Concentrations of Nb95 in maternal and fetal organs of rats (5 dams, 25 fetuses each group) and rabbits (3 dams, 9 fetuses each group) on comparative stages of the total gestational period. Animals were injected after having passed 82% (a) and 90% (b) of the full duration of pregnancy. Values are expressed as % of injected dose per g tissue (mean ± SE). The ratios of fetal and maternal tissue concentrations are given as C_f/C_m

		Rats			Rabbits		
		Dam	Fetus	C_f/C_m	Dam	Fetus	C_f/C_m
a)	Blood	0.63 ± 0.03	0.04 ± 0.008	0.06	0.17 ± 0.01	0.001 ± 0.0002	0.06
	Liver	0.29 ± 0.01	0.02 ± 0.003	0.07	0.07 ± 0.008	0.001 ± 0.0001	0.01
	Kidney	0.82 ± 0.07	0.02 ± 0.002	0.02	0.12 ± 0.004	0.006 ± 0.001	0.05
	Femur	0.36 ± 0.15	0.21 ± 0.01	0.60	0.07 ± 0.01	0.14 ± 0.007	1.8
	Whole-body	0.28 ± 0.02	0.05 ± 0.004	0.2	0.024 ± 0.002	0.018 ± 0.001	0.8
	Placenta	0.91 ± 0.02	–	–	0.14 ± 0.004	–	–
b)	Blood	0.41 ± 0.003	0.01 ± 0.003	0.02	0.11 ± 0.09	0.001 ± 0.0004	0.01
	Liver	0.21 ± 0.01	0.004 ± 0.0007	0.02	0.03 ± 0.004	0.0006 ± 0.0002	0.02
	Kidney	0.54 ± 0.04	0.01 ± 0.002	0.02	0.08 ± 0.002	0.004 ± 0.001	0.05
	Femur	0.38 ± 0.06	0.23 ± 0.03	0.6	0.02 ± 0.006	0.07 ± 0.003	3.5
	Whole-body	0.27 ± 0.02	0.03 ± 0.003	0.1	0.026 ± 0.003	0.019 ± 0.01	0.7
	Placenta	1.17 ± 0.09	–	–	0.15 ± 0.02	–	–

very extensive bone formation and ossification, a process which occurs in rats during the early postnatal period.

Summarizing the findings of this report, the results clearly illustrate the problem of introducing serious errors in theoretical calculations of radiation dosages to maternal and fetal tissues by using data obtained only from one species.

1 The work was supported by a grant of the Bundesministerium des Innern F.R.G..

2 Norman, C., Science *215* (1982) 22.
3 McDonald, N.S., Hamel, R., Hepler, M., and James, E., Proc. Soc. expl Biol. *119* (1965) 148.
4 Schneidereit, M., Senekowitsch, R., and Kriegel, H., Radiat. Envir. Biophys. *24* (1985) 125.
5 Moll, W., Die Placenta des Menschen, p. 153. Georg Thieme Verlag, 1981.
6 McAfee, J.G., and Subramanian, G., 3rd Int. Radiopharmaceutical Symposium Oak Ridge, Tenn., 1981. USA HHS-Publication, FDA 81–8166.
7 Zipkin, J., Biological Mineralization, p. 320. John Wiley & Sons, New York 1973.
8 Minot, Ch.S., and Taylor, E., Heft 5 (1905), and Henneberg, B., Heft 15 (1937), in: Normentafeln zur Entwicklungsgeschichte der Wirbeltiere. Ed. F. Keibel. Gustav-Fischer, Jena.

0014-4754/86/060619-02$1.50 + 0.20/0

A blue light-reversible reaction in an animal system *(Daphnia pulex)*

E. A. Davison Jr and R. G. Stross*

Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany (New York 12201, USA), and Department of Biological Sciences, State University, Albany (New York 12222, USA), 8 July 1985

Summary. A photoreversible reaction, previously found only in plants and fungi, has now been found in an animal system. Activation of development in diapausing embryos of *Daphnia pulex*, induced with white light, was suppressed with subsequent exposure to narrow-band (470 nm) or wide-band (410–525 nm) blue light. Pulses of wide-band blue light repeatedly reversed white light activation.

Key words. Daphnia pulex; waterflea; photoreversibility; blue light; embryonic diapause.

Photoreversible reactions have been reported for plants and fungi[1–4]. Phytochrome, a red/far-red reversible receptor, senses shade[5] and regulates development in plants[6,7]. Mycochrome, a UV-B/blue reversible receptor, regulates sporulation in certain fungi[2,8,9]. Development in animals may also be regulated by light, especially blue light[10,11]. Unfortunately, most reported light reactions in animals are complicated by the coupling of a circadian oscillator with photoinduction[12,13]. However, in the waterflea *(Daphnia)*, a single pulse of light may reinitiate development following an embryonic diapause[14,15]. Near-UV and blue light activate most effectively, as shown by action spectra[16,17]. This evidence, in conjunction with reported blue-light reactions in fungi, prompted us to examine embryonic development in *Daphnia* for photoreversibility. In this paper we show that embryonic development, activated by white light, is repeatedly reversed by blue light. This is the first demonstration of the photoreversal of a light-induced reaction in an animal[18].

Materials and methods. Sediments containing diapausing embryos of *Daphnia pulex* Leydig were dredged from Saratoga Lake, N.Y. (43° N. Lat., 73°45′ W. Long.) from a depth of 27 m. The embryos exist at densities of 10^4 m^{-2} and appear permanently trapped in diapause by lack of light and oxygen. Egg pods, each containing two embryos, were separated from the sediments and stored in lake water in 20-ml serum bottles under one atmosphere of 5.14% CO_2 with a balance of N_2. Collections were made under natural night light and the embryos were held in the laboratory in the dark at 4 °C.

Aerated embryos, within their egg pods, were exposed from above for 30 min to white light from a 150 W xenon lamp. Immediately following, they were exposed for 60 min to one of 11 narrow-bands of light, as provided by interference filters (Schott) with a 7 nm half-band width (table). The embryos, submersed beneath 4 cm of water, were irradiated at 4 °C and transferred immediately thereafter to 15 °C. Photoactivation was

evaluated from the mean hatch of 10 replicates of 80 embryos each. Means were compared for significance ($p < 0.05$) using the T-Method of Unplanned Comparisons[19].

Results. An attempt was made to identify a spectral region of maximum sensitivity for photoreversibility. Of the 11 narrow-band interference filters employed within the range of 349–756 nm, repeated trials revealed that only 470 nm narrow-band light significantly reversed activation. In a typical trial, the hatch after exposure to 470 nm light was only 23% of the controls. Embryos exposed to white light alone or held in the dark yielded responses that were not significantly different, with a pooled mean hatch of 14.2 young (fig. 1). In other trials, hatching was significantly greater in white-light controls compared to dark controls. In all trials, however, a subsequent exposure to narrow-band 470 nm light reversed white light activation; it also reversed dark activation when white light and dark treatments yielded similar numbers of young. Thus, blue light prevented hatching of embryos whether the hatching was activated by exposure to white light or was light-independent, i.e., activated by re-aeration only.

A wide-band blue (WBB) filter was developed for experiments requiring simultaneous exposures to blue light. It consisted of a 10 cm column of 0.293 M $CuCl_2$, having a half-band width of 410–525 nm and a transmission maximum at 468 nm. WBB light repeatedly reversed development. Alternating exposure to white and WBB light either significantly increased or decreased the mean hatch, depending on the final exposure (fig. 2). The reversing action of WBB light clearly required a preliminary exposure to white light. In fact, a preliminary exposure to WBB light alone, or followed by an additional pulse of WBB light, actually stimulated embryonic development relative to the response in dark controls. Reversal also required that blue light follow immediately after the priming pulse of white light. A dark interval of only 60 min rendered WBB light ineffective (fig. 3).

Discussion. Photoregulation of development may be similar in *Daphnia* and in fungi. First, there is a correspondence in the wavelengths of sensitivity, as summarized by Ninnemann[20]. In fact, the peak sensitivities of *Daphnia* and *Neurospora* are to nearly identical wavelengths in the blue[21,22]. Secondly, reversal may require a priming pulse of light[23]. Although 300 nm is known to prime blue-light reversal of the mycochrome system in certain fungi, activating wavelengths within the priming pulse for *Daphnia* appear to lie within the region of 370–450 nm[16,17]. Thirdly, the reversing stimulus must immediately follow the priming pulse[8,9].

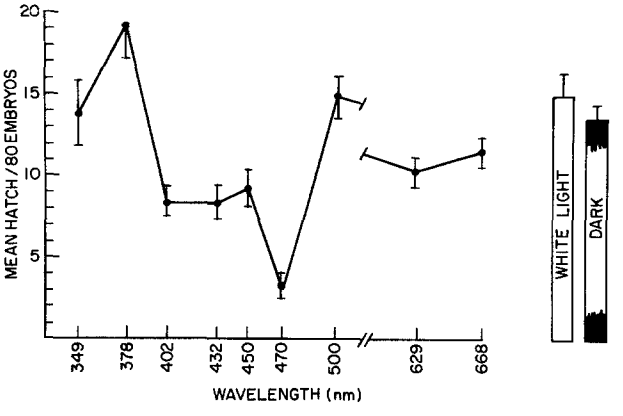

Figure 1. A typical spectrum of hatching in response to 60 min narrow-band light following a 30-min priming pulse of white light. Bars indicate ± SEM.

Fluence rates of light utilized in exposures. Narrow-band fluence rates were measured at the level of the embryos with an amplified silicon-diode detector (UV-PIN 10, United Detector Technologies). White and wide-band blue fluence rates were measured with a Licor quantum photometer (LI-185). Rates were adjusted using neutral density filter. Throughout all experiments, rates varied no more than 5% of the values listed.

Narrow-band (nm)	Fluence rate (nEin $m^{-2}sec^{-1}$)
349	1.71
378	1.33
402	1.95
432	1.71
450	1.58
470	1.57
500	3.10
629	3.82
668	2.88
725	2.63
756	3.04
Wide-band blue light	800.0
White light	4500.0

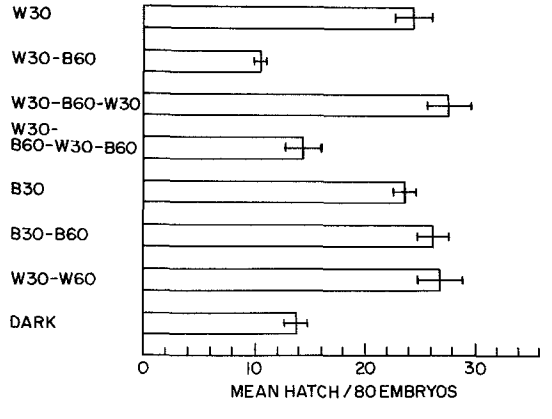

Figure 2. Suppression of embryonic development with wide-band blue (B) light following a white (W) light priming pulse. Numbers indicate minutes of irradiation. Bars indicate ± SEM.

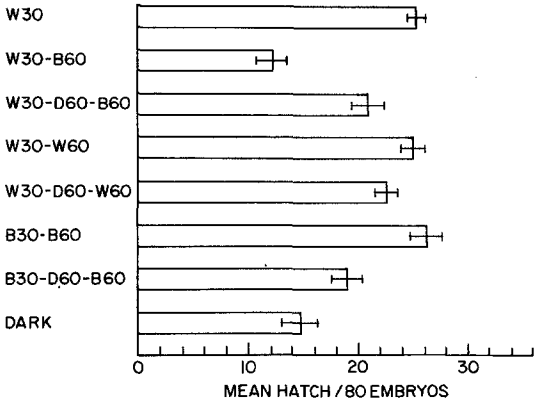

Figure 3. Hatching in response to a sequence of treatments involving dark (D), white (W) and wide-band blue (B) light. Numbers indicate minutes of irradiation. Bars indicate ± SEM.

Several possible mechanisms might explain blue-light reversibility in *Daphnia*. The most obvious possibility is the presence of a photochromic molecule analogous to phytochrome[6]. Equally plausible is the modification of a photosensitive molecule within an electron transport chain[23]. The least likely possibility is a long day/short day alternative reaction[10], although the blue light receptor could be involved in a photoperiodic mechanism. The photoreversal mechanism in *Daphnia* appears to be distinct from well known photoperiodic reactions in other animals since it is independent of daylength, sensitive to low fluence rates, and transitory.

A photoreversible reaction, such as exists in *Daphnia,* is likely to be found in other arthropods, and indeed in all organisms known to be sensitive to blue light. The search for similar blue light-reversible reactions in other animal systems will prove very exciting and will greatly aid in understanding the relevance of such reactions.

Acknowledgment. The authors thank S.S. Bowser, W.R. Colquhoun, and C.L. Rieder for their helpful criticisms. Supported in part by USEPA Grant 3898537.

* Person to whom all correspondence should be sent.

1 Borthwick, H.A., Hendricks, S.B., Parker, M.W., Toole, E.H., and Toole, V.K.,, Proc. natn. Acad. Sci. USA *38* (1952) 662.
2 Kumagai, T., and Bjorn, L.O., Physiologia Pl. *60* (1984) 449.
3 Löser, G., and Schäfer, E., in: The Blue Light Syndrome, p. 244. Ed. H. Senger. Springer-Verlag, Berlin 1980.
4 Erlanger, B.F., and Wassermann, N.H., in: Trends in Photobiology, p. 81. Eds C. Hélène, M. Charlier, Th. Montenay-Garestier and G. Laustriat. Plenum Press, New York 1982.
5 Smith, H., Symp. Soc. expl Biol. *36* (1983) 81.
6 Smith, H., Phytochrome and Photomorphogenesis. McGraw-Hill, Maidenhead 1975.
7 Hendricks, S.B., Proc. natn. Acad. Sci. USA *58* (1967) 2125.
8 Kumagai, T., Physiologia Pl. *57* (1983) 468.
9 Kumagai, T., Physiologia Pl. *59* (1983) 590.
10 Lees, A.D., J. Insect Physiol. *27* (1981) 761.
11 Klemm, E., and Ninnemann, H., Photochem. Photobiol. *24* (1976) 369.
12 Danilevskii, A.S., Photoperiodism and Seasonal Development in Insects. Oliver and Boyd, London 1965.
13 Saunders, D.S., An Introduction to Biological Rhythms. J. Wiley and Sons, New York 1977.
14 Pancella, J.C., and Stross, R.G., Chesapeake Sci. *4* (1963) 135.
15 Stross, R.G., Ecology *47* (1966) 368.
16 Davison, J., J. gen. Physiol. *53* (1969) 562.
17 Stross, R.G., unpublished.
18 Davison, E.A. Jr, A Blue Light-Reversible Photoresponse Controlling Embryonic Development in Daphnia pulex. Thesis, Library State Univ. at Albany, New York 1984.
19 Sokal, R., and Rohlf, J., Biometry, 2nd edn. W.H. Freeman and Co., San Francisco 1981.
20 Ninnemann, H., Bioscience *30* (1980) 166.
21 deFabo, E., Harding, R.W., and Shropshire, W. Jr,, Pl. Physiol. *57* (1976) 440.
22 Muñoz, V., and Butler, W.L., Pl. Physiol. *55* (1975) 421.
23 Kumagai, T., Photochem. Photobiol. *27* (1978) 371.

0014-4754/86/060620-03$1.50 + 0.20/0

Karyological conservatism in South American camelids[1,2]

N.O. Bianchi, M.L. Larramendy, M.S. Bianchi and L. Cortés

Instituto Multidisciplinario de Biología Celular (IMBICE), C.C. 403, 1900 La Plata (Argentina), 7 August 1985

Summary. Llama, guanaco, vicuna and alpaca show similar diploid numbers, gross chromosomal morphology and homologous G, C and NOR banding patterns. This chromosomal homology is also found in the two-humped camel and very probably in the one-humped camel as well. These findings indicate that the camelid karyotype can probably be traced back to early Miocene times. This probably represents the most extreme case of chromosomal conservatism among mammals.
Key words. Karyological conservatism; camelids; camelids karyology.

Taylor et al. in 1968[3] described similar diploid numbers and gross chromosome morphology for *Lama glama* (llama), *Camelus bactrianus* (two-humped camel) and *C. dromedarius* (one-humped camel). In 1983 Larramendy et al.[4] reported that *L. guanicoe* (guanaco) and *L. glama* showed a complete homology of G banding patterns. This homology was recently confirmed by Bunch et al.[5] who also extend the studies to *C. bactrianus* and to C- and NOR banding patterns.

In this report we present information on G, C and NOR banding patterns for the four species forming the group of South American camelids. Our data show the presence of a complete karyological conservatism for *L. guanicoe, L. glama, L. pacos* (alpaca) and *L. vicugna* (vicuna).

Material and methods. A total of two llamas, four guanacos, three vicunas and three alpacas were studied. Sex and origin of animals was as follows: llama, one male and one female from La Plata Zoo; guanaco, three males from La Plata Zoo and one female from the reserve of Trelew, Province of Chubut (Argentina); vicuna and alpaca, one male and two females from the reserve of Abra Pampa, Province of Jujuy (Argentina).

Blood cultures were set up according to Halman[6]. Culture medium contained 20% of fetal calf serum and was supplemented with L-cysteine 0.02%, L-glutamine 0.01% and L-tyrosine 0.03%. Harvesting was performed at 72 h. Colchicine treatments (0.1 µg/ml) lasted 3 h. Chromosome spreads were obtained by air drying.

C-banding was induced with the BaOH technique of Sumner[7]. Trypsin digestion was employed for G banding[7]. The silver method was used for identification of NOR[9].

A total of five to ten karyotypes per animal and per cytogenetic method were employed for karyological characterization of each species.

Results. Modal chromosome number in the four species was 2n = 74. The four species also showed similar chromosome morphology and similar NOR, C and G banding patterns.

According to gross morphology the autosomal pairs were arranged in two groups and within each group by order of decreasing size. Pairs 1–20 were acrocentric-subterminal; pairs 21–36 metacentric-submetacentric. X chromosomes were the longest metacentric of the set, while the Y chromosome was the smallest acrocentric. Pairs 1 and 18 could also be identified with accuracy on morphological grounds. Chromosomes 1 were the longest subterminal elements in the complement; pair 18 showed a constant and marked secondary constriction in the short arm producing the appearance of satellites.

All chromosomes showed positive C banding. Pairs 22–25 and