S. Marc Breedlove
Department of Psychology and Graduate Groups Neuroscience, Endocrinology, 3210 Tolman Hall, MC 1650, University of California, Berkeley, California 94720-1650, USA
e-mail: breedsm@socrates.berkeley.edu

1. Manning, J. T., Scutt, D., Wilson, J. & Lewis-Jones, D. I. *Hum. Reprod.* **13,** 3000–3004 (1998).
2. Blanchard, R. *Annu. Rev. Sex Res.* **8,** 27–67 (1997).
3. Breedlove, S. M., Cooke, B. M. & Jordan, C. L. *Brain Behav. Evol.* **54,** 8–14 (1999).
4. Manning, J. T., Trivers, R .L., Singh, D. & Thornhill, R. *Nature* **399,** 214–215 (1999).
5. McFadden, D. & Pasanen, E. *Proc. Natl Acad. Sci. USA* **95,** 2709–2713 (1998).
6. James, W. H. *Hum. Biol.* **59,** 721–752 (1987).
7. Bogaert, A. F. *Behav. Neurosci.* **111,** 1395–1397 (1997).
8. Bailey, J. M. & Pillard, R. C. *Arch. Gen. Psychiatry* **48,** 1089–1096 (1991).
9. Hamer, D. D., Hu, S., Magnuson, V. L., Hu, N. & Pattatucci, A. M. L. *Science* **261,** 321–327 (1993).
10. Gorman, M. R. *Persp. Biol. Med.* **38,** 61–81 (1994).
11. Brodie, H. K. H. *et al. Am. J. Psychiatry* **131,** 82–83 (1974).
12. Mayer-Bahlburg, H. F. L. *Progr. Brain Res.* **61,** 375–398 (1984).
13. Bogaert, A. F. & Hershberger, S. *Arch. Sexual Behav.* **28,** 213–221 (1999).
14. McFadden, D. & Champlin, C. A. *J. Ass. Res. Otolaryngol.* (in the press).

Drosophila cryptochromes

A unique circadian-rhythm photoreceptor

Cryptochrome proteins are critical for circadian rhythms, but their function(s) is uncertain. Here we show that a mutation in a cryptochrome (dCRY) from the fruitfly *Drosophila* blocks an essential photoresponse of circadian rhythms, namely arrhythmicity under constant light conditions. We conclude that dCRY acts as a key photoreceptor for circadian rhythms and that there is probably no other comparable photoreceptor in this species.

Constant light causes the intrinsic circadian period of diurnal animals to shorten and that of nocturnal animals to lengthen (Aschoff's rule). More intense light produces more extreme effects, ultimately resulting in arrhythmicity[1] in most mammals and birds. The circadian period of arthropods generally lengthens in constant light, whether the animal is nocturnal or diurnal[1]. *Drosophila melanogaster* is no exception and intense constant illumination leads to arrhythmicity[2].

The cryptochrome family includes blue-light photoreceptors[3]. The single known *Drosophila* cryptochrome is thought to be a circadian photoreceptor: flies carrying a mutant allele, cry^b, have severely decreased circadian photoresponses[4], whereas overproduction of dCRY causes increased photosensitivity[5]. In mammals, however, mCRY1 and mCRY2 are more likely to be involved in the central clock mechanism[6–8].

This raises the possibility that dCRY effects on photosensitivity reflect a role downstream of circadian photoreception, somewhere along the circadian light-input pathway or within the *Drosophila* central clock itself. This fits with the fact that cry^b flies are still able to reset their circadian rhythm (entrain) to new light–dark cycles[2]. We find, however, that cry^b flies remain behaviourally rhythmic in intense constant light, in contrast to wild-type flies and many other species which are arrhythmic under such conditions (Fig. 1a,b)[1,2].

The arrhythmicity of cry^b flies must be a property of the *cry* gene, because the normal phenotype can be rescued by expressing wild-type dCRY in rhythm-generating cells of cry^b flies. In intense constant light, the cry^b mutant's behaviour is strikingly similar to that of wild-type flies in constant darkness (Fig. 1c). An identical 24.7-hour period is also recorded under constant-darkness conditions, indicating that this slightly longer period is a characteristic of the background genotype. Thus, there is not even a detectable lengthening of period in the cry^b mutant strain under constant light conditions[2]. The free-running, circadian nature of cry^b flies' constant-light behaviour is further demonstrated by the 19.2-h period observed for flies with cry^b in combination with a short allele of the *period* gene (per^s; Fig. 1a).

Our results show that the cry^b mutation impairs the circadian photoreception pathway so profoundly that the fly cannot 'see' constant light. This mutant also responds very poorly to short light pulses[4]; by these criteria, this circadian photoreceptor must be unique in *Drosophila*. How then can cry^b flies entrain to different 24-h light–dark cycles? The missense cry^b mutation might generate a protein with weak activity that would be sufficient for light–dark entrainment but not for a normal arrhythmic behavioural response to constant illumination. However, our previous results suggest a different explanation: entrainment of cry^b flies is through a second, completely separate light-input pathway[4]. Visual photoreception[4,9] may even directly influence locomotor activity, which then affects circadian rhythms only indirectly through a non-photic phase-resetting pathway.

Our results indicate that dCRY is an important circadian photoreceptor and

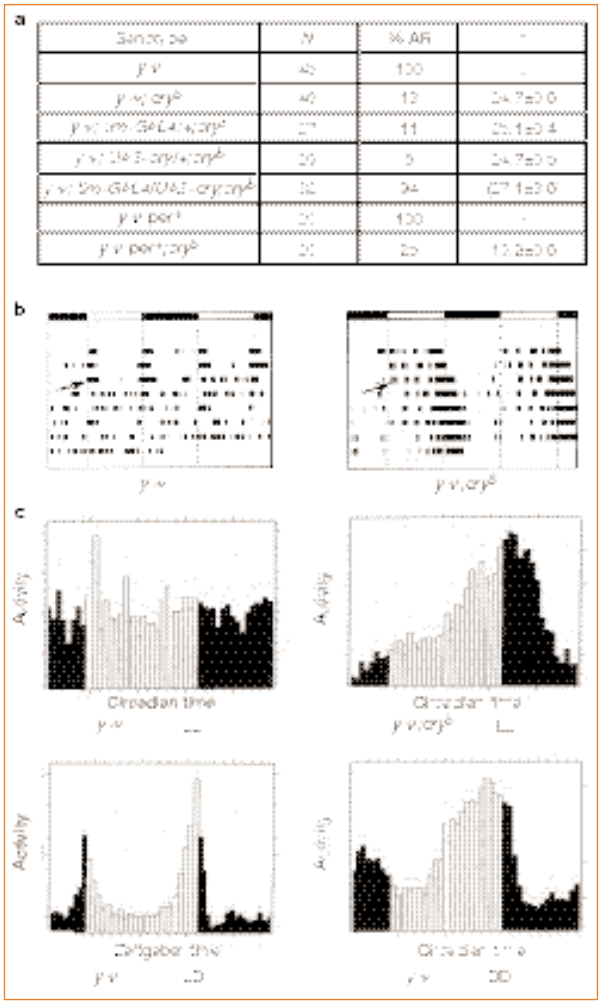

Figure 1 cry^b circadian rhythms free-run under intense constant light. **a,** cry^b behavioural rhythmicity and rescue of wild-type arrhythmicity. Flies were entrained under a normal 12-h light:12-h dark regime for 3 d. At the end of the fourth light period, the light was left on at saturating light intensities (2,000 lux) and activity monitored over the next 6 d. The last 5 d were used to analyse locomotor activity rhythms. *tim-GAL4* and *UAS-cry* are two transgenes[5] used to express wild-type CRY in circadian-rhythm-generating cells of cry^b flies. *N*, number of flies analysed; % AR, percentage of arrhythmic flies (cut off, 'power' < 10 or 'width' < 2; ref. 10); τ, period length of the circadian behavioural rhythms in hours. Value in parentheses is based on only two weakly rhythmic flies. **b,** Representative *y w* and *y w*;cry^b actograms; data are double-plotted. After the light is permanently left on (arrow), cry^b fly behaviour starts to free-run, whereas wild-type flies become arrhythmic. The first day of entrainment is not shown. White boxes, days or subjective days; black boxes, nights or subjective nights. **c,** Average activity plots of 14 *y w* and 16 *y w*;cry^b flies under constant light conditions (LL), and 16 *y w* flies under light–dark (LD) or constant-darkness conditions (DD). White bars, days or subjective days (zeitgeber or circadian time, 0–12); black bars, nights or subjective nights (zeitgeber or circadian time, 12–24). Each bar represents the 5-day average activity of a pool of flies during a specific 30-min period of the day (for example, the first white bar of an LD plot is the average activity of a pool of flies between zeitgeber time 0 and 0.5 during the 5 d of measurement). Dots, standard deviation.

probably the only dedicated one in *Drosophila*. Although we cannot yet exclude additional central-clock functions for dCRY, the abrogation of constant-light effects in *cry*^{b} mutant flies indicates that this cryptochrome makes a unique contribution to *Drosophila* circadian photoreception.

Patrick Emery*, Ralf Stanewsky‡, Jeffrey C. Hall*, Michael Rosbash*†

**Department of Biology, NSF Center for Biological Timing, †Howard Hughes Medical Institute, Brandeis University, Waltham, Massachusetts 02454, USA*

‡Zoologisches Institut, Universität Regensburg, D93040 Regensburg, Germany

e-mail: rosbash@brandeis.edu

1. Aschoff, J. *Z. Tierpsychol.* **49,** 225–249 (1979).
2. Konopka, R. J., Pittendrigh, C. & Orr, D. *J. Neurogenet.* **6,** 1–10 (1989).
3. Cashmore, A. R., Jarillo, J. A., Wu, Y. J. & Liu, D. *Science* **284,** 760–765 (1999).
4. Stanewsky, R. *et al. Cell* **95,** 681–692 (1998).
5. Emery, P., So, W. V., Kaneko, M., Hall, J. C. & Rosbash, M. *Cell* **95,** 669–679 (1998).
6. Kume, K. *et al. Cell* **98,** 193–205 (1999).
7. van der Horst, G. T. *et al. Nature* **398,** 627–630 (1999).
8. Vitaterna, M. H. *et al. Proc. Natl Acad. Sci. USA* **96,** 12114–12119 (1999).
9. Ohata, K., Nishiyama, H. & Tsukahara, Y. in *Biological Clocks: Mechanisms and Applications* (ed. Touitou, Y.) 167–170 (Elsevier, Amsterdam, 1999).
10. Ewer, J., Frisch, B., Hamblen-Coyle, M. J., Rosbash, M. & Hall, J. C. *J. Neurosci.* **12,** 3321–3349 (1992).

Structural colour

Colour mixing in wing scales of a butterfly

Green coloration in the animal kingdom, as seen in birds' feathers and reptile integument, is often an additive mixture of structurally effected blue and pigmentary yellow[1]. Here we investigate the origin of the bright green coloration of the wing scales of the Indonesian male *Papilio palinurus* butterfly, the microstructure of which generates an extraordinary combination of both yellow and blue iridescence. The dual colour arises from a modulation imposed on the multilayer, producing the blue component as a result of a previously undiscovered retro-reflection process.

Scanning electron micrographs of scales taken from the wings' coloured regions show that their surfaces comprise a regular two-dimensional array of concavities, of about 4–6 μm in diameter and 0.5–3 μm at the greatest depth. Transmission electron micrographs of these scales in cross-section reveal the multilayering that causes the iridescence as well as a modulation that leads to retro-reflection(Fig. 1a).

The variation in colour across each concave surface modulation is evident from optical microscopy. In reflection, for normally incident light, the flat regions between and

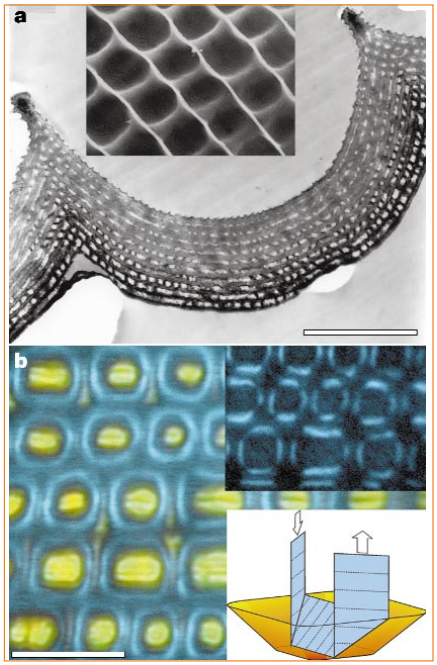

Figure 1 Modulated multilayering leads to dual colour in *P. palinurus*. **a**, Transmission electron microscope image showing a cross-section through one concavity on a *P. palinurus* iridescent scale. Inset; a scanning electron microscope image of the surface of an iridescent scale. Scale bar, 1 μm (inset, 7 μm). **b**, Real-colour image showing the dual-colour nature of the reflectivity from the surface of the *P. palinurus* iridescent scale, taken using unpolarized light in an optical microscope. Top inset, image of the same region taken with crossed polarizers. Bottom inset, illustration of the mechanism by which polarization is converted through double reflection from orthogonal sides of a concavity. Scale bar, 12 μm (inset, 6 μm).

in each concavity appear yellow, and the inclined sides of each concavity appear blue (Fig. 1b). It is the juxtaposition of these yellow and blue regions that synthesizes the green coloration perceived by the human eye, as they are too small to be resolved individually. Such spatial-averaging colour-stimulus synthesis[2] has been reported in beetles[3], and it also forms the basis of colour-television pictures and pointillistic painting.

The blue component cannot be back-reflected from a single multilayer system inclined at 45∞ to the incident direction. The effect is in fact caused by a pair of orthogonal multilayer surfaces that lie on opposite sides of each concavity. Light incident along the scale perpendicular, reflected from one surface inclined at 45∞ , is directed across the concavity to the opposite, orthogonal surface, where it is returned back along the incident direction. These pairs of inclined surfaces with almost identical multilayering have matched spectral reflectivity characteristics; this causes intense blue reflectivity through this double reflection.

Support for this retro-reflection mechanism comes from evidence of polarization conversion in the reflection from these scales. When we cross an input linear polarizer with an exit analyser while viewing the sample under normally incident light, all yellow reflected light is extinguished, but a substantial amount of blue reflected light remains, indicating that the blue reflected light has undergone polarization conversion. This effect occurs after double reflection from a pair of orthogonal surfaces when the wavevector of the incident light is at 45∞ to the reflecting surface and the polarization vector is at 45∞ to the plane of incidence.

Under diffuse white light, humans see the wing's green coloration in a limited solid angle about the wing normal. Outside this perspective, the wing colour changes predictably, becoming bluer as observation approaches grazing incidence[4]. The retro-reflection from pairs of opposite sides of each concavity is then less effective because their angle-dependent spectral reflectivity characteristics become mismatched. However, increasingly non-normal incidence observation is facilitated through large-angle reflections from the bottom and single sides of each concavity.

The purpose of this mechanism of colour generation is unclear. Structural colours can provide higher visibility[5] than pigmentary colours and can, given the appropriate microstructure[6], create colour-dependent polarization and angle effects. Conspecific and predator photoreceptor sensitivity must also be considered. Species whose spectral-vision sensitivity spans the two reflected structural colours may perceive a third by colour-stimulus synthesis. Polarization sensitivity associated with such photoreceptors[7] would provide further detail from wing reflectivity about species type and even wing orientation.

The mechanism by which *P. palinurus* produces its bi-colour and polarization effects is optically rare (although similar but less pronounced polarization effects and colour-stimulus synthesis of green have been identified in the other *Papilio* butterflies, *P. crino*, *P. buddha* and *P. blumei*). Through simple modulation of an otherwise uniform multilayer system, it synthesizes a very different colour stimulus in certain visual systems. The structure shows strong local polarization conversion of only one of the colours, through the mechanism we term orthogonal-surface retro-reflection.

P. Vukusic*, J. R. Sambles*, C. R. Lawrence†

**Thin Film Photonics, School of Physics, University of Exeter , Exeter EX4 4QL, UK*

†Mechanical Sciences Sector, DERA, Farnborough, GU14 0LX, UK

e-mail: P.Vukusic@ex.ac.uk

1. Fox, D. L. *Animal Biochromes and Structural Colours* (Univ. California Press, Berkeley, 1976).
2. Burnham, R. W., Hanes, R. M. & Bartleson, C. J. *Color* (Wiley, New York, 1963).
3. Schultz, T. D. & Bernard, G. D. *Nature* **337,** 72–73 (1989).
4. Huxley, J. *J. Entomol. A* **50,** 9–22 (1975).
5. Vukusic, P., Sambles, J. R., Lawrence, C. R. & Wootton, R. J. *Proc. R. Soc. Lond. B* **266,** 1402–1411 (1999).
6. Ghiradella, H. *Microsc. Anat. Invert. A* **11,** 257–287 (1998).
7. Kelber, A. *Nature* **402,** 251 (1999).