Specialized bird perch aids cross-pollination

A plant scores by providing an access point for visiting sunbirds to feed on its nectar.

Birds may hover over or perch on flowers when feeding on nectar[1], and this assists cross-pollination if they then visit other plants. Here we investigate the curious sterile inflorescence axis of the South African Cape endemic 'rat's tail' plant (*Babiana ringens*, Iridaceae), whose function — unlike in other bird-pollinated plants — is exclusively to provide a perch for foraging birds. We find that this structure promotes the plant's mating success by causing the malachite sunbird (*Nectarinia famosa*), its main pollinator, to adopt a position ideal for the cross-pollination of its unusual ground-level flowers.

The Cape naturalist Rudolf Marloth was the first to propose that the rat's tail of *B. ringens* (Fig. 1a) could function as a perch to facilitate cross-pollination by visiting sunbirds (cited in ref. 2). We investigated this proposal in two populations of *B. ringens* near Mamre, Western Cape Province, from August to October in 2003 and 2004. Our observations indicated that the plants' only pollinator was the malachite sunbird, the largest of the sunbirds that visit species of Iridaceae in the Cape[3].

We observed birds alighting on the sterile axis of the plant and rotating upside down before probing the flowers for nectar (Fig. 1b). Because the floral tube in *B. ringens* curves upwards, rather than downwards as in most bird-pollinated species, a sunbird accesses nectar by inserting its curved beak from above. The plant's sexual organs contact the bird's breast, enabling pollen to be transferred between plants.

To test the function of the bird perch, we compared fitness components in unmanipulated plants with those from which we had removed the sterile axis just before flowering. Perch removal did not alter the floral display or the intrinsic ability of plants to set seed. We found no significant difference in seed set between plants with and without perches following supplemental cross-pollination (repeated measures analysis of variance, specific contrast: $t = -0.87$, $P = 0.39$). Perch removal therefore does not interfere with seed provisioning, and the perch is not a significant source of photosynthetic carbon for seed development. (For methods, see supplementary information.)

Although perch removal did not preclude visitation by sunbirds (Fig. 1c), they preferred plants with intact perches. Of 93 visits recorded after perches were removed from half the plants, 59 were made to plants with perches and 34 were made to plants without perches ($G = 6.80$, $P = 0.009$). Female and male birds showed different preferences for plants with and without perches: only males had a strong preference for plants with perches. Males visited significantly more plants (total visits by males: perch present, 24; perch removed, 11; $G = 4.95$, $P = 0.026$; total visits by females: perch present, 35; perch removed, 23; $G = 2.50$, $P = 0.113$; NS). Males spent longer feeding from flowers (time spent foraging by males: perch present, 53.95 s (s.e. 9.42); perch removed, 12.4 s (s.e. 4.72) (paired *t*-test, $t = 3.92$, d.f. = 19, $P = 0.0005$); time spent foraging by females: perch present, 23.88 s (s.e. 4.87); perch removed, 13.34 s (s.e. 4.18) ($t = 1.52$, d.f. = 31, $P = 0.07$)).

This sex difference in preference may be associated with the much longer tail feathers of males, which could interfere with ground landings and suffer damage. Males may also be more reluctant to land because of the predation risk associated with their bright colours, or because perching in prominent places may be linked to territoriality.

Perch removal reduced female fertility: plants without perches produced 47% fewer seeds on average than unmanipulated plants (Fig. 2a). Plants without perches set considerably more seed than bagged inflorescences, however, so sunbird-mediated self-pollination seems likely to have occurred in these plants. We confirmed this by analysing mating patterns. Perch removal increased self-fertilization by an average of 35% (Fig. 2b), indicating that perch specialization reduces the genetic costs of self-pollination[4].

Fitness advantages to mating and fertility arise because the perch manipulates the position of foraging sunbirds for better pollen dispersal. The specialized bird perch of *B. ringens* is a novel example of a structural adaptation that promotes cross-pollination in angiosperms.

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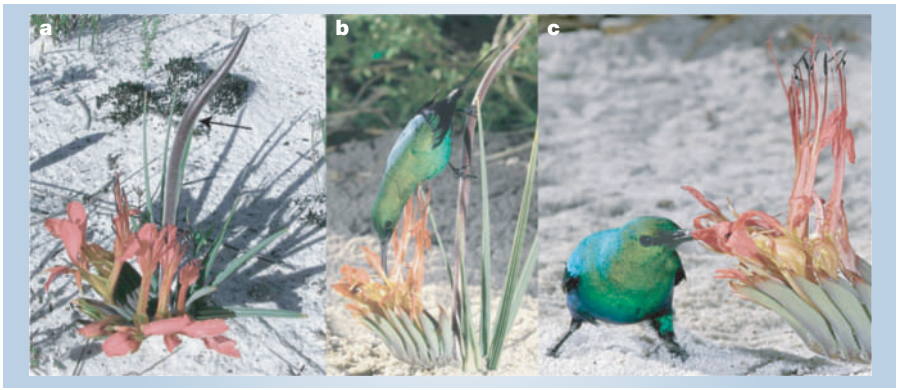

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Figure 1 Features of the South African plant *Babiana ringens*. **a,** The specialized bird perch (arrow) and ground-based flowers of *B. ringens*. **b, c,** Male sunbirds (*Nectarinia famosa*) visiting the plant adopt different positions to feed according to whether the perch is **b**, present, or **c**, absent.

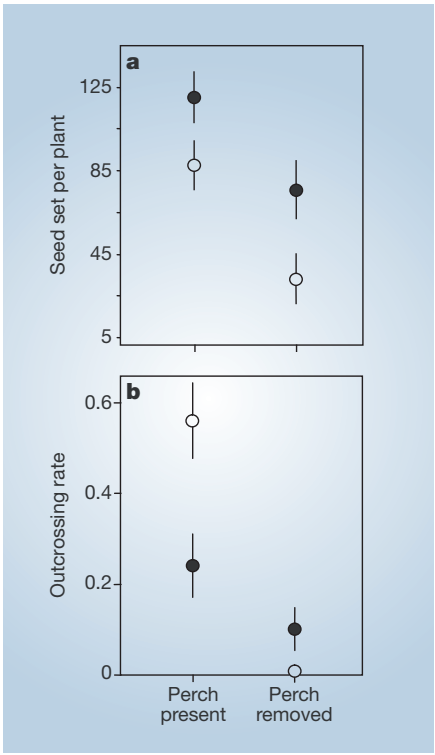

Figure 2 The specialized bird perch of *Babiana ringens* increases mating success. A fraction of each of two plant populations (A and B, represented by filled and open circles) located about 5 km apart had the bird perch removed (in 11 of 25 and 19 of 38 plants, respectively) and the seed set and outcrossing rate were compared with those of unmanipulated plants. **a,** Perch removal decreases seed production per plant (treatment: $F_{1,52} = 15.05$, $P < 0.001$; no significant treatment × population interaction: $F_{1,52} = 0.16$, $P = 0.692$). **b,** Perch removal increases the mean (±95% confidence interval) frequency of self-fertilization (population A, $P = 0.019$; population B, $P < 0.001$). Genotype scoring at five allozyme loci was used to determine the outcrossing rates, with the following sample sizes for each population: families from A, 25; families from B, 30; seeds per family from A, 8.4; seeds per family from B, 8.5. A family is the collection of seeds from one maternal parent. For details, see supplementary information.

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Biochemistry

A cadmium enzyme from a marine diatom

The ocean biota contains a vast reservoir of genomic diversity[1]. Here we present the sequence and preliminary characterization of a protein that is a cadmium-containing carbonic anhydrase from the marine diatom *Thalassiosira weissflogii*. The existence of a cadmium enzyme in marine phytoplankton may indicate that there is a unique selection pressure for metalloenzymes in the marine environment[2], and our discovery provides a long-awaited explanation for the nutrient-like behaviour of cadmium in the oceans[3].

In marine diatoms, the metals cadmium, cobalt and zinc can functionally substitute for one another to maintain optimal growth rates. This effect is at least partly due to metal replacement in the metal-binding site of the enzyme carbonic anhydrase[4], which is involved in the acquisition of inorganic carbon for photosynthesis. In addition to a zinc carbonic anhydrase that can substitute cobalt in its active site *in vivo*[5], *T. weissflogii* has a putative cadmium carbonic anhydrase that is also involved in acquiring inorganic carbon[6].

We purified a protein, CDCA1, from this organism that has carbonic anhydrase activity and contains cadmium (Fig. 1a, b; Genbank accession number AY772014). Determination of its sequence was complicated by the presence of a triple repeat (see supplementary information): the three amino-acid sequences are virtually identical (about 85% identity), but there is more variation in their encoding DNA (about 78% identity). Contrary to an earlier estimate[6], we have determined the relative molecular mass of CDCA1 as about 69K (for methods, see supplementary information). CDCA1 has probably not been sequenced before, as there are no hits in the NCBI database. It is significantly different from any of the known major classes of carbonic anhydrases[7,8], and therefore represents the first member of a new class of carbonic anhydrases, the ζ class.

The genome of *T. pseudonana* contains a single sequence that is highly homologous to the three repeats in *T. weissflogii*, corresponding to a protein of 25.5K (see supplementary information). The presence of expressed-tag sequences shows that this putative cadmium-containing carbonic anhydrase is expressed, indicating that only a single repeat of CDCA1 may be necessary for activity. The amino-terminal sequence of the *T. pseudonana* enzyme (not available for CDCA1) has only 15 amino acids preceding the homologous sequence from *T. weissflogii*.

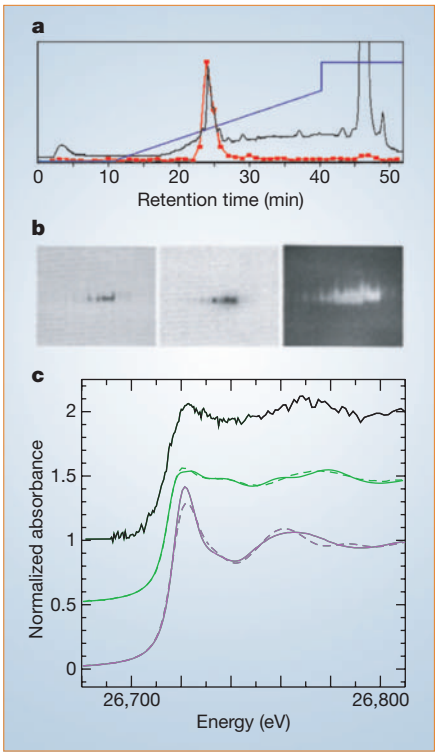

Figure 1 Characterization of the carbonic anhydrase CDCA1 from a marine diatom. **a,** High-pressure liquid chromatography (HPLC) of the CDCA1 enzyme, showing co-elution of purified CDCA1 (black trace, absorbance at 280 nm) and of ^{109}Cd-radiolabelled enzyme (red, c.p.m., 330 c.p.m at peak) at 24 min. Blue line, NaCl gradient. **b,** Non-denaturing gel electrophoresis of HPLC fractions containing the main peak of protein and radiolabel shows an intense Coomassie-blue-stained protein band (left) that co-migrates with the ^{109}Cd radiolabel (centre) and carbonic anhydrase activity (right). **c**, Cadmium K-edge X-ray absorption spectra from purified CDCA1 (top) compared with two tetrahedral, thiolate-coordinated species, $[Cd(SPh)_4](Me_4N)_2$ (green solid line), cadmium phytochelatin (green broken line), and with two octahedral species $[Cd(H_2O)_6]^{2+}$ (purple solid line) and $[Cd(imidazole)_6](NO_3)_2$ (purple broken line). The similarity of the CDCA1 spectrum to that of the tetrahedral species suggests that the metal site has tetrahedral symmetry and involves cysteinyl ligands to the metal. (For methods, see supplementary information.)

X-ray absorption near-edge spectroscopy of the purified protein confirms the presence of the cadmium-binding site (Fig. 1c). Comparison with standards[9] indicates that the site probably has a roughly tetrahedral geometry and that the cadmium ion is bound by two or more thiolates — as in the zinc-containing β class of carbonic anhydrases in higher plants, which have two cysteines and a histidine at the metal-binding site[10]. The spectra are also consistent with an active site containing an activated water molecule, as in other carbonic anhydrases.

Gene-expression analysis shows that there is an increase in the abundance of *cdca1* transcripts within 1 hour of increasing the concentration of cadmium or of decreasing the partial pressure of carbon dioxide in the medium (results not shown). Expression of CDCA1 may therefore be controlled in part by the availability of cadmium and carbon dioxide in sea water.

High-throughput sequencing of a seawater sample has revealed that the marine environment may contain unique genes[1]. We have identified and partially characterized one such gene — for a carbonic anhydrase from a marine diatom that, to our knowledge, is the first native enzyme so far discovered to contain cadmium. Because of the extraordinarily low concentrations of many essential trace metals in sea water, it is likely that there are other metalloenzymes in marine organisms that use unusual metals for activity and contribute to trace-metal geochemical cycling in the oceans.

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Corrigendum

Animal behaviour: Elephants are capable of vocal learning

J. H. Poole, P. L. Tyack, A. S. Stoeger-Horwath, S. Watwood *Nature* **434,** 455–456 doi:10.1038/434455a (2005)

The African elephant Calimero spent 18 years with two Asian elephants at Bioparco in Rome, Italy, not the Basel Zoo, Switzerland. He was later transferred to Basel, where the recordings described in this Brief Communication were made.