Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Phenylthiocarbamide: A 75-Year Adventure in Genetics and Natural Selection

Stephen Wooding[1]

Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112-5330

VARIATION in taste sensitivity to the bitter compound phenylthiocarbamide (PTC) is one of the best known Mendelian traits in human populations, ranking alongside eye color and blood types in the canon of classic examples. Much of PTC's appeal arises from the fact that it is nearly impossible to guess one's phenotype until explicitly tested, yet, when tested, the phenotype is so striking as to be amusing. This property is important, particularly in education, because it can spice up lessons on inheritance. Less obvious, especially today, is PTC's appeal as an easily typed yet highly informative genetic marker. It was this aspect of the trait that made PTC an important instrument in the earliest efforts to dissect the human genome.

Variation in PTC sensitivity has also had long-standing appeal from an evolutionary standpoint. The connection of variable taste sensitivity with an aspect of behavior so obviously connected to fitness—diet choice—has long raised a basic question: Has natural selection been acting on this trait? It was this question that motivated the great statistician, R. A. Fisher, and his friends E. B. Ford and Julian Huxley, to perform what appears to be the first test for natural selection in a specific human gene. In an often-overlooked article published in 1939 (listed by Science Citation Index as having been cited <50 times in the ensuing 65 years), Fisher *et al.* (1939) reported that chimpanzees, like humans, show variable sensitivity to PTC. This, they argued, is strong evidence that balancing natural selection must have actively maintained variation at the locus from a time prior to the human–chimp divergence. While little-known today, this article set the stage for what has become an industry in the field of genetics.

[1]*Address for correspondence:* Department of Human Genetics, University of Utah, 15 North 2030 East, Salt Lake City, UT 84112-5330. E-mail: swooding@genetics.utah.edu

THE DISCOVERY OF VARIABLE PTC SENSITIVITY

Variation in PTC sensitivity was first discovered in the early 1930s. In a laboratory incident that would curl the toes of the most stoic OSHA officer, Arthur L. Fox was pouring some PTC (a white powder) into a bottle when some "flew around in the air" (Figure 1) (Fox 1932). A co-worker nearby, C. R. Noller, complained that the dust tasted bitter, but Fox insisted that he could taste nothing. The two then took turns tasting the powder and found that they really did differ dramatically in sensitivity. Determined to find out whether he and Noller were unique, Fox set about testing "a large number" of people and found that distinct variation was common regardless of age, sex, and ethnicity. Most interestingly, Fox found that most people fall into just two categories: those able to taste the compound even at very low concentrations, whom he referred to as "tasters," and those unable to taste the compound except at very high concentrations, whom he referred to as "nontasters" or "taste blind."

Fox's finding received immediate attention, appearing in brief news stories in both Science and The Scientific News Letter (Anonymous 1931a,b). These stories focused on the curiosity of the finding; however, they also caught the attention of geneticists, who were beginning to explore the organization of the human genome. T. H. Morgan's earlier work with Drosophila, which would earn him the Nobel Prize in 1933, had shown that Mendelian markers could inform genomic organization: studies of these markers in flies had led to the discovery of "linkage groups." The availability of a similar set of markers in humans would be similarly useful; however, they were difficult to find. Further, while novel Mendelian markers in flies could be driven rapidly to usefully high frequencies through selective breeding, human variants had to be used at their natural frequencies.

In mid-1931, Fox's work came to the attention of L. H. Snyder, who had been working on Mendelian markers

VOL. 18, 1932 GENETICS: A. L. FOX 115

THE RELATIONSHIP BETWEEN CHEMICAL CONSTITUTION AND TASTE

BY ARTHUR L. FOX

JACKSON LABORATORY, E. I. DU PONT DE NEMOURS & CO., WILMINGTON, DELAWARE

Read before the Academy Tuesday, November 17, 1931

Some time ago the author had occasion to prepare a quantity of phenyl thio carbamide, and while placing it in a bottle the dust flew around in the air. Another occupant of the laboratory, Dr. C. R. Noller, complained of the bitter taste of the dust, but the author, who was much closer, observed no taste and so stated. He even tasted some of the crystals and assured Dr. Noller they were tasteless but Dr. Noller was equally certain it was the dust he tasted. He tried some of the crystals and found them extremely bitter. With these two diverse observations as a starting point, a large number of people were investigated and it was established that this peculiarity was not connected with age, race or sex. Men, women, elderly persons, children, negroes, Chinese, Germans and Italians were all shown to have in their ranks both tasters and non-tasters.

FIGURE 1.—Facsimile of the heading and first paragraph of Arthur Fox's first article on PTC sensitivity in the Proceedings of the National Academy of Sciences (Fox 1932). This article was accompanied by Albert Blakeslee's article on the inheritance of PTC sensitivity (BLAKESLEE 1932b).

in human populations. At the time, the number of such traits was small. SNYDER (1931b) listed just six, including dubious entries like the direction of the whorl of hair on the back of the head and the presence of hair on the second joint of the fingers and toes. Primed to recognize more such traits, Snyder siezed upon the utility of Fox's finding right away, reporting later that on learning of Fox's results, he "immediately wrote Dr. Fox asking for some of the compound with which to investigate the possible inheritance of this taste deficiency" (SNYDER 1931a, p. 151). Snyder confirmed Fox's basic results and also tested a number of families, which led him to the conclusion that nontaster status is conferred by the recessive allele at a single locus (SNYDER 1931a).

Fox's findings also caught the attention of Albert F. Blakeslee. Blakeslee had achieved fame for his work on the genetics of plants, but his interest in Fox's work seemed to stem more from the fact that it involved variation in the human senses. Blakeslee had long been interested in sensory variation. In 1918, for instance, he had reported that humans vary in sensitivity to the scent of certain flowers, with some people being "blind" to the smell of some strains of verbena (BLAKESLEE 1918, 1935b). Blakeslee, like SNYDER (1931a), immediately replicated Fox's basic results, along with the finding that PTC blindness appears to be a Mendelian recessive (BLAKESLEE and SALMON 1931). Blakeslee's interest in the chemosenses would persist for some decades, during which he would publish a number of articles on human variation in taste and smell (BLAKESLEE and SALMON 1931, 1935; BLAKESLEE 1932a,b, 1935a,b; BLAKESLEE and FOX 1932; SALMON and BLAKESLEE 1935; BLAKESLEE and CAMPBELL 1948).

In 1932, Fox published the definitive description of the PTC sensitivity polymorphism in the Proceedings of the National Academy of Sciences (most likely at the invitation of Blakeslee, who was a member) (Fox 1932). In this article, Fox described his initial discovery of the polymorphism along with his early efforts to determine whether variation in PTC sensitivity is rare or common. The article went on to show that sensitivity to PTC is correlated with sensitivity to a variety of related compounds characterized by the presence of a distinctive N=S moiety. Further, Fox found that the bitterness of many of these compounds could be eliminated by substituting the sulfur (S) with an oxygen. One of these compounds (para-ethoxy-phenylthiocarbamide) differed from Dulcin, an artificial sweetener, by this simple substitution. However, Dulcin does not show variation in taste as PTC does (Fox 1932).

In an article immediately following Fox's in the Proceedings of the National Academy of Sciences, Blakeslee described the first large-scale study of PTC inheritance in families and also explored the threshold of PTC sensitivity (the minimum concentration at which PTC can be detected), discovering the almost incredible fact that sensitivity can vary by almost five orders of magnitude (BLAKESLEE 1932b). This study supported earlier results, but it also suggested that while the vast majority of variance in PTC sensitivity must be accounted for by a single locus, other genes are likely involved as well. Thus, PTC sensitivity is not a simple Mendelian trait. It is (in today's parlance) complex. Nonetheless, inheritance of PTC sensitivity is so close to simple Mendelian that it rightly retained its use as a marker for decades.

The identification of variable PTC sensitivity, its (nearly) simple Mendelian pattern of inheritance, and the relative ease of PTC phenotyping using treated blotter paper led to an explosion of studies of PTC sensitivity in human populations. In the 10 years following Fox's initial findings, sample sizes reached the thousands, with estimates of the nontaster allele frequency ranging from 13 to 63% with an average of ~50% (BLAKESLEE and FOX 1932; FERNBERGER 1932; LEVINE and ANDERSON 1932; BLAKESLEE 1935a,b; BLAKESLEE and SALMON 1935; SALMON and BLAKESLEE 1935; RIKIMARU 1936; STRANDSKOV 1941). This number would eventually grow to include tens of thousands of subjects in hundreds of studies (CAVALLI-SFORZA *et al.* 1994; GUO and REED 2001) (Figure 2).

FISHER, FORD, AND HUXLEY

In August 1939, the Seventh International Congress of Genetics convened in Edinburgh, Scotland (CREW 1939). This meeting had suffered an inauspicious start, with its location having been moved from Moscow due to disputes with the Soviet government, which was under a strong Lysenkoist influence (SOYFER 2003). To make matters worse, Europe stood on the brink of war.

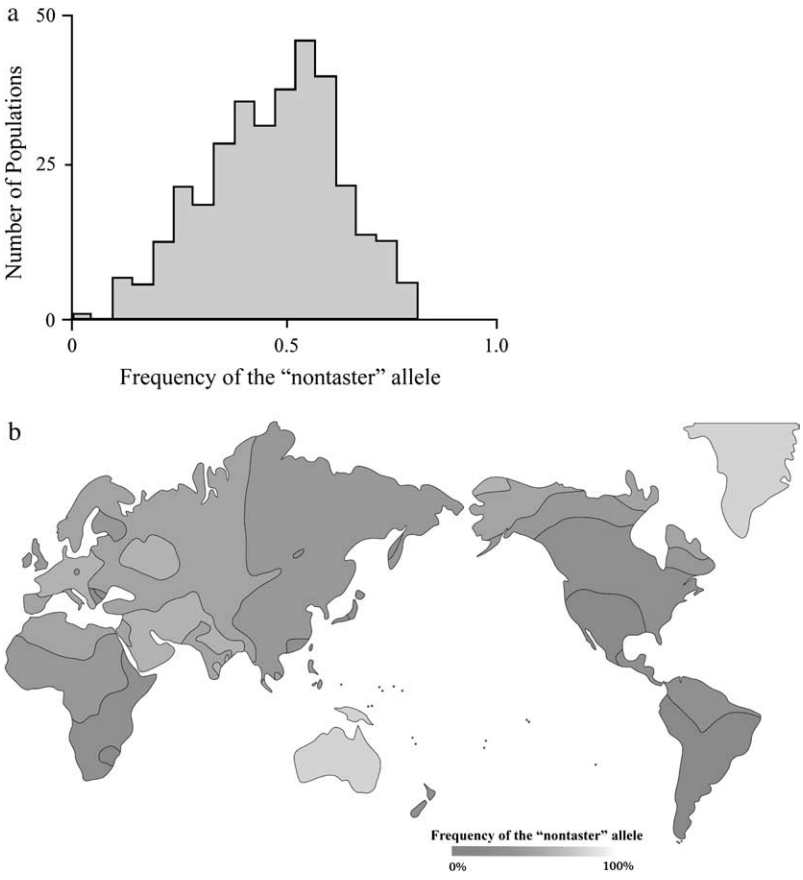

FIGURE 2.—(a) Frequency distribution of "nontaster" allele frequencies reported by GUO and REED (2001). The mean frequency across the 344 samples examined is 0.48 (modified from WOODING *et al.* 2004). (b) Geographical distribution of PTC allele frequencies (modified from CAVALLI-SFORZA *et al.* 1994).

The Congress started as planned, but the war was a major disruption. First the German delegation, and then the Dutch, were forced to leave prematurely. Immediately following the meeting, on September 3, several American participants trying to return home were among those on board the *Athenia*, the first ship sunk in the war by a German submarine (CREW 1939; CAULFIELD 1959). Among the *Athenia* survivors were Charles Cotterman and Bronson Price, who were rescued by the *City of Flint*—which happened to be transporting James Neel and George Beadle (OPITZ 1989; SCHULL 2002; BERG and SINGER 2003, pp. 127–128). This meeting was later described as being "of no great significance" scientifically (PUNNETT 1941); however, from this meeting emerged a remarkable experiment involving a remarkable cast of characters: R. A. Fisher, E. B. Ford, and Julian Huxley (YATES and MATHER 1963; BAKER 1976; CLARKE 1995) (Figure 3).

At the time of the Seventh Congress, Fisher, Ford, and Huxley had been friends for many years. Ford recalled that he and Fisher had first been introduced by Huxley in 1923, when Fisher came to see him at Oxford to learn more about his ideas on genetics and evolution, in spite of Ford's being a mere undergraduate at the time (FORD 2005). By Ford's account, the three were united in their interest in the possibility that natural selection, which was viewed as being "of minor importance, only capable, at most, of bringing about small adaptations," could be

FIGURE 3.—(Left to right) R. A. Fisher, E. B. Ford, and Julian Huxley. Portraits are from YATES and MATHER (1963), CLARKE (1995), and BAKER (1976).

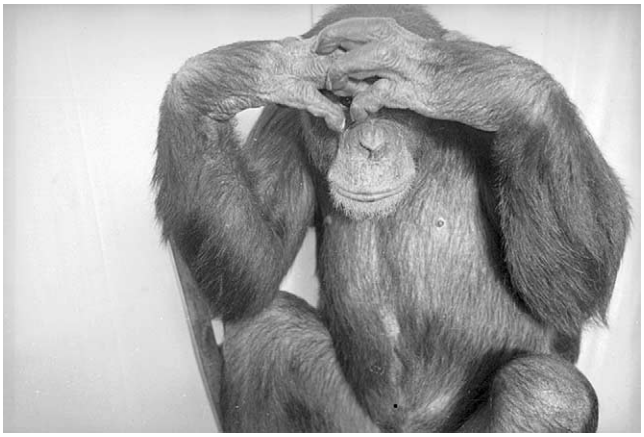

Figure 4.—A possible participant, Jackie (Jacqueline), who is mentioned in Fisher *et al.*'s (1939) article on the first tests of PTC sensitivity. Photograph from Helton Archives/Getty Images.

an important driving force in evolution (Ford 2005, p. 416). While theoretical work on the relationship between genetics, natural selection, and evolution by then was well developed (Haldane 1924; Fisher 1930), the genetic effects of selection in humans were yet to be demonstrated empirically. However, Fisher *et al.* (1939, p. 750) later related, "in the course of discussions on the possibility that the blood-group frequencies found in man were determined by a balance of selective influences, it occurred to one of the authors that evidence on the parallel possibility for taste-[sensitivity] could be obtained by testing the anthropoid apes." Thus, the three recognized that PTC sensitivity represented a unique opportunity to test the hypothesis that natural selection has acted on a specific human gene.

With the aid of a "Dr. Riddell" from Glasgow, Fisher was able to procure graduated solutions composed of "2% sugar in all, and either none, 6 1/4, 50, or 400 parts per million P.T.C." (Fisher 1939a). Then, with the assistance of F. A. E. Crew and a "Dr. Gillespie," these solutions were presented to eight chimpanzees and one orangutan at the Edinburgh Zoo, apparently as a drink (Figure 4). These tests were not without incident. David Finney was in attendance at the Seventh Congress when Fisher *et al.* made an expedition to the Edinburgh Zoo to test the animals there. Finney did not go along himself, but remembers the group coming back with the story that one of the chimps had taken a dislike to Fisher, "and spat at him or even tried to grab him" (A. Edwards, personal communication). Luca Cavalli-Sforza recalls the same incident, which was related to him by either Ken Mather or E. B. Ford. Apparently, there was some initial concern about whether it would be possible to determine from an animal's reaction whether it was a taster or not; however, "the first animal they tested took them out of any embarrassment, because it looked at Fisher in his eyes, and spit at him" (L. Cavalli-Sforza, personal communication).

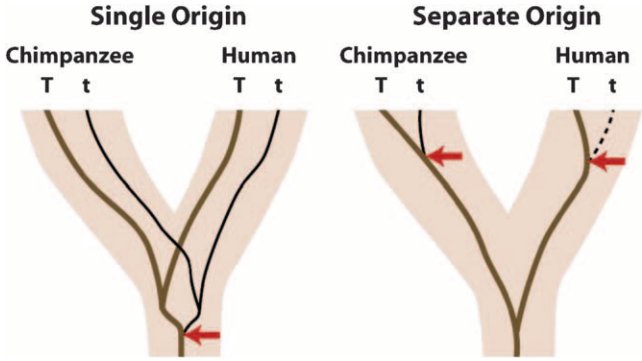

Figure 5.—Hypotheses for the origin of PTC taster and nontaster alleles. (Left) Fisher *et al.*'s (1939) "Single Origin" hypothesis. Under this hypothesis, the taster (T) and nontaster (t) alleles diverged prior to the human–chimpanzee species divergence. Then both alleles were maintained separately in each species up to the present time. The maintenance of both alleles for such an extended period [Fisher (1939b) thought that it must be ~1 million generations, or 20–30 million years] is unlikely if balancing selection has not been active because one allele or the other would be expected to go to fixation. (Right) Wooding *et al.*'s (2006) "Separate Origin" hypothesis. Under this hypothesis, nontaster alleles were derived from taster alleles twice—once in each species—after the human–chimpanzee species divergence. Arrows indicate divergence events.

The results of the Edinburgh experiment were remarkable: six of the eight tested chimpanzees appeared to be tasters and two appeared to be nontasters. Under the assumption that PTC sensitivity is conferred by the dominant allele in a single-locus two-allele system, these results implied that the taster and nontaster alleles were present at 50:50 frequencies strikingly similar to those estimated in humans (Fisher 1939a; Fisher *et al.* 1939). Fisher immediately grasped the significance of the finding, writing to Ford, "It seems to me to be immensely exciting that there should be polymorphism with a gene ratio apparently stable for millions of years in a quantity which we had originally thought of as quite without selective effects." That is, Fisher argued, if variation in PTC sensitivity is sufficiently complex to have evolved just once, then the presence of polymorphism both within humans and chimpanzees can be explained only by a single origin of PTC taster and nontaster alleles prior to the evolutionary divergence of these two species, followed by the maintenance of both alleles within each species (Figure 5).

This finding was of such obvious importance that Fisher, Ford, and Huxley immediately agreed to submit it as a letter to Nature (Fisher 1939a; Ford 1939b). Before doing so, however, they decided to expand the project to include animals at the London Zoo, where tests were conducted on September 25, and the Whipsnade Zoo, on October 1 (Fisher 1939a,b). A series of letters among Fisher, Ford, and Huxley reveal escalating enthusiasm in the course of these experiments (Fisher 1939a,b,c,d; Ford 1939a,b; Huxley 1939). Upon hearing about the

Edinburgh results, Ford wrote to Fisher, "What exciting possibilities arise! I do hope it will be possible to obtain enough material to make a fair estimate of the proportions of tasters and non-tasters in chimps" (Ford 1939b). Fisher responded, exclaiming that Huxley was, "all on fire to test all sorts of other monkeys."

Unfortunately, in the midst of this excitement came turmoil as Great Britain prepared for its fight with Germany. Many university facilities were turned over to the war effort at this time and Fisher, at University College London's Galton Laboratory, was affected immediately. In a September 26 letter to Ford, Fisher worried, "My Lab. [sic] is practically in a state of siege, evacuation having been ordered without provision for alternative accommodation. ... Actually my assistants are now threatened that, if they come to their work here it will be regarded as a breach of the College regulations" (Fisher 1939b). In spite of these difficulties, the experiment continued, although Ford lamented, "I dont [sic] find it too easy to work, somehow" (Ford 1939b). Fisher and Huxley discussed the possibility of doing more detailed work on sensitivity thresholds (Fisher 1939c), but the necessities of war soon prevailed. Fisher was forced out of the city and the project drew to a close.

Despite its early end, the results of the PTC project were better than Fisher, Ford, and Huxley could ever have hoped, and their article on the chimpanzee tests was published in the October 28, 1939, issue of Nature (Fisher *et al.* 1939). All told, PTC sensitivity was measured in every subject approached, "[w]ith the exception of one chimpanzee, which was too shy" (Fisher *et al.* 1939, p. 750). Sample sizes for four of the tested species were disappointing and yielded ambiguous results: the two gorillas appeared to be tasters; two of the three orangutans were tasters; the gibbon sample had two tasters and two nontasters, but this result was complicated by the fact that two species were included, and one of these was represented by two subspecies (Table 1). However, the results in chimpanzees were beyond doubt: of the 27 individuals tested, 20 were tasters and 7 were nontasters, implying allele frequencies of 49 and 51% for the taster and nontaster alleles, respectively—frequencies nearly identical to several studies in humans (Strandskov 1941). Fisher *et al.* (1939, p. 750) concluded,

> Without the conditions of stable equilibrium it is scarcely conceivable that the gene ratio should have remained over the million or more generations which have elapsed since the separation of the anthropoid and hominid stocks. The remarkable inference follows that over this period the heterozygotes for this apparently valueless character have enjoyed a selective advantage over both the homozygotes, and this, both in the lineage of the evolving chimpanzees and in that of evolving man. Wherein the selective advantages lie, it would at present be useless to conjecture, but of the existence of a stably balanced and enduring polymorphism determined by this gene there can be no room for doubt.

TABLE 1

Summary of results of PTC tests in apes by Fisher *et al.* (1939)

	Tested	Taster	Nontaster
Chimpanzee	27	20	7
Gorilla	2	2	0
Orangutan	3	2	1
Gibbon[a]	4	2	2

[a] Included two species.

A ROLE FOR PLANTS?

As acknowledged in their article, Fisher, Ford, and Huxley had little idea how bitter-taste sensitivity might be driven by natural selection. However, as early as 1931, variation in bitter-taste sensitivity had been suggested as a possible source of food preferences in humans and that these variations might provide insight into variation in drug response (Williams 1931). It was also recognized that a common source of bitter compounds in nature was found in plants, which produce a diversity of bitter toxins to discourage herbivores (Dunstan *et al.* 1906; Sadtler 1929). Some of these, such as strychnine, quinine, and ricin, are notorious. The widespread use of toxins by plants would seem to suggest a mechanism that might be driving the evolution of the PTC gene; however, PTC is synthetic and no compounds found naturally were known to correlate with PTC sensitivity.

The first compelling connection between plant toxicity and PTC sensitivity was finally made in 1950, when William Boyd reported that taste sensitivity to *l*-5-vinyl-2-thio-oxazolidone correlates nearly perfectly with taste sensitivity to PTC (Boyd 1950). This compound, which is found in common cultivars like cabbage and rapeseed, was recognized as a cause of goiter (Astwood *et al.* 1949; Greer 1950). Thus, a mechanism through which natural selection might act on the PTC gene was suggested: individuals better able to taste such toxic compounds might better regulate intake and thereby avoid poisoning (Boyd 1950). This was a compelling suggestion, but it failed to explain the observation of both tasters and nontasters in chimpanzees and humans, for if the ability to taste such compounds were favored, then the taster allele should rapidly have reached fixation.

In spite of lingering problems in explaining Fisher *et al.*'s (1939) original observation, the potential role of PTC sensitivity in shaping diet spawned a rich diversity of fields focused on understanding the psychophysiology of taste, along with its behavioral and ecological consequences. In 1949, Harris and Kalmus established the "threshold" procedure that was to become standard in measures of bitter-taste sensitivity (Harris and Kalmus 1949), and a large body of subsequent work tested various hypotheses about the relationship between taste sensitivity and specific phenotypes, such as cancer, smoking behavior, and body mass index

(Fischer *et al.* 1963; Kaplan *et al.* 1964; Milunicova *et al.* 1969; Drewnowski *et al.* 1998; Enoch *et al.* 2001; Tepper and Ullrich 2002; Yackinous and Guinard 2002; Bartoshuk *et al.* 2004; Cannon *et al.* 2005; Goldstein *et al.* 2005). A battery of new and more subtle assays for taste sensitivity were developed during this time (Bartoshuk and Beauchamp 1994), and the anthropological implications of variable PTC sensitivity were explored as well (Green 1973, 1974). However, missing in these studies was a firm grasp of the molecular genetics of bitter-taste sensitivity, which, despite almost 70 years of interest, remained largely unknown.

THE PTC GENE: *TAS2R38*

Major steps toward understanding the sources of genetic variation in bitter-taste sensitivity were taken in 1999, when Hoon *et al.* (1999) identified two proteins expressed at the apex of taste receptor cells. Subsequent work revealed that one of these acts as a receptor for bitter ligands and that it is just one of many such receptors found in mammals (Adler *et al.* 2000; Chandrashekar *et al.* 2000; Shi *et al.* 2003). These small proteins (most <400 amino acids in length) are encoded by the *TAS2R* gene family. Expressed in the apical microvillae of bitter-taste receptor cells, the *TAS2R* receptors are exposed to the oral cavity through a small opening called the taste pore (Hoon *et al.* 1999), where they are positioned to come into contact with the many compounds that enter the mouth. Each of these compounds represents a potential ligand, capable of binding to a receptor and stimulating bitter-taste perception. These receptors are capable of responding to a diversity of plant toxins (Bufe *et al.* 2002, 2005; Behrens *et al.* 2004; Pronin *et al.* 2004; Nelson *et al.* 2005; Soranzo *et al.* 2005).

While some early studies had observed that PTC sensitivity was correlated with variation in the Kell blood groups, little progress was made beyond that in mapping a PTC locus (Conneally *et al.* 1976; Spence *et al.* 1984). However, the discovery of the bitter-taste receptors as a group led immediately to a series of studies of the inheritance of PTC sensitivity. An early effort by Reed *et al.* (1999) with a closely related phenotype (sensitivity to 6-*n*-propylthiouracil) yielded encouraging results, identifying significant linkage near a putative bitter-taste receptor (*TAS2R01*) on chromosome 5. However, definitive results were obtained by Kim *et al.* (2003) in an association analysis and by Drayna *et al.* (2003) in a linkage analysis of the Utah CEPH pedigrees. These analyses revealed that variation at the *TAS2R38* locus accounts for 50–80% of phenotypic variance in PTC sensitivity and that most of this variance is accounted for by the presence of just two common haplotypes: a "taster" haplotype and a "nontaster" haplotype. Further, the frequencies of these haplotypes in human populations worldwide correspond well to frequencies estimated from phenotype data (Cavalli-Sforza *et al.* 1994; Guo and Reed 2001; Wooding *et al.* 2004). The identification of a major locus for PTC sensitivity provided information much needed for investigating human sensitivity to this peculiar compound in greater detail than previously possible. For example, association tests for relationships between phenotypes and the *TAS2R38* genotype, rather than between phenotypes and PTC sensitivity, are becoming the norm (*e.g.*, Duffy *et al.* 2004; Cannon *et al.* 2005; Timpson *et al.* 2005).

One of the most important consequences of the mapping of *TAS2R38* has been the change in perspective on the nontaster allele. Beginning with the very earliest findings, PTC sensitivity has been described in terms of "taster" and "nontaster" alleles, with little thought given to the molecular mechanisms underlying the differences between them. The tacit assumption has been that the nontaster allele is somehow broken, or nonfunctional. However, molecular studies of variation at *TAS2R38* suggest that this assumption could be wrong. The major taster and nontaster haplotypes differ from each other by just three amino acid substitutions; no premature stop codons, frameshifts, insertions, deletions, or other obviously catastrophic mutations are present (Drayna *et al.* 2003; Kim *et al.* 2003; Wooding *et al.* 2004; Kim *et al.* 2005). Further, while haplotypes intermediate to the taster and nontaster haplotypes show attenuated response to PTC, response is not abolished completely (Bufe *et al.* 2005). Thus, the human nontaster allele may be a functional receptor for some family of compounds that does not include PTC. No specific ligand for the PTC nontaster allele has yet been described; however, two studies have reported that the fruits of the plant *Antidesma bunius* taste bitter to PTC nontasters, but sweet to PTC tasters, raising the possibility that it contains such a ligand (Henkin and Gillis 1977; Tharp *et al.* 2005). The molecular assays of Bufe *et al.* (2005) seem likely to resolve this problem soon.

Evidence that the *TAS2R38* nontaster allele is functional suggests an immediate mechanism through which heterozygote advantage might arise at this locus. If the taster allele confers sensitivity to PTC and its chemical relatives, and the nontaster allele confers sensitivity to some other set of compounds, then heterozygotes should be able to taste both sets of compounds. Thus, they might garner a fitness advantage by being able to regulate the intake of a greater diversity of bitter compounds than can homozygotes.

REVISITING SELECTION AT THE PTC LOCUS

The identification of the *TAS2R38* genotype as a major determinant of PTC sensitivity has recently allowed the long-standing hypothesis of Fisher, Ford, and Huxley to be revisited using molecular population genetic analyses not available at the time of their project. These analyses rest on the same basic principles as those used by Fisher *et al.* (1939), but are able to capitalize on

patterns of variation at the nucleotide level (Bamshad and Wooding 2003).

Wooding *et al.* (2004) analyzed patterns of DNA sequence variation at *TAS2R38* in a sample of Africans, Asians, and Europeans. This analysis revealed that nucleotide diversity at *TAS2R38* is significantly greater than expected, given the number of variable nucleotide positions, when human population growth (which tends to reduce relative diversity) is taken into account. In addition, levels of differentiation at this locus are lower than average for humans ($F_{ST} = 0.056$ among continents), indicating that the high nucleotide diversity cannot be explained by differences among populations. These patterns were interpreted by Wooding *et al.* (2004) as evidence that balancing natural selection has, as first suggested by Fisher *et al.* (1939), actively maintained two distinct alleles in human populations. However, patterns of variation in chimpanzees suggest that Fisher *et al.* (1939) were not entirely correct in their interpretation of this shared polymorphism.

To determine whether PTC sensitivity in chimpanzees is, as in humans, controlled by two major alleles at *TAS2R38*, Wooding *et al.* (2006) analyzed patterns of variation in a captive population. DNA sequences in this sample revealed that chimpanzees do indeed harbor two common alleles at *TAS2R38*, and an experiment presenting PTC-soaked apples to the chimps showed that these alleles are strongly associated with PTC sensitivity. However, unlike the common human taster and nontaster alleles, which differ by three amino acid substitutions, the chimpanzee taster and nontaster alleles were found to differ by a single nucleotide substitution in the second position of the start codon, changing it from ATG to AGG. This change interferes with production of functional protein product by the AGG allele, which is, consequently, the nontaster form. Further, a phylogenetic analysis revealed that the taster and nontaster alleles in chimpanzees are much more similar to each other than either is to the alleles found in humans.

Taken together, the findings of Wooding *et al.* (2006) support Fisher *et al.*'s (1939) finding that both humans and chimpanzees harbor taster and nontaster alleles and that these alleles are found at similar frequencies in each species; however, they reject the hypothesis that these alleles were derived once, prior to the human–chimpanzee divergence. Rather, the nontaster alleles, which confer their phenotypic effects through entirely different molecular mechanisms, appear to have twice evolved independently. The details of the selective pressures underlying this more complex process remain a matter of conjecture.

SEVENTY-FIVE YEARS LATER

Arthur Fox could not have anticipated, in 1931, the impact that his chance discovery would have over the next 75 years. Over the decades, his observation was recruited as a fundamental tool in fields as diverse as genetics, psychophysiology, ecology, evolution, nutrition, and even science education. The role of this trait in shaping R. A. Fisher's perspective on natural selection, and his design of the first-ever test for the effects of natural selection in a specific human gene, is testament to the fundamental importance of the trait in allowing us to understand the origins of genetic variation in humans.

In many respects, the revolution started by Fox (1932) and Fisher *et al.* (1939) is just beginning. Just as the discovery of variable PTC sensitivity by Fox opened the door for Fisher, Ford, and Huxley to take early steps toward understanding the evolutionary origins of genetic variation, the recent discovery of dozens more such genes has opened many such doors (Kim *et al.* 2005; Soranzo *et al.* 2005). Equally promising are the possibilities offered by the increasing availability of information from whole genomes, which can offer perspectives on the origins of the genes themselves (Shi *et al.* 2003; Parry *et al.* 2004; Wang *et al.* 2004; Fischer *et al.* 2005; Go *et al.* 2005). One has little doubt that the early pioneers would be delighted with the progress that we have made and excited by our prospects.

Luca Cavalli-Sforza, Anthony W. F. Edwards, and Jon Seger provided helpful comments and insights.

LITERATURE CITED

Adler, E., M. A. Hoon, K. L. Mueller, J. Chandrashekar, N. J. Ryba *et al.*, 2000 A novel family of mammalian taste receptors. Cell **100:** 693–702.

Anonymous, 1931a Science news: tasteblindness. Science (Suppl.)**73:** 14a.

Anonymous, 1931b Six in ten "tasteblind" to bitter chemical. Sci. News Lett. **9:** 249.

Astwood, E. B., M. A. Greer and M. G. Ettlinger, 1949 L-5-vinyl-2-thio-oxazolidone, an antithyroid compound from yellow turnip and from brassica seeds. J. Biol. Chem. **181:** 121.

Baker, J. R., 1976 Julian Sorell Huxley, 22 June 1887–14 February 1975. Biogr. Mem. Fellows R. Soc. **22:** 206–238.

Bamshad, M., and S. P. Wooding, 2003 Signatures of natural selection in the human genome. Nat. Rev. Genet. **4:** 99–111.

Bartoshuk, L. M., and G. K. Beauchamp, 1994 Chemical senses. Annu. Rev. Psychol. **45:** 419–449.

Bartoshuk, L. M., K. Fast, D. J. Snyder and V. B. Duffy, 2004 Genetic differences in human oral perception: advanced methods reveal basic problems in intensity scaling, pp. 1–42 in *Genetic Variation in Taste Sensitivity*, edited by J. Prescott and B. J. Tepper. Marcel Dekker, New York.

Behrens, M., A. Brockhoff, C. Kuhn, B. Bufe, M. Winnig *et al.*, 2004 The human taste receptor hTAS2R14 responds to a variety of different bitter compounds. Biochem. Biophys. Res. Commun. **319:** 479–485.

Berg, P., and M. Singer, 2003 *George Beadle: An Uncommon Farmer.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Blakeslee, A. F., 1918 Unlike reactions of different individuals to fragrance in verbena flowers. Science **48:** 298–299.

Blakeslee, A. F., 1932a Are freesias fragrant? Natl. Hortic. Mag. **11:** 211–212.

Blakeslee, A. F., 1932b Genetics of sensory thresholds: taste for phenyl-thio-carbamide. Proc. Natl. Acad. Sci. USA **18:** 120–130.

Blakeslee, A. F., 1935a A dinner demonstration of threshold differences in taste and smell. Science **81:** 504–507.

Blakeslee, A. F., 1935b Demonstration of differences between people in the sense of smell. Sci. Monthly **41:** 72–84.

Blakeslee, A. F., and W. I. Campbell, 1948 Would a rose smell so sweet by any other name? Horticulture **24:** 333.

Blakeslee, A. F., and A. L. Fox, 1932 Our different taste worlds: P.T.C. as a demonstration of genetic differences in taste. J. Hered. **23:** 97–107.

Blakeslee, A. F., and M. R. Salmon, 1931 Odor and taste blindness. Eugen. News **16:** 105–109.

Blakeslee, A. F., and M. R. Salmon, 1935 Genetics of sensory thresholds: individual taste reactions for different substances. Proc. Natl. Acad. Sci. USA **21:** 84–90.

Boyd, W. C., 1950 Taste reactions to antithyroid substances. Science **112:** 153.

Bufe, B., T. Hofmann, D. Krautwurst, J. D. Raguse and W. Meyerhof, 2002 The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nat. Genet. **32:** 397–401.

Bufe, B., P. A. Breslin, C. Kuhn, D. R. Reed, C. D. Tharp *et al.*, 2005 The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr. Biol. **15:** 322–327.

Cannon, D. S., T. B. Baker, M. E. Piper, M. B. Scholand, D. L. Lawrence *et al.*, 2005 Associations between phenylthiocarbamide gene polymorphisms and cigarette smoking. Nicotine Tob. Res. **7:** 853–858.

Caulfield, M., 1959 *Tomorrow Never Came: The Story of the S. S. Athenia.* W. W. Norton, New York.

Cavalli-Sforza, L. L., P. Menozzi and A. Piazza, 1994 *The History and Geography of Human Genes.* Princeton University Press, Princeton, NJ.

Chandrashekar, J., K. L. Mueller, M. A. Hoon, E. Adler, L. Feng *et al.*, 2000 T2Rs function as bitter taste receptors. Cell **100:** 703–711.

Clarke, B. C., 1995 Edmund Brisco Ford: 23 April 1901–21 January 1988. Biogr. Mem. Fellows R. Soc. **41:** 146–168.

Conneally, P. M., M. Dumont-Driscoll, R. S. Huntzinger, W. E. Nance and C. E. Jackson, 1976 Linkage relations of the loci for Kell and phenylthiocarbamide taste sensitivity. Hum. Hered. **26:** 267–271.

Crew, F. A. E., 1939 Seventh international genetical congress. Nature **144:** 496–498.

Drayna, D., H. Coon, U. K. Kim, T. Elsner, K. Cromer *et al.*, 2003 Genetic analysis of a complex trait in the Utah Genetic Reference Project: a major locus for PTC taste ability on chromosome 7q and a secondary locus on chromosome 16p. Hum. Genet. **112:** 567–572.

Drewnowski, A., S. A. Henderson and A. Barratt-Fornell, 1998 Genetic sensitivity to 6-n-propylthiouracil and sensory responses to sugar and fat mixtures. Physiol. Behav. **63:** 771–777.

Duffy, V. B., A. C. Davidson, J. R. Kidd, K. K. Kidd, W. C. Speed *et al.*, 2004 Bitter receptor gene (*TAS2R38*), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. Alcohol Clin. Exp. Res. **28:** 1629–1637.

Dunstan, W. R., T. A. Henry and S. J. M. Auld, 1906 Cyanogenesis in plants. Part V. The occurrence of phaseolunatin in cassava (*Manihot aipi* and *Manihot utilissima*). Proc. Roy. Soc. Ser. B. **78:** 145–158.

Enoch, M. A., C. R. Harris and D. Goldman, 2001 Does a reduced sensitivity to bitter taste increase the risk of becoming nicotine addicted? Addict. Behav. **26:** 399–404.

Fernberger, S. W., 1932 A preliminary study of taste deficiency. Am. J. Psychol. **44:** 322–326.

Fischer, A., Y. Gilad, O. Man and S. Paabo, 2005 Evolution of bitter taste receptors in humans and apes. Mol. Biol. Evol. **22:** 432–436.

Fischer, R., F. Griffin and A. R. Kaplan, 1963 Taste thresholds, cigarette smoking, and food dislikes. Med. Exp. Int. J. Exp. Med. **210:** 151–167.

Fisher, R. A., 1930 *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

Fisher, R. A., 1939a Personal letter to Edmund Brisco Ford, September 22, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1,* University of Adelaide Library, Adelaide, Australia.

Fisher, R. A., 1939b Personal letter to Edmund Brisco Ford, September 26, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1,* University of Adelaide Library, Adelaide, Australia.

Fisher, R. A., 1939c Personal letter to Julian Sorell Huxley, October 26, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1.* University of Adelaide Library, Adelaide, Australia.

Fisher, R. A., 1939d Personal letter to Julian Sorell Huxley, October 27, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1.* University of Adelaide Library, Adelaide, Australia.

Fisher, R. A., E. B. Ford and J. Huxley, 1939 Taste-testing the anthropoid apes. Nature **144:** 750.

Ford, E. B., 1939a Personal letter to Ronald Aylmer Fisher, October 17, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1.* University of Adelaide Library, Adelaide, Australia.

Ford, E. B., 1939b Personal letter to Ronald Aylmer Ford, September 24, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1.* University of Adelaide Library, Adelaide, Australia.

Ford, E. B., 2005 R. A. Fisher: an appreciation. Genetics **171:** 415–417.

Fox, A. L., 1932 The relationship between chemical constitution and taste. Proc. Natl. Acad. Sci. USA **18:** 115–120.

Go, Y., Y. Satta, O. Takenaka and N. Takahata, 2005 Lineage-specific loss of function of bitter taste receptor genes in humans and nonhuman primates. Genetics **170:** 313–326.

Goldstein, G. L., H. Daun and B. J. Tepper, 2005 Adiposity in middle-aged women is associated with genetic taste blindness to 6-n-propylthiouracil. Obes. Res. **13:** 1017–1023.

Green, L. S., 1973 Physical growth and development, neurological maturation and behavioral functioning in two Ecuadorian Andean communities in which goiter is endemic. I. Outline of the problem of endemic goiter and cretinism. Physical growth and neurological maturation in the adult population of La Esperanza. Am. J. Phys. Anthropol. **38:** 119–134.

Green, L. S., 1974 Physical growth and development, neurological maturation and behavioral functioning in two Ecuadorian Andean communities in which goiter is endemic. II. PTC taste sensitivity and neurological maturation. Am. J. Phys. Anthropol. **41:** 139–152.

Greer, M. A., 1950 Nutrition and goiter. Physiol. Rev. **30:** 513–548.

Guo, S. W., and D. R. Reed, 2001 The genetics of phenylthiocarbamide perception. Ann. Hum. Biol. **28:** 111–142.

Haldane, J. B. S., 1924 A mathematical theory of natural and artificial selection. Part I. Trans. Cambridge Philos. Soc. **23:** 19–41.

Harris, H., and H. Kalmus, 1949 The measurement of taste sensitivity to phenylthiourea (P. T. C.). Ann. Eugen. **15:** 24–31.

Henkin, R. I., and W. T. Gillis, 1977 Divergent taste responsiveness to fruit of the tree Antidesma bunius. Nature **265:** 536–537.

Hoon, M. A., E. Adler, J. Lindemeier, J. F. Battey, N. J. Ryba *et al.*, 1999 Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell **96:** 541–551.

Huxley, J. S., 1939 Personal letter to Ronald Aylmer Fisher, October 26, 1939, in *Sir Ronald Aylmer Fisher, Papers 1911–94, Correspondence Series 1.* University of Adelaide Library, Adelaide, Australia.

Kaplan, A. R., E. V. Glanville and R. Fischer, 1964 Taste thresholds for bitterness and cigarette smoking. Nature **202:** 1366.

Kim, U. K., E. Jorgenson, H. Coon, M. Leppert, N. Risch *et al.*, 2003 Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science **299:** 1221–1225.

Kim, U., S. Wooding, D. Ricci, L. B. Jorde and D. Drayna, 2005 Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. Hum. Mutat. **26:** 199–204.

Levine, P., and A. S. Anderson, 1932 Observations on taste blindness. Science **75:** 497–498.

Milunicova, A., A. Jandova and V. Skoda, 1969 Phenylthiocarbamide tasting ability and malignant tumours. Hum. Hered. **19:** 398–401.

Nelson, T. M., S. D. Munger and J. D. Boughter, Jr., 2005 Haplotypes at the Tas2r locus on distal chromosome 6 vary with quinine taste sensitivity in inbred mice. BMC Genet. **6:** 32.

Opitz, J. M., 1989 Charles W. Cotterman. Am. J. Med. Genet. **34:** 149–154.

Parry, C. M., A. Erkner and J. le Coutre, 2004 Divergence of T2R chemosensory receptor families in humans, bonobos, and chimpanzees. Proc. Natl. Acad. Sci. USA **101:** 14830–14834.

Pronin, A. N., H. Tang, J. Connor and W. Keung, 2004 Identification of ligands for two human bitter T2R receptors. Chem. Senses **29:** 583–593.

Punnett, R. C., 1941 *Proceedings of the VII International Genetical Congress, Edinburgh, Scotland (August 23–30, 1939).* Cambridge University Press, Cambridge, UK.

Reed, D. R., E. Nanthakumar, M. North, C. Bell, L. M. Bartoshuk *et al.*, 1999 Localization of a gene for bitter-taste perception to human chromosome 5p15. Am. J. Hum. Genet. **64:** 1478–1480.

Rikimaru, J.-Y., 1936 Taste deficiency of Japanese and other races in Formosa. Am. J. Psychol. **48:** 649–653.

Sadtler, R. E., 1929 The chemistry of the formation of poisons in plants. Scientific Monthly **29:** 153–154.

Salmon, T. M., and A. F. Blakeslee, 1935 Genetics of sensory thresholds: variations within single individuals in taste sensitivity for PTC. Proc. Natl. Acad. Sci. USA **21:** 78–83.

Schull, W. J., 2002 James Van Gundia Neel (1915–2000). Biogr. Mem. Natl. Acad. Sci. **81:** 214–233.

Shi, P., J. Zhang, H. Yang and Y. P. Zhang, 2003 Adaptive diversification of bitter taste receptor genes in mammalian evolution. Mol. Biol. Evol. **20:** 805–814.

Snyder, L. H., 1931a Inherited taste deficiency. Science **74:** 151–152.

Snyder, L. H., 1931b Linkage in man. Eugen. News **16:** 117–119.

Soranzo, N., B. Bufe, P. C. Sabeti, J. F. Wilson, M. E. Weale *et al.*, 2005 Positive selection on a high-sensitivity allele of the human bitter-taste receptor *TAS2R16*. Curr. Biol. **15:** 1257–1265.

Soyfer, V. N., 2003 Tragic history of the VII International Congress of Genetics. Genetics **165:** 1–9.

Spence, A.-M., C. T. Falk, K. Neiswanger, L. L. Field, M. L. Marazita *et al.*, 1984 Estimating the recombination frequency for the PTC-Kell linkage. Hum. Genet. **67:** 183–186.

Strandskov, H. H., 1941 The distribution of human genes. Scientific Monthly **52:** 203–215.

Tepper, B. J., and N. V. Ullrich, 2002 Influence of genetic taste sensitivity to 6-n-propylthiouracil (PROP), dietary restraint and disinhibition on body mass index in middle-aged women. Physiol. Behav. **75:** 305–312.

Tharp, C. D., A. Tharp, S. M. Alarcon, D. R. Reed and P. A. Breslin, 2005 PTC non-tasters find the fruit of Antidesma bunius bitter, while PTC tasters find it sweet, pp. 23 in *Proceedings of the Association for Chemoreception Sciences (April 13–17, 2005)*, Sarasota, FL.

Timpson, N. J., M. Christensen, D. A. Lawlor, T. R. Gaunt, I. N. Day *et al.*, 2005 TAS2R38 (phenylthiocarbamide) haplotypes, coronary heart disease traits, and eating behavior in the British Women's Heart and Health Study. Am. J. Clin. Nutr. **81:** 1005–1011.

Wang, X., S. D. Thomas and J. Zhang, 2004 Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes. Hum. Mol. Genet. **13:** 2671–2678.

Williams, R. J., 1931 "Taste deficiency" for creatine. Science **74:** 597–598.

Wooding, S., U. K. Kim, M. J. Bamshad, J. Larsen, L. B. Jorde *et al.*, 2004 Natural selection and molecular evolution in *PTC*, a bitter-taste receptor gene. Am. J. Hum. Genet. **74:** 637–646.

Wooding, S., B. Bufe, C. Grassi, M. T. Howard, A. C. Stone *et al.*, 2006 Independent evolution of bitter-taste sensitivity in humans and chimpanzees. Nature (in press).

Yackinous, C. A., and J. X. Guinard, 2002 Relation between PROP (6-n-propylthiouracil) taster status, taste anatomy and dietary intake measures for young men and women. Appetite **38:** 201–209.

Yates, F., and K. Mather, 1963 Ronald Aylmer Fisher (1890–1962). Biogr. Mem. Fellows R. Soc. **9:** 91–129.