

## Domatia mediate plant-arthropod mutualism

Leaf domatia are small hair-tufts or pockets on the lower surface of leaves, and are exceedingly common among perennial angiosperms, having been reported in 277 plant families and nearly 2,000 species<sup>1</sup>. Domatia seem to provide refuges for predatory arthropods. Here we show that cotton plants with experimentally added leaf domatia host larger populations of predatory arthropods and smaller populations of herbivorous mites than control plants. Total fruit production was increased by 30 per cent in plants with domatia—the first demonstration that plants benefit from their presence.

Leaf domatia are ubiquitously inhabited by primarily predatory (rather than herbivorous) arthropods (Fig. 1), leading to the conclusion that there is mutualism between arthropods and plants<sup>2,3</sup>. Previous studies have shown that blockage or addition of domatia results in positive associations between domatia and populations of predatory arthropods<sup>2,4,5</sup>, but to comprise true mutualism the fitness of the plant must increase as a result of a reduction in the populations of herbivorous arthropods or other plant parasites.

We simulated the presence of domatia on cotton plants (*Gossypium hirsutum*), close relatives of which have naturally occurring leaf domatia<sup>6</sup>, by attaching a small tuft of cotton fibres to the abaxial surface of leaves where the veins meet. Among the most severe pests in the San Joaquin Valley, California, are herbivorous spider mites<sup>7</sup>. They became established early in the season on the cotyledons of experimental plants grown near Chowchilla, but we found that there were consistently fewer spider mites on plants with domatia (Fig. 2a). Predatory arthropods were found in the simulated domatia, indicating that suppression of spider mite populations might be related to the presence of domatia.

The western flower thrips, *Frankliniella occidentalis*, an opportunistic predator of mites<sup>8,9</sup>, was more abundant on plants with artificial domatia (Fig. 2b), and thrips were usually found in domatia. Other predators of spider mites, the big-eyed bug (*Geocoris* spp.) and the minute pirate bug (*Orius tristicolor*), were five times more abundant on domatia-bearing plants (Fig. 2c), and their eggs and nymphs were found almost exclusively within domatia.

We found that the addition of leaf domatia early in the season improved the overall yield (number of bolls) of cotton by almost 30 per cent (Fig. 2d;  $t = -2.63$ , d.f. = 103.1,  $P = 0.01$ ). This increase in yield was found



**Figure 1** Adult big-eyed bug (*Geocoris* sp.), a voracious predator of herbivorous spider mites.

in a species known to tolerate high levels of herbivory without reductions in yield<sup>10</sup>.

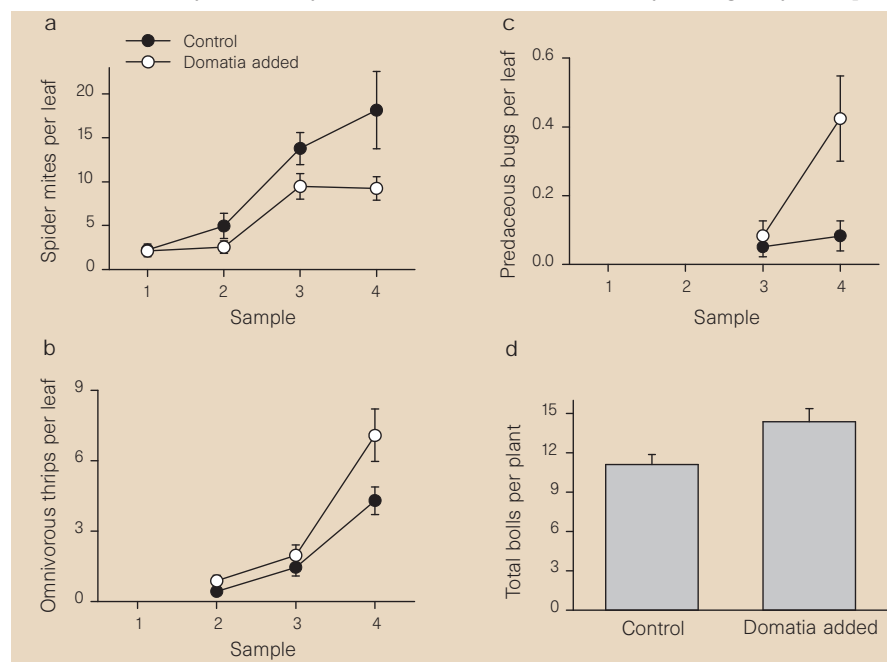
Later in the growing season we ran an identical experiment in Davis, California. The density of spider mites was below 'pest status' on six sampling dates (mean  $\pm$  s.e.m.,  $4.3 \pm 0.43$  mites per leaf). Populations of thrips were also low ( $1.5 \pm 0.11$  thrips per leaf) and were only affected by domatia in

the early part of the season. Because we initiated this experiment in the mid-season, arthropods that colonized early in the season were less affected by domatia than in the previous experiment.

Arthropods whose populations peak later in the season, such as big-eyed bugs and minute pirate bugs, showed strong responses to leaf domatia in Davis plots (big-eyed bugs,  $0.5 \pm 0.05$  per leaf with domatia and  $0.1 \pm 0.02$  without,  $F_{1,117} = 43.20$ ,  $P < 0.001$ ; minute pirate bugs,  $0.6 \pm 0.06$  per leaf with domatia,  $0.2 \pm 0.03$  without,  $F_{1,117} = 32.52$ ,  $P < 0.001$ , repeated measures ANOVA). The number of bolls produced was slightly, but not significantly, higher in plants that had leaf domatia added later in the season ( $t = -1.34$ , d.f. = 117,  $P = 0.18$ ).

There appears to be a mutually beneficial effect of leaf domatia on predatory arthropods and plants. Where spider mite densities are high, domatia favour predatory arthropods and increase plant reproduction. Such benefits were not species specific as leaf domatia benefit predatory mites<sup>2,4,5</sup>, thrips and bugs. Predation by thrips and bugs, mediated by leaf domatia, seems to reduce the population of herbivorous spider mites and increases plant reproduction.

Our results are promising for a natural biological control strategy as an alternative to the use of pesticides. Several crop species that are commonly damaged by mite pests



**Figure 2** We attached leaf domatia to most newly expanded leaves of 60 plants during the first two months of the growing season at an organic farm near Chowchilla. **a–c**, The number of arthropods inhabiting cotton leaves with and without domatia was sampled on four occasions: 1, 10 May; 2, 24 May; 3, 7 June; 4, 21 June, on these and 60 control plants (mean  $\pm$  s.e.m.). **a**, Herbivorous spider mites established early in the season and were reduced by leaf domatia ( $F_{1,114} = 7.85$ ,  $P = 0.006$ ). **b**, Thrips appeared in the second sample and were increased with domatia ( $F_{1,114} = 6.64$ ,  $P = 0.01$ ). **c**, Predaceous bugs appeared in the third sample in strongly increased numbers with domatia ( $F_{1,116} = 6.46$ ,  $P = 0.01$ ). As the season progressed the positive effect of domatia on predators and the negative effect of domatia on herbivores increased. **d**, Mean  $\pm$  s.e.m. number of cotton bolls produced by plants with and without domatia.

have wild progenitors or relatives with leaf domatia<sup>1,2,11,12</sup>. It may be possible to breed or engineer plants for better expression of leaf traits such as domatia that increase predator populations and efficacy. For plants that already have leaf domatia, these traits may mediate an important, yet poorly documented, mutualism.

**Anurag A. Agrawal**  
**Richard Karban**

Department of Entomology,  
Center for Population Biology,  
University of California at Davis,  
Davis, California 95616-8584, USA  
e-mail: aaagraval@ucdavis.edu

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## Salt enhances flavour by suppressing bitterness

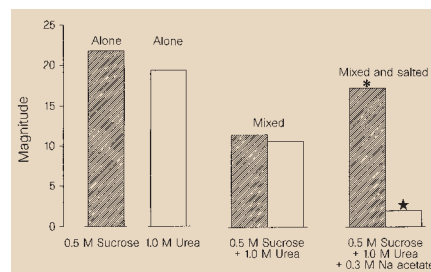
Salts are used as flavouring agents in the cuisines of many cultures<sup>1</sup>, the most commonly used being NaCl. They impart their own salty taste and enhance other flavours. The apparent ability to increase the intensity of other desirable flavours<sup>2,3</sup> is puzzling as virtually all published psychophysical studies show that NaCl either suppresses or has no effect on other flavours<sup>3,4</sup>. To reconcile this contradiction we have proposed<sup>5</sup> that salts selectively filter flavours, such that unpleasant tastes (such as bitterness) are more suppressed than palatable ones (such as sweetness) thereby increasing the salience and/or intensity of the latter. We now present evidence to support this idea.

We used mixtures of aqueous solutions of a bitter substance, urea, which is strongly suppressed by sodium-containing compounds<sup>6</sup>; a sweetener, sucrose; and a salt, sodium acetate, which has a fairly mild taste<sup>9</sup> and so is suitable for studying the flavour-modifying effects of sodium ions. Subjects (21 volunteers) were required to judge the extent of bitterness, sweetness and 'otherness' of all possible combinations of three concentrations of urea (0.0, 0.5, 1.0

M), four of sucrose (0.0, 0.1, 0.3, 0.5 M) and three of salt (0.0, 0.1, 0.3 M) using the method of magnitude estimation<sup>6</sup>. We evaluated the solutions, 12 per day (twice) over three consecutive days, in a counterbalanced order. Data were standardized and normalized<sup>6</sup>.

As predicted, there was a selective suppression of the taste components by sodium acetate (Fig. 1). The bitterness of urea was suppressed much more by the salt than was the sweetness of sucrose. Consequently, the sucrose–urea mixtures with added salt were relatively less bitter and more sweet than when sodium acetate was not added. Moreover, at the higher concentrations of sucrose (0.3, 0.5 M) and both concentrations of urea (0.5, 1.0 M), the absolute sweetness intensity was increased by adding either 0.1 or 0.3 M sodium acetate compared with when no sodium acetate was added (one example is shown in Fig. 1). This presumably occurred by releasing sweetness from suppression by the bitterness of urea<sup>7</sup>. As expected<sup>8</sup>, the addition of sodium acetate to sucrose in the absence of urea never had an enhancing effect on sweetness (data not shown).

Although this simple three-component aqueous system does not fully mimic the complex food systems in which salts are used, it illustrates at least one mechanism by which a salt increases both the relative and absolute intensity of palatable components of foods. This mechanism has not commonly been considered in taste mixture studies, which have tended to concentrate either on two-component mixtures, or on complex foods where interpretations are difficult.



**Figure 1** The normalized reported magnitude of the taste of various solution mixtures is shown. The intensity of urea and sucrose at the highest concentrations were roughly the same (left). Statistical analysis revealed that in mixtures, the highest concentrations of sucrose and urea (without sodium acetate), mutually and roughly equally suppressed their intensities (centre). When sodium acetate was added, also at the highest concentration, intensity of the bitterness greatly decreased, being suppressed by sodium ions<sup>6</sup>, whereas the sweetness intensity increased to levels that approximated the sweetness in pure deionized water (right). Relative to binary mixture levels, asterisk denotes increase ( $P < 0.0001$ ) and star denotes decrease ( $P < 0.0001$ ). These trends were evident for other concentrations tested. Detailed analyses available from the authors.

Our data show that, in addition to adding desired saltiness to food, salts potentiate flavour<sup>9</sup> through the selective suppression of bitterness (and perhaps other undesirable flavours), and the release from suppression of palatable flavours such as sweetness. The desire for NaCl and other salts in foods as diverse as (often bitter) vegetables, oily foods and meats may be due in part to their ability to suppress unpleasant flavours<sup>10</sup>. This may explain why it is difficult to make low-sodium foods acceptable.

Biophysical evidence<sup>11</sup> implies that it will be extremely difficult to develop a salty-tasting sodium-free substitute for NaCl. However, the multiple sensory functions of salts in foods should be considered, as the differential flavour-suppressing effect shown here might be duplicated by non-sodium substances, such as bitterness blockers.

**P. A. S. Breslin**

**G. K. Beauchamp**

Monell Chemical Senses Center,  
3500 Market Street, Philadelphia,  
Pennsylvania 19104-3308, USA  
e-mail: beaucham@pobox.upenn.edu

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## Structures of mollusc shell framework proteins

Mollusc shells consist of the nacreous mother-of-pearl layer and the prismatic layer. Both layers are microlaminate composites of CaCO<sub>3</sub> crystals (aragonite in the nacre and calcite in the prismatic layer) and biopolymers. The main biopolymers are structural proteins, insoluble in water and methanoic acid, which determine the framework of each shell layer<sup>1–4</sup> and bind soluble polyanionic proteins<sup>2–4</sup> which determine the type of CaCO<sub>3</sub> crystal that grows<sup>5,6</sup>. Here we report the sequences and structures of the framework proteins for the nacreous and prismatic layers of the pearl oyster, *Pinctada fucata*.

We ground and decalcified the nacreous layer of the mollusc shell using 50 per cent methanoic acid, and cleaved the protein by treatment with cyanogen bromide (CNBr),