Unraveling Traveling

Charalambos P. Kyriacou

Science **325**, 1629 (2009);
DOI: 10.1126/science.1178935

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this infomation is current as of February 12, 2011):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/content/325/5948/1629.full.html

A list of selected additional articles on the Science Web sites **related to this article** can be found at:
http://www.sciencemag.org/content/325/5948/1629.full.html#related

This article **cites 13 articles**, 3 of which can be accessed free:
http://www.sciencemag.org/content/325/5948/1629.full.html#ref-list-1

This article appears in the following **subject collections:**
Physiology
http://www.sciencemag.org/cgi/collection/physiology

Downloaded from www.sciencemag.org on February 12, 2011

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2009 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

PHYSIOLOGY

Unraveling Traveling

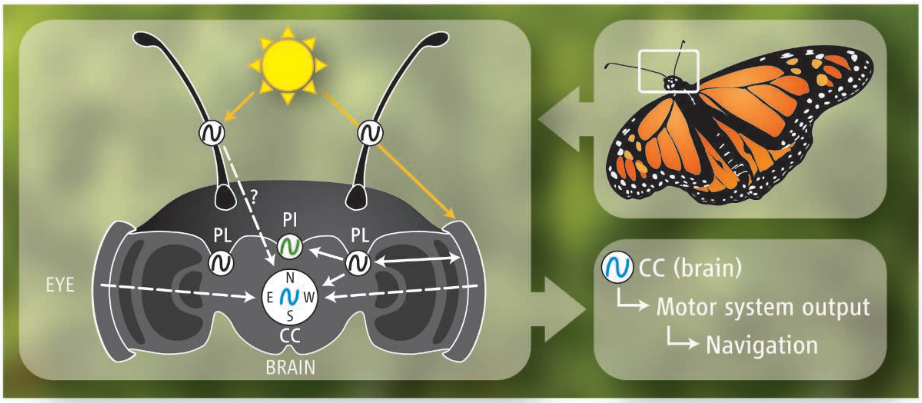
Charalambos P. Kyriacou

The monarch butterfly uses a time-compensated clock in its antennae to calculate seasonal migration routes relative to the Sun's position.

There are few more awesome sights in the animal world than the seasonal mass migrations of the monarch butterfly, *Danaus plexippus*, from the northern United States and southern Canada to its overwintering grounds in central Mexico (*1*). As with other insect orientations, the monarch uses the position of the Sun to calculate where it should be going. However, as the Sun moves across the sky during the day, the monarch must continuously adjust its calculations, which it does by using its 24-hour circadian clock. So where is this time-compensated clock located? On page 1700 of this issue, Merlin *et al*. reveal that it's in the antennae (*2*).

The monarch's ~3200-km journey is initiated by a photoperiodic response to the shorter daylengths of the fall, whereby the individual moves into a reproductive diapause to conserve resources, enhance longevity, and carry it through to the following spring. Then, after mating, eggs are laid in milkweed, and the few survivors and the new generation begin their journey home, laying eggs progressively further northward with the advancing growth of milkweed. After two or three such nomadic generations, the butterfly reaches its northern home, before the cycle begins again in the fall (*1*).

If the circadian clock is disrupted by environmental manipulations, such as constant bright light or phase-shifting of the light-dark cycle (12 hours:12 hours) by a few hours, the monarchs get lost (*3*). Merlin *et al*. observed that after surgical removal of the antennal flagellae, the butterfly's normal southwestern orientation is lost, even though the brain's canonical circadian clock keeps normal time (as indicated by the abundance of clock molecules during 24-hour cycles). In the intact animals, these molecules—*period*, *timeless*, and *cryptochrome2*—also keep time in the antennae with the same phase as in the brain. Even when explanted, the antennal clock keeps on ticking and can be reset by light, thereby maintaining a precise synchrony with the environmental light-dark cycle.



The monarch antennal clock. Circadian clocks are located in the antennae, pars lateralis (PL), and pars intercerebralis (PI). Retinal photoreceptors that respond to polarized light connect to the PL clock through neuronal fibers expressing the clock protein CRY1 and to the central complex (CC) in a clock-independent pathway. CRY1-expressing fibers connect the PL and PI clocks, whereas CRY2-expressing fibers connect the PL and CC clocks. The putative Sun compass resides in the CC and connects to the motor output. A key question is whether the antennal clocks are directly connected to the CC Sun compass.

This light-dependent resetting of the antennal clock was challenged by blackening the antennae with enamel paint and maintaining the butterflies for >10 days in light-dark cycles (thus, only the antennae are effectively under constant darkness). Under these conditions, the antennal clocks started to drift and their molecular cycles were dampened. However, the brain clocks maintained their synchrony with the light-dark cycle, underscoring the independence of the two oscillators. Monarchs whose antennae were covered in clear enamel paint had no such problems, with antennal and brain clocks maintaining the same phase in light-dark cycles. In flight orientation tests, the clear-painted animals gave the expected southerly flight, whereas the blackened butterflies flew predominantly in a northerly direction. This ~180° shift in direction in the blackened monarchs would be expected if the clock free-ran in the dark with a period either 1 hour shorter or longer than 24 hours, because after 12 to 14 days of the experiment, these animals' antennal clocks would be in approximate antiphase (180°) relative to those that were entrained to the light-dark cycle. Indeed, the free-running endogenous eclosion rhythms of these butterflies are closer to 23 hours than to 24 (*4*). Consequently, the blackened monarchs revealed that their phase-shifted but functioning antennal clock dominated any navigational input into the time-compensated "Sun compass" that might come from the brain clock.

The antennal clock is therefore rather like a standalone global positioning system that one might use while driving, which now eclipses the paper map (brain clock). This result is surprising, given that several studies have set the stage for a brain clock to mediate navigation. Monarchs have polarized light receptors in the dorsal rim area of the retina and these are connected to the presumed "clock" region, which is represented by four neurons in the pars lateralis (PL) of the brain (which express cycling clock molecules) (*5*, *6*) (see the figure). Neuronal fibers from the PL that express CRY1 [which is encoded by the gene *cryptochrome*, the ortholog of *cry*, the circadian blue light photoreceptor of the fly *Drosophila melanogaster* (*7*)] extend to the optic medulla, where axons of the dorsal rim photoreceptors terminate, which suggests a putative circadian modulation of polarized light input (*6*). Other neurons that express CRY1 connect the PL with neurons of the pars intercerebralis (PI), another clock gene–expressing region (*6*) that has been implicated in diapause and aging in *Drosophila* (*8*)—clearly important phenotypes for monarch migration. CRY2-expressing neuronal fibers that may arise from the PI and PL are also found in the central complex (CC) region of the brain, where this canonical clock molecule shows rhythmic oscillations in expression (*4*). In locusts, polarized light information from dedicated photoreceptors is integrated into the CC, which is believed to house the Sun compass (*9*). Thus, all the connections are present in the monarch for a brain clock in the PL to connect to skylight photoreceptors, to diapause, and to the putative Sun compass in the CC—a putative migratory network. Yet the antennal clock appears to override any input from the brain clock for naviga-

CREDIT: M. TWOMBLY/SCIENCE

Department of Genetics, University of Leicester, Leicester LE1 7RH, UK. E-mail: cpk@leicester.ac.uk

Downloaded from www.sciencemag.org on February 12, 2011

Published by AAAS

tion. However, much of this network is dedicated to aspects of migration (e.g., reproductive diapause and longevity) that are regulated separately from navigation (*10*).

It may be that the CRY2-expressing neurons of the brain clock (PL) that connect to the CC are a red herring in terms of navigation, and these are simply there to mediate the diurnal rhythmic flight patterns of the butterflies. In *Drosophila*, locomotor activity is maintained by the CC (*11*) that, intriguingly, receives input from nonclock neurons that express CRY (*12*). Clarification of how the monarch antennal clock connects to the putative Sun compass in the CC is certainly required. The possibility that antennal clocks are used in similar ways in other insects that use Sun compass orientation should also be entertained. Finally, *Drosophila* CRY behaves as a putative magnetoreceptor (*13*, *14*), with the clear implication that these molecules could provide additional orientation cues for butterfly migration.

References

1. F. Urquhart, *The Monarch Butterfly: International Traveler* (Nelson-Hall, Chicago, 1987).
2. C. Merlin *et al.*, *Science* **325**, 1700 (2009).
3. O. Froy *et al.*, *Science* **300**, 1303 (2003).
4. H. Zhu *et al.*, *PLoS Biol.* **6**, e4 (2008).
5. S. M. Reppert *et al.*, *Curr. Biol.* **14**, 155 (2004).
6. I. Sauman *et al.*, *Neuron* **46**, 457 (2005).
7. R. Stanewsky *et al.*, *Cell* **95**, 681 (1998).
8. S. Broughton, L. Partridge, *Biochem. J.* **418**, 1 (2009).
9. S. Heinze, U. Homberg, *J. Neurosci.* **29**, 4911 (2009).
10. H. Zhu *et al.*, *BMC Biol.* **7**, 14 (2009).
11. J. R. Martin, T. Raabe, M. Heisenberg, *J. Comp. Physiol. A* **185**, 277 (1999).
12. T. Yoshii *et al.*, *J. Comp. Neurol.* **508**, 952 (2008).
13. R. J. Gegear, A. Casselman, S. Waddell, S. M. Reppert, *Nature* **454**, 1014 (2008).
14. T. Yoshii, M. Ahmad, C. Helfrich-Forster, *PLoS Biol.* **7**, e1000086 (2009).

10.1126/science.1178935

CHEMISTRY

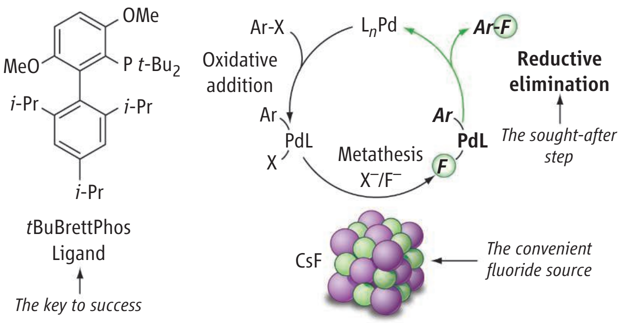
A New Departure in Fluorination Chemistry

A method for fluorinating a wide range of aromatic molecules will find immediate application in pharmaceutical research and may facilitate access to medical imaging reagents.

Véronique Gouverneur

Medicinal chemists often consider fluorine substitution of aromatics as a strategy for lead optimization, because the introduction of fluorine helps to rapidly improve pharmacokinetic and toxicological properties (*1*). Moreover, "editing" a lead structure with fluorine substitution can improve binding efficacy and selectivity. Traditional methods such as halogen exchange or diazonium-based transformations usually require reaction conditions that are too harsh for many aromatic molecules. These considerations have fueled interest in methodologies that are tolerant to functional group diversity, enabling fluorination of aromatics late in a synthetic sequence. On page 1661 of this issue, Watson *et al*. (*2*) report a novel palladium-catalyzed reaction that can be used to fluorinate a wide range of aromatics.

The recent success of transition metal catalysis for carbon-heteroatom bond formation suggests that the use of organometallic complexes for C–F bond construction could be central to future synthetic fluorine chemistry. Several laboratories have contributed to the development of metal–mediated fluorination to create aryl fluorides (ArF). It became quickly apparent that the most difficult elementary reaction of a transition metal-catalyzed process for aryl fluoride formation is the reductive elimination step, an event that releases the reduced metallic catalytic species and the product from the organometallic complex.



A new route to aryl fluorides. Reductive elimination from LPd(II)Ar(F) complexes has failed for many years as a route to aryl fluorides. Watson *et al.* solved this problem by using *t*BuBrettPhos as the ancillary ligand to force Ar–F bond formation; CsF is used as the fluoride source. The method may yield products with applications in pharmaceutical research and imaging.

In search of the elusive C–F reductive elimination event, Hull *et al.* (*3*) and Furuya and Ritter (*4*) used Pd(IV) intermediates to construct Ar–F bonds. These transformations (not always catalytic in palladium) used an electrophilic source of fluorine (F$^+$) as the oxidant. These studies were viewed as a major advance, even though the use of F$^+$-type reagent might be restrictive. In earlier studies, Grushin (*5*) prepared L$_{it}$Pd(II)Ar(F) complexes from a nucleophilic fluoride (F$^-$) source, but reported complications with the reductive elimination step necessary to form the Ar–F bond. Yandulov and Tran documented the formation of *p*-fluoronitrobenzene from a dimeric 16-electron Pd(II) complex; however, the low yield (10%) and the failure to extend this chemistry to unactivated or electron-rich aromatics cast doubt on whether the product resulted from reductive elimination (*6*).

Reasoning that dimeric Pd complexes may impede aryl fluoride formation, Watson *et al.* have now prepared the monomeric complex LPd(II)Ar(F)—where L is the easily accessible ligand BrettPhos (see the figure)—and show that this complex remains monomeric in solution. The new complex finally enabled the sought-after reductive elimination step, delivering the desired aryl fluoride (see the figure). A catalytic variant (Pd precatalyst and BrettPhos) was also successfully implemented with AgF as the fluoride source, and control experiments ruled out other possible mechanisms.

Through further fine-tuning of the experimental conditions, the authors show that *t*BuBrettPhos is the ideal ligand for fluorinating aryl triflates (functional group CF_3SO_3), using CsF or spray-dried KF as the fluorine source. These data indicate that the correct choice of ligand is decisive for a successful outcome, allowing for the preparation of aryl fluoride from a stabilized 14-electron Pd(II) complex. The steric size of *t*BuBrettPhos may also compress the ArPdF angle, thereby forcing reductive elimination by bringing closer the aryl and fluorine substituents.

The chemistry reported by Watson *et al.* provides access to fluorinated heteroaromatics, as well as various aromatics with

University of Oxford, Chemistry Research Laboratory, Oxford OX1 3TA, UK. E-mail: veronique.gouverneur@chem.ox.ac.uk

Published by AAAS

Downloaded from www.sciencemag.org on February 12, 2011