the UV-sensitive type 2 cone, have linear dichroism similar to that of other vertebrates.

Another property of the type 2 outer segment is its sensitivity to actinic "bleaching" lights. Both the alpha-band A and LD peaks diminish in size in response to such lights. The resulting bleaching difference spectrum has a transient new peak at about 440 nm with a corresponding negative LD (arrow in Fig. 2E). Therefore, when the light-absorbing substance in type 2 cells is bleached, the resulting photoproducts share the dichroic characteristics of photoproducts formed in the other cones of ugui and other vertebrates.

Although the wavelength ranges and cell distribution in Table 1 may imply more, there are probably only five opsins present in ugui photoreceptors. Most of the observed λ_{max} variations could be simulated by the use of various proportions of the two known chromophores and five opsins. The much narrower ranges and shorter λ_{max} values found in the regeneration experiments support this conclusion. The larger numbers of long-single than long-double and short-double than medium-single cones found at longer wavelengths probably have no significance. Although much is yet to be discovered, the high blue sensitivity in physiological responses can now be explained, at least qualitatively, from spectral determinations. Whether ugui can perceive UV light and, if so, what benefit it derives from that ability are not known. Ugui, however, has been reported to be a fast and powerful swimmer that tends to feed at dusk and that sights its prey of aquatic and terrestrial insects from below, catching them in an upward move at the surface of the water (*15*). Broader spectral coverage may be helpful in detecting insects against sky light as background illumination, or it may be used in detecting special markings for identifying conspecifics.

Not only ugui, but also the roaches (*16*), goldfish (*17*), pigeons (*4*), hummingbirds (*5*), and chickens (*18*) seem good candidates for having UV-absorbing cones. Even the human retina may have UV receptors, in view of the high UV sensitivity of the aphakic human observer (*1, 19*). Although the yellow lenses of adults normally block the penetration of UV light to the depth of the retina (thus apparently rendering UV receptors useless), such receptors may nevertheless be present either vestigially or to serve some function in ontogenetic development.

The tetrachromatic cone system (or pentachromatic eye) of ugui does not contradict current color vision theories (*20*). Combining Young's three-receptor theory with Hering's opponent color theory, Svaetichin concluded, "Three is the minimum number of receptors on which the Hering opponent system can be based; four would also do, but nature designs economically. This is the only magic of the number three in vision. The finding of a fourth cone in the periphery of the human retina would not at all reduce the value of Young's idea" (*21*). Depending on nature's original intent, tetra or even higher chromaticity may be economical (*22*). Since ugui is an ordinary fish and not apparently a singular creation of nature, we expect other animals to be similarly endowed.

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Pinosylvin Methyl Ether Deters Snowshoe Hare Feeding on Green Alder

Abstract. *Pinosylvin methyl ether (PME), a toxic phenol, is a potent deterrent to showshoe hare feeding on green alder. Concentrations of PME found in green alder parts can account for the low palatability of winter-dormant foliar buds and staminate catkins but cannot affect internode palatability. The lack of a PME-related defense system in internodes suggests that green alder has at least a two-level defense system: defense of growth stages and defense of parts within growth stages.*

Herbivores do not feed on all parts of a plant; they usually eat specific parts (*1*). For example, when feeding upon winter-dormant green alder (*Alnus crispa*), snowshoe hares (*Lepus americanus*) eat internodes and reject foliar buds and staminate catkins (Fig. 1). Foliar buds and catkins contain high concentrations of nutrients and nonstructural carbohydrates and low concentrations of fiber and methanol-soluble phenolic constituents as compared to internodes (Table 1). Thus factors other than these constituents must influence snowshoe hare preferences for green alder parts. We

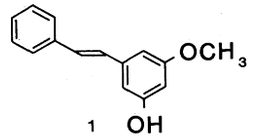

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Table 1. Chemical characteristics of winter-dormant green alder twigs. Values are percent (dry weight); means and standard errors are given; $N = 5$.

	Nitrogen	Phosphorus	TNC*	Cellulose	Hemicellulose	Lignin	Total phenols†	Total resin‡	PME
				Juvenile growth form					
Bud	2.08 ± 0.06	0.28 ± 0.01	12.6 ± 0.5	9.9 ± 0.6	4.3 ± 0.2	6.5 ± 0.5	6.6 ± 0.2	28.5 ± 1.2	1.4 ± 0.04
Internode	1.39 ± 0.03	0.16 ± 0.01	8.2 ± 0.6	27.2 ± 0.5	11.1 ± 0.6	12.8 ± 0.4	7.2 ± 0.3	4.4 ± 6.1	0.06 ± 0.01
				Mature growth form					
Bud	2.33 ± 0.19	0.30 ± 0.01	11.7 ± 1.1	10.2 ± 0.3	2.8 ± 0.3	6.5 ± 0.4	6.0 ± 0.1	34.3 ± 2.8	2.6 ± 0.2
Staminate catkin	2.48 ± 0.03	0.30 ± 0.01	11.2 ± 0.7	14.4 ± 0.5	0.0 ± 0.0	5.3 ± 0.5	4.3 ± 0.4	25.2 ± 2.3	1.7 ± 0.1
Internode	1.32 ± 0.02	0.13 ± 0.01	8.3 ± 0.4	23.3 ± 0.7	8.6 ± 0.5	13.9 ± 0.7	7.2 ± 0.3	4.8 ± 0.6	0.05 ± 0.01

*Total nonstructural carbohydrate. †Expressed as tannic acid equivalents. ‡Material extractable by diethyl ether.

found that pinosylvin methyl ether (PME) (**1**), a low molecular weight phenolic substance, present in the buds and catkins, is highly repellent to snowshoe hares and can partially account for differential feeding by snowshoe hares upon green alder parts during winter.

Small-diameter (< 4 mm) twigs from the height range available to snowshoe hares were collected during midwinter (January) from mature and juvenile green alder growing near Fairbanks, Alaska. Collections were divided into "subsamples" for chemical analysis and a preference bioassay on each was made with snowshoe hares (*2*). Material to be bioassayed and analyzed chemically was stored in tightly sealed plastic bags at −40°C until used.

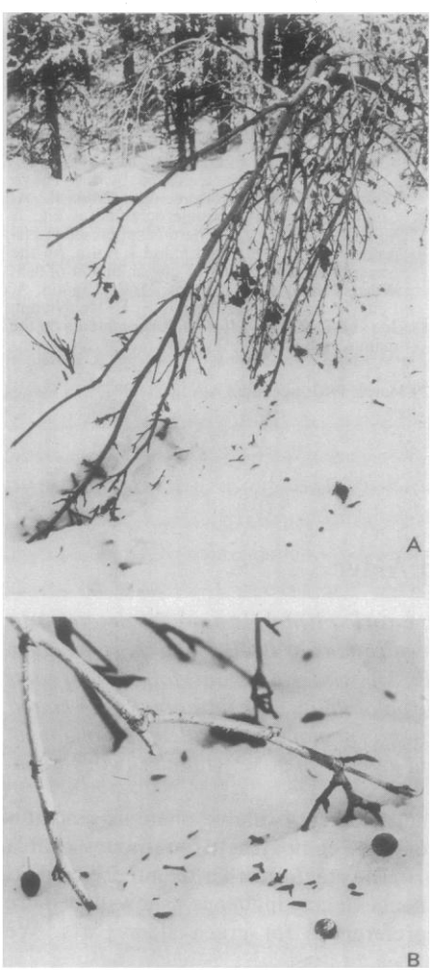

Fig. 1. (A) Site at which free-ranging snowshoe hares have fed on mature green alder, showing rejected foliar buds. (B) Close-up of the site shown in (A).

Bioassay with both free-ranging and captive hares confirmed that, during winter, snowshoe hares reject green alder foliar buds and staminate catkins (*3*) and that juvenile green alder is less palatable to snowshoe hares than mature green alder (*4*) (Table 2). Removal of foliar buds and staminate catkins from mature and juvenile green alder twigs prior to bioassay did not alter snowshoe hare preferences for mature twigs as compared to juvenile growth form twigs (Table 2). Thus snowshoe hare preferences for growth stage of internode (juvenile compared to mature) may be controlled by factors other than those controlling preferences for plant parts within a growth stage (internodes compared to buds and catkins).

Preliminary chemical analysis of foliar buds, staminate catkins, and internodes suggested that snowshoe hare preferences for green alder parts are negatively correlated with the resin concentration of these parts (Table 1). Fractionation of the diethyl ether extract by column chromatography on silica gel (step gradients of mixtures of petroleum ether, diethyl ether, and methanol) yielded fractions that elicited varying avoidance reponses in bioassays with free-ranging hares. Further chromatography (silica gel; petroleum ether and ethyl acetate) of the most repellent fraction provided PME as a major component.

Purified (recrystallized and sublimed) PME had physical and spectral properties consistent with those reported for pinosylvin methyl ether (*5*). The repellency of PME, a previously recognized toxic secondary metabolite (*6*) present in the bud and staminate catkin resins of *Alnus pendula* (*7*), was demonstrated by offering oatmeal, a preferred commercial food of snowshoe hares, impregnated with PME to captive snowshoe hares for a 24-hour period (*2*). This bioassay demonstrated the deterrent properties of PME (Table 3).

Measured (*8*) concentrations of PME in winter-dormant foliar buds and staminate catkins (Table 1) were comparable to those causing strong avoidance when applied to oatmeal, but concentrations of PME found in both juvenile and mature internodes (Table 1) elicited no response in the bioassay. Thus, while PME can account for the low palatability of buds and catkins, it does not appear to be a factor in the preference shown by snowshoe hares for mature as compared to juvenile green alder internodes.

To determine if deterrency in this bioassay is a property of all secondary metabolites, β-sitosterol (a ubiquitous nontoxic phytochemical) was also of-

Table 2. Effect of bud and staminate catkin removal on snowshoe hare use of alder. Values are means ± standard errors for grams of dry matter eaten per hare in a 24-hour period ($N = 25$). Values with the same superscript do not differ at $P \leq 0.05$; values with different superscripts differ at $P \leq 0.001$ (F test).

Growth stage	Intact twig	Buds and catkins removed
Mature	8.65 ± 1.12^a	10.76 ± 1.58^a
Juvenile	2.04 ± 0.38^b	1.89 ± 0.44^b

Table 3. Effects of pinosylvin methyl ether (PME) and β-sitosterol on hare feeding behavior. Values are means ± standard errors ($N = 10$ hares).

Compound	Concentration (%)*	PI†
PME	0.05	0.97 ± 0.15
	1.5	0.07 ± 0.03
	4.0	0.09 ± 0.05
β-Sitosterol	4.0	1.02 ± 0.09

*Samples prepared according to (*2*). †PI (preference index) is the ratio of the treated food consumed (percent) to the untreated oatmeal consumed (percent).

fered to the same hares in a similar feeding trial. The lack of response to β-sitosterol (Table 3) demonstrates that hares do not avoid all secondary metabolites.

Thus a single identifiable secondary plant metabolite can function as a deterrent to snowshoe hare browsing. The concentrations of PME found in catkins and foliar buds of winter-dormant green alder are sufficient to deter feeding by snowshoe hares. However, the low levels of PME found in internodes suggest that hare preferences for mature over juvenile internodes are controlled by factors other than those leading to avoidance of buds and catkins. Thus green alder may have at least a two-level defense system during winter—defense of growth stages (mature and juvenile internodes) and parts within growth stages (buds and catkins compared to internodes).

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9. Supported by NSF grants DEB-8207170 and DEB-7823919.

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Identification of Presynaptic Neurons by Laser Photostimulation

Abstract. *An optical method involving the use of a laser and a novel fluorescent dye as a photostimulation probe has been developed to identify presynaptic neurons in a large ensemble of cells. Illumination of an extracellularly stained neuron by the laser microbeam evokes action potentials. With this technique an interneuron connecting identified leech neurons was quickly located. The method speeds up the elucidation of neuronal networks, especially when small cells are involved.*

Understanding the cellular basis of any central nervous system function requires mapping the neuronal networks controlling that function. Much progress has been made toward this end, especially in the evaluation of simple networks in some invertebrate ganglia that control behavioral reflexes or more complex behavioral acts such as swimming, feeding, defensive withdrawal, and simple learning (*1, 2*).

A microelectrode search for the neu-

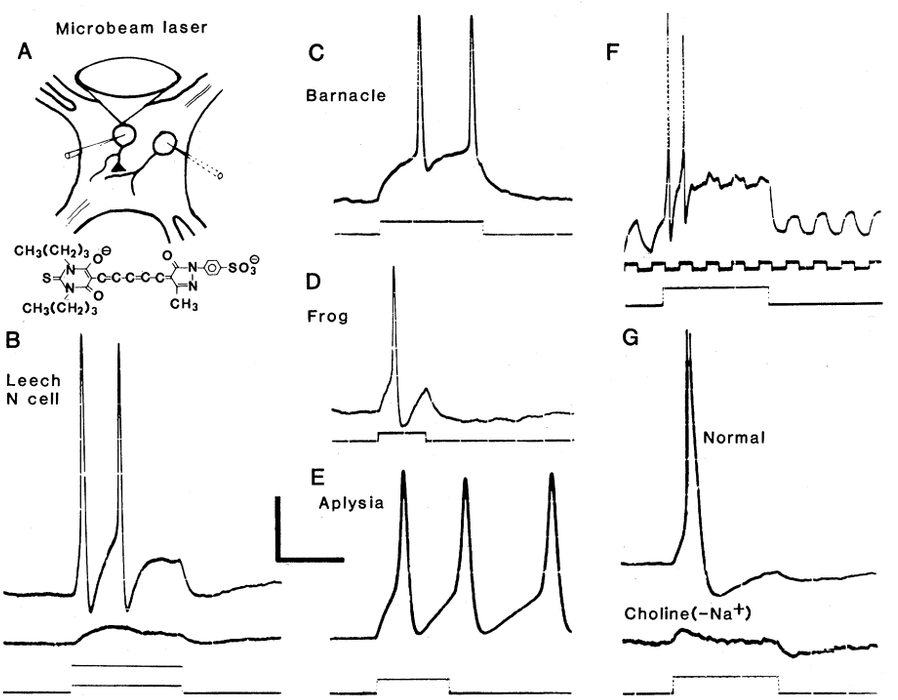

Fig. 1. Photostimulation of nerve cells. (A) Simplified scheme of the experimental arrangement for detecting presynaptic neurons. For more details on the microbeam optics, see Grinvald and Farber (*13*). The structure of RGA-30 is also shown. (B) Subthreshold and suprathreshold stimulation of the leech N sensory cell. Square pulses denote duration and relative amplitude of laser light. Subthreshold stimulation required 75 percent light attenuation. Scale is 16 mV (vertical) and 40 msec (horizontal). (C to E) Photostimulated action potentials from barnacle (C), frog (D), and *Aplysia* (E) neurons. Scale is 40 mV and 40 msec. (F and G) Biophysics of photostimulation. (F) Measurement of membrane resistance during photostimulation of the leech P cell. Injected current (3 nA) is indicated by the small, periodic square pulses. (The time constant for the resulting change in membrane potential is approximately proportional to the membrane resistance.) Note the recovery immediately after illumination. Scale bar is 20 mV and 100 msec. (G) Two photostimulations of the same leech N cell. The first stimulation was in choline-substituted, Na^+-free saline (scale, 4 mV and 40 msec); the second, in normal saline (scale, 15 mV and 40 msec).