senses cooling, whereas the latter senses warming. The two proteins are 54% similar throughout their length, so it is not obvious which domains are crucial for the warm or cold response. But analysis of chimaeric mouse and *Drosophila* ANKTM1 proteins should help in the mapping of temperature-activation domains of these TRP channels.

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1. Caterina, M. J. & Julius, D. *Annu. Rev. Neurosci.* **24,** 487–517 (2001).
2. Guler, A. D. *et al. J. Neurosci.* **22,** 6408–6414 (2002).
3. Peier, A. M. *et al. Science* **296,** 2046–2049 (2002).
4. McKemy, D. D., Neuhausser, W. M. & Julius, D. *Nature* **416,** 52–58 (2002).
5. Peier, A. M. *et al. Cell* **108,** 705–715 (2002).
6. Story, G. M. *et al. Cell* **112,** 819–829 (2003).
7. Sayeed, O. & Benzer, S. *Proc. Natl Acad. Sci. USA* **93,** 6079–6084 (1996).
8. Liu, L., Yermolaieva, O., Johnson, W. A., Abboud, F. M. & Welsh, M. J. *Nature Neurosci.* **6,** 267–273 (2003).
9. Tobin, D. *et al. Neuron* **35,** 307–318 (2002).
10. Tracey, W. D., Wilson, R. I., Laurent, G. & Benzer, S. *Cell* **113,** 261–273 (2003).
11. Walker, R. G., Willingham, A. T. & Zuker, C. S. *Science* **287,** 2229–2234 (2000).

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RNA interference

Producing decaffeinated coffee plants

The demand for decaffeinated coffee is increasing because the stimulatory effects of caffeine can adversely affect sensitive individuals by triggering palpitations, increased blood pressure and insomnia[1]. Three *N*-methyltransferase enzymes are involved in caffeine biosynthesis in coffee plants — CaXMT1, CaMXMT1 (theobromine synthase) and CaDXMT1 (caffeine synthase), which successively add methyl groups to xanthosine in converting it into caffeine[2–4]. Here we describe the construction of transgenic coffee plants in which expression of the gene encoding theobromine synthase (*CaMXMT1*) is repressed by RNA interference (RNAi). The caffeine content of these plants is reduced by up to 70%, indicating that it should be feasible to produce coffee beans that are intrinsically deficient in caffeine.

Specific sequences in the 3′ untranslated region (UTR) of *CaMXMT1* messenger RNA were selected for construction of RNAi short and long fragments (Fig. 1). We transformed *Agrobacterium tumefaciens* EHA101 cells with these constructs and then used them to transform *Coffea canephora*[5]. After 2–4 months of culture, most infected tissues turned brown and necrotic; however, it was possible to regenerate hygromycin-resistant cells from these tissues. Seedlings were then cultured as described[5].

More than 35 transgenic somatic seedlings were obtained from each transformant, each containing short or long RNAi fragments or a control gene encoding green fluorescent protein (GFP). The phenotypes were apparently normal when compared with the wild-type plant (Fig. 1a).

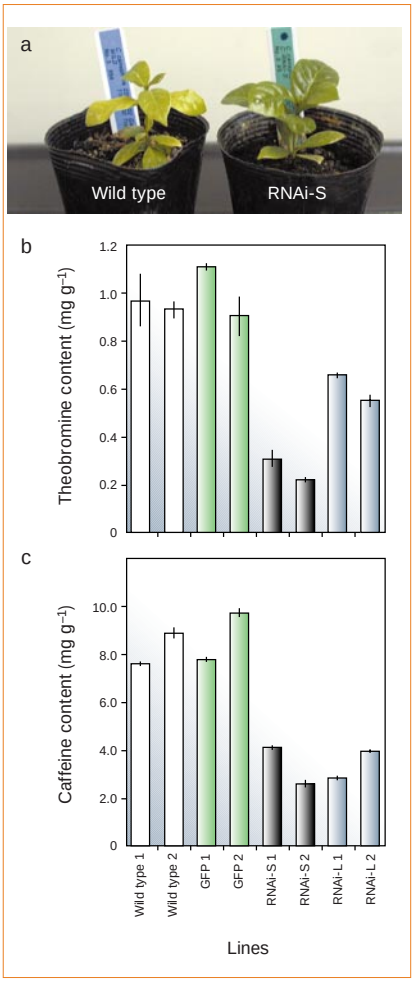

Figure 1 Properties of decaffeinated transgenic coffee leaves. **a,** One-year-old somatic seedlings of *Coffea canephora* from wild-type (left) and RNAi-transgenic (right) plants. **b, c,** Endogenous theobromine and caffeine, respectively, in mg per g of fresh plant tissue, in different somatic seedlings of *C. canephora*, as detected by high-performance liquid chromatography[2]. Mean values were calculated from six independent measurements per line. Short RNAi fragments (RNAi-S) were constructed using 139-base-pair (bp; corresponding to nucleotide positions 1,139–1,277) and 161-bp (positions 1,117–1,277) sequences of *CaMXMT1* (GenBank accession number AB048794), with an intervening 517-bp β-glucuronidase (*GUS*) fragment as spacer; long RNAi fragments (RNAi-L) contained two identical sequences of 332 bp (positions 946–1,277) separated by a 517-bp *GUS* fragment. The resulting constructs were inserted into a pBIH1-IG vector[7]; the control construct contained a green fluorescent protein gene (*GFP*). Somatic embryos of *C. canephora* were grown on modified half-strength Murashige and Skoog medium containing 20 μM 2-isopentenyladenine.

Young leaves of one-year-old seedlings were collected 2–3 weeks after flushing and their purine alkaloid content was measured. The wild-type and transgenic lines that expressed GFP contained similar amounts of endogenous theobromine and caffeine (about 1 and 8 mg per g of fresh plant tissue, respectively; Fig. 1b, c). By contrast, young leaves of transgenic lines expressing RNAi showed a 30–80% reduction in theobromine content (Fig. 1b) and a 50–70% reduction in caffeine content (Fig. 1c) in comparison with the controls.

At present, coffee is decaffeinated industrially, but the process is expensive and the flavour of the product is poor[6] — problems that could potentially be overcome by the genetic engineering of coffee plants[3,6]. As *CaMXMT1* is expressed in young leaves, buds and immature fruits[4], the transgenic plants described here should yield coffee beans that are essentially normal apart from their low caffeine content at maturity.

We are now applying this RNAi-based technique to *C. arabica*, which produces high-quality Arabica coffee and accounts for roughly 70% of the world market. Our method not only shortens the breeding period, which is more than 25 years for conventional crossing, but also opens the way to develop new species of coffee plant.

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1. www.ico.org/frameset/coffset.htm
2. Ashihara, H., Monteiro, A. M., Gillies, F. M. & Crozier, A. *Plant Physiol.* **111,** 747–753 (1996).
3. Ogawa, M., Herai, Y., Koizumi, K., Kusano, T. & Sano, H. *J. Biol. Chem.* **276,** 8213–8218 (2001).
4. Uefuji, H., Ogita, S., Yamaguchi, Y., Koizumi, N. & Sano, H. *Plant Physiol.* **132,** 372–380 (2003).
5. Hatanaka, T., Choi, Y. E., Kusano, T. & Sano, H. *Plant Cell Rep.* **19,** 106–110 (1999).
6. Ashihara, H. & Crozier, A. *Trends Plant Sci.* **6,** 407–413 (2001).
7. Ohta, S., Mita, S., Hattori, T. & Nakamura, K. *Plant Cell Physiol.* **31,** 805–813 (1990).

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COMMUNICATIONS ARISING

Ecology

Mycorrhizal weathering in base-poor forests

Minerals in soil contain many small pores, which has led to the suggestion that trees are able to ‘mine’ essential nutrients such as calcium through their association with symbiotic mycorrhizae, thereby bypassing the exchangeable calcium pool in the soil[1,2]. On the basis of the calcium-to-strontium (Ca/Sr) ratios in the foliage of trees at Hubbard Brook (an experimental forest), Blum *et al.* suggest that these trees have direct access to calcium from apatite (calcium phosphate) in lower soil horizons

Table 1 Mean molar Ca/Sr ratios in tree parts

	Roots	*Wood*	*Branches*	*Bark*	*Foliage*
White pine	396 (148)	199 (78)	880 (432)	918 (130)	3,175 (1,858)
Eastern hemlock	814 (312)	275 (55)	756 (201)	831 (233)	2,655 (1,367)
White cedar	1,198 (661)	135 (8)	1,029 (200)	1,009 (421)	334 (123)
Black spruce	369 (151)	131 (27)	330 (90)	503 (154)	569 (189)
Balsam fir	511 (237)	188 (69)	402 (93)	500 (108)	802 (192)
Red maple	348 (128)	118 (22)	220 (31)	330 (59)	640 (273)
Red oak	349 (96)	126 (21)	800 (86)	629 (186)	1,626 (660)
Large tooth aspen	395 (255)	91 (18)	212 (17)	345 (91)	358 (64)
White birch	330 (81)	99 (16)	236 (61)	325 (50)	335 (108)

Data are from nine tree species at Plastic Lake, Ontario (45° 11′, 78° 50′). Latin names for these species (top to bottom) are *Pinus strobus, Tsuga canadensis, Thuja occidentalis, Picea mariana, Abies balsamea, Acer rubrum, Quercus rubra, Populus grandidentata* and *Betula papyrifera*. Standard deviations are given in parentheses (*n*, 5–10).

through their mycorrhizal associations[3]. But because Ca/Sr ratios can vary greatly in different parts of a plant, Ca/Sr ratios in foliage are poor indicators of the source of calcium for trees. Differences in Ca/Sr ratios in tree foliage should be interpreted with caution until more is known about the cycling of these elements in forests.

Depletion of the exchangeable calcium pool in soil is of widespread concern and may have serious implications for future forest health and productivity[4–8]. Blum *et al.* propose that apatite-derived calcium is used largely by ectomycorrhizal tree species, and that mycorrhizae may weather apatite and absorb the released ions directly, without ions entering the exchangeable pool[3].

The mineralogy of the shallow soils and bedrock at Plastic Lake (referred to here as PC1) in south-central Ontario is dominated by quartz, plagioclase, K-feldspar, hornblende, vermiculite, imogolite and goethite; there is no evidence that apatite is present at PC1 (ref. 9). Furthermore, base-cation mass-balance budgets that consider only silicate weathering are consistent with the measured changes in stream water and soil chemistry, which together indicate that substantial calcium losses have occurred from the exchangeable pool over the past two decades[10].

Despite the absence of calcium-rich minerals at PC1, molar Ca/Sr ratios in tree foliage at PC1 vary widely and range from about 330 to 3,200 (Table 1), which is similar to the range (about 500 to 2,200) reported at Hubbard Brook[3]. As shown previously[11], we found that Ca/Sr ratios vary widely across plant parts within a single tree species (Table 1).

The annual cycling of calcium in the above-ground biomass (litter fall plus foliar leaching) of temperate forests in eastern North America is typically an order of magnitude greater than the rate of calcium weathering expected in base-poor forests[12,13]. Because the 'mining' of calcium cannot supply a large proportion of a forest's annual calcium demand, the direct uptake of calcium from minerals will not protect trees from the negative effects of low calcium or high aluminium availability that may occur in acidified soils[1,3]. The ecological significance of direct calcium uptake through symbiotic mycorrhizal association lies in whether this process increases the annual calcium weathering rate, which will determine the size of the exchangeable calcium pool under given acid-deposition and harvesting conditions.

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1. Jongmans, A. G. *et al. Nature* **389,** 682–683 (1997).
2. Van Breemen, N. *et al. Biogeochemistry* **49,** 53–67 (2000).
3. Blum, J. D. *et al. Nature* **417,** 729–731 (2002).
4. Likens, G. E., Driscoll, C. T. & Buso, D. C *Science* **272,** 244–246 (1996).
5. Johnson, C. E., Driscoll, C. T., Siccama, T. G. & Likens, G. E. *Ecosystems* **3,** 159–184 (2000).
6. Federer, C. A. *et al. Environ. Mgmt* **13,** 593–601 (1989).
7. Sverdrup, H. & Rosen, K. *For. Ecol. Mgmt* **110,** 221–236 (1998).
8. Lawrence, G. B., David, M. B., Bailey, S. W. & Shortle, W. C. *Biogeochemistry* **38,** 19–39 (1997).
9. Kirkwood, D. E. & Nesbitt, H. W. *Geochim. Cosmochim. Acta* **55,** 1295–1308 (1991).
10. Watmough, S. A. & Dillon, P. J. *For. Ecol. Mgmt* **177,** 155–177 (2003).
11. Poszwa, A., Dambrine, E., Pollier, B. & Atteia, O. *Plant Soil* **225,** 299–310 (2000).
12. Likens, G. E. *et al. Biogeochemistry* **41,** 89–173 (1998).
13. Duchesne, L., Ouimet, R., Camiré, C. & Houle, D. *Can. J. For. Res.* **31,** 333–334 (2001).

Blum et al. *reply* — We identified apatite as an important reservoir of calcium in the soil horizons termed Bs and C at Hubbard Brook experimental forest (HBEF) and suggested that it could exceed the size of the soil-exchange pool[1,2]. Apatite has high calcium-to-strontium (Ca/Sr) and phosphorus-to-calcium ratios, distinct from silicate mineral and atmospheric sources of calcium. The higher Ca/Sr ratios in foliage of ectomycorrhizal tree species (compared with non-ectomycorrhizal species) implied that calcium in apatite might be more accessible to ectomycorrhizal species[1]. Some preferential plant uptake of calcium over strontium was expected[1,3], so we reported the proportions of apatite-derived foliar calcium as maximum values[1]. Previous work indicated that species differences in Ca/Sr were more directly related to soil mineralogy (silicate versus calcite) than to preferential uptake that depended on species[3,4].

Ca/Sr ratios in HBEF roots and wood also reveal higher Ca/Sr in ectomycorrhizal trees (J.D.B. *et al.*, unpublished results), whereas Watmough and Dillon do not find consistent variations in the Ca/Sr ratio of various tree parts from different species at Plastic Lake, Ontario. Further work is needed to understand these intra-species variations, and we agree that Ca/Sr ratios in foliage should be interpreted with caution, particularly where detailed soil chemical and mineralogical data are not available.

Watmough and Dillon show that pine, hemlock and red oak (all ectomycorrhizal species) at Plastic Lake have high foliar Ca/Sr ratios. Although apatite has not been identified as a major soil mineral at Plastic Lake[5], virtually all crystalline silicate rocks contain small amounts of apatite[6] and/or calcite[7], including those throughout Ontario[6]. Soil phosphorus concentrations and/or cathodoluminescence images have not been reported from Plastic Lake and would be necessary to identify minute inclusions of apatite and/or calcite, which are easily weathered trace minerals with high Ca/Sr ratios.

Our results show that apatite accounts for about 20% of the calcium in the HBEF soil parent material[1], and more than 20% of the calcium released by weathering since deglaciation was derived from apatite. Nevertheless, most of the annual calcium uptake by trees is recycled through detritus, with only a small annual contribution of newly weathered calcium. Therefore, although the apatite calcium pool in HBEF soils changes our view of 'plant-available' calcium, direct uptake of calcium from minerals will not fully protect trees from the negative effects of calcium depletion in acidified soils. We agree that the ecological significance of calcium release by ectomycorrhizal-driven weathering lies in its effect on calcium-weathering rates — indeed, organic acids of low relative molecular mass (which are released by ectomycorrhizal hyphae[8]) accelerate apatite weathering markedly compared with inorganic acids[9].

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1. Blum, J. D. *et al. Nature* **417,** 729–731 (2002).
2. Hamburg, S. P., Yanai, R. D., Arthur, M. A., Blum, J. D. & Siccama, T. G. *Ecosystems* (in the press).
3. Blum, J. D., Taliaferro, H., Weisse, M. T. & Holmes, R. T. *Biogeochemistry* **49,** 87–101 (2000).
4. Poszwa, A., Dambrine, E., Pollier, B. & Atteia, O. *Plant Soil* **225,** 299–310 (2000).
5. Kirkwood, D. E & Nesbitt, H. W. *Geochim. Cosmochim. Acta* **55,** 1295–1308 (1991).
6. Shaw, D. M., Reilly, G. A., Muysson, J. R., Pattenden, G. E. & Cambell, F. E. *Can. J. Earth Sci.* **4,** 829–853 (1967).
7. White, A. F., Bullen, T. D., Vivit, D. V., Schulz, M. S. & Clow, D. W. *Geochim. Cosmochim. Acta* **63,** 1939–1953 (1999).
8. Van Breeman, N. *et al. Biogeochemistry* **49,** 53–67 (2000).
9. Welch, S. A., Taunton, A. E. & Banfield, J. F. *Geomicrobiol. J.* **19,** 343–367 (2002).