Of pungency, pain, and naked mole rats: chili peppers revisited

Capsaicinoids are unique to the chili pepper genus *Capsicum* within the plant family Solanaceae. The family is also known for species that produce powerful alkaloids such as nicotine (tobacco) and atropine (*Atropa*). This is the same family to which tomatoes, potatoes, and aubergines belong. Members of this family have a diversity of fruit types ranging from dry dehiscent capsules to fleshy drupes and berries. Botanically speaking, chili peppers are berries (Knapp 2002) and capsaicinoids are synthesized mostly, if not exclusively, in the interlocular septum that separates seeds in the fruit (Stewart *et al.* 2007).

Capsaicin and dihydrocapsaicin are the most abundant of the capsaicinoids, which are vanillin alkaloids responsible for the hot or burning sensation experienced on contact with chili peppers. This sensation is transduced by TRP (transient receptor potential) ion channels, specifically TRPV1 (transient receptor potential, vanilloid). TRP channels are nociceptors or noxious stimulus detectors on specialized sensory neurons. They are usually polymodal in their activation, responding to stimuli such as heat, chemicals, and protons (pH), but only at high thresholds (e.g. 42°C and pH 4) (Woolf and Ma 2007; Patapoutain *et al.* 2009). Consequently, nociceptors have considerable survival value since they signal the presence of extreme external and internal threats; internally they respond to the danger of tissue inflammation and ensuing damage. They are also believed to be relatively ancient receptors, although they have not yet been found in bacteria, archaebacteria or in plants; *trp*-related genes have been found in fungi and the most ancient *trp* genes in animals have been discovered in unicellular choanoflagellates (Damann *et al.* 2008). The ecology of pungency in *Capsicum* as well as the behavioural response of organisms to capsaicin and other irritants transduced by TRP receptors provide extremely interesting insights into the evolution of plant secondary metabolites (*see* Borges 2001) and also into why some special higher mammals such as naked mole rats are unresponsive to these irritants.

The centre of origin of pungent chili peppers is now believed to be the desert *chaco* of Bolivia and Peru (Walsh and Hoot 2001; Tewksbury *et al.* 2006). Pungency is a monophyletic derived trait, and a non-pungent species *Capsicum ciliatum* is regarded to be basal to all pungent taxa (Walsh and Hoot 2001). Contrary to popular conception, all wild *Capsicum* species are not pungent, and more importantly, non-pungent populations of pungent species also occur (Tewksbury *et al.* 2006). In the highlands of Bolivia, there appears to be an altitudinal gradient in pungency with the most pungent populations of at least one species, *C. chacoense*, occurring at the highest altitudes (Tewksbury *et al.* 2006). While the degree of pungency is encoded by several QTLs (Ben-Chaim *et al.* 2006), double recessive mutants at the *Pun1* locus, which probably codes for an acyltransferase, are always non-pungent (Stewart *et al.* 2005).

The pungent chili species are dispersed by birds since mammals are deterred by the pungency; moreover, mammalian guts destroy chili seeds and consequently mammals are not suitable seed dispersers for these species (Tewksbury and Nabhan 2001). The TRPV1 nociceptor in birds is insensitive to capsaicin although it demonstrates sensitivity to pH and heat (Jordt and Julius 2002). Non-pungent seeds of *C. chacoense* were found to be 12% thicker than pungent seeds, indicating a possible trade-off between the production of capsaicin and seed coat lignin in the phenylpropanoid pathway (Tewksbury *et al.* 2008b); however, since capsaicin had a constipative effect on birds, affecting the retention of seeds in their guts, pungent seeds with thinner seed coats were retained longer and suffered more damage in the gut. Yet, the longer that seeds were retained in the guts of dispersal agents, the farther they may move from their parent plants. This movement away from parental sites is usually considered an advantage since it affords escape

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from rivalry between germinating siblings, and also from pathogens and predators that may be attracted to the large quantity of fruit on the parent. Therefore, a tradeoff also appears to exist between gut retention time and seed viability.

Besides its role as a deterrent to mammalian seed dispersal agents, capsaicin in *C. chacoense* was also found to be extremely effective against seed-attacking fungi. The polymorphism in pungency observed in wild *C. chacoense* populations in Bolivia was found to be related to geographical variation in population densities of fruit-piercing hemipteran bugs whose mouthpart insertions provide the entry points for the seed-attacking *Fusarium* fungus (Tewskbury *et al.* 2008a). Seed infection rates by the fungus were found to be twice as high in non-pungent chili peppers compared to pungent ones at a given hemipteran feeding pressure. Furthermore, *Fusarium* growth was significantly reduced in tests with artificial media containing the principal capsaicinoids capsaicin and dihydrocapsaicin (Tewskbury *et al.* 2008a). Thus the pungency of the chili pepper in this species was directly related to seed protection against fungal attack. The evolutionary ecology of pungency in wild chili pepper appears to involve direct deterrence of unwanted mammalian dispersers that destroy chili seeds in their guts (Tewksbury and Nabhan 2001) as well as prevention of fungal attack on the seeds (Tewskbury *et al.* 2008a). Future research is certain to reveal additional selection pressures on such a potent trait.

The acquisition of capsaicin sensitivity in the TRPV1 nociceptor by mammals has been considered by some to be a late event in evolution (Jordt and Julius 2002); however another possibility is that birds have lost sensitivity to capsaicin. Rigorous testing of capsaicin sensitivity across the evolutionary ladder is needed to resolve such gain and losses of function issues. Endogenous capsaicin analogs (*e.g.* the endocannabinoid N-arachidonoyldopamine) have also been found in mammalian brains and these compounds may explain pleasurable sensations derived from chili pepper consumption or even a liking for chili pepper in humans (Huang *et al.* 2002; Cota *et al.* 2006). Recently, capsaicin has been making the news since a most unusual mammal, the subterranean eusocial naked mole rat (*Heterocephalus glaber*) was found to lack behavioural response to capsaicin and acid as well as to demonstrate extreme tolerance to ammonia fumes (Park *et al.* 2008; LaVinka *et al.* 2009). This virtually blind and hairless rat occurs in East Africa and is the mammalian counterpart of the honeybee; in this species only certain females, i.e. queens, breed. In these naked mole rats, the primary afferent nociceptors are insensitive to acid, and these animals appear to be the only known vertebrate to exhibit such insensitivity. However, while their TRPV1 receptors are sensitive to capsaicin, the capsaicin-sensitive nociceptor neurons bearing these receptors (the C-fibres) have unusual connections in the dorsal horn ganglia of the spinal cord (the ganglia where the cell bodies of pain-responsive neurons are located). These unusual connections may result in failure to activate pain circuits and may explain the observed lack of capsaicin-induced pain (Park *et al.* 2008). However, capsaicin-insensitive nociceptor neurons in this species make regular connections in the dorsal horn ganglia, and elicit pain-induced behavioural responses to mechanical and thermal noxious stimuli. Therefore, while naked mole rats are capable of detecting capsaicin and ammonia, these substances do not elicit the usual behavioural responses that indicate irritation or pain on experimental exposure to these substances via nasal or cutaneous routes (LaVinka *et al.* 2009). A lack of production of neuropeptides such as Substance P (belonging to the tachykinin class) from the trigeminal C-fibres as well as from primary sensory fibres in the skin was postulated as an additional reason for the lack of behavioural response to noxious stimuli in this unusual species (Park *et al.* 2003; LaVinka *et al.* 2009). While capsaicin is not a normal compound that these animals would encounter in their environment, why should these animals be unresponsive to acid and ammonia? The reasons postulated relate to the unusual ecology of these animals; they live underground in dense colonies, and are probably subject to high levels of $CO_{2,}$ resulting in accumulation of acid in body tissues, as well as to high loads of urine-derived ammonia. Such living conditions may have resulted in a need to disable nocifensive responses to such stimuli in these animals (Park *et al.* 2008). Not surprisingly, the naked mole rat is now an important model organism in pain research.

Chili peppers have evolved, maintained or lost capsaicinoids under their own particular selection pressures. These secondary metabolites and the responses of organisms to them, particularly those invoking pain, are now being exploited by pain researchers in the hope of developing better pain management therapies. Whether nociception and pain are synonymous is also being debated (Bateson 1991; Barr *et al.* 2007). Such investigations have universally important implications for understanding the evolution of survival strategies that include nocifensive behaviour in response to noxious stimuli.

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