant receptor genes, far more than has been proposed for vertebrates or *Drosophila.*

Odorant receptor genes are expressed in zones in both vertebrates and *Drosophila.* In mammals, odorant receptor genes are expressed in one of four broad zones in the olfactory epithelium, and expression of a single gene is scattered randomly within a particular zone [Ressler et al., 1993; Vassar et al., 1993; Strotmann et al., 1994]. In the olfactory rosette of zebrafish, each receptor gene is expressed in one of three concentric, slightly overlapping zones [Weth et al., 1996]. The odorant receptor genes in *Drosophila* are expressed in a variable number of sensillae in a bilaterally symmetrical pattern on the antennae. The number and position of the sensillae in which a receptor is expressed appear to be consistent across individuals [Vosshall et al., 2000]. Again, the olfactory system of *C. elegans* appears to be organized differently, as there is no evidence of a zonal pattern of expression of odorant receptor genes [Troemel et al., 1995].

The ligand specificity of the odorant receptors may be similar across phyla. The first demonstration of the function of a putative odorant receptor was accomplished in *C. elegans,* using a behavioral assay of odorant sensitivity in animals with mutations in the *odr-10* gene. Of the seven odorants tested, the animals failed to respond only to diacetyl, suggesting that the gene codes for a very narrowly-tuned odorant receptor [Sengupta et al., 1996]. When expressed in a human cell line, however, the ODR-10 receptor responded to diacetyl and to one of several structurally-related compounds that were tested, as well as to one structurally dissimilar chemical [Zhang et al., 1997]. Thus, the ODR-10 receptor is fairly narrowly tuned, but can interact with more than one ligand. In rats, when the I7 odorant receptor gene was overexpressed in the olfactory epithelium, the response to octyl aldehyde was greatly enhanced, responses to the related compounds heptaldehyde, nonyl aldehyde, and decyl aldehyde were increased, and responses to 70 other odorants were unchanged [Zhao et al., 1998]. Studies of mammalian odorant receptors in heterologous expression systems have found that the cells respond to a subset of the odorants tested, and show some selectivity for families of structurally similar compounds [Krautwurst et al., 1998; Murrell and Hunter, 1999]. A goldfish odorant receptor expressed in *Xenopus* oocytes responded to basic and neutral aliphatic L-amino acids, with a high affinity for arginine and lysine, and did not respond to 13 other amino acids, 10 amino acid derivatives and neurotransmitters, or to other behaviorally important odorants such as bile acids, prostaglandins, and sex steroids [Speca et al., 1999]. Relatively broad ligand binding has also been suggested by studies in which the *Drosophila* Or43a receptor gene was overexpressed in antennal olfactory receptor neurons or expressed in *Xenopus* oocytes [Störtkuhl and Kettler, 2001]. Based on the data available, it appears that odorant receptors in *C. elegans, Drosophila,* and vertebrates respond to multiple odorants, and in some cases appear to respond preferentially to groups of structurally similar compounds.

Why do we find G protein-coupled odorant receptors in *C. elegans, Drosophila,* and vertebrates? In principle, other types of receptors, such as ligand-gated ion channels, could be used as odorant receptors. Is the presence of G protein-coupled odorant receptors in these phyla an example of evolutionary convergence that informs us about the workings of the olfactory system, or are alternative hypotheses also tenable?

Homology

GPCRs may have been co-opted once for use in odorant detection, in which case the odorant receptors in *C. elegans, Drosophila,* and vertebrates would be descended from, and therefore homologous with, these original odorant receptors. Given the very low level of sequence similarity among odorant receptor gene families in these different phyla, we can rule out this possibility, and conclude that the odorant receptors were co-opted independently out of the larger family of GPCRs. Furthermore, the status of GPCRs as a monophyletic family is questionable [Josefsson, 1999].

Convergence

If odorant receptors have been co-opted from GPCR families that arose independently, they could not be considered homologous, but are examples of structural convergence [Doolittle, 1994; Zakon, 2002]. Are they adaptations, or responses to constraints?

Adaptation. The occurrence of G protein-coupled odorant receptors in nematodes, insects, and vertebrates may constitute independent adaptations for transducing odorant stimuli. If so, why? Is there something about these receptors that makes them particularly suitable for use in olfaction? Based on a consideration of the properties of GPCRs, three explanations seem plausible. These

hypotheses are not mutually exclusive, and all these properties of GPCRs could have contributed to their adoption as odorant receptors. (1) The use of GPCRs allows for amplification of small signals [Selbie and Hill, 1998], a feature that would be adaptive in situations in which few odorant molecules reach the olfactory epithelium. Although the lower limit of detectability has been debated, some studies have suggested that olfactory receptor neurons in both insects and vertebrates may be able to respond to single odorant molecules [Kaissling, 1986; Menini et al., 1995]. (2) Another potential functional advantage to the use of GPCRs is that different odorants could bind to a single receptor type and activate different, interacting signaling pathways through one or more G proteins [Selbie and Hill, 1998]. Such flexibility may provide individual olfactory receptor neurons with the ability to respond to different odorants in different ways, even if each neuron expresses only a single receptor gene. (3) GPCRs can form homo- and heterodimers [Bouvier, 2001], and recent studies suggest that G protein-coupled sweet taste receptors form dimers [Max et al., 2001; Nelson et al., 2001]. If odorant receptors can also form heterodimers, the number of combinations that could be formed from the large families of odorant receptor genes is virtually limitless. The ability of GPCRs to form dimers may make them particularly suitable for transducing or coding the broad array of odorants that many animals encounter.

Constraint. Perhaps the repeated deployment of GPCRs for use in olfaction reflects the action of a constraint, such as a developmental or genetic constraint. For example, perhaps members of this gene family are particularly easily duplicated, and this creates a bias such that these receptor genes are more likely than others to be coopted for novel functions. I know of no data that would support such a scenario, but it cannot be dismissed at present.

The Null Hypothesis

Finally, we must consider the possibility that the discovery of G protein-coupled odorant receptors in vertebrates, *Drosophila,* and *C. elegans* is misleading. One could argue that because researchers found G protein-coupled odorant receptors in *Drosophila* and *C. elegans* only by combing through genomic data seeking receptors similar to those found in vertebrates, the results are biased, the similarity is superficial, and that other receptor types also serve as odorant receptors in these animals but have not been found because we have not looked for them. This position seems untenable: the large numbers of G protein-coupled odorant receptor genes found in *Drosophila* and *C. elegans* suggest that these genes code for all or almost all the odorant receptors in these animals; further, members of both gene families have been demonstrated to function in odorant transduction, as described above.

In summary, the use of GPCRs as odorant receptors in *C. elegans, Drosophila,* and vertebrates appears to be an example of evolutionary convergence, and the use of these receptors may constitute an adaptation for the processing of odorant signals, although the functional advantages of their use are not yet clear. GPCRs seem to be evolutionarily and physiologically flexible, as they can interact with each other and with a variety of different intracellular signaling pathways.

Signal Transduction Strategies

The binding of odorants to receptors can cause olfactory receptor neurons to depolarize or hyperpolarize, and can cause changes in baseline membrane conductance. These effects are mediated by a variety of different signal transduction pathways, which might co-exist within individual cells, in teleosts, amphibians, rodents, lobsters, squid, and *C. elegans* [Schild and Restrepo, 1998; Ache and Restrepo, 2000]. In this paper, I will concentrate on one particular olfactory transduction strategy, as it is unusual among sensory systems and has been well documented in olfactory receptor neurons in two phyla: receptor binding initiates a two-step signal transduction cascade in which cation channels open, and the cations that enter the cell then gate additional ion channels, contributing to depolarization of the cell [Ache and Restrepo, 2000].

A two-step signal transduction pathway has been described in detail in rats *(R. norvegicus)* and mice *(M. musculus domesticus)* in electrophysiological, biochemical, and molecular/genetic studies. Odorant binding activates an olfactory-specific G protein, stimulating type III adenylyl cyclase, which increases cAMP levels inside olfactory receptor neurons [Sklar et al., 1986; Jones and Reed, 1989; Bakalyar and Reed, 1990; Belluscio et al., 1998; Wong et al., 2000]. cAMP gates a non-selective cation conductance that is permeable mainly to calcium, and the calcium that enters through these channels gates calcium-dependent chloride channels [Dhallen et al., 1990; Brunet et al., 1996]. Because intracellular chloride levels are relatively high in olfactory receptor neurons, Cl^- flows outward, depolarizing the cell [Lowe and Gold, 1993; Reuter et al., 1998]. Components of this pathway have been described in humans and in cows *(Bos taurus),* suggesting

that this transduction strategy might be common among mammals [Ludwig et al., 1990; Gomez et al., 2000].

In other classes of vertebrates, this two-step transduction pathway appears to be present, although it has not been characterized as fully as in rodents. For example, electrophysiological experiments demonstrate that these signaling mechanisms are also active in olfactory receptor neurons of salamanders *(Ambystoma tigrinum, Cynops pyrrhogaster,* and *Necturus maculosus)* and anurans *(Bufo marinus, Rana catesbeiana, R. pipiens, R. 'esculenta', R. ridibunda,* and *Xenopus laevis)* [Sklar et al., 1986; Nakamura and Gold, 1987; Firestein et al., 1991; Frings and Lindemann, 1991; Kleene and Gesteland, 1991; Dubin and Dionne, 1993; Kurahashi and Yau, 1993; Zhainazarov and Ache, 1995a]. Studies with olfactory receptor neurons from catfish *(Ictalurus punctatus),* zebrafish *(B. rerio),* and carp *(Cyprinus carpio)* demonstrate that a cyclic nucleotide-gated cation channel is present, that odorant binding elevates cAMP levels, and that cAMP activates a cation current [Goulding et al., 1992; Kolesnikov and Kosolapov, 1993; Ma and Michel, 1998], indicating that the first step in a two-step transduction cascade is present. The second step might also be present in teleosts: in rainbow trout *(Oncorhynchus mykiss)* a calcium-dependent chloride conductance is activated by odorants [Sato and Suzuki, 2000]. Taken together, these data indicate that the two-step signal transduction pathway that has been described in rodents is also present in amphibians and teleost fishes, suggesting that it is widely used in vertebrates.

Studies from Ache's laboratory have elucidated a different two-step transduction pathway that is activated by odorant binding in Caribbean spiny lobsters *(Panulirus argus).* Although the links among steps in this pathway are not as firmly established as in rodents, odorants have been demonstrated to activate a depolarizing cation current in spiny lobster olfactory receptor neurons [Anderson and Ache, 1985]. The underlying mechanism involves odorant activation of a G_q, a G protein that is associated with phospholipase C [Fadool et al., 1995]. The second messenger that activates the cation current seems to be IP_3, as odorant exposure elevates IP_3 levels, receptors for IP_3 are found in the ciliary membrane, and IP_3 activates a depolarizing cation current [Fadool and Ache, 1992; Boekhoff et al., 1994; Hatt and Ache, 1994; Munger et al., 2000; Zhainazarov et al., 2001]. Thus, although the second messenger in the first step of the transduction pathway in spiny lobsters is IP_3, rather than cAMP as in vertebrates, the result is the same: odorant exposure causes a cation channel to be opened. Interestingly, sodium-dependent non-selective cation channels are also present in outer dendrites of spiny lobster olfactory receptor neurons. Depolarizing odorant responses are reduced by drugs that block the sodium-dependent cation channel and by the substitution of other cations for sodium in the extracellular fluid, suggesting that the sodium-dependent cation channel in spiny lobster olfactory receptor neurons plays a role analogous to that of the calcium-activated chloride channel in vertebrate olfactory receptor neurons [Zhainazarov and Ache, 1995b, 1997; Zhainazarov et al., 1998]. Thus, it appears that in spiny lobsters, odorant binding elevates IP_3 levels, gating a cation channel, and the sodium that enters the cell through this cation channel gates a second, non-selective cation channel.

Can we find components of a two-step transduction pathway in other groups of animals? The pathways used in both vertebrates and spiny lobsters possess some common features: odorant binding increases levels of a second messenger that gates a cation channel, and the entering cations are involved in gating an additional channel. In *C. elegans,* the first step in such a pathway appears to be present. The *C. elegans* genome project has revealed the existence of 20 genes coding for alpha subunits of G proteins, two of which are necessary for chemotaxis to some volatile odorants [Jansen et al., 1999]. Cyclic nucleotide-gated cation channels are present in a subset of olfactory receptor neurons, are gated by cGMP, and are necessary for chemotaxis [Coburn and Bargmann, 1996; Komatsu et al., 1996, 1999]. Data concerning a second step in the transduction pathway, such as an additional cation-activated channel, are lacking.

In insects, a depolarizing odorant-induced current appears to be mediated by G protein-activated stimulation of IP_3, as has been demonstrated in American cockroaches *(Periplaneta americana),* migratory locusts *(Locusta migratoria),* sphinx moths *(M. sexta),* and fruit flies *(D. melanogaster)* [Boekhoff et al., 1990a, b; Breer et al., 1990; Stengl, 1994; Riesgo-Escovar et al., 1995]. Stengl's description of odorant-induced currents in cultured *Manduca* olfactory receptor neurons suggests the presence of a two-step transduction process: odorants increase levels of IP_3, which stimulates an IP_3-dependent calcium current; the influx of calcium then stimulates a calcium-dependent cation current [Stengl, 1994]. Although the details of the channels and second messengers involved are not known, these data hint that different two-step processes might mediate odorant transduction in vertebrates, spiny lobsters, and *Manduca.*

Figure 3 illustrates the phylogenetic distribution of animals in which the presence of a two-step odorant trans-

duction pathway is indicated. A transduction strategy that involves the use of external cations to gate additional ion channels is unusual in sensory receptor cells. Why do we find this transduction strategy in the olfactory systems of spiny lobsters, *Manduca,* and vertebrates? Is this an adaptation for processing odorant stimuli, or should we consider other hypotheses as well?

Homology

In theory, the presence of a two-step transduction strategy in these groups could be due to inheritance from a common ancestor, and represent an example of homology. This hypothesis is simply not supportable: the components of the pathway differ greatly among *Manduca,* spiny lobsters, and vertebrates, including the type of G-protein, the second messenger used, the primary cation channel, and the second ion channel that is activated.

Convergence

The separate origins of two-step transduction pathways may be due to evolutionary convergence, and might constitute an adaptive mechanism that helps us understand the ways in which olfactory information is processed by the nervous system.

Adaptation. Several hypotheses concerning the adaptive function of a two-step transduction strategy have been proposed. (1) The use of a second ion channel that opens as an indirect result of odorant binding may serve to amplify the signal. This feature would be particularly useful in a sensory system such as olfaction that detects low-concentration or infrequent stimuli [Kleene, 1993; Lowe and Gold, 1993; Zhainazarov and Ache, 1995b]. (2) A variant on this hypothesis states that the use of a second ion channel might allow for *regulation* of amplification of the signal. For example, the degree of amplification achieved by opening chloride channels in vertebrate olfactory receptor neurons could be regulated by altering the sensitivity of the channel to calcium, or by regulating the concentration of calcium inside the cell [Ache and Restrepo, 2000]. (3) The contribution of the chloride current in vertebrate olfactory receptor neurons depends on the internal concentration of chloride. If the concentration is not as high as some have suggested [Reuter et al., 1998], then rather than contributing to depolarization, the chloride current might limit depolarization or contribute to the repolarization of the cell [Kleene and Gesteland, 1991; Kleene, 1993]. This argument seems to apply only to the transduction pathway described in vertebrates, as the ion channels involved in transduction in spiny lobsters and *Manduca* pass external cations.

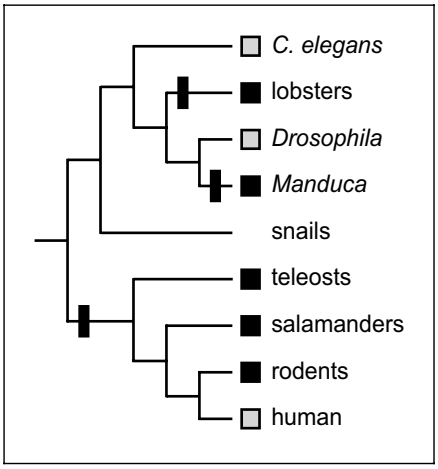

Fig. 3. Phylogenetic distribution of two-step odorant transduction processes among common model animals used in olfactory research. Taxa in which a two-step transduction process has been demonstrated are indicated with solid boxes; gray boxes indicate taxa for which odorant-gated cation currents or cation channels, the first step in such pathways, have been described. Based on these data, a hypothesis concerning the evolutionary history of this transduction strategy is illustrated. Although data from suitable outgroups are lacking, the components of the pathways differ greatly among groups, suggesting that this transduction strategy evolved independently in the ancestors of vertebrates, lobsters, and *Manduca.* The strategy used by *Manduca* and *Drosophila* may be the same, but the available data are too sparse for hypotheses to be proposed.

Constraint. Two-step transduction pathways might have arisen because of a physical constraint that is unique to olfactory receptor neurons. Indeed, such a hypothesis has been proposed to explain the use of a secondary step involving chloride ions in vertebrate olfactory receptor neurons. If the ionic composition of the mucus layer is difficult to regulate, the ionic environment of olfactory receptor neurons could be somewhat unpredictable, particularly for aquatic organisms. Thus, it is not always certain that enough external cations will be available to depolarize the cell; however, depolarization can be ensured through the use of a step that involves the efflux of anions [Kurahashi and Yau, 1993]. This hypothesis is attractive, but only applies when the second ion channel in the transduction pathway passes internal anions. That is, the hypothesis might explain the evolution of a two-step transduction pathway in vertebrates, but cannot explain the evolution of the strategy used by spiny lobsters or *Manduca.*

The Null Hypothesis

Perhaps the presence of a two-step transduction pathway in different taxa is coincidental, and any similarity is merely superficial. It may be misleading to focus on the observation that some olfactory receptor neurons contain ion channels that are gated by ions that enter during the transduction process, because a great diversity of transduction pathways has been described in olfactory receptor neurons [Dionne and Dubin, 1994; Schild and Restrepo, 1998; Ache and Restrepo, 2000]. We cannot at present dismiss the possibility that the presence of such a strategy in disparate groups simply reflects the fact that olfactory receptor neurons contain myriad ion channels that are directly or indirectly activated by odorants.

In summary, it is possible that a two-step transduction cascade is an adaptation for amplifying (or regulating the amplification of) small odorant signals, and has evolved independently in vertebrates, spiny lobsters, and *Manduca* for this purpose. However, we cannot conclude with any certainty that the use of two-step odorant transduction pathways constitutes an adaptation, because the function of this mechanism might differ among groups, and it seems possible that any similarity is coincidental.

Glomerular Neuropils

The axons of olfactory receptor cells terminate in glomeruli, large bundles of tangled neuropil, in the olfactory bulb of vertebrates, the olfactory lobe of lobsters, the antennal lobe of insects, and the tentacle ganglion in snails. These glomerular tangles usually have an overall round shape, and are encircled by glial cells.

Glomeruli in the vertebrate olfactory bulb consist of fibers emanating from peripheral receptor neurons, periglomerular interneurons, and various types of output cells. The cellular elements of glomerular circuits in some neopteran insects, such as *Manduca,* are organized in a remarkably similar fashion, comprising fibers from receptor neurons, interneurons, and antennal lobe output neurons [Hildebrand and Shepherd, 1997; Strausfeld and Hildebrand, 1999]. Many structural and functional features of glomeruli appear to be shared by vertebrates and insects, and I will describe these below. Nevertheless, it should be noted that most of the data available to date are drawn from studies of a small number of species: rats *(R. norvegicus),* mice *(M. musculus domesticus), Manduca,* and *Drosophila.* One feature that might be unique to insects is that chemosensory neurons from different portions of the body may send axons to glomerular structures in the antennal lobe; for example, in *Drosophila,* the axons of olfactory receptor neurons on the antennae and the maxillary palps both project to antennal lobe glomeruli, as do axons of olfactory receptor neurons on the antennae and in the labial pit organ of lepidopterans [Singh and Nayak, 1985; Kent et al., 1986].

In visual, auditory, and somatosensory systems, stimulus coding often involves the use of topographic maps in the central nervous system. This does not seem to be true of olfactory systems in any group examined to date: in both vertebrates and neopteran insects, there is no obvious relationship between the location of a glomerulus and the odorant stimuli to which it responds. Each glomerulus responds to a particular subset of odorants, such that a given odorant at a given concentration will evoke activity in the same subset of glomeruli in different individuals within a species, as has recently been demonstrated in imaging studies using rats, mice, zebrafish *(B. rerio),* honeybees *(Apis mellifera),* and two species of noctuid moths *(Heliothis virescens* and *H. zea)* [Friedrich and Korsching, 1997; Vickers et al., 1998; Galizia et al., 1999; Rubin and Katz, 1999; Wachowiak and Cohen, 2001]. In addition, inputs from the receptor epithelium to the glomerular layer appear to be tightly controlled and reproducible among members of a species. For example, in *Drosophila* as well as in rats and mice, axons of receptor cells that express a particular receptor or subset of receptors converge at the same glomerulus, which is in the same location in different individuals within a species [Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Vosshall et al., 2000; Schaefer et al., 2001]. As described above, receptors are expressed in a few large zones in the vertebrate olfactory epithelium and on *Drosophila* antennae. A given glomerulus receives input from receptor neurons scattered throughout one of these zones, but there is no fine-scale topographical organization of inputs from the sensory periphery to glomeruli [Stocker et al., 1983; Clancy et al., 1994; Schoenfeld et al., 1994; Vosshall, 2001]. Thus, although we do not completely understand the role of glomeruli in coding odorant information, they appear to play a similar role in insects and vertebrates.

Finally, even the cellular interactions involved in the development of glomeruli are similar in insects and vertebrates. Studies of developing *Manduca* demonstrate that ingrowing axons from olfactory receptor neurons interact with each other to form small protoglomeruli, which do not require interaction with potential synaptic targets, such as the output cells of the antennal lobe, to develop [Oland and Tolbert, 1998]. Nevertheless, unless the pro-

toglomerulus is rapidly surrounded by glial cells, it will dissolve and the projections will become diffuse [Oland and Tolbert, 1988; Oland et al., 1988; Baumann et al., 1996]. In developing and adult mice, axons of olfactory receptor cells will form glomeruli and interact with neurons in ectopic locations, and the development of glomerular tangles has been thought to be an intrinsic property of olfactory receptor cell axons [Graziadei and Kaplan, 1980; Graziadei and Samanen, 1980; Graziadei and Monti Graziadei, 1986]. Recent studies of rat embryos demonstrate that axons of receptor neurons initially form protoglomeruli, then interact with glia to form glomerular boundaries, and later interact with dendrites of interneurons and output neurons, just as in *Manduca* [Valverde et al., 1992; Bailey et al., 1999; Treloar et al., 1999].

Glomeruli in decapod crustaceans share similarities with those of insects and vertebrates, but also possess some unique features. In decapods, the axons of olfactory receptor neurons interact with fibers from interneurons and projection neurons in unusual cone-shaped glomeruli located in the olfactory lobe, as has been demonstrated in the Australian yabby *(Cherax destructor),* red swamp crayfish *(Procambarus clarkii),* American lobster *(Homarus americanus),* and Caribbean spiny lobster *(P. argus)* [Sandeman and Luff, 1973; Mellon and Munger, 1990; Schmidt et al., 1992a; Helluy et al., 1995]. In lobsters, the glomeruli of the olfactory lobe comprise three distinct horizontal zones or compartments that are innervated by different interneurons [Schmidt et al., 1992b; Langworthy et al., 1997; Schmidt and Ache, 1997]. Intraglomerular compartments have also been described in rats and mice, and may correlate with dendritic fields of different classes of periglomerular interneurons [Treloar et al., 1996; Kosaka et al., 1998; Kasowski et al., 1999]; if so, the subdivision of glomeruli into different compartments in which receptor and output neurons interact with different groups of interneurons might constitute another feature of olfactory glomeruli that is shared across phyla. In addition to this anatomical feature, a physiological characteristic of glomeruli is also shared in vertebrates and crustaceans: presynaptic inhibition of sensory input to glomeruli occurs in both vertebrates (turtles, *Terapene carolina*) and lobsters *(P. argus),* but the underlying mechanisms differ in the two groups [Wachowiak and Cohen, 1999]. One unique feature of the olfactory system in lobsters and crayfish is the presence of small, round glomeruli in the accessory lobe and other secondary olfactory regions of the deutocerebrum [Sandeman and Luff, 1973; Blaustein et al., 1988; Helluy et al., 1993]. This feature appears to be an evolutionary innovation, as the accessory lobe is only present in a subset of malacostracan crustaceans [Sandeman et al., 1993].

Although phylogenetically widespread in olfactory targets in the central nervous system, glomeruli are not a universal feature. For example, although glomeruli are present in the olfactory bulbs of all craniates examined to date, including Pacific hagfish *(Eptatretus stouti)* [Wicht and Northcutt, 1992], they are not present in near outgroups to craniates, such as amphioxus or larval tunicates [Bone, 1960; Vorontsova et al., 1997].

Among molluscs, olfactory pathways have been described in detail only in gastropods (snails and slugs), and the phylogenetic distribution of glomeruli is not clear. Glomeruli may not be present in cephalopods: the olfactory lobe of *Nautilus* is layered, and that of *Octopus* is smaller and has no apparent large-scale organization [Young, 1965, 1971]. Although glomeruli are present in the first olfactory target in snails, not all olfactory axons terminate in these glomeruli. In the giant African snail *Achatina fulica*, the axons of some olfactory receptor neurons project past the glomerular neuropils of the digit to terminate in the body of the tentacle ganglion, or even in the cerebral ganglion [Chase and Tolloczko, 1993]. The functional significance of extra-glomerular olfactory projections is not understood, but primary olfactory projections that bypass the olfactory bulb have also been described in most classes of vertebrates [Eisthen, 1997].

The distribution of olfactory glomeruli among arthropods is variable, and has been examined in detail by Strausfeld and colleagues [Strausfeld et al., 1995, 1998; Strausfeld, 1998; Strausfeld and Hildebrand, 1999]. Glomeruli are broadly present in neopteran insects, such as *Drosophila* and *Manduca,* but are not present in those that have secondarily lost odorant-sensitive antennae, such as diving beetles *(Dytiscus marginalis)* [Strausfeld et al., 1998]. Glomeruli are also absent in members of the sister groups to neopterans, such as mayflies, dragonflies, and damselflies [Strausfeld, 1998; Strausfeld and Hildebrand, 1999]. Glomeruli are not present in antenno-recipient areas in basal hexapods, such as silverfish, firebrats, or bristletails [Strausfeld and Hildebrand, 1999]. Among crustaceans, the sister group to hexapods, the presence of glomeruli is variable. The presence of cone-shaped glomeruli in the olfactory lobe is a feature unique to decapod crustaceans. In contrast, the olfactory lobe of isopods contains large, round glomeruli like those typically seen in neopteran insects, and basal branchiopods such as *Triops* appear to completely lack a specialized antennal neuropil [Strausfeld, 1998; Strausfeld et al., 1998]. The other major groups of arthropods are myriapods and chelicerates, and

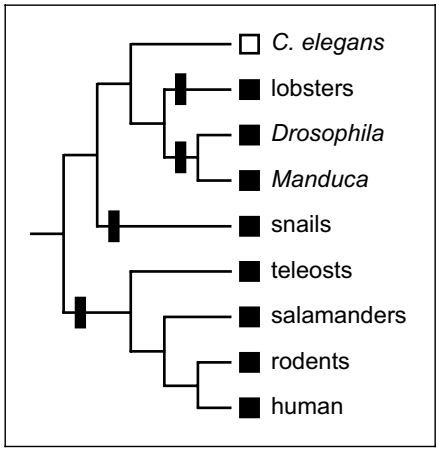

Fig. 4. Phylogenetic distribution of glomerular structures in the first central olfactory target in common model animals used in olfactory research. Taxa in which glomeruli have been described are indicated with solid boxes; the empty box signifies that *C. elegans* lacks glomeruli. Given that glomeruli are known to be lacking in outgroups relative to vertebrates, terrestrial insects, and lobsters, the hypothesis illustrated here postulates that glomerular structures evolved independently at least four times.

olfactory glomeruli are clearly present in at least some species in these groups. Among myriapods, glomeruli have been described in olfactory regions in chilopods (the centipede *Lithobius variegatus*) and diplopods (the desert millipede *Orthoporus ornatus*) [Strausfeld et al., 1995; Strausfeld, 1998]. Among chelicerates, olfactory glomeruli have been identified in various groups, including pycnogonids (sea spiders, *Lecythorhynchus hilgendorfii*), solpugids (sun spiders, *Eremobates pallipes*), scorpions (the bark scorpion *Centruroides sculpturatus*), opilionids (the group of false spiders containing the familiar 'daddy longlegs'), uropigids (vinegaroons or whip scorpions, *Mastigoproctus giganteus*), and amblypygids (tailless whip scorpions, *Tarantula*) [Strausfeld et al., 1998]. Glomeruli are not universally present within this group, and are lacking in the basal spider *Heptathela kimurai* [Strausfeld et al., 1998]. Interestingly, chelicerates have repeatedly coopted various legs for use as olfactory organs, and rather than being located in the most anterior segment, glomeruli tend to be present in the segmental ganglion that receives input from olfactory sensillae [Strausfeld et al., 1998]. Finally, olfactory glomeruli have been described in onychophorans (velvet worms), the sister group to arthropods: in *Euperipatoides leukartii,* afferents from antennal chemosensory neurons terminate in glomerular structures lateral to the mushroom bodies [Schürmann, 1995; Strausfeld et al., 1995].

The olfactory neurons of the amphid organ in *C. elegans* do not form glomerular structures, and seem to participate in circuits that are quite different from those in other phyla. Each olfactory neuron forms electrical and/or chemical synapses with interneurons and sometimes with other chemosensitive neurons, including, in some cases, its paired equivalent from the contralateral side [White et al., 1986]. The phylogenetic distribution of glomerular neuropils in olfactory regions of the central nervous system is illustrated in figure 4.

The similarity of architecture in the central olfactory targets of various animals is compelling, but a few notes of caution are in order. First, not all olfactory receptor neurons project to glomerular structures, and one must avoid circularity in identifying the olfactory component of chemosensory systems. For example, an insect may have chemosensitive sensillae on its legs, wings, mouth parts, and antennae. Which are 'olfactory'? Traditionally, the sensillae that respond to chemicals emanating from distant sources would be labeled olfactory, but making this functional determination can be difficult, and it is tempting to assume that the sensillae containing neurons with axons that project to glomerular structures constitute the olfactory system. This assumption can be misleading, as illustrated by studies of spiny lobsters *(P. argus).* Spiny lobsters have chemosensitive sensillae over most of their bodies, including the medial and lateral antennules, the antennae, and walking legs [Laverack, 1988]. The axons of neurons innervating aesthetasc sensillae on the medial antennule project to the 'olfactory lobe', which contains glomeruli, and those innervating non-aesthetasc sensillae on the medial and lateral antennules project to the 'lateral antennular neuropil', which lacks glomeruli [Schmidt et al., 1992a; Schmidt and Ache, 1996]. Despite the suggestive names of their central targets, both types of sensillae can mediate olfactory tasks, such as searching for and localizing food [Steullet et al., 2001].

In addition to the definition of 'olfaction', the definition of a 'glomerulus' can be problematic. Although the term was originally applied to structures in the olfactory bulb [Cajal, 1890], it has been widely used to refer to tangles of fibers [Pinching and Powell, 1971], or, even less specifically, to any 'synaptic complex enclosed in glial membranes or otherwise set apart' [Shepherd, 1974, p. 191]. By these definitions, many regions of the central nervous system might be interpreted as containing glomeruli [Leise, 1990]. Nevertheless, olfactory glomeruli possess unique features. Olfactory glomeruli are strikingly large, with diameters of 50–120 μm in rats *(R. norvegicus),* 45–100 μm in sphinx moths *(M. sexta),* and roughly 40–100 μm in snails *(Acha-*

tina fulica); in spiny lobsters *(P. argus),* the cone-shaped olfactory lobe glomeruli are 40–100 μm in diameter and 250 μm long [Pinching and Powell, 1971; Chase, 1985; Schmidt et al., 1992b; Rospars and Hildebrand, 2000]. Another key difference between glomeruli in olfactory centers and glomerulus-like structures in other portions of the brain, such as the large 'barrels' in the whisker region of rat somatosensory cortex, is that olfactory glomeruli do not receive topographically-organized afferent input, but instead seem to receive input from groups of axons expressing identical receptor genes. Thus, olfactory glomeruli possess features that distinguish them from other types of compartments in the central nervous system.

Overall, glomeruli are broadly present in olfactory targets in the central nervous system. Why? Is the presence of these odd structures significant for our understanding of olfactory function?

Homology

Given their widespread phylogenetic distribution glomeruli could have evolved once in the common ancestor of arthropods, molluscs, and craniates. However, given that glomeruli are not present in outgroups relative to craniates or neopteran insects, and are absent in basal crustaceans, glomeruli must have arisen independently at least three times, and cannot be considered homologous across groups. Further, the phylogenetic distribution of glomeruli among arthropods suggests that glomeruli have evolved independently several times within this group [Strausfeld et al., 1995, 1998; Strausfeld, 1998; Strausfeld and Hildebrand, 1999]. We can therefore reject the hypothesis that glomeruli are homologous across phyla.

Convergence

The repeated evolution of olfactory glomeruli might be due to evolutionary convergence. If so, are glomeruli a functional adaptation for processing odorant information, or a response to a constraint?

Adaptation. If glomeruli arose many times independently as an adaptation related to olfactory information processing, their presence in diverse taxa is an important clue to understanding the neurobiology of olfaction. The repeated evolution of these enigmatic structures has fascinated olfactory researchers, most of whom accept the hypothesis that glomeruli play a crucial role in the coding of odorant information [Hildebrand and Shepherd, 1997; Strausfeld and Hildebrand, 1999; Christensen and White, 2000]. Unlike visual or auditory stimuli, olfactory stimuli cannot be arrayed along a small number of linear dimensions; unlike the visual or auditory systems, the olfactory system does not use topographic maps to represent the stimulus, but may instead use glomerular structures for a similar purpose. The exact function of glomeruli, and their role in odorant information coding, remains unclear, but two fundamental roles for glomeruli have been proposed. First, the architecture of glomeruli might function to amplify odorant signals, as has been demonstrated in studies with both insects and vertebrates [Duchamp-Viret et al., 1989, 1990; Hartlieb et al., 1997]. Another hypothesis holds that glomeruli and their associated interneurons may provide for a form of lateral inhibition, or could serve to sharpen the contrast among different odorants [Shepherd, 1974, 1992]. These hypotheses are not mutually exclusive, and both mechanisms could work together to enhance the signal-to-noise ratio between the olfactory periphery and the central nervous system.

Constraint. The presence of glomeruli in olfactory targets may reflect the operation of physical constraints imposed by the need to have similarly-tuned receptor neurons scattered throughout regions of the olfactory epithelium, either to protect against complete loss or to ensure that the odorant signal is averaged across as large an epithelial sheet as possible. The axons of these scattered neurons then must converge on a small number of locations in the central nervous system. In this view, glomerular structures could simply be a space-efficient method of bringing together axons of similarly-tuned receptor neurons and segregating them from other aggregations of axons. Hildebrand and Shepherd [1997] argue persuasively that if this were the case, interneurons between glomeruli would be unnecessary; nevertheless, such neurons are found in vertebrates, insects, and lobsters.

The Null Hypothesis

Although it is conceivable that the similar organization of central olfactory targets into glomerular structures in different taxa is coincidental, or that the similarities are superficial, the many shared morphological and physiological features of glomeruli make this hypothesis seem unlikely. It is also possible that glomeruli serve different functions in different groups; again, the many similarities make this seem unlikely, but until we develop a better understanding of the function of glomeruli, we cannot completely dismiss this hypothesis.

In summary, glomeruli have arisen repeatedly many times in the olfactory systems of a wide array of animals. Data currently available suggest that glomeruli constitute a functional adaptation for processing odorant informa-

tion, although their precise role in olfactory information coding remains unclear.

Implications for Olfactory Research

Researchers studying the neurobiology of olfaction commonly assume that features that are present in distantly-related animals are functional adaptations for carrying or processing odorant information. The analysis presented here demonstrates that other hypotheses can rarely be ruled out and that in many cases convergent features could have been shaped by constraints as well as adaptation. The preceding analysis suggests that a consideration of alternative hypotheses based on an explicit evolutionary framework is a useful method for determining which hypotheses logically compete with each other and for developing new hypotheses.

For example, odorant binding proteins (OBPs) may be present in terrestrial animals because such animals face the constraint of having to detect hydrophobic odorants in air; conversely, aquatic animals may be constrained from using OBPs because of the high metabolic cost of producing soluble proteins that could diffuse away into the environment. In addition, OBPs might serve one or more functional roles in carrying odorants to the receptor, interacting with odorant receptors, or removing odorants from the sensory epithelium. Because the function of OBPs is not yet known, we cannot dismiss the hypothesis that the presence of these proteins in insects and mammals is coincidental, or that OBPs serve a role not specific to olfaction in one or both groups.

The presence of a two-step signal transduction strategy in vertebrates, spiny lobsters, and sphinx moths *(Manduca)* could represent an adaptation for detecting small quantities of odorants. However, given the great diversity of transduction mechanisms employed in olfactory receptor neurons, the similarity may be coincidental, and these mechanisms might also have arisen for different reasons in different taxa.

The clearest examples of adaptive convergence in olfactory systems appear to be the use of G protein-coupled receptors (GPCRs) as odorant receptors in *C. elegans, Drosophila,* and vertebrates, and the presence of glomerular structures in the first olfactory relay in the central nervous systems of many animals. GPCRs and their underlying genes seem to be remarkably flexible, which might explain why they been co-opted repeatedly for use in olfaction. The repeated evolutionary origin of olfactory glomeruli strongly suggests that this anatomical arrangement serves a valuable and specific function in olfactory information processing, although an understanding of the precise function of these structures remains elusive.

Implications for the Use of Convergence in Neurobiological Research

Many neurobiologists use a 'model organism' approach in which similarity is emphasized without consideration of the evolutionary processes that may have given rise to these similarities. Similar features can be present due to inheritance from a common ancestor or may represent examples of convergent evolution. In the latter case, these features may represent responses to similar constraints, or could constitute adaptations to similar demands. The interpretation of similar features depends on an explicit or implicit hypothesis about the underlying cause of similarity, and an explicit attention to evolutionary processes would enrich our understanding of many problems in neurobiology.

The goal of this symposium was to draw attention to examples of convergent evolution, which can serve as valuable clues to understanding strategies for coding information in the nervous system. As demonstrated in this paper, adaptation and constraints may operate together to shape many features, and unless we know the function of a feature we often cannot exclude the possibility that any similarity is superficial or misleading. Thus, we should not simply assume that features that have arisen independently constitute adaptations that will prove informative about mechanisms of neural processing.

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