

Corrections and Retraction

CORRECTIONS

IMMUNOLOGY. For the article “Gene therapy with a single chain interleukin 12 fusion protein induces T cell-dependent protective immunity in a syngeneic model of murine neuroblastoma” by Holger N. Lode, Torsten Dreier, Rong Xiang, Nissi M. Varki, Angray S. Kang, and Ralph A. Reisfeld, which appeared in number 5, March 3, 1998, of *Proc. Natl. Acad. Sci. USA* (**95**, 2475–2480), the authors note the following correction. Figs. 1*A* and *B*, the legend to Fig. 1*B*, and lines 5–9 on page 2476 erroneously indicate that an OKT3 leader sequence was introduced upstream from the p35 gene to assure secretion in eukaryotic cells. The κ chain leader sequence of the anti-HIV-1 p24 monoclonal antibody was used instead. The leader sequence is as follows.

```
          10          20          30
ATGAGTGTGC CCACTCAGGT CCTGGGGTTG
          40          50          60
CTGCTGCTGT GGCTTACAGA TGCCAGATGT
```

NEUROBIOLOGY. For the article “Functional identification and reconstitution of an odorant receptor in single olfactory neurons” by Kazushige Touhara, Shintaro Sengoku, Koichiro Inaki, Akio Tsuboi, Junzo Hirono, Takaaki Sato, Hitoshi Sakano, and Tatsuya Haga, which appeared in number 7, March 30, 1999, of *Proc. Natl. Acad. Sci. USA* (**96**, 4040–4045), the authors note the following correction. In Fig. 4, the methyl group at the 3'

position is missing in the chemical structures of hydroxycitronellol (HC) and hydroxycitronellal dimethyl acetal (HCA). The International Union of Pure and Applied Chemistry (IUPAC) nomenclature for HC and HCA is 3,7-dimethyloctan-1,7-diol and 3,7-dimethyl-7-hydroxyoctan-1-al dimethyl acetal, respectively.

Correction published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.070046597. Text and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.070046597

PHYSIOLOGY. For the article “Corticotropin-releasing hormone deficiency unmasks the proinflammatory effect of epinephrine” by Katia P. Karalis, Effie Kontopoulou, Louis J. Muglia, and Joseph A. Majzoub, which appeared in number 12, June 8, 1999, of *Proc. Natl. Acad. Sci. USA* (**96**, 7093–7097), the authors note that in addition to those mentioned in the *Acknowledgments*, Dr. K. Rice is thanked for his synthesis of the corticotropin-releasing hormone antagonist antalarmin.

RETRACTION

ECOLOGY. For the article “Monarch butterflies (*Danaus plexippus* L.) use a magnetic compass for navigation” by Jason A. Etheredge, Sandra M. Perez, Orley R. Taylor, and Rudolf Jander, which appeared in number 24, November 23, 1999, of *Proc. Natl. Acad. Sci. USA* (**96**, 13845–13846), the authors note the following, “The positive response to magnetic fields in two experiments cannot be repeated. Further experiments show the false positives in these tests result from a positive taxis by the butterflies to the light reflected off the clothing of the observers. We therefore retract our report. We regret the inconvenience that publication of this study may have caused.”

Monarch butterflies (*Danaus plexippus* L.) use a magnetic compass for navigation

Jason A. Etheredge*, Sandra M. Perez†, Orley R. Taylor**‡, and Rudolf Jander*

*Department of Entomology, University of Kansas, Lawrence, KS 66045; and †Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

Edited by May R. Berenbaum, University of Illinois, Urbana, IL, and approved August 10, 1999 (received for review March 10, 1999)

Fall migratory monarch butterflies, tested for their directional responses to magnetic cues under three conditions, amagnetic, normal, and reversed magnetic fields, showed three distinct patterns. In the absence of a magnetic field, monarchs lacked directionality as a group. In the normal magnetic field, monarchs oriented to the southwest with a group pattern typical for migrants. When the horizontal component of the magnetic field was reversed, the butterflies oriented to the northeast. In contrast, nonmigratory monarchs lacked directionality in the normal magnetic field. The results are a direct demonstration of magnetic compass orientation in migratory insects.

Each fall, monarch butterflies migrate up to 4,000 km from their breeding grounds in the northeastern United States and Canada to overwintering sites in the transvolcanic mountain range of central Mexico. These insects, with a mass of 0.5 g, are descendants, 3–5 generations removed, of monarchs that migrated north from Mexico the previous March. There are many unanswered questions concerning this migration; among them, how do naive autumnal migrating monarchs navigate as they cross the continent to the few mountainsides on which they overwinter? Monarchs are not strong fliers and they use winds and thermals to move in a southwestern direction (1). They are easily blown off course, and, to reach overwintering sites, monarchs would seem to need a general geographic sense (2, 3), perhaps not present in nonmigratory generations. To adjust for changes in location, the butterflies need the ability to set a compass heading based on their present location and to reset or remodel the compass direction should they be blown off course. Although the overall pattern of migration within the United States, as determined from vanishing bearings (3) and mark and recapture records (2), suggests that monarchs adopt regional headings consistent with magnetic compass orientation, magnetic orientation per se has not been established for this species.

In a previous orientation study, to determine whether monarchs use celestial information, Perez *et al.* (4) showed that clock shifted monarchs used the position of the sun to orient themselves. Orientation by means of a sun compass, however, does not address the question of how butterflies navigate on days when the sun is not visible. Such ability could be explained by magnetic compass orientation (3). Monarchs are thought to contain magnetite (5, 6), a magnetically active, biosynthesized mineral suspected to mediate orientation in organisms that respond to magnetic fields (6). To determine whether migratory monarchs respond to magnetic fields, Perez *et al.* (7) subjected the butterflies to a magnetic pulse. As in birds, (8–10), orientation was altered after this treatment. These results demonstrate a sensitivity to magnetic fields, but they do not directly implicate use of a magnetic compass.

Here we present direct experimental evidence that monarch butterflies use an internal magnetic compass to maintain their migratory direction. Compass headings of field-collected fall migratory monarchs were recorded in amagnetic, normal, and reversed magnetic environments with the use of a circular (0.5 m radius and 0.43 m sidewall) arena. Butterflies entered the arena from below by climbing up a central tube (10 cm i.d. and

40 cm long). Upon reaching the top of the tube, they hesitated, often rotated, and took flight. The point of contact by the butterfly with the off-white paper-covered sidewall of the arena, estimated to the nearest 10° interval, was recorded as the compass heading. For the amagnetic environment, we used a room encased in Mu-metal, a nickel-iron alloy (77% Ni, 15% Fe, plus Cu and Mo), which provided an effective magnetic screen and an internal volume with an extremely low residual magnetic field. The reversed magnetic environment was created by using a set of Helmholtz coils to cancel out the normal magnetic field and then recreate it in the opposite direction at the same strength (11). All experiments were conducted in enclosed rooms. The only source of light was a 120-watt bulb placed 40 cm above the arena to give an omnidirectional lighting effect. The top of the arena was covered with Plexiglass with a 10-cm cardboard disc

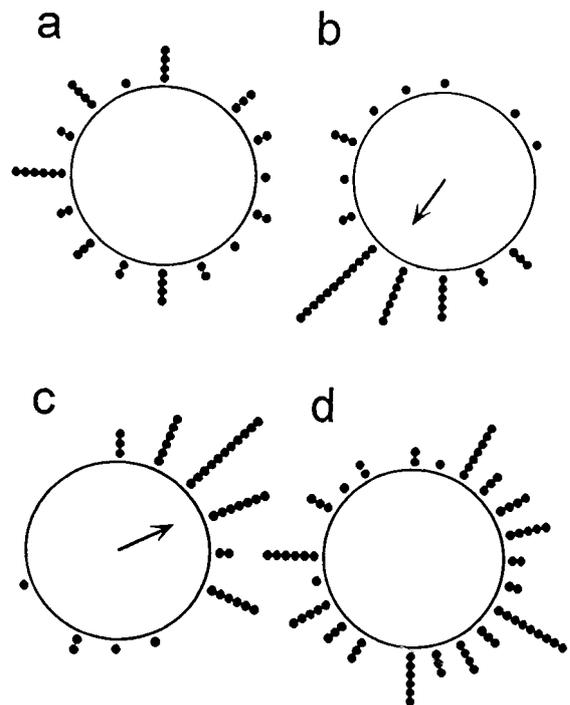


Fig. 1. Mean laboratory heading data giving resultant vector direction (μ) and length (r) for subjects in three environments (a) amagnetic (no mean heading for random distribution; $n = 39$); (b) normal magnetic field ($\mu = 213.98^\circ$; $r = 0.60$; $n = 40$); (c) reversed magnetic field ($\mu = 61.15^\circ$; $r = 0.65$; $n = 40$); (d) reproductive, non-migratory, subjects > 7 days of age (no mean heading for random distribution; $n = 75$). Each circle represents one subject.

This paper was submitted directly (Track II) to the PNAS office.

†To whom reprint requests should be addressed at Department of Entomology, 7005 Haworth Hall, University of Kansas, Lawrence, KS 66045. E-mail: monarch@ukans.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

in the center to prevent direct light from entering the entrance tube.

Butterflies tested under amagnetic conditions showed no consistent directionality as a group (Fig. 1a) (Rayleigh test of uniformity, $P = 0.23$, $n = 39$), which is indicative of the absence of orientation stimuli. The mean direction under normal magnetic conditions (Fig. 1b) was southwest ($\mu = 213.98$; $r = 0.60$; $n = 40$) and did not differ significantly from the average direction ($\mu = 200$) of naturally migrating monarchs ($F = 3.51$; $P > 0.05$) in eastern Kansas (4). Butterflies tested in a reversed magnetic field (Fig. 1c) flew toward the northeast ($\mu = 61.15$; $r = 0.65$; $n = 40$), that is, in the reverse direction of the normal migratory behavior. The results from this reversed group are significantly different from the normal field group (Watson's F -test, $F = 121.36$; $P < 0.01$). These tests demonstrate that monarchs navigate by means of a magnetic compass even in the absence of celestial information. Whether monarchs use the magnetic dip angle or horizontal polarity, or both, is an open question. The site of magnetoreception may be the thorax, which contains 65% of the magnetite (5), rather than the head, where in rainbow trout, it is associated with olfactory lamellae (12).

The butterflies used in these tests were fall migrants that remain nonreproductive throughout the migration. A different set of monarchs that were reproductive, nonmigratory, and at

least 7 days post eclosion were also tested in the normal magnetic field. Headings of this reproductive group were randomly distributed (Rayleigh test of uniformity, $P = 0.07$, $n = 75$, Fig. 1d), indicating that they lacked a consistent response to the normal magnetic field. Reproduction, however, does not appear to limit response to the magnetic field because monarchs are reproductive on the return spring migration. Thus, the response to the magnetic field appears to be coupled with the migratory condition but not the reproductive state. The external cues and/or physiological changes that trigger migration and cause monarchs to respond to magnetic information have not been determined.

Although monarchs use both magnetic and sun compass information to orient and navigate, the system is probably more complex. Once monarchs reach Mexico, the predominant course changes from southwest to southeast along the Sierra Madre Oriental (13), suggesting that structural features or other cues are used for navigation.

We thank the University of Kansas Department of Geology and Paul Montgomery for use of the amagnetic room and Bill McGregor for assistance in the design and construction of the arena. Support for this study was provided by Monarch Watch, The George E. Gould Student Assistance Fund, The University of Kansas Division of Biology, and a National Science Foundation Postdoctoral Fellowship to S.M.P.

1. Gibo, D. L. & Pallett, M. J. (1979) *Can. J. Zool.* **57**, 1393–1401.
2. Rogg, K. A., Taylor, O. R. & Gibo, D. L. (1999) *North American Conference on the Monarch Butterfly*, eds. Hoth, J., Merino, L., Oberhauser, K., Pisanty, I., Price, S. & Wilkinson, T. (Commission for Environmental Cooperation, Montreal), in press.
3. Schmidt-Koenig, K. (1993) in *Biology and Conservation of the Monarch Butterfly*, eds. Malcom, S. B. & Zaluki, M. P. (Natural History Museum of Los Angeles County, Los Angeles), pp. 275–283.
4. Perez, S. M., Taylor, O. R. & Jander, R. (1997) *Nature (London)* **387**, 29.
5. MacFadden, B. J. & Jones, D. S. (1985) in *Magnetite Biomineralization and Magnetoreception in Organisms*, eds. Kirschvink, J. L., Jones, D. S. & MacFadden, B. J. (Plenum, New York), pp. 407–415.
6. Jungreis, S. A. (1987) *Florida Entomologist* **70**, 277–283.
7. Perez, S. M., Taylor, O. R. & Jander, R. (1999) *Naturwissenschaften* **86**, 140–143.
8. Kirschvink, J. L. & Gould, J. L. (1981) *BioSystems* **13**, 181–201.
9. Wiltshcko, W., Munro, U., Beason, R. C., Ford, H. & Wiltshcko, R. (1994) *Experientia* **50**, 697–700.
10. Beason, B. C., Wiltshcko, R. & Wiltshcko, W. (1997) *Auk* **114**, 405–415.
11. Wiltshcko, R. & Wiltshcko, W. (1995) *Magnetic Orientation in Animals*. (Springer, Berlin).
12. Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C. & Green, C. R. (1998) *Nature (London)* **320**, 371–376.
13. Rzedowski, J. (1957) *Acta Zool. Mex.* **2**, 1–4.