if we also include body mass, $F = 15.8$, $P < 0.0001$). However, as one of the referees observed, there are concerns about the validity of this analysis because non-random covariation exists between the independent variable (heterozygosity) and 'population', hence, the relationship across population means.

A particular problem presented by UK rabbits is that their populations are genetically structured at extremely fine scales: sites in East Anglia separated by a few hundred metres are as different as those separated by 125 kilometres [4–6]. Such extreme differentiation means that insufficient genetic variation exists within discrete 'populations' to explore fitness relationships, with potential non-independence due to close relatedness.

Our results clearly indicate the established importance [7] of sampling very low heterozygosities for revealing possible fitness correlations: the relationship is heavily influenced by a small number (~12) of strongly homozygous males (one homozygous at all 29 loci). These individuals will be rare in the wild in general, and even rarer within one discrete population that holds individuals with a high degree of heterozygosity. Without an isolation-by-distance relationship, such fine-scale structuring also means that our sampling sites contain numerous 'populations'. As sampling was spread throughout most areas by several kilometres, we can be confident that most individuals represent single samples from different 'populations' by those criteria.

A key consideration is what constitutes a discrete population? There are arguments for designating the UK to be a population at one level of interpretation: rabbits colonised the wild recently, in the 18th century [4,8], providing us with a natural experiment. However, we agree that we cannot eliminate all potential confounds. Although our study was founded upon evidence that heavily inbred big cats have unusually elevated sperm abnormalities [9], we cannot exclude additional interpretations, such as that proposed by Slate and Pemberton [1]: abnormal sperm constrain reproductive output which leads to inbreeding. Ideally, we would match our natural environment correlations with experiments that applied tight 'environmental' control. However, complementary approaches are important because inbreeding depression could be influenced by both genetic and environmental variation [10].

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Variability in a taste-receptor gene determines whether we taste toxins in food

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TAS2R bitter receptors are thought to have evolved to detect toxins in plants and foods and to modulate ingestion of them [1]. Indeed, virtually every plant, edible or otherwise, contains toxins. Although this toxin-detector hypothesis of bitter taste is prevalent, there is no indication that TAS2R receptors detect specific toxins enmeshed within natural foods, a necessary link for the natural selection argument. Several expressed TAS2R receptors, however, are known to respond to pure solutions of toxins. For example, some variants of the antithyroid-toxin receptor hTAS2R38 respond to phenylthiocarbamide (PTC) and propylthiouracil (PROP) [2,3], compounds which contain a thiourea (N-C=S) moiety. We report here that genotypes of *hTAS2R38* specifically determine humans' bitterness perception of plants that synthesize glucosinolates, a class of anti-thyroid compounds that also contain the thiourea moiety.

The natural selection argument for detecting thyroid toxins in plants is supported by data in which sensitivity to PTC was shown to be associated with decreased risk of both goiter (thyroid enlargement) and central neural defects in an Andean community with endemic goiter; no such association was found in a neighboring community that was treated en masse with iodine injections [4]. Endemic goiter arises under conditions of low iodine ingestion as an adaptive response to maintain levels of thyroid hormones, which incorporate inorganic iodine in a process facilitated by thyroid peroxidase [5].

Ingestion of glucosinolates in plants exacerbates the hormone problem by inhibiting thyroid peroxidase activity, as well as blocking active transport of iodide into the thyroid [6]. Thus, one would expect that the expression of a 'sensitive' allele of *hTAS2R38* would convey an important reproductive advantage to individuals in such environments.

The most common sensitive allele of *hTAS2R38* is labeled PAV and the insensitive allele is AVI after amino acid identities at positions 49, 262, and 296 [3]. While PTC is the 'defining' ligand for the PAV-hTAS2R38 receptor, it is a synthetic antithyroid medicine and does not occur in nature. However, many Cruciferous vegetables of the geni Brassica, Raphanus, and Nasturtium, such as kale, radish, and watercress respectively, are well known to contain PTC-like glucosinolates [7].

Here we divided a large test array of foods into glucosinolate-containing vegetables and those vegetables that lack known glucosinolates based on the extant literature and asked subjects to rate them all for bitterness (see the Supplemental data available on line with this issue, in particular Tables S1–3, Figure S2 and the experimental procedures) [7].

Overall, sensitive (PAV/PAV) subjects rated the glucosinolate-generating vegetables as 60% more bitter than did the insensitive (AVI/AVI) subjects, while these two genotype groups found the nonglucosinolate-generating vegetables equally bitter overall (Figure 1 A,B). Heterozygotic subjects (PAV/AVI) gave intermediate results, as expected [2] (Figure 1A).

All three genotype groups differed significantly from each other in their perception of the glucosinolate vegetables, but not for the non-glucosinolates. Without exception, every individual glucosinolate-producing vegetable was rated as more bitter by the PAV/PAV than the AVI/AVI subjects (Figure 1C). The difference in ratings

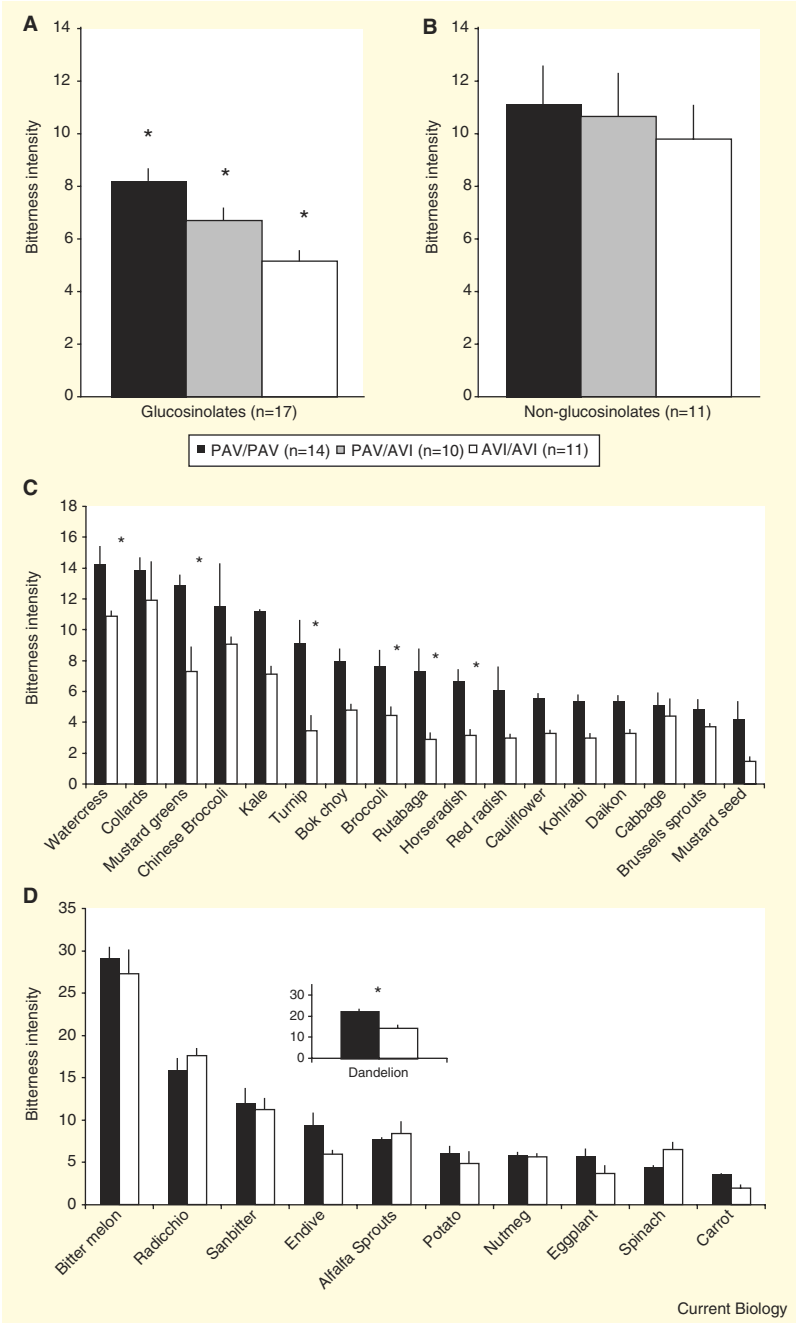

Figure 1. Bitterness ratings by *hTAS2R38* genotype groups of vegetable classes.

(A,B) The ratings of the glucosinolate-producing vegetables (A) and nonglucosinolate-producing vegetables (B) are separated. Data are presented as mean bitterness ratings of all sessions (n = 3) + standard error (SE). Ratings were made on the general labeled magnitude scale (gLMS). The effect of genotype on gLMS bitterness ratings was significant for the glucosinolate vegetables (A) [$F_{(2,102)} = 35.99$, $p < 0.001$] but not for non-glucosinolate vegetables (B) [$F_{(2,60)} = 1.072$, $p = 0.35$]. Significant differences (Tukey HSD test, $\alpha = 0.05$) among genotypes within a vegetable class (A) are marked with asterisks. Black bars = PAV/PAV; Grey bars = PAV/AVI; White bars = AVI/AVI. (C,D) Bitterness ratings of individual vegetables by the PAV/PAV and AVI/AVI groups. Vegetables are presented in descending order of bitterness sorted by the PAV/PAV group ratings. (C) compares PAV/PAV (black bars) and AVI/AVI (white bars) gLMS ratings of the glucosinolate-producing vegetables and (D) compares the two groups' gLMS ratings of the nonglucosinolate-producing vegetables. Dandelion is presented separately as an inset because it is not known to contain glucosinolates, but dandelion gLMS bitterness ratings by the genotype groups perfectly reflected the ratings of the glucosinolate-synthesizing plants (see also Figure S1 in the Supplemental data). Asterisks indicate significant differences in bitterness ratings between the two genotype groups for the particular vegetable ($\alpha = 0.05$).

reached significance for six of these vegetables: watercress, mustard greens, turnip, broccoli, rutabaga and horseradish ($p < 0.05$) (Figure 1C).

Consistent with their intermediate sensitivity, heterozygous PAV/AVI subjects rated glucosinolate vegetables as more bitter than did AVI/AVI subjects for a clear majority of species but not all of them, one of which reached significance: watercress ($p < 0.05$) (Supplemental data, Figure S1A).

In contrast, neither of the PAV-genotype groups rated non-glucosinolate vegetables as more bitter than did the AVI/AVI group with the intriguing exception of dandelion greens (Figure 1D and Supplemental data, Figure S1B and inset). At present dandelion is not known to contain glucosinolates.

Among cruciferous vegetables the ratios of bitterness ratings between homozygous PAV and AVI subjects were greatest for rutabaga and turnip, and smallest for cabbage, which suggests that rutabaga and turnip contain the most specific ligands for the PAV-hTAS2R38 receptor (Figure 1C, black-to-white bar ratios).

These observations correspond well with the inhibition of radioactive iodine (I^{131}) uptake into thyroids of living humans after single vegetable meals. Meals consisting only of rutabaga or turnip very strongly inhibited iodine uptake by the thyroid, while cabbage meals inhibited iodine uptake only weakly [8]. The agreement between which vegetables activate bitterness via the PAV-hTAS2R38 receptor and the capacity of these vegetables to inhibit iodine uptake supports the idea that the human TAS2R38 receptor responds to thyroid toxins proportionately to their anti-thyroid activity.

The approximately two hundred TAS2R alleles that humans possess should detect the thousands of toxins encountered in our evolutionary diet, but little is known of the relationships between diet, toxin, and receptors [1,9,10]. Presently, vegetable bitterness ratings were related to *hTAS2R38* genotypes and the synthesis of glucosinolate toxins by Brassicaceae vegetables specifically. Glucosinolates in our vegetables block the formation of organic iodine and the transport of iodine into the thyroid, resulting in both retarded sexual maturation as well as mental retardation in low iodine regions [11,12].

Despite the existence of modern diets, over one billion people are presently at risk of thyroid insufficiency, which creates a strong selective pressure to detect these compounds in our diet and minimize their intake especially in low iodine regions [12,13]. This study demonstrates the importance of individual human taste gene alleles for the perception of food and illustrates how possession of even a single allele of a bitter taste receptor gene may greatly impact how a sub-population perceives an entire family of vegetables.

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Supplemental data

Supplemental data including experimental procedures, tables listing vegetable species, and figures depicting ratings of individual vegetables by genotype groups as well as photographs of the vegetables are available at http://www.current-biology.com/cgi/content/full/16/18/R792/DC1/

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