

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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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 insomnia¹. 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

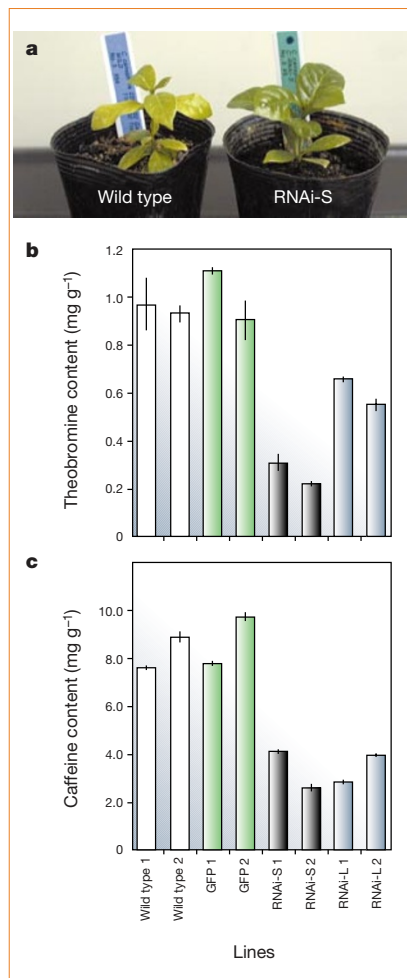


Figure 1 Properties of decaffeinated transgenic coffee leaves. **a**, One-year-old somatic seedlings of *Coffea canephora* from wild-type (left) and RNAi-transgenic (right) plants. **b**, **c**, Endogenous theobromine and caffeine, respectively, in mg per g of fresh plant tissue, in different somatic seedlings of *C. canephora*, as detected by high-performance liquid chromatography². 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 vector⁶; 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 μ M 2-isopentenyladenine.

and necrotic; however, it was possible to regenerate hygromycin-resistant cells from these tissues. Seedlings were then cultured as described⁵.

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).

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 poor⁶ — 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 fruits⁴, 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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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 (65)	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 associations³. 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 pool⁵.

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 decades¹⁰.

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 Brook³. As shown previously¹¹, 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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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 species¹. Some preferential plant uptake of calcium over strontium was expected^{1,3}, so we reported the proportions of apatite-derived foliar calcium as maximum values¹. 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 Lake⁵, virtually all crystalline silicate rocks contain small amounts of apatite⁶ and/or calcite⁷, including those throughout Ontario⁶. 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 material¹, 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 hyphae⁸) accelerate apatite weathering markedly compared with inorganic acids⁹.

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Coalition among male fiddler crabs

Seeing off a neighbour's intruder may be easier than negotiating with a larger usurper.

Until now, no compelling evidence has emerged from studies of animal territoriality to indicate that a resident will strategically help a neighbour to defend its territory against an intruder^{1,2}. We show here that territory-owning Australian fiddler crabs will judiciously assist other crabs in defending their neighbouring territories. This cooperation supports the prediction³ that it is sometimes less costly to assist a familiar neighbour than to renegotiate boundaries with a new, and possibly stronger, neighbour⁴.

In Darwin, Australia, the sexually dimorphic fiddler crab *Uca mjoebergi* lives in mixed-sex colonies on intertidal mudflats. Males have one greatly enlarged claw (Fig. 1), which is used both for attracting females and as a weapon^{5,6}. Each crab defends an all-purpose territory containing a central burrow that is used as a refuge during high tide. Males mainly fight other males and vigorously defend their territory against wandering 'floaters' that are seeking a new burrow. They also repel neighbours that encroach on their territory.

Fights involving three males have been recorded, but the territorial status of the participants was unknown⁵. We therefore tracked 268 floaters until we saw them fighting a territorial male. We recorded 17 cases in which a resident that was fighting an intruding floater was joined by an immediate neighbour (henceforth called an 'ally'). These fights always occurred at the resident's burrow entrance so the ally had to vacate his own territory temporarily to join the fight. The two neighbours never fought each other. They pushed or grappled only with the intruder.

Allies helped when they were most likely to have a beneficial effect. In unassisted fights, the smaller the resident was compared with the floater, the greater was the likelihood that he would be evicted (logistic regression, $\chi^2_1 = 36.4$, $P < 0.001$, $n = 251$). In 94% of cases when assistance was provided, the resident was smaller than the intruding floater, but this was only true in 51% of cases when no assistance was given (Fisher's exact test, $P = 0.001$, 16/17 compared with 128/251). Males therefore provided assistance when their neighbour was more likely to lose his territory.

In addition, the ally was generally larger than the floater (binomial test, $P = 0.013$, 14/17; mean \pm s.d. for claw length, 21.0 ± 2.9 mm compared with 18.5 ± 4.0 mm) and therefore more likely than his smaller



Figure 1 The Australian fiddler crab uses its enlarged claw to defend non-threatening neighbours from stronger, encroaching males.

neighbour (14.8 ± 4.0 mm) to defeat the floater. The ally was always larger than the assisted neighbour (binomial test, $P < 0.0001$, 17/17). The net result was that assisted residents were evicted during the contests significantly less often than unassisted residents (logistic regression, $\chi^2_1 = 9.6$, $P = 0.002$, $n = 268$, 12% compared with 29%).

Helping is potentially costly. Allies leave their territory, which increases the risk that their own burrow will be usurped⁷, and fighting is energetically expensive and can result in claw loss^{5,6}. So why do territory owners help neighbours? First, we can exclude direct reciprocal altruism⁸ because allies were always larger than the males they assisted. Second, there is no evidence that floaters pose a delayed threat to allies. When we continued to track a floater after his first fight, we never saw him fight his previous opponent's neighbour; floaters moved 73.7 ± 34.9 cm between successive fights, compared with a mean distance between neighbours of ± 10 cm ($n = 30$ floaters).

It has been predicted that residents will form territorial coalitions when losing neighbours is costly⁹. To test this, we located 20 large males with a smaller neighbour (size-matched to observed helper-resident pairs) and watched them for 15 minutes. In no case did we observe threat displays or fights between neighbours. To mimic neighbour eviction or retention, we then either replaced the smaller neighbour with an intermediate-sized male ($n = 10$), or caught the smaller

neighbour and then returned him to his territory ($n = 10$). Once both territory owners were present on the surface, we watched them for another 15 minutes. We observed no threat displays or fights when the smaller neighbour stayed. After replacement, however, the larger male approached and fought the unfamiliar neighbour in nine out of ten cases (Fisher's exact test, $P < 0.001$).

Territorial coalitions in *U. mjoebergi* seem to be due to by-product mutualism⁹: the ally pays to retain an established neighbour and the neighbour keeps his territory. The circumstances under which assistance was provided appeared to involve judicious decision-making. That this occurs in an invertebrate, but has still not been reported in birds or mammals, suggests that territorial coalitions depend more on appropriate circumstances than on advanced cognitive skills.

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Corrigendum

Producing decaffeinated coffee plants

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It has been drawn to *Nature's* attention that S. O., N. K. and H. S. are named as the inventors on a patent relevant to this work (WO2004006658, filed in July 2002), which should therefore have been declared as a competing financial interest.

brief communications arising online

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Evolutionary genetics: Ambiguous role of CCR5 in *Y. pestis* infection

S. J. Elvin, E. D. Williamson, J. C. Scott, J. N. Smith, G. Pérez de Lema, S. Chilla, P. Clapham, K. Pfeffer, D. Schlöndorff & B. Luckow (doi:10.1038/nature02822)