

the modern form and is evidence of a roughly 120-Myr morphological stasis in ovulate organs of *Ginkgo* (Fig. 1e). It also suggests that ovulate organs of the *G. biloba* type could have originated by heterochrony (peramorphosis)<sup>10</sup> from the Jurassic *G. yimaensis* type<sup>7</sup>.

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Ion channels

Opposite thermosensor in fruitfly and mouse

Several members of the TRP (for transient receptor potential) family of ion channels act as physiological temperature sensors in mammals<sup>1–6</sup>, but it is not known whether the invertebrate TRP subfamilies that are found in the fruitfly *Drosophila* and the roundworm *Caenorhabditis elegans* can be directly activated by temperature. Here we show that the *Drosophila* orthologue of ANKTM1, which is a cold-activated ion channel in mammals, responds to a warming rather than a cooling stimulus. The thermosensing function of these channels is therefore evolutionarily conserved, and they show a surprising flexibility in their response to different temperature ranges.

In mammals, four TRPVs (members of the vanilloid subfamily of TRP channels) are activated at distinct heat thresholds (33–52 °C), whereas TRPM8 (of the melastatin subfamily) and ANKTM1 are activated at cold (17–25 °C) temperatures. However, the molecular mechanisms that underlie thermal preference in *Drosophila* are not well understood<sup>7,8</sup>. To identify potential invertebrate temperature-activated ion channels, we analysed the sequences of predicted TRP channels from *Drosophila* and *C. elegans*, focusing on orthologues of the mammalian thermosensitive TRPs. We operationally defined orthologues as reciprocal best BLAST hits on a comparison of the two genomes, which might also be indicative of a common ancestry. By this definition, mammalian TRPM8 and TRPV1–4 channels do not have invertebrate orthologues (results not shown).

Consistent with this, the *C. elegans* TRPV homologues, which have diverse sensory functions, are not directly activated by temperature<sup>9</sup>. Mammalian ANKTM1 belongs to a branch of TRP channels that includes four *Drosophila* and two *C. elegans*

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predicted proteins (Fig. 1a). One of these proteins, Painless, is required for response to noxious thermal and mechanical stimuli in *Drosophila* larvae, although it is unclear whether this channel is a direct sensor<sup>10</sup>.

Another of these relatives is a sequence orthologue of mouse ANKTM1 (mANKTM1; GenBank accession number AY231177); we term this *Drosophila* ion channel dANKTM1 (GenBank accession number CG5751). It is more similar to mANKTM1 (32% identical, 54% similar) than to Painless (GenBank accession number CG15860; 22% identical, 39% similar) and has 13 predicted amino-terminal ankyrins and six transmembrane domains. We amplified a full-length, 3.5-kilobase dANKTM1

complementary DNA from adult *Drosophila* RNA by using the polymerase chain reaction with reverse transcription (sequence deposited under GenBank accession number AY302598), and analysed its behaviour as an ion channel.

Cooling temperature steps did not elicit currents at –70 mV from oocytes expressing dANKTM1, but they elicited strong inward currents in those that expressed mANKTM1 and human ANKTM1 (Fig. 1b, and data not shown). However, transient currents were consistently activated in response to warming in oocytes expressing dANKTM1, with a threshold of 24–29 °C ( $n = 18$ ) (Fig. 1c). The heat-activated dANKTM1 current was outwardly rectifying and reversed near –30 mV (Fig. 1d), indicating that dANKTM1 is a relatively non-selective cation channel, as is mANKTM1 (ref. 6). In calcium-imaging experiments, dANKTM1-transfected Chinese hamster ovary cells also responded to a warming stimulus, with an activation threshold of about 27 °C (results not shown)<sup>6</sup>.

To our knowledge, this is the first characterization of an invertebrate temperature-activated ion channel. Given that *Drosophila* strongly prefers a temperature of 24 °C (ref. 7), the activation threshold of dANKTM1 at about 24–29 °C suggests that this ion channel might have a physiological role in heat sensing. Although mANKTM1 and dANKTM1 are sequence orthologues, they do not seem to be functional orthologues — the former

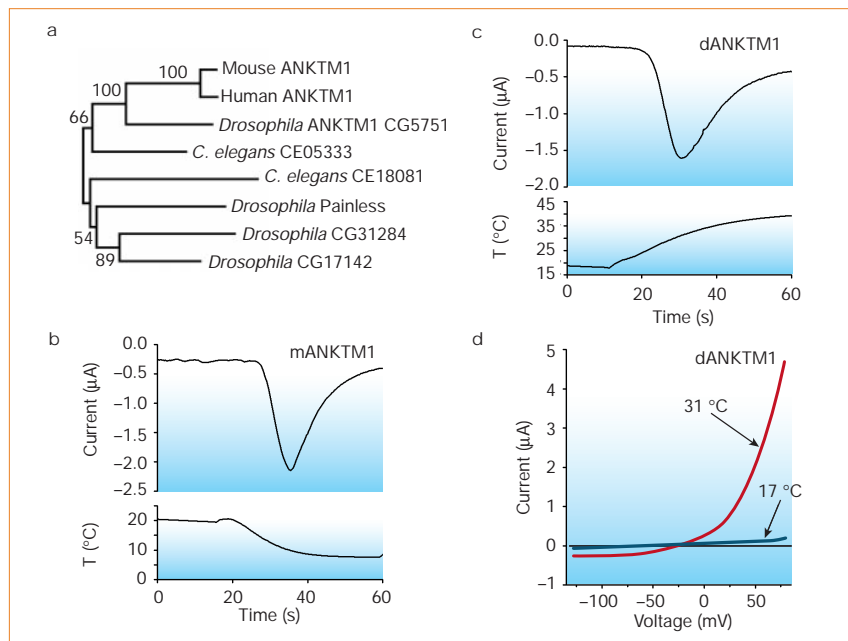


Figure 1 *Drosophila* orthologue of the mammalian ion channel ANKTM1 is activated by warm, not cold, temperatures. a, Phylogeny of human, mouse, *Drosophila* and *C. elegans* members of the ANKTM1 gene family. Branch lengths reflect the degree of divergence and the numbers indicate the percentage replication of branch points in bootstrap tests. NOMP, a TRP channel involved in *Drosophila* mechanosensation<sup>11</sup>, defines a separate subfamily and so is not included here. b–d, Temperature activation of mouse (b) and *Drosophila* (c, d) ANKTM1 in transfected *Xenopus* oocytes. Whole-cell currents were recorded at –70 mV in response to cooling and warming steps. Currents recorded in response to 2-s voltage ramps from –130 to 80 mV are shown in d. For mouse ANKTM1, warming from 20 to 40 °C resulted in almost no change in current, whereas *Drosophila* ANKTM1 produced no current increase in response to cooling from 20 to 7 °C (data not shown).

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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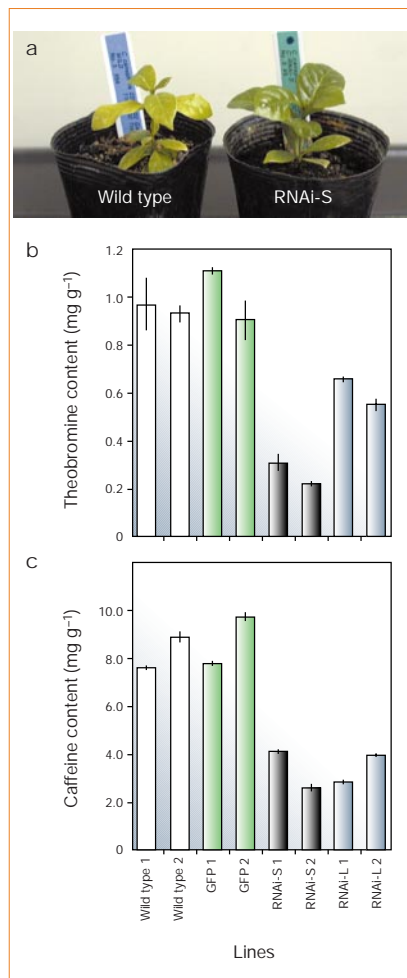
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RNA interference

## 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<sup>1</sup>. 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<sup>2–4</sup>. 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*<sup>5</sup>. 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 chromatography<sup>2</sup>. 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  $\beta$ -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<sup>7</sup>; 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  $\mu$ M 2-isopentenyladenine.

and necrotic; however, it was possible to regenerate hygromycin-resistant cells from these tissues. Seedlings were then cultured as described<sup>5</sup>.

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 poor<sup>6</sup> — problems that could potentially be overcome by the genetic engineering of coffee plants<sup>3,6</sup>. As *CaMXMT1* is expressed in young leaves, buds and immature fruits<sup>4</sup>, 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<sup>1,2</sup>. 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