Caffeine in Floral Nectar Enhances a Pollinator's Memory of Reward

G. A. Wright,[1]* D. D. Baker,[2] M. J. Palmer,[3] D. Stabler,[1,2] J. A. Mustard,[4] E. F. Power,[1,2] A. M. Borland,[2] P. C. Stevenson[5,6]

Plant defense compounds occur in floral nectar, but their ecological role is not well understood. We provide evidence that plant compounds pharmacologically alter pollinator behavior by enhancing their memory of reward. Honeybees rewarded with caffeine, which occurs naturally in nectar of *Coffea* and *Citrus* species, were three times as likely to remember a learned floral scent as were honeybees rewarded with sucrose alone. Caffeine potentiated responses of mushroom body neurons involved in olfactory learning and memory by acting as an adenosine receptor antagonist. Caffeine concentrations in nectar did not exceed the bees' bitter taste threshold, implying that pollinators impose selection for nectar that is pharmacologically active but not repellent. By using a drug to enhance memories of reward, plants secure pollinator fidelity and improve reproductive success.

Many drugs commonly consumed by humans are produced by plants as a form of toxic defense against herbivores (*1*, *2*). Although plant-derived drugs like caffeine or nicotine are lethal in high doses (*3*–*5*), at low doses they have pharmacological effects on mammalian behavior. For example, low doses of caffeine are mildly rewarding and enhance cognitive performance and memory retention (*6*). Caffeine has been detected in low doses in the floral nectar and pollen of *Citrus* (*7*), but whether it has an ecological function is unknown.

Two caffeine-producing plant genera, *Citrus* and *Coffea*, have large floral displays with strong scents and produce more fruits and seeds when pollinated by bees (*8*, *9*). If caffeine confers a selective advantage when these plants interact with pollinators, we might expect it to be commonly encountered in nectar. We measured caffeine in the nectar of three species of *Coffea* (*C. canephora*, *C. arabica*, and *C. liberica*) and four species of *Citrus* (*C. paradisi*, *C. maxima*, *C. sinensis*, and *C. reticulata*) using liquid chromatography–mass spectrometry (*10*) (fig. S1A). When caffeine was present, its concentration ranged from 0.003 to 0.253 mM. The median caffeine concentration in both genera was not significantly different (Fig. 1A, Mann-Whitney, $Z = -1.09$, $P = 0.272$). Caffeine was more common in the nectar of *C. canephora* than in that of *C. arabica* or *C. liberica* (*Coffea*: logistic regression $\chi_2^2 = 11.1$, $P = 0.004$); it was always present in *Citrus* nectar. The mean total nectar sugar concentration ranged from 0.338 to 0.843 M (Fig. 1B; see fig S1B for individual sugars). Caffeine concentration in nectar did not correlate with total sugar concentration (Pearson's $r = 0.063$, $P = 0.596$).

We hypothesized that caffeine could affect the learning and memory of foraging pollinators. To test this, we trained individual honeybees to associate floral scent with 0.7 M sucrose and seven different concentrations of caffeine and tested their olfactory memory. Using a method for classical conditioning of feeding responses (proboscis extension reflex) (*11*), we trained bees for six trials with 30 s between each pairing of odor with reward. This intertrial interval approximated the rate of floral visitation exhibited by honeybees foraging from multiple flowers on a single *Citrus* tree (see methods). The presence of low doses of caffeine in reward had a weak effect on the rate of learning (Fig. 2A), but it had a profound effect on long-term memory. When rewarded with solutions containing nectar levels of caffeine, three times as many bees remembered the conditioned scent 24 hours later and responded as if it predicted reward (Fig. 2B, logistic regression, $\chi_7^2 = 41.9$, $P < 0.001$). Twice as many bees remembered it 72 hours later (Fig. 2C). This improvement in memory performance was not due to a general increase in olfactory sensitivity resulting from caffeine consumption (fig. S2A). Indeed, the effect of caffeine on long-term

[1]Centre for Behaviour and Evolution, Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE1 7RU, UK. [2]School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK. [3]Division of Neuroscience, Medical Research Institute, Ninewells Medical School, University of Dundee, Dundee DD1 9SY, UK. [4]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA. [5]Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3AB, UK. [6]Natural Resources Institute, University of Greenwich, Chatham, Kent ME4 4TB, UK.

*To whom correspondence should be addressed. E-mail: jeri.wright@ncl.ac.uk

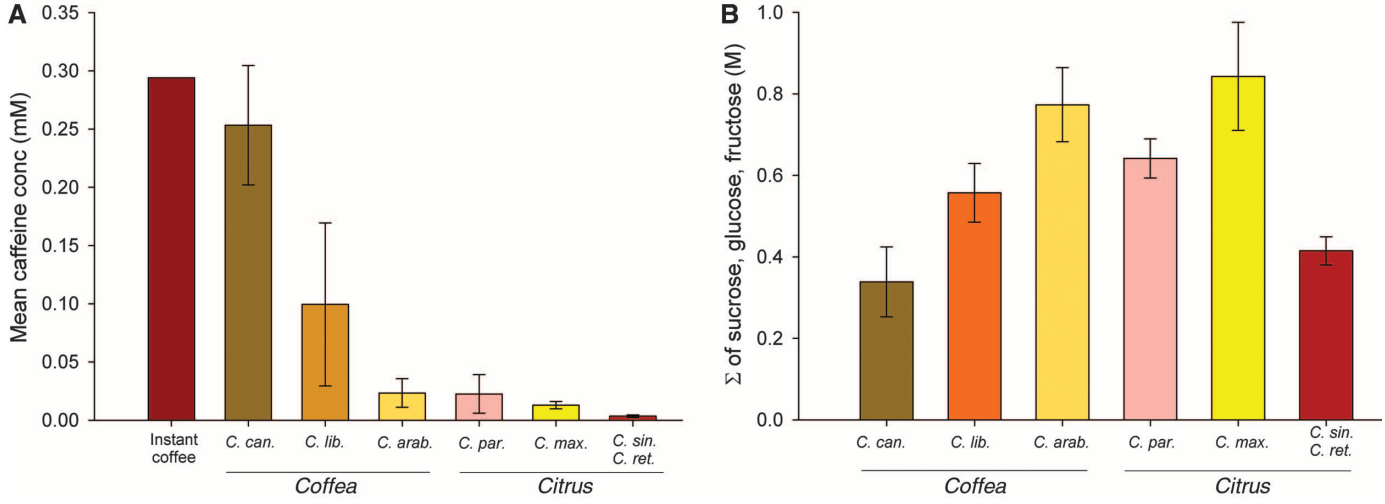

Fig. 1. (**A**) Caffeine concentration in *Coffea* and *Citrus* spp. and a cup of instant coffee. Caffeine concentration depended on species within each genus (*Coffea*: Kruskal-Wallis, $\chi_2^2 = 28.1$, $P < 0.001$; *Citrus*: Kruskal-Wallis, $\chi_2^2 = 6.98$, $P = 0.030$); *C. canephora* had the highest mean concentration of all species sampled. (**B**) The sum of the concentration of sucrose, glucose, and fructose (total nectar sugars) depended on species (one-way analysis of variance: $F_{5,\ 161} = 4.64$, $P < 0.001$) and was greatest in *Citrus maxima* and hybrids (citron, lemons, clementines). [*C. can.*, *Coffea canephora*, $N = 34$; *C. lib.*, *Coffea liberica*, $N = 31$; *C. arab.*, *Coffea arabica*, $N = 27$; *C. par.*, *Citrus paradisi* and hybrids, $N_{cp} = 17$; *C. max.*, *Citrus maxima* and hybrids, $N = 5$; *C. sin.* and *C. ret.*, *Citrus sinensis* and *Citrus reticulata*, $N_{CS} = 7$, $N_{CR} = 5$ (data for these two species were pooled).] Mean responses ± SE.

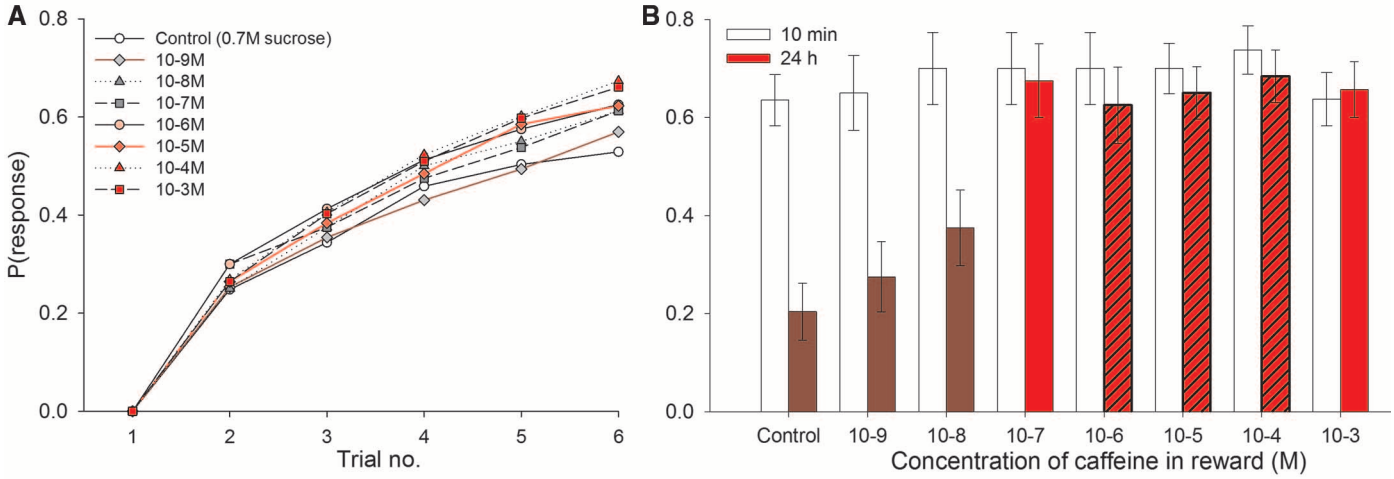

Fig. 2. (**A**) The rate of learning of bees conditioned with an odor stimulus paired with a 0.7 M sucrose reward containing caffeine. The rate of learning was slightly greater for the bees fed caffeine in reward during conditioning (logistic regression, $\chi_1^2 = 4.85$, $P = 0.028$). $N \geq 79$ for all groups. (**B**) Memory recall test for odors at 10 min (white bars) or 24 hours (red bars) after bees had been trained as in (A). Bright red bars indicate that the response at 24 hours was significantly different from the control (0.7 M sucrose) (least-squares contrasts: $P < 0.05$); dark red bars were not significantly different. Nectar levels of caffeine are indicated by hatching. $N > 79$ for each group. (**C**) Bees fed 0.1 mM caffeine in sucrose (orange bars) were more likely to remember the conditioned odor than sucrose alone (white bars) (logistic regression, $\chi_1^2 = 9.04$, $P < 0.003$) at 24 hours and 72 hours after conditioning. $N = 40$ per group.

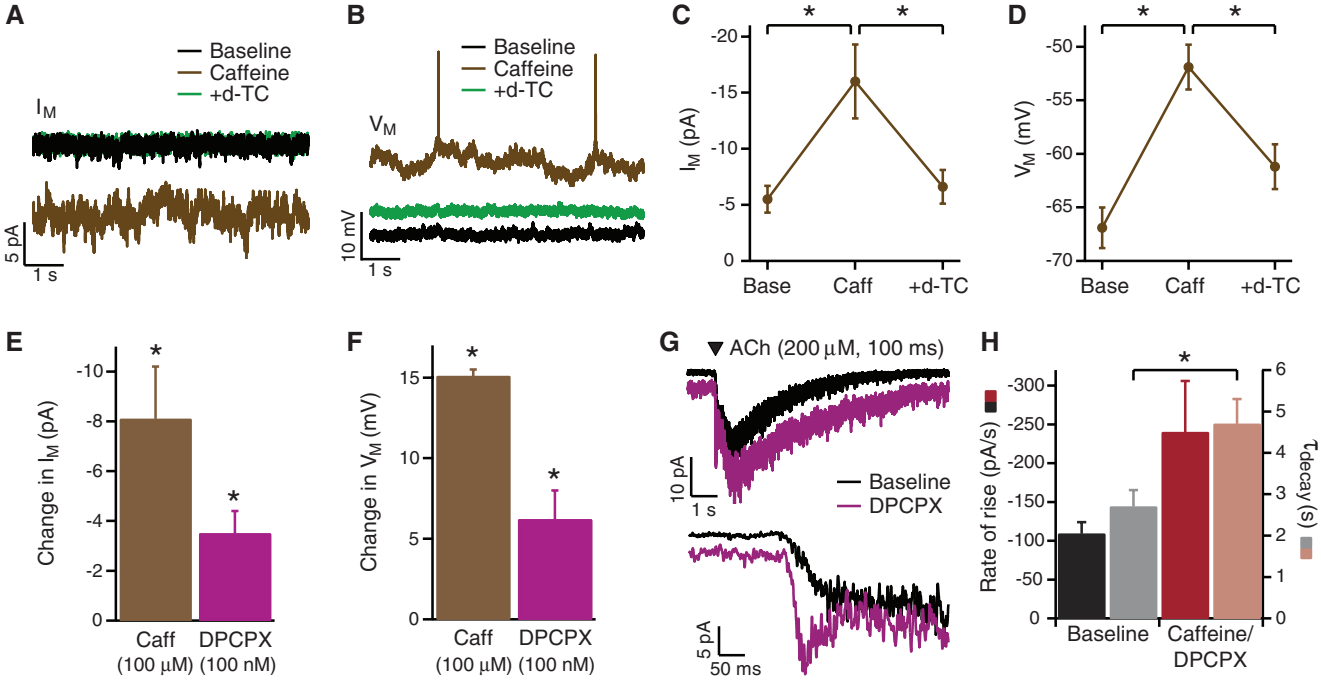

Fig. 3. The effect of caffeine on Kenyon cells. (**A** and **B**) Example traces from a KC in intact honeybee brain recorded under voltage-clamp [(A), $V_H = -73$ mV) and current-clamp [(B), at resting V_M), showing the increase in I_M and depolarization evoked by bath application of caffeine (100 µM) and subsequent reversal by the nAChR antagonist d-TC (500 µM). (**C** and **D**) Mean data showing the reversal by d-TC (500 µM) of the effect of caffeine (Caff; 100 µM) on I_M [(C); $N = 6$, $t_5 = 4.03$, $P = 0.010$; $t_5 = 4.07$, $P = 0.010$] and V_M [(D); $N = 6$, $t_5 = 34.1$, $P < 0.001$; $t_5 = 12.0$, $P < 0.001$]. (**E** and **F**) Comparison of the mean effects of caffeine and DPCPX on I_M [(E); Caff: $N = 10$, $t_9 = 3.84$, $P = 0.004$; DPCPX: $N = 6$, $t_5 = 4.04$, $P = 0.010$] and V_M [(F) Caff: $N = 6$, $t_5 = 34.1$, $P < 0.001$; DPCPX: $N = 6$, $t_5 = 3.39$, $P = 0.019$]. (**G** and **H**) Example traces [(G); rising phase shown on an expanded time scale below] and mean data [(H); rate of rise: $N = 6$, $t_5 = 2.20$, $P = 0.079$; τ_{decay}: $N = 9$, $t_8 = 3.54$, $P = 0.008$] showing that DPCPX (100 nM) and caffeine (100 µM) slowed the decay and, in six of nine KCs, potentiated the fast component of the response evoked by exogenous ACh. (Student's paired *t* test used in all comparisons.) Mean responses ± SE.

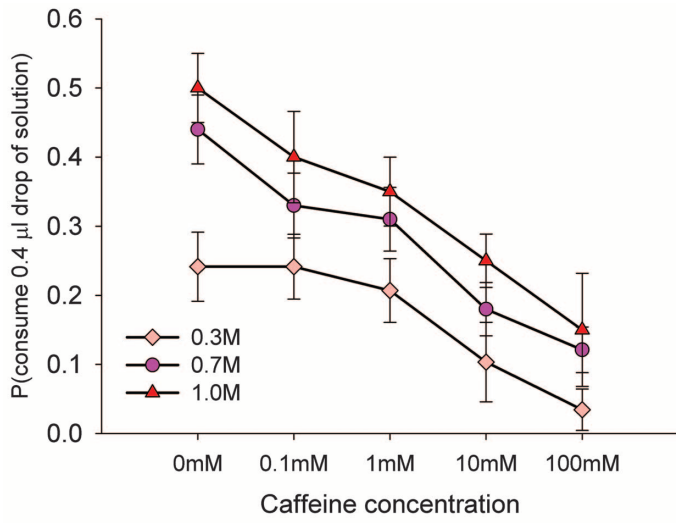

Fig. 4. Bees are more likely to reject sucrose solutions containing caffeine at concentrations greater than 1 mM (logistic regression, χ_4^2 = 23.4, $P < 0.001$; for 0.7 and 1.0 M, 1 mM caffeine versus sucrose post hoc, $P < 0.05$; for 0.3 M, 100 mM caffeine versus sucrose post hoc, $P < 0.05$). Bees were less likely to drink 0.3 M sucrose (pale pink diamonds) than 0.7 M (pink circles) or 1.0 M solutions (red triangles) (logistic regression, $\chi_2^2 = 8.69$, $P = 0.013$). Mean responses ± SE. $N_{0.3M} = 29$, $N_{0.7M} = 100$, $N_{1.0M} = 20$.

olfactory memory in bees was greater than that produced by high concentrations of sucrose when the same experimental methods were used (e.g., 2.0 M, fig. S2B).

Caffeine's influence on cognition in mammals is in part mediated by its action as an adenosine receptor antagonist (*6*). In the hippocampal CA2 region, inhibition of adenosine receptors by caffeine induces long-term potentiation (*12*), a key mechanism of memory formation (*13*). The Kenyon cells (KCs) in mushroom bodies of the insect brain are similar in function to hippocampal neurons: They integrate sensory input during associative learning, exhibit long-term potentiation, and are involved in memory formation (*14*–*16*). To determine whether nectar-caffeine doses affect mushroom body function, we made whole-KC recordings in the intact honeybee brain. Caffeine (100 µM) evoked a small increase in the holding current (I_M) and depolarized KC membrane potential (V_M) toward the action potential firing threshold, by increasing nicotinic acetylcholine receptor (nAChR) activation (Fig. 3, A to D). To determine whether the observed effects of caffeine were due to interactions with adenosine receptors, we applied the adenosine receptor antagonist DPCPX and observed that it similarly increased I_M and depolarized V_M, but to a lesser extent (Fig. 3, E and F). Both caffeine and DPCPX affected KC response kinetics evoked by brief, local application of ACh, increasing the activation rate and slowing the decay (Fig. 3, G and H). Our data show that caffeine modulates cholinergic input via a postsynaptic action, but could act via presynaptic adenosine receptors to potentiate ACh release (*17*). The resulting increase in KC excitability should lead to an increased probability of action potential firing in response to sensory stimulation (*18*), thereby facilitating the induction of associative synaptic plasticity in KCs (*19*). The enhanced activation of KCs may also facilitate plasticity at synapses with mushroom body extrinsic neurons (*20*), which exhibit spike-timing–dependent plasticity (*21*). In this way, a "memory trace" could be formed for the odor associated with reward during and after conditioning (*22*, *23*).

Caffeine is bitter tasting to mammals and is both toxic (*24*) and repellent to honeybees at high concentrations (*25*, *26*). If bees can detect caffeine, they might learn to avoid flowers offering nectar containing it (*27*). We found that honeybees were deterred from drinking sucrose solutions containing caffeine at concentrations greater than 1 mM (Fig. 4); they also have neurons that detect caffeine in sensilla on their mouthparts (fig. S3). However, nectar concentrations did not exceed 0.3 mM (0.058 mg/ml), even though levels of caffeine in vegetative and seed tissues of *Coffea* have been reported to be as great as 24 mg/ml (*28*). This implies that pollinators drive selection toward concentrations of caffeine that are not repellent but still pharmacologically active.

Our data show that plant-produced alkaloids like caffeine have a role in addition to defense: They can pharmacologically manipulate a pollinator's behavior. When bees and other pollinators learn to associate floral scent with food while foraging (*29*), they are more likely to visit flowers bearing the same scent signals. Such behavior increases their foraging efficiency (*30*) while concomitantly leading to more effective pollination (*31*, *32*). Our experiments suggest that by affecting a pollinator's memory, plants reap the reproductive benefits arising from enhanced pollinator fidelity.

References and Notes

1. R. J. Sullivan, E. H. Hagen, P. Hammerstein, *Proc. Biol. Sci.* **275**, 1231 (2008).
2. J. B. Harborne, *Introduction to Ecological Biochemistry* (Academic Press, London, ed. 4, 1993).
3. R. G. Hollingsworth, J. W. Armstrong, E. Campbell, *Nature* **417**, 915 (2002).
4. M. Okamoto, T. Kita, H. Okuda, T. Tanaka, T. Nakashima, *Pharmacol. Toxicol.* **75**, 1 (1994).
5. T. Rudolph, K. Knudsen, *Acta Anaesthesiol. Scand.* **54**, 521 (2010).
6. A. Nehlig, *Neurosci. Biobehav. Rev.* **23**, 563 (1999).
7. J. A. Kretschmar, T. W. Baumann, *Phytochemistry* **52**, 19 (1999).
8. T. H. Ricketts, G. C. Daily, P. R. Ehrlich, C. D. Michener, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12579 (2004).
9. D. W. Roubik, *Nature* **417**, 708 (2002).
10. Materials and methods are available in the supplementary materials in *Science* Online.
11. M. E. Bitterman, R. Menzel, A. Fietz, S. Schafer, *J. Comp. Psychol.* **97** 107–119 (1983).
12. S. B. Simons, D. A. Caruana, M. L. Zhao, S. M. Dudek, *Nat. Neurosci.* **15**, 23 (2012).
13. R. G. M. Morris, E. Anderson, G. S. Lynch, M. Baudry, *Nature* **319**, 774 (1986).
14. M. Heisenberg, *Nat. Rev. Neurosci.* **4**, 266 (2003).
15. S. Oleskevich, J. D. Clements, M. V. Srinivasan, *J. Neurophysiol.* **78**, 528 (1997).
16. N. J. Strausfeld, L. Hansen, Y. S. Li, R. S. Gomez, K. Ito, *Learn. Mem.* **5**, 11 (1998).
17. B. Sperlágh, E. S. Vizi, *Curr. Top. Med. Chem.* **11**, 1034 (2011).
18. J. Perez-Orive *et al.*, *Science* **297**, 359 (2002).
19. P. Szyszka, A. Galkin, R. Menzel, *Front. Syst. Neurosci.* **2**, 3 (2008).
20. R. Menzel, G. Manz, *J. Exp. Biol.* **208**, 4317 (2005).
21. S. Cassenaer, G. Laurent, *Nature* **482**, 47 (2012).
22. D. S. Galili, A. Lüdke, C. G. Galizia, P. Szyszka, H. Tanimoto, *J. Neurosci.* **31**, 7240 (2011).
23. P. Szyszka *et al.*, *J. Neurosci.* **31**, 7229 (2011).
24. A. Detzel, M. Wink, *Chemoecology* **4**, 8 (1993).
25. J. A. Mustard, L. Dews, A. Brugato, K. Dey, G. A. Wright, *Behav. Brain Res.* **232**, 217 (2012).
26. N. Singaravelan, G. Nee'man, M. Inbar, I. Izhaki, *J. Chem. Ecol.* **31**, 2791 (2005).
27. G. A. Wright *et al.*, *Curr. Biol.* **20**, 2234 (2010).
28. H. Ashihara, A. Crozier, *Trends Plant Sci.* **6**, 407 (2001).
29. G. A. Wright, F. P. Schiestl, *Funct. Ecol.* **23**, 841 (2009).
30. L. Chittka, A. Gumbert, J. Kunze, *Behav. Ecol.* **8**, 239 (1997).
31. R. Hopkins, M. D. Rausher, *Science* **335**, 1090 (2012).
32. W. E. Kunin, *Ecology* **74**, 2145 (1993).

Acknowledgments: We thank staff at Centro Agronomico Tropicale de Investigacion et Ensenanza in Costa Rica for access to the *Coffea* collections and at Technological Education Institute of Crete for access to *Citrus* orchards; M. Thomson, K. Smith, F. Marion-Poll, and A. Popescu for help with the data collection; M. Thompson for beekeeping; and J. Harvey and C. Connolly for project support. This work was funded in part by the Linnean Society of London and by a UK government Insect Pollinators Initiative grant BB/I000968/1 to G.A.W. and a separate grant to C. Connolly (BB/1000313/1). Methods and additional data are available in the online supplementary materials. All data are archived on the Natural Environment Research Council Environmental Information Data Centre.

Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6124/1202/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S3
References (*33*–*36*)

14 August 2012; accepted 21 December 2012
10.1126/science.1228806

Caffeine in Floral Nectar Enhances a Pollinator's Memory of Reward

G. A. Wright *et al.*

Science **339**, 1202 (2013);
DOI: 10.1126/science.1228806