10. Mao, K.S., Hao, G., Liu, J.Q., Adams, R.P., and Milne, R.I. (2010). Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. New Phytol. *188*, 254–272.
11. Wagstaff, S.J. (2004). Evolution and biogeography of the austral genus *Phyllocladus* (Podocarpaceae). J. Biogeogr. *31*, 1569–1577.
12. Gernandt, D.S., Magallon, S., Lopez, G.G., Flores, O.Z., Willyard, A., and Liston, A. (2008). Use of simultaneous analyses to guide fossil-based calibrations of Pinaceae phylogeny. Int. J. Pl. Sci. *169*, 1086–1099.
13. Biffin, E., Brodribb, T.J., Hill, R.S., Thomas, P., and Lowe, A.J. (2011). Leaf evolution in Southern Hemisphere conifers tracks the angiosperm ecological radiation. Pro. R. Soc. Biol. Sci. 10.1098/rspb.2011.0559.
14. Wei, X.X., Yang, Z.Y., Li, Y., and Wang, X.Q. (2010). Molecular phylogeny and biogeography of *Pseudotsuga* (Pinaceae): Insights into the floristic relationship between Taiwan and its adjacent areas. Mol. Phyl. Evol. *55*, 776–785.
15. Treutlein, J., and Wink, M. (2002). Molecular phylogeny of cycads inferred from *rbcL* sequences. Naturwissenschaften *89*, 221–225.
16. Hill, R.S., and Brodribb, T.J. (1999). Turner Review No. 2-Southern conifers in time and space. Austral. J. Bot. *47*, 639–696.
17. Zachos, J., Pagani, M., Sloan, L., Thomas, E., and Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. Science *292*, 686–693.
18. Crepet, W.L., and Niklas, K.J. (2009). Darwin's second "abominable mystery": Why are there so many angiosperm species? Am. J. Bot. *96*, 366–381.
19. Brenner, E.D., Stevenson, D.W., and Twigg, R.W. (2003). Cycads: evolutionary innovations and the role of plant-derived neurotoxins. Trends Plant Sci. *8*, 446–452.
20. Hermsen, E.J., Taylor, T.N., Taylor, E.L., and Stevenson, D.W. (2006). Cataphylls of the Middle Triassic cycad *Antarcticycas schopfii* and new insights into cycad evolution. Am. J. Bot. *93*, 724–738.

Department of Organismic and Evolutionary Biology, Harvard University, Herbaria, 22 Divinity Avenue, Cambridge, MA 02138, USA.
*E-mail: cdavis@oeb.harvard.edu

DOI: 10.1016/j.cub.2011.11.020

Chemoreception: Identifying Friends and Foes

The vomeronasal organ detects chemical cues that trigger sexual, aggressive and defensive behaviors. An *in situ* hybridization analysis has identified the specificities of nearly a hundred VNO receptors and elucidated the logic by which they encode these cues.

Tong-Wey Koh and John R. Carlson

In his seminal essay 'The Hedgehog and the Fox', Sir Isaiah Berlin divided thinkers into two categories. Plato, Pascal, and Dostoevsky are like hedgehogs, which 'know one big thing', whereas Aristotle, Montaigne, and Goethe are like foxes, which 'know many things'. While Berlin later revealed that he had intended this celebrated distinction as a kind of game, the ability to distinguish hedgehogs and foxes is not a game for mice. It can mean the difference between life and death. A remarkable new study by Catherine Dulac and colleagues [1] has provided new insight into the molecular mechanisms by which mice make such distinctions.

How do mice identify the presence of animals that pose a threat, and of those that present opportunities for food or reproduction? Animals can be identified on the basis of pheromones (conspecific cues) or kairomones (heterospecific cues that benefit the recipient) [2]. The vomeronasal organ (VNO) is exquisitely sensitive to pheromones, containing neurons that are narrowly tuned to specific ligands [3]. Genetic or surgical disruption of VNO function in mice leads to profound but specific alterations of social behaviors [4]. The VNO acts in the sensing of individual differences, sex and the physiological status of conspecifics, in addition to the detection of kairomones from predators [5–7].

The VNO expresses more than 250 putative chemoreceptors. Most of these receptors belong to two families of heterotrimeric G-protein-coupled receptors, the V1Rs and the V2Rs [8–12]. V1Rs and V2Rs are thought to be expressed in a one receptor–one neuron pattern, with the exception of the broadly -expressed V2R2 clade [13,14]. Expression of V1R and V2R members are spatially segregated, with V1Rs expressed in the apical layer of the VNO neuroepithelium and V2Rs in the basal layer.

For only a very few of these receptors have ligands been previously identified. Efforts to map ligands to VNO receptors have focused on a single receptor or a single ligand [15,16]. Isogai *et al.* [1], in a *tour de force*, systematically characterized the functional specificities of nearly a hundred VNO receptors [1]. To accomplish this, they first improved an existing method of detecting VNO responses. Immediate early genes are induced in vomeronasal neurons by chemical cues. Isogai *et al.* [1] screened a panel of immediate early genes in the VNO of female mice that had been exposed to bedding used by male mice. One gene, *Egr1,* was found to be induced particularly strongly. *Egr1* induction was confirmed to reflect neuronal activation, as determined by calcium imaging and patch-clamp electrophysiology. Thus, by performing double labeling with *Egr1* and VNO receptor probes, it was possible to detect the activation of neurons expressing particular receptors by particular chemical cues.

Mice were exposed to a panel of ~30 chemical cues, including conspecific scents derived from the same or opposite sex, and heterospecific scents representing predators, prey, or neutral species. The heterospecific cues were derived from: mammalian predators, including foxes, ferrets, and bobcats; avian predators, including hawks and owls; reptilian predators, including snakes and alligators; potential prey, represented by insect larvae; related rodents that are sympatric with the wild ancestors of laboratory mice; and presumably neutral species such as woodchucks. Using probes that target 139 VNO receptors, Isogai *et al.* [1] were able to identify 88 receptors (56 V1Rs and 32 V2Rs) that responded to subsets of this panel of animal scents.

Isogai *et al.* [1] found that 28 receptors responded to mouse cues, and 26 of these responded to sex-specific cues. Some receptors were activated by female-specific cues in both males and females, while other receptors were activated by female-specific cues only in males. Some receptors responded to male-specific cues in males only, other receptors responded in females only, and still others responded in both.

Approximately 71 receptors were activated by heterospecific scents, indicating that the detection of other species is a major role of the VNO. Only 11 of these receptors also responded to mouse cues, consistent with the different behavioral effects of pheromones and kairomones. Intriguingly, some receptors were activated specifically by classes of predators: two receptors responded only to snake scents, while another group of receptors showed responses only to owl scents. Each predator species tested activated a distinct subset of receptors, which the authors interpret as evidence that the mouse VNO has the capacity to discriminate predators.

How does *Mus musculus* discriminate conspecifics from the closely related sympatric species *Mus spicilegus*, with which it does not breed? Scents of the two species activated different subsets of VNO receptors. In fact, members of one clade of receptors showed mutually exclusive activation patterns when exposed to scents from the two species. These results suggest that activation of VNO receptors contributes towards the reproductive isolation of the two species.

In addition to animal scents, which are complex mixtures, Isogai *et al.* [1] tested sulfated steroids, which are believed to account for most of the VNO activity that is elicited by female mouse urine [6]. A subset of V1Rs was found to distinguish classes of steroids, including estrogens, androgens, and corticosteroids. Because the levels of these steroids reflect the physiological status of animals, these V1Rs may help a mouse identify the physiological state of another mouse during a social encounter.

How are responses distributed among classes of VNO receptors? Most stimuli activated both V1Rs and V2Rs. However, hawk stimuli activated only V1Rs, while fox and rat scents stimulated only V2Rs. Male mouse scents activated only V2Rs in females. Most V2Rs are activated only by stimuli with seemingly consistent biological significance; for example, most V2Rs are activated uniquely by the scents of either predators, male mice, female mice, or neutral species. Surprisingly, many individual V1Rs responded to multiple cues of apparently conflicting ethological salience, such as mouse and predator cues, raising interesting questions about the processing of information transmitted by neurons expressing these receptors.

The cue-to-receptor map in the VNO provided by Isogai *et al.* [1] will be immensely useful in elucidating the circuitry by which animal cues are translated into behavioral output. A number of receptors may activate dedicated circuits, as in the case of ESP1, a male-secreted peptide pheromone that increases female receptivity in mice [16]. Other receptors, such as V1Rs that detect signals of apparently conflicting behavioral significance, may influence behavior through a more complicated logic. VNO neurons expressing the same receptor project to multiple glomeruli in the accessory olfactory bulb, where they synapse with mitral neurons. Some mitral neurons receive information solely from a single vomeronasal receptor, but others receive converging streams of information from multiple receptors [17,18]. To understand the flow of information through this circuitry, it may be especially revealing to combine the rich database of Isogai *et al.* [1] with analysis of concentration coding. In the case of some chemical cues, different VNO neurons are recruited at different concentrations [19], and a recent breakthrough in the functional expression of V2Rs in a heterologous system provides a new approach to investigating such concentration coding [20]. In any case, the spectacular advances of Isogai *et al.* [1] in functional characterization of VNO receptors, together with genetic and anatomical analysis, will surely lead to further major advances in our understanding of the mechanisms by which animals sense and respond to each other.

References

1. Isogai, Y., Si, S., Pont-Lezica, L., Tan, T., Kapoor, V., Murthy, V.N., and Dulac, C. (2011). Molecular organization of vomeronasal chemoreception. Nature *478*, 241–245.
2. Ferrero, D.M., and Liberles, S.D. (2010). The secret codes of mammalian scents. Wiley Interdiscip. Rev. Syst. Biol. Med. *2*, 23–33.
3. Leinders-Zufall, T., Lane, A.P., Puche, A.C., Ma, W., Novotny, M.V., Shipley, M.T., and Zufall, F. (2000). Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature *405*, 792–796.
4. Keverne, E.B. (2002). Mammalian pheromones: from genes to behaviour. Curr. Biol. *12*, R807–R809.
5. He, J., Ma, L., Kim, S., Nakai, J., and Yu, C.R. (2008). Encoding gender and individual information in the mouse vomeronasal organ. Science *320*, 535–538.
6. Nodari, F., Hsu, F.F., Fu, X., Holekamp, T.F., Kao, L.F., Turk, J., and Holy, T.E. (2008). Sulfated steroids as natural ligands of mouse pheromone-sensing neurons. J. Neurosci. *28*, 6407–6418.
7. Papes, F., Logan, D.W., and Stowers, L. (2010). The vomeronasal organ mediates interspecies defensive behaviors through detection of protein pheromone homologs. Cell *141*, 692–703.
8. Dulac, C., and Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. Cell *83*, 195–206.
9. Herrada, G., and Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell *90*, 763–773.
10. Matsunami, H., and Buck, L.B. (1997). A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell *90*, 775–784.
11. Ryba, N.J., and Tirindelli, R. (1997). A new multigene family of putative pheromone receptors. Neuron *19*, 371–379.
12. Rodriguez, I., Del Punta, K., Rothman, A., Ishii, T., and Mombaerts, P. (2002). Multiple new and isolated families within the mouse superfamily of V1r vomeronasal receptors. Nat. Neurosci. *5*, 134–140.
13. Mombaerts, P. (2004). Genes and ligands for odorant, vomeronasal and taste receptors. Nat. Rev. Neurosci. *5*, 263–278.
14. Martini, S., Silvotti, L., Shirazi, A., Ryba, N.J., and Tirindelli, R. (2001). Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ. J. Neurosci. *21*, 843–848.
15. Boschat, C., Pelofi, C., Randin, O., Roppolo, D., Luscher, C., Broillet, M.C., and Rodriguez, I. (2002). Pheromone detection mediated by a V1r vomeronasal receptor. Nat. Neurosci. *5*, 1261–1262.
16. Haga, S., Hattori, T., Sato, T., Sato, K., Matsuda, S., Kobayakawa, R., Sakano, H., Yoshihara, Y., Kikusui, T., and Touhara, K. (2010). The male mouse pheromone ESP1 enhances female sexual receptive behaviour through a specific vomeronasal receptor. Nature *466*, 118–122.
17. Del Punta, K., Puche, A., Adams, N.C., Rodriguez, I., and Mombaerts, P. (2002). A divergent pattern of sensory axonal projections is rendered convergent by second-order neurons in the accessory olfactory bulb. Neuron *35*, 1057–1066.
18. Wagner, S., Gresser, A.L., Torello, A.T., and Dulac, C. (2006). A multireceptor genetic approach uncovers an ordered integration of VNO sensory inputs in the accessory olfactory bulb. Neuron *50*, 697–709.
19. He, J., Ma, L., Kim, S., Schwartz, J., Santilli, M., Wood, C., Durnin, M.H., and Yu, C.R. (2010). Distinct signals conveyed by pheromone concentrations to the mouse vomeronasal organ. J. Neurosci. *30*, 7473–7483.
20. Dey, S., and Matsunami, H. (2011). Calreticulin chaperones regulate functional expression of vomeronasal type 2 pheromone receptors. Proc. Natl. Acad. Sci. USA *108*, 16651–16656.

Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
E-mail: john.carlson@yale.edu

DOI: 10.1016/j.cub.2011.10.038