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Chemical Signals and Repellency: Problems and Prognosis

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ABSTRACT

The chemical senses (olfaction, gustation, and chemical irritation or pain) were likely the first to evolve. Their functions are among the most basic—to attract and to repel. Attracting compounds often signal food or sex; repelling compounds presumably signal danger. Among the chemical senses, only olfaction appears to have several functional roles, two of which are modulation of social behaviors and identification of food. Whether an odor attracts or repels often depends to a large degree on learning. Consequently, dissociated olfactory stimuli may be relatively poor candidates for repellents since, after repeated exposure, pest animals are likely to ignore them. Taste, in contrast, is primarily related to the identification of food and the avoidance of poisons. Learning, here, appears to be less important although still a significant factor. Therefore, dissociated bitter tastes are good candidates for repellents. The development of effective, specific, bitter repellents, however, is complicated by the fact that herbivores tend to be relatively insensitive to many bitter compounds. Chemical irritants warn of danger; painful stimuli, almost by definition, are to be avoided. Although irritants may be the most potent of repellents, the extent to which a compound is irritating varies for many different species, pest and nonpest alike, which makes them difficult to use in certain situations. Finally, it is likely that the most effective strategy in the formulation of effective repellents will employ stimuli that activate a combination of the chemical (and other) senses. Research should focus in this area.

KEY WORDS

taste, olfaction, irritation, chemoreception, repellent, vertebrates

INTRODUCTION

This paper focuses on the role that three classes of chemical signals (smells, tastes, chemical irritants) might play in repellents for animals (see Table 1). Much of it is speculative and relies upon research that is primarily laboratory-based. Only field testing can determine whether a repellent actually works. However, good laboratory work, based on solid, biologically-based knowledge and reasoning, can go a long way toward identifying both effective repellents and repellent strategies.

The three major chemical senses will be discussed in turn. However, it should be noted at the onset that there are more than three such systems. In particular, the vomeronasal/accessory olfactory system, which is involved in pheromone reception (Wysocki and Meredith 1987), might be exploited in repellency. For example, if signals involved in mediating intraspecies aggression

Table 1. Chemical Senses and Repellency

Chemical Sense	Qualities	Importance of Learning	Potential for Repellency
Olfaction	Hundreds or thousands	High	Possible
Taste	Sweet, sour, salty, bitter, a few others	Medium-Low	Good
Chemesthesis (Trigeminal)	Pain and related sensations	Low	Excellent
Vomeronasal	Unknown	Unknown	Unknown

are detected by this system, they could be used as repellents. But we know so little about this that one can only speculate at this point.

OLFACTORY STIMULI AS REPELLENTS

It is generally believed that olfactory perception encompasses a very large number of unique sensory experiences. For humans, this numbers hundreds or thousands, although there are no real data to support these estimates. The discovery of a family of genes numbering 1,000 or more that purportedly code for olfactory receptors is consistent with the belief that there are many olfactory qualities, at least as far as mammals are concerned (Buck and Axel 1991). This may not be the case for insects or aquatic species; in catfish, far fewer olfactory genes have been identified, indicating that fish olfactory responsiveness may be limited (although still exquisitely sensitive) to, for example, amino acids and related compounds (Ngai et al. 1993).

In many species, olfaction serves at least three general functions: social (e.g., sexual, maternal, aggression), environmental/informative, and nutritive. This contrasts with taste and sensory irritation, which are presumably dedicated to guiding acceptance or rejection of food and other stimuli.

Given this multitude of qualities and the combinatorial properties potentially available with odors and the multiple functions this sensory system subserves, it is not surprising that it is viewed as the most plastic of the chemical senses (Engen 1982, Bartoshuk 1990, Bartoshuk and Beauchamp 1994). Evidence that particular odors are inherently or innately attractive or repellent is sparse. Instead, it is believed that the attractiveness or repellency of most odors is learned during prenatal and postnatal development. However, there may be some cases where attractiveness or repellency is, at least in part, innate.

What odors could be candidates for repellents, given this role for learning? Put another way, what classes of odors have animals been selected to avoid? The most obvious class is one that denotes danger, such as predator odors; and experiments to examine the efficacy of predator odors as repellents are discussed by others in this symposium.

There are other possibilities that have elicited less attention. Odors associated with poisons or stress could be inherently repellent. For example, if certain poisonous plants had unique odors, it is possible that animals would be selected to avoid these odors. However, given the flexibility conferred by being able to learn such a connection (via conditioned aversion), it would seem that the plant would need to be extremely poisonous indeed for selection to operate at the level of olfaction.

It is conceivable that there are common physiological changes that accompany disease that could also be repellent. We have found (K. Yamazaki et al., Monell Chemical Senses Center, unpubl. data) recently that mice infected with a virus (Mammary Tumor Virus) that will eventually cause breeding females to develop mammary tumors (and which is passed to offspring via mother's milk) have a unique smell long before tumors develop. Whether this odor is repellent needs investigation.

Even if one were to find that natural compounds from predators, poisonous foods, stressful situations, or diseased animals are repellent, a procedural problem arises in using the compounds underlying repellency. Although in theory, one could use natural products without identifying active components, this can be impractical and even unethical, for example, with the stress- or illness-induced odors. Thus, one would like to identify the active compounds and use these in a repellent formulation. This, however, may be extremely difficult in many cases. This problem is probably best exemplified by the remarkable difficulty in chemically identifying pheromones in mammals. It is safe to say that in 25 years of fairly intense investigation of mammalian pheromones, fewer than a handful have been chemically specified; and many of these identifications are currently disputed. The one bright note here is the work on predator odors, which is guided by a theory that the odors derive from breakdown products of the prey. This theory has directed chemical studies to examine a class of compounds containing sulfur (Mason 1994).

But even if we were to successfully identify and chemically characterize an olfactory repellent, another problem is likely to be encountered. To the extent that the odor is actually repellent, both individual experience and genetic selection may operate to limit long-term usefulness. For innately repellent odors, learning could operate to alter and reduce repellency. We have seen this phenomenon clearly with attractive odors (Beauchamp et al. 1979). For adult male guinea pigs reared from birth in total isolation, the urine odor of a female conspecific is highly attractive on first presentation. But learning plays a role in modifying or focusing the response, as indicated by studies that (a) compare response of wild, domestic, and F_1 animals and (b) the results of fostering studies where relative preference can be changed by experience during rearing.

In addition to learning effects, selective pressures could also reduce the proportion of animals repelled in succeeding generations. This latter process could occur quickly in many rapidly breeding species.

Furthermore, one of the hallmarks of the olfactory system is its rapid adaptation or loss of sensitivity with repeated or continuous exposure (Engen 1982). Thus, if one were to broadcast an aversive odor to, for example, protect plants from herbivores, it may be that the animals would quickly adapt to it and hence no longer detect it. Moreover, it has recently been shown in human studies that long-term exposure to odors—over a period of weeks—leads to a long-term decline in sensitivity to that odor that also lasts for several weeks (Dalton and Wysocki 1996. Presumably a similar phenomenon exists for other vertebrates. Thus, for predator (or stress/illness) odors to work for some species, they would probably have to mimic the natural context in which they would be detected—i.e., they would be encountered rarely and in very select locations. For many repellency applications, these characteristics may limit usefulness, particularly over the long term.

In summary, although there are some potentially attractive avenues for research in olfactory repellents, the plasticity of the system may limit their usefulness. A combination of repellent odors with repellents detected by other sensory systems may provide a synergistic basis to improve repellent performance, as will be discussed subsequently.

GUSTATORY STIMULI AS REPELLENTS

Compared with olfaction, there appear to be a very limited number of so-called primary tastes, the main ones being sweet, sour, salty, bitter, and perhaps a few others (e.g., "umami" or the unique taste of monosodium glutamate) (Bartoshuk and Beauchamp 1994). Although these categories are derived mainly from human studies, it appears that, broadly speaking, they also apply to many other mammals (Beauchamp and Mason 1991). However, there are substantial species differences in relative sensitivity to many taste compounds. Furthermore, there may be compounds that are not detected by humans but are highly salient taste stimuli to other organisms (Sclafani 1990).

In contrast to olfaction, there is no evidence that taste plays any role other than in regulating nutrient intake and utilization. In fact, it has been argued that taste preferences and presumably taste hedonics evolved exclusively to insure adequate consumption of nutrients and avoidance of poisons (Beauchamp and Mason 1991).

Given this dedicated function, it is not surprising that the consensus is that taste preference and aversions are often innately determined with little input from experiences required for an organism to appropriately respond. A vast literature on taste aversion learning clearly demonstrates that positive or neutral tastes can become aversive if associated with negative postingestive consequences (Garcia et al. 1974). The reverse is also the case, although less research has been done here (e.g., Sclafani 1990, Provenza 1995). Nevertheless, the initial hedonic response to tastes appears to be innately determined.

For herbivorous and omnivorous animals, carbohydrates are often highly acceptable ("sweet"); sweet things are generally calorie-rich and perhaps vitamin- and mineral-rich as well. Although very few strict carnivores have been tested, experiments indicate that species of the family Felidae do not prefer carbohydrate sweeteners (Beauchamp et al. 1977). In fact, they may not even be able to taste them. They do prefer amino acids that humans describe as sweet (e.g., L-proline) (White and Boudreau 1975, Beauchamp et al. 1977).

Salts (NaCl being the prototype) are often also highly acceptable, perhaps because the Na^+ ion plays such an important physiological role and because in nature, particularly for herbivores, it is often in short supply and is patchily distributed. Interestingly, other mineral salts at very low concentrations are also quite acceptable; whether each of these is detected by specific taste receptors or whether at low concentrations they all taste like NaCl and hence are attractive is not known (Tordoff 1994).

There is relatively little behavior work with acids (sour), but it is assumed that they are often avoided by animals. It is thought that this may be due to the damage that acids can cause in the oral cavity. However, acids are also irritating at higher concentrations so that sour taste per se may not be generally aversive, as is often assumed. In fact, in studying taste preferences in several inbred mouse strains, we have recently found very high preference for citric acid at levels that are sour but probably not irritating (Bachmanov et al. 1996). It would not appear that sour taste per se would be an effective repellent; however, higher concentrations of acids (when they are irritating) may be useful.

Bitter compounds are the logical candidate for repellents. It has been argued that this is their function in nature: bitter perception evolved to protect animals from ingesting poisons, mainly alkaloids, and to protect plants from being eaten. Yet, here too are problems that must be considered.

Quinine hydrochloride, a prototypic bitter compound, is bitter to humans, and rejected by many species; however, the rejection threshold exhibits marked interspecific variation as shown by comparing studies of cats, rats, and guinea pigs (Jacobs et al. 1978). Although methodological differences could account for some of this variation, the importance of bitter tastes, and thus sensitivity of the bitter system, may depend upon the feeding ecology of the species in question. In particular, the guinea pig is a strict herbivore and, as such, is confronted with the problem of consuming sufficient calories from plants, most of which taste bitter to humans. Given this problem, if guinea pigs had a sensitive bitter-rejection mechanism, they would have substantial difficulty in finding acceptable foods. At the other extreme, the carnivore is much less likely to encounter bitter compounds in its natural diet and can "afford" to be very sensitive to bitter compounds. This rough correlation between bitter sensitivity and likelihood of confronting bitter compounds in the food supply has been greatly extended by Glendinning (1994) who reported a similar relationship when many more species were studied.

If some herbivorous mammals have a blunted bitter-rejection system, what is to prevent them from ingesting excessive poisonous compounds? While there apparently is a rough correlation between bitterness and toxicity, this correlation is not perfect. A more flexible mechanism to avoid toxins is the formation of conditioned aversions to other flavor components of a toxic plant. If there is no innate bitter-alkaloid-based rejection of plants, the animal is free to sample and, in effect, meter the intake of toxic plants at an acceptable level. An interesting example of this can be found in the work of Jacobs and Labows (1979) with wild guinea pigs. They found that the animals' natural neophobia resulted in consumption of very small amounts of novel plants. However, over the course of several days, the animals began eating more of some plants, never ate much of one—honeysuckle, and exhibited a bell-shaped intake of another one—nightshade. Moreover, those animals that continued to eat nightshade adopted a novel strategy of eating stem first, often eating only the stem. Subsequent toxicological analyses revealed that the stems contained far less alkaloids than did the blades or petioles. The finding that these animals rejected honeysuckle from the very beginning suggests that this plant may contain compounds that wild guinea pigs, and perhaps other herbivores, find inherently unpleasant. It would be instructive to identify these compounds and perhaps to use this strategy generally to identify nontoxic repellents. On the other hand, if the compounds were extremely toxic, even one mouthful might be sufficient to establish a conditioned aversion.

Herbivorous animals could also develop lower sensitivity to the toxic effects of these compounds and/or detoxifying mechanisms. In one interesting example of this strategy, Glendinning (1990, Glendinning et al. 1990) has studied mouse predation on monarch butterflies *(Danaus plexippus)* at the butterflies' wintering grounds in Mexico. Of the four species of mice present in the area, only one makes extensive use of the vast protein store potentially available from these butterflies. Since the butterflies contain toxic cardiac glycosides that are bitter and can cause emesis, how is this ecological challenge met by the mouse species? Experimental studies demonstrated that the one species known to prey upon monarchs in the field was the only one which (1) extensively ate monarchs in test situations, (2) gained weight, and (3) selectively ate those parts which were least toxic. It is suggested that the predatory species is probably less sensitive (though not completely insensitive) to the taste of cardiac glycosides, a result confirmed in formal taste studies, is able to withstand ingestion of relatively high levels of toxic compounds (i.e., has a superior detoxifying ability) and is likely to have well-developed behavioral techniques for assessing the danger associated with a food, for example, the ability to form conditioned aversions.

Up to this point, bitter has been discussed as almost a unitary phenomenon. Yet, in humans and other animals, studies of genetic control over bitter perception by inbred strains of mice have demonstrated the existence of several genes determining sensitivity to specific bitter substances (e.g., Whitney et al. 1990). Behavioral, electrophysiological, and breeding studies all support the idea that these genes are involved in coding for specific receptor-associated proteins. These studies lead to the conclusion that bitter perception is not a unitary phenomenon. This is not surprising if one believes that perception and rejection of toxic compounds is the "function" of a bitter taste system. To date, there is no agreed-upon chemical basis for bitterness, and this may be because no common molecular configuration exists for this taste. Instead, as plants (and perhaps insects) have evolved substances that are potentially harmful to invertebrates and vertebrates alike, these latter organisms have evolved means to detect the substances. Specific taste mechanisms may have, as a result, developed as a family of different protein-based receptors (or other mechanisms of detection), all of which are connected to genetic rejection mechanisms and which elicit a more or less common sensory experience that humans label as bitter and unpleasant.

What implications do these considerations have for using bitter compounds as repellents? First, it would seem that for herbivores bitter compounds may be relatively ineffective. However, given the evidence for multiple bitter transduction mechanisms, it is possible that novel bitter substances may be effective for herbivores if there have been no selective pressures to reduce sensitivity. This is purely speculative at this time. However, a recent paper by Nolte et al. (1994), also using guinea pigs, suggested that the insensitivity of this species to bitter stimuli was consistent across many different compounds. More comparative studies are needed to investigate this further. At the least, the potential use of a bitter compound as a repellent for herbivores must be tested with the target species.

The converse of this argument is that bitter compounds may be of particular value in repelling carnivores. The obvious difficulty here is that to detect a bitter repellent, an animal actually has to have it in its mouth; and for some uses of carnivore repellency, this may be too late. For others (e.g., keeping animals away from garbage, etc.), bitter compounds could, at least theoretically, be effective.

A broad comparative approach to evaluating sensitivity to a variety of bitter compounds among many species—carnivores, omnivores, and herbivores—is still needed to test these generalizations. For example, it would be of particular interest to study bitter repellency among mustelids which include species that are apparently strictly carnivorous as well as omnivorous species.

IRRITATING STIMULI AS REPELLENTS

The qualities of sensations transmitted over the fifth cranial nerve (trigeminal) have been described by humans as tingling, itching, burning, pain, cooling, etc. Little is known about how compounds that elicit these various sensations are transduced, although this is now an active area of investigation (see Bryant, Chapter 3, this symposium). This sensitivity remains among the most enigmatic of all those common to most vertebrates.

Regardless of the stimulus, strong sensations elicited by trigeminal sensations are almost universally regarded as unpleasant and are avoided. Presumably, such signals imply extreme danger. Pain, by definition, is a markedly unpleasant sensation.

There are, of course, some exceptions to this general rule. In humans, low to moderate levels of pain are often sought out (e.g., hot peppers, CO_2) and, perhaps less commonly, a similar situation may exist in other species. Factors underlying the positive hedonic judgments of presumably painful stimuli remain a puzzle (Rozin 1984), although it seems obvious that learning is involved; there is no evidence that infant animals, human or otherwise, will seek out, move toward, or innately appreciate pain.

Other papers in this symposium deal in great detail with irritants as repellents. Irritants clearly have some very positive characteristics that make them excellent candidates. They are innately unpleasant, and their unpleasantness does not extinguish with repeated presentation. Thus, they theoretically remain effective, even after many exposures. Moreover, oral irritants, at least, tend to sensitize with repeated stimulation—i.e., the sensation of pain often <u>increases</u> with repeated exposures rather than decreasing as is often the case for sensations associated with odorants and tastants (Green 1991). However, this may not always be the case since desensitization is observed for some oral irritants under some conditions (Green 1991). For volatile irritants, little is yet known about the dynamics of sensation following repeated stimulation; anecdotal information in humans does indicate that there may be <u>desensitization</u> following repeated or continuous stimulation. If this is the case, this could compromise the long-term effectiveness of such irritants as repellents.

Another virtue of irritants is that some can work from a distance. Many volatile compounds are also irritating to the nose, eyes, and other sensitive skin areas. Thus, repellents that are volatile irritants can theoretically ensure that the animal does not contact the repellent source. One difficulty here, however, is that repellents are likely to be at least class-specific: what repels a deer may also repel a cow or a person. Apparently, they are not necessarily specific across classes, as research with bird repellents has made abundantly clear (see other papers in this symposium).

A MULTIPLE SENSES APPROACH

In all likelihood, the classic repellent, skunk spray, uses multiple chemical signals (and associated visual signals) to work. The spray is itself irritating to the eyes and other parts of the face of an animal who is unfortunate enough to experience it. It may also taste bad! Finally, it has a distinct odor. Although this odor could be innately avoided, it is most likely that if other species, such as humans, avoid this odor and find it offensive, this is due to learning.

In devising repellents for vertebrates, it would seem to be a good idea to mimic the skunk. Using a multisensory array of substances may be the most effective strategy. And although this paper has concentrated exclusively on chemical signals, it is obvious that auditory and visual stimuli should not be ignored.

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