

- Ross, J. F. *Science* **146**, 1170–1172 (1964).
 5. Harkness, D. D. *Nature* **240**, 302–303 (1972).
 6. Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H. & Frisén, J. *Cell* **122**, 133–143 (2005).
 7. Nolla, C. M. *J. Dent. Child.* **27**, 254–266 (1960).
 8. Bolanos, M. V., Manrique, M. C., Bolanos, M. J. & Briones, M. T. *Foren. Sci. Int.* **110**, 97–106 (2000).
 9. Ritz-Timme, S. et al. *Int. J. Legal Med.* **113**, 129–136 (2000).

10. Otlet, R. L., Walker, A. J., Fulker, M. J. & Collins, C. *J. Envir. Radioact.* **34**, 91–101 (1997).

Supplementary information accompanies this communication on Nature's website.

Competing financial interests: declared (see online version of the communication).

doi:10.1038/437333a

BOTANY

Floral fluorescence effect

The way flowers appear to insects is crucial for pollination^{1–3}. Here we describe an internal light-filtering effect in the flowers of *Mirabilis jalapa*, in which the visible fluorescence emitted by one pigment, a yellow betaxanthin, is absorbed by another, a violet betacyanin, to create a contrasting fluorescent pattern on the flower's petals. This finding opens up new possibilities for pollinator perception as fluorescence has not previously been considered as a potential signal in flowers.

We investigated the spectra and distribution of the pigments in the multicoloured, strikingly patterned flowers of *M. jalapa* (Nyctaginaceae), which open only in the late afternoon. This and related plants, such as *Bougainvillea*, *Celosia*, *Gomphrena* and *Portulaca*, contain pigments known as betalains. These comprise the yellow, fluorescent betaxanthins⁴ and violet betacyanins, of which betanin (betanidin-O-β-glucoside) is the most common.

We extracted and purified the pigments of *M. jalapa* flowers and analysed them by high-performance liquid chromatography, as previously described⁵. The analysis confirmed that the pigmentation pattern on the flowers was due to a mixture of betaxanthins and betanins. Measurement of the fluorescence-emission

spectrum of dopaxanthin and the absorbance spectrum of betanin indicates that the light emitted by the fluorophore is strongly re-absorbed (Fig. 1). Addition of increasing concentrations of betanin to the dopaxanthin solution reduced the intensity of its fluorescence, until only 30% of the initial fluorescence was detectable at a ratio of 8.5:1. (For details and methods, see supplementary information.)

This internal light-filtering effect between the two types of betalain plant pigment causes a fading of visible fluorescence on parts of the flower where both types are present; areas containing only betaxanthins appear yellow under white light because of a combination of fluorescence and reflectance of non-absorbed radiation (Fig. 2a). The effect can be demonstrated in a system designed to visualize green fluorescence, which filters the incident light to blue and causes betaxanthins in the flower to fluoresce by emitting green light (Fig. 2b).

Detailed images of different zones of petal coloration were obtained by using light and fluorescence microscopy. A brightfield image under white light shows some cells containing only betaxanthins (Fig. 2c, yellow), others with betacyanins (Fig. 2c, deep-red spots), and some with both pigments together (Fig. 2c, orange).

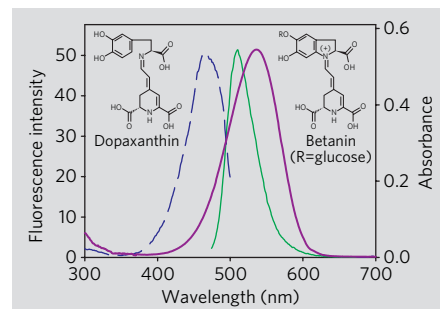


Figure 1 | Spectra of dopaxanthin and betanin.

Dopaxanthin is used as a model betaxanthin because of its structural (insets) and biochemical similarity to betacyanins. When excited by blue light, betaxanthins emit green fluorescence⁴. Fluorescence spectra (blue line, excitation spectrum; green line, emission spectrum) for natural dopaxanthin (6.0 μM, in water) are shown; violet line, absorbance spectrum of pure betanin (8.4 μM, in water). Note the overlap of the emission and absorbance spectra of the pigments.

The fluorescence micrograph shows that fluorescence is inhibited in areas where betaxanthins coexist with betanin (Fig. 2d) — the dark area corresponds to the orange area in Fig. 2c.

Fluorescence can be an important signal in mate choice for budgerigars⁶ and possibly in mantis shrimp⁷, and it may be that in flowers it attracts pollinators. The patterns arising from the internal light-filtering effect between betalain pigments described here could encourage bees¹ and bats⁸, which have visual receptors that are sensitive to green light and can detect bright targets better than dim ones⁹. Variation in light emission by flowers at visible wavelengths also modifies their colour, which would enhance their visibility to pollinators¹⁰.

Fernando Gandia-Herrero,

Francisco Garcia-Carmona, Josefa Escribano

Departamento de Bioquímica y Biología Molecular A, Unidad Docente de Biología, Facultad de Veterinaria, Universidad de Murcia, 30100 Espinardo, Murcia, Spain
 e-mail: gcarmona@um.es

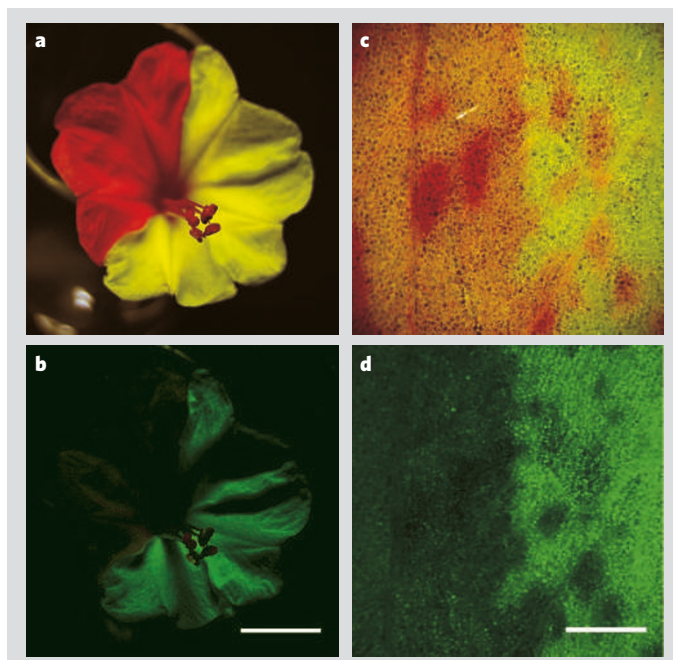


Figure 2 | Visible fluorescence in *Mirabilis jalapa* petals.

a, b, Flower with areas of red or yellow coloration under white light (**a**); only the yellow areas emit green fluorescence when excited by blue light (**b**) (scale bar, 1.5 cm).
c, d, Light micrographs of a section of a single red-and-yellow petal, showing brightfield (**c**) and fluorescent (**d**); excitation wavelength, 450–490 nm) images (scale bar, 500 μm). Green fluorescence is due to betaxanthins; dark areas correspond to orange areas in **c**, where light emitted from the fluorescent pigment is absorbed by betanin.

- Gumbert, A. *Behav. Ecol. Sociobiol.* **48**, 36–43 (2000).
- Giurfa, M., Eichmann, B. & Menzel, R. *Nature* **382**, 458–461 (1996).
- Heiling, A. M., Herberstein, M. E. & Chittka, L. *Nature* **421**, 334 (2003).
- Gandia-Herrero, F., Garcia-Carmona, F. & Escribano, J. *J. Chromatogr. A* **1078**, 83–89 (2005).
- Gandia-Herrero, F., Escribano, J. & Garcia-Carmona, F. *Plant Physiol.* **138**, 421–432 (2005).
- Arnold, K. E., Owens, I. P. F. & Marshall, N. J. *Science* **295**, 92 (2002).
- Mazel, C. H., Cronin, T. W., Caldwell, R. L. & Marshall, N. J. *Science* **303**, 51 (2004).
- Winter, Y., Lopez, J. & von Helversen, O. *Nature* **425**, 612–614 (2003).
- De Ibarra, N. H., Vorobyev, M., Brandt, R. & Giurfa, M. *J. Exp. Biol.* **203**, 3289–3298 (2000).
- Vorobyev, M., Marshall, J., Osorio, D., De Ibarra, N. H. & Menzel, R. *Color Res. Appl.* **26** (suppl.), 214–217 (2001).

Supplementary information accompanies this communication on Nature's website.

Competing financial interests: declared none.

doi:10.1038/437333a

BRIEF COMMUNICATIONS ARISING online
 ▶ www.nature.com/bca see Nature contents.