Title

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Journal

Chemical Senses, 43(7)

ISSN

0379-864X

Author

Wooding, Stephen P

Publication Date

2018-08-24

DOI

10.1093/chemse/bjy044

Peer reviewed

Print ISSN: 0379 864X CODEN CHSED8
Online ISSN: 1464 3553

Volume 43 Number 7
September 2018

Chemical Senses

In this issue:

In association with
Association for Chemoreception Sciences
European Chemoreception Research Organization
Japanese Association for the Study of Taste and Smell
Australasian Association for ChemoSensory Science
Korean Society for Chemical Senses and Ingestive Behavior

https://academic.oup.com/chemse

OXFORD
UNIVERSITY PRESS

Chemical Senses, 2018, Vol 43, 447–450
doi:10.1093/chemse/bjy044
Commentary
Advance Access publication 5 July 2018

Commentary

Bitter Fruit: Inverse Associations Between PTC and *Antidesma bunius* Perception

Stephen P. Wooding

University of California, Merced 5200 North Lake Road Merced, CA 95343, USA

Correspondence to be sent to: Stephen P. Wooding, University of California Merced, 5200 North Lake Road, Merced, CA 95343, USA. e-mail: swooding@ucmerced.edu

Abstract

Ability to perceive the bitter compound phenylthiocarbamide (PTC) is inherited via a dominant "taster" allele of the *TAS2R38* gene, whereas inability is inherited via a recessive "non-taster" allele. This raises a question: Is the non-taster allele functionless, or does it mediate perception of compounds other than PTC? New evidence supports speculation that it is indeed functional. Associations between *TAS2R38* mutations and bitter sensitivity to the tropical berry *Antidesma bunius* are the inverse of those PTC, suggesting that the non-taster allele enables perception to compounds in the fruit.

Variation in bitter taste sensitivity has long been recognized as an important determinant of diet and health. By shaping behaviors such as diet choice and smoking habits, it exerts downstream effects on health measures such as body mass index, cardiovascular function, hormonal processes, and possibly even cancer susceptibility (Duffy 2007). Much interest stems from the fact that bitter sensitivity varies profoundly from person to person and is highly heritable (Kim et al. 2004). This has spurred efforts to detect gene mutations shaping responses to specific compounds, with the aim of better understanding the mechanistic underpinnings of taste–health connections. A consistent finding as these efforts have progressed is that mutations in *TAS2R* genes, which encode G protein-coupled receptors (GPCRs) controlling the initial stages of the bitter perception process, are major contributors (Hayes et al. 2013).

This issue of *Chemical Senses* features a new study of *TAS2R38*, which is famous for its role in shaping PTC sensitivity (Risso et al. 2018). Here, Risso et al. investigate associations between mutations in *TAS2R38* and taste responses to fruit from a little known tree found in southeast Asia, *Antidesma bunius*. Risso et al. ask, does variation in *TAS2R38* predict bitter taste perception of *A. bunius* fruit, as it does in PTC? The short answer is, yes. However, the association is the opposite of PTC's: mutations associated with high PTC sensitivity are associated with low *A. bunius* sensitivity and, conversely, mutations associated with low PTC sensitivity are associated with high *A. bunius* sensitivity. The pattern is striking, and it is more than a novelty. It bears on our understanding of not just PTC and *A. bunius* perception, but bitter perception in general.

Though little known outside the region, *A. bunius* is a familiar sight in south and southeast Asia, and into the Malay archipelago, the Philippines, Borneo, Papua New Guinea, and most regions of Melanesia and Micronesia. *A. bunius* goes by many common names, most commonly bignay or bignai, Chinese laurel, salamander tree, or variants of these (Morton 2013). It is a leafed evergreen with characteristics similar to those of oak, with a large central trunk and limbs branching above, and heights reaching 30 m (Figure 1A). It is frequently cultivated as an ornamental due to its attractive appearance, ability to provide shade, and yield of edible fruit.

A. bunius fruit is small (~0.5 cm), round, consists of a single seed surrounded by a thin layer of pulp, and grows in clusters like grapes (Figure 1B). The fruits ripen at different rates, giving maturing clusters a striking appearance with white, red, and black berries. *A. bunius* fruit is generally not eaten raw due to its sourness and astringency, but it is a popular ingredient in sweetened, cooked, or fermented products such as jellies, juices, and wine. *A. bunius* tea, another popular product, is derived from the tree's bark.

A. bunius first garnered attention in the taste sciences in 1977. At that time, the strongly dichotomous bitter responses evoked by PTC were well documented, and all signs indicated that they were controlled by alleles of a single gene (Blakeslee 1931). This immediately raised questions about whether bitter responses to other compounds are also dichotomous and genetically controlled. By happy accident, *A. bunius* was discovered to have taste properties relevant to both of these issues (Henkin and Gillis 1977). R.I. Henkin, coauthor of the paper, explains:

> The story began at The Fairchild Garden in Miami Florida, in 1976. A group of scientists from various universities converged on the garden at various times to study the flora present

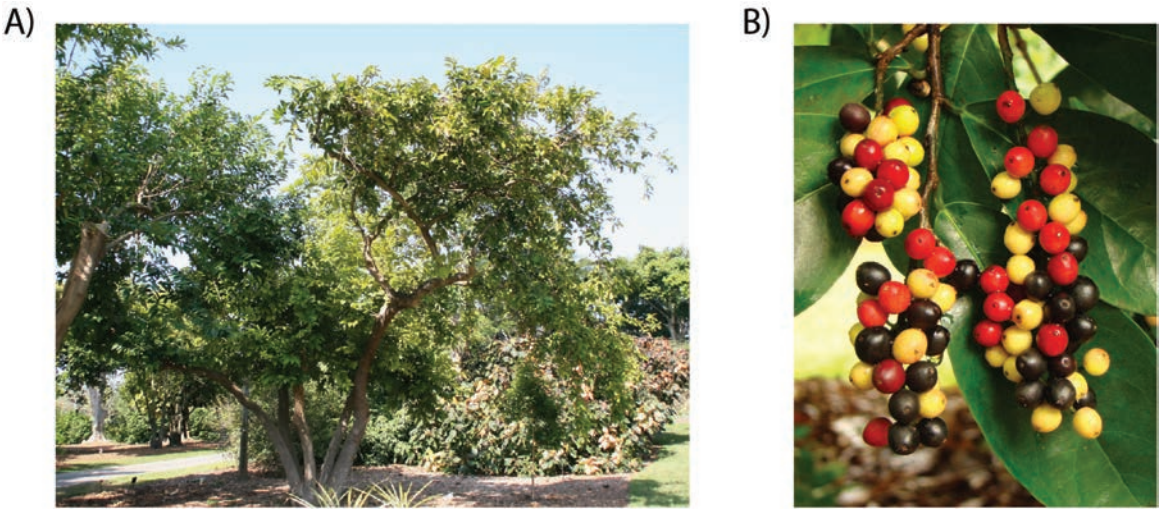

Figure 1. *A. bunius* **tree and fruits. (A)** *A. bunius* **tree heights reach 30 m. Credit: D.J. Stang 2007—CC BY-SA IGO 4.0. (B) Fruits grow in clusters and ripen at different rates, giving them a vivid appearance. Credit: Asit K. Ghosh 2010 - CC BY-SA IGO 3.0.**

there as it was such a unique place. We had lunch there on a regular basis. They offered a pie made of *Antidesma* berries at the cafe, as one might expect at this unusual kind of place, and all participants at the lunch said that the pie was very good. However, one, my eventual coauthor [W.T. Gillis], declared that the pie was bitter and he could not eat it. On the basis of this discrepancy and my interest in the garden, the participants, and the fruit, I studied the relationship between the bitter taste of PTC and *Antidesma*. Hence, the paper.

Henkin and Gillis evaluated subjects' responses to 2 solutions: PTC at saturation in water and juice pressed from *A. bunius* fruits. Subjects' responses to PTC were consistent with previous studies, with 68% of subjects reporting bitterness ("responders") and 32% reporting no taste ("non-responders"). Patterns of response to *A. bunius* were different, with 15% of subjects being responders and 85% being non-responders. Strikingly, responses to PTC and *A. bunius* were negatively associated. Of the 115 PTC responders, none were *A. bunius* responders; however, of the 25 *A. bunius* responders, none were PTC responders. Henkin and Gillis concluded from this that bitter perception of PTC and *A. bunius* are mediated by related factors, probably on a genetic level, although the specific mechanism accounting for this remained speculative.

Risso et al.'s new study capitalizes on current understanding of the molecular genetics of bitter perception to revisit the *A. bunius* puzzle. It is now firmly established that the inheritance of PTC sensitivity is controlled principally by 2 alleles at a single locus, *TAS2R38* (Kim et al. 2003). Thus, it seemed likely that these might account for the patterns observed by Henkin and Gillis. To find out, Risso et al. first replicated Henkin and Gillis' earlier taste tests. Perception was ascertained in subjects evaluating 2 solutions: PTC and *A. bunius* juice. Risso et al. then went farther, obtaining DNA sequences from *TAS2R38* in subjects. These allowed, for the first time, a test of whether the specific mechanism underlying variability in PTC perception also accounts for variation in *A. bunius* perception.

Risso et al.'s results are consistent with those of Henkin and Gillis and shed new light on the mechanisms underlying the PTC/*A. bunius* relationship. Risso et al. found that taste responses to PTC are indeed predictive of responses to *A. bunius*: only 11% of PTC responders were *A. bunius* responders, whereas 16% of *A. bunius* responders were PTC responders. Thus, there is an unambiguous inverse relationship between sensitivity to PTC and sensitivity to *A. bunius*. Risso et al.'s data further show that variation in *TAS2R38* does show an association with *A. bunius* sensitivity, and it is strong. More than 30% of Risso et al.'s subjects harbored the *TAS2R38*-PAV/PAV genotype, yet none were *A. bunius* responders; conversely, 100% of *A. bunius* responders harbored *TAS2R38*-AVI. These results are consistent with the longstanding hypothesis that perception of both PTC and *A. bunius* is mediated by alleles of *TAS2R38*, with PAV conferring PTC sensitivity and AVI conferring *A. bunius* sensitivity. They are also consistent with computational structure-function analyses suggesting that the A262V mutation causes a shift in ligand specificity, but does not disable the receptor (Tan et al. 2012). Thus, the simplest explanation for Risso et al.'s findings is that *A. bunius* harbors an agonist compound specific to AVI, much as PTC is an agonist of PAV, and homozygotes can only perceive one compound or the other while heterozygotes can perceive both (Figure 2).

Although the findings of Henkin and Gillis and Risso et al. only pertain directly to perception of PTC and *A. bunius*, they have implications for bitter perception in general. Most TAS2Rs are responsive to multiple substances, and it is already known that PAV mediates responses not just to PTC, but to a constellation of related compounds as well (Figure 3) (Meyerhof *et al.* 2010). If AVI is a functional receptor, it would be remarkable if were mediating responses to just a single compound found in relatively obscure plant. What is it mediating responses to? The obvious strategy for answering this question is to take the approach of (Meyerhof et al. 2010) and systematically test AVI for responses to libraries of compounds in vitro. A sensible starting point would be to focus on compounds already known to be present in *A. bunius*, which are receiving attention for their bioactive properties (Jorjong *et al.* 2015).

Evidence that both of *TAS2R38*'s common alleles contribute to bitter responses also raises the prospect that similar patterns occur in other *TAS2R*s. *TAS2R* genes are highly diverse, harboring significantly more variation than average for the human genome (Kim et al. 2005). In a study analyzing genetic diversity in all known *TAS2R* genes in 55 subjects, Kim et al. found an average of 6 coding haplotypes per locus. In larger sample, (Campbell et al. 2012) found that *TAS2R38* alone has >20 coding haplotypes. If divergent functionality among TAS2R alleles is the norm rather than the exception, or even

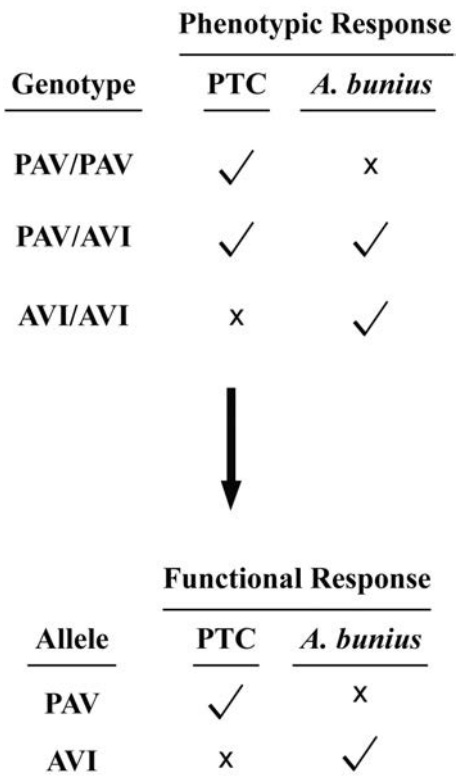

Figure 2. Simplified depiction of Risso *et al.*'s result. Check marks indicate response, x's indicate no response. Top: Phenotypic responses associated with the three common *TAS2R38* genotypes. Bottom: Implied functional responses of the two common TAS2R38 alleles.

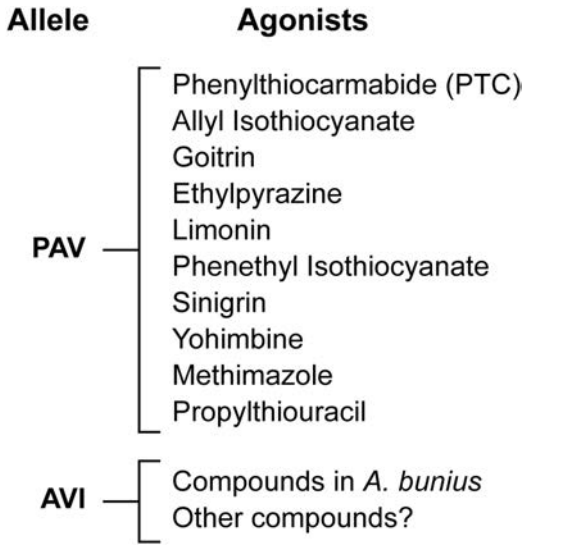

Figure 3. TAS2R38 alleles and agonists. TAS2R38-PAV is responsive not just to PTC, but to a constellation of structurally related compounds. Specific TAS2R38-AVI agonists are not known; however, Risso *et al.*'s findings point to such compounds being present in *A. bunius*.

merely common, it would point to the presence of major phenotypic variation arising from effectively unlimited combinatorial diversity.

An additional point made by Risso et al. is that evidence for functionality of AVI provides an explanation for signatures of balancing natural selection at *TAS2R38*. If PAV enables perception of one set of compounds, and AVI enables perception of another, then heterozygotes should be able to perceive both. This could provide a selective advantage to heterozygotes, resulting in the maintenance of both alleles human populations. Evidence that AVI is functional also explains an intriguing but little discussed aspect of variation in *TAS2R38*, which is that while coding variants of AVI do exist, they are found only at low frequencies, and no profound mutations such as premature stop codons or frame shifts have been reported on the AVI background (Campbell et al. 2012). Both of these observations are consistent with the AVI sequence being conserved by selective processes, which could only occur if it has some type of function.

It is crucial to recognize that while the simplest explanation for Risso et al.'s findings is that AVI is a functional receptor, it is not the only mechanism through which AVI might mediate taste responses to *A. bunius*. A compelling alternative is protein–protein interaction. Protein–protein interactions such as heterodimerization are ubiquitous among members of the GPCR superfamily, and frequently alter the function of the participating molecules (Gurevich and Gurevich 2008). Indeed, the human umami and sweet receptors are both GPCR heterodimers: TAS1R1+TAS1R3 and TAS1R2+TAS1R3, respectively (Zhao et al. 2003). One explanation for the association of AVI with *A. bunius* perception is that AVI interacts with a second TAS2R, forming a heterodimer responsive to *A. bunius* constituents. In this scenario, AVI would be functional, but as a cofactor rather than a receptor. Support for this possibility comes from computational and experimental evidence that the A/V and V/I mutations abolish TAS2R38's activation by ligands, but do not have major effects on the protein's overall structure (Marchiori *et al.* 2013). Thus, while the present findings suggest that AVI is acting as a receptor for constituents of *A. bunius*, they do not demonstrate it definitively. Isolating AVI's function will require identification of the specific compounds involved and establishing their interactions.

The possibility that the AVI allele is mediating responses to unknown substances suggests that caution should be taken in efforts to understand associations between *TAS2R* variation and responses to complex aspects of diet and other orosensation driven behaviors. In particular, it raises questions about the directionality and strength of genotype–phenotype associations. In the case of *TAS2R38*, the prevailing focus is currently on the fact that genotype predicts high, medium, and low sensitivity to known agonists, such as goitrogens found in cruciferous vegetables. The new findings raise the question of whether sensitivity to some unknown compound is commensurately increasing, and whether it might be an equally important driver of observed associations. Both could even occur simultaneously, which could weaken or obscure associations. Dissecting such effects, even if just to rule them out, will require substantial effort from all chemosensory perspectives: molecular, psychophysical, and behavioral.

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