increased by 0.4 g per day, which corresponds to the daily mass increase found in other, similar bird species preparing to cross the desert[15]. After about 12 days food was again given ad libitum.

During the period preceding food deprivation, migratory restlessness had a value of about 19 half-hours/night (median). During food deprivation this value increased in all birds slightly, but significantly (sign-test, $p < 0.01$) to 21 half-hours/night. As soon as food was given again 8 birds immediately stopped showing migratory restlessness. In two birds it took three days before migratory restlessness ceased. There was practically no migratory restlessness while the birds deposited fat, until they reached a body mass between 16 and 17 g. From then onwards migratory restlessness developed gradually and eventually reached the initial level although body mass still increased.

Discussion. Two factors seem to be critical for the suppression of migratory restlessness during the night:

1) the body mass or the fat reserves and 2) the possibility of foraging or of gaining mass during the day.

The data on experimental and free-living birds are in accordance: in the field most birds (94%) arriving in the oasis with a body mass above 15 g continued to migrate the following night; in the laboratory, birds above 16–17 g showed migratory restlessness. Birds between 12 and 15 g at the oasis with the possibility of foraging interrupted migration and had a mean stopover period of nine days; the experimental birds with body masses between 13.5 and 16 g terminated migratory restlessness when food was given. This suggests that migratory timing in the flycatcher is not rigidly, endogenously fixed, but rather that the birds are equipped with a mechanism which allows adjustment to different environmental and physiological factors.

The time- and energy-related decisions can be summarized by the following hypothesis: An endogenous program has different setpoints of fat reserves during the migratory period in autumn. A bird with fat reserves above the setpoint will fly during the nights and rest during the days.

If the bird falls short of the setpoint it will interrupt migration provided that it has the chance to refill the fat depots. As soon as fat levels are above the setpoint it continues to migrate. If the feeding conditions are not sufficient for fat accumulation it will not stop for more than one day, and will continue to migrate until an adequate stopover place is reached. Superimposed on such a mechanism may be an endogenous program for the amount of migratory activity and a changing responsiveness to weather conditions. Besides the ecological context of these findings we now have, for the first time, a tool to manipulate migratory activity of captive long-distance migrants. This will enable researchers to tackle the problem of the internal mechanisms involved during the interaction of migratory activity, fat deposition and feeding.

1 I thank W. Friedrich, G. Heine, R. Mitreiter, J. Resch, K. Roth, D. Schmidl and H. Schüle for assistance in the field; Said Abdel Razik for permission to work in the oasis in Egypt and for logistic help; K. Pfeiffer for providing the experimental birds; H. Wegner for running the experiment and E. Gwinner, J. Dittami and R. Stevenson for reading and commenting on the manuscript.

2 Gwinner, E., Animal Orientation and Navigation, p. 321. NASA, Washington 1972.
3 Berthold, P., and Querner, U., Science *212* (1981) 77.
4 Biebach, H., Auk *100* (1983) 601.
5 Schindler, J., Berthold, P., and Bairlein, F., Vogelwarte *31* (1981) 14.
6 Lofts, B., Marshall, A.J., and Wolfson, A., Ibis *105* (1963) 99.
7 Berthold, P., Vogelwarte *29* (1977) 113.
8 Berthold, P., Naturwissenschaften *62* (1975) 399.
9 Berthold, P., Vogelwarte *28* (1976) 263.
10 Richardson, W.J., Oikos *30* (1978) 224.
11 Rappole, J.H., and Warner, D.W., Oecologia *26* (1976) 193.
12 Cherry, J.D., Auk *99* (1982) 725.
13 McCabe, T.T., Auk *60* (1943) 550.
14 Moody, D.T., Auk *87*(1970) 579.
15 Biebach, H., Wegner, H., and Habersetzer, J., Experientia, in press.
16 Fry, C.H., Ash, J.S., and Ferguson-Less, I.J., Ibis *112* (1970) 58.

0014-4754/85/050695-03$1.50 + 0.20/0

Natural selection for resistance to mercury pollution

R. Baker, B. Lavie and E. Nevo

Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31999 (Israel), 12 June 1984

Summary. The survival under conditions of mercury pollution of two natural populations of the marine gastropod *Cerithium rupestre*, derived from mercury-polluted and mercury-free sites, was tested in the laboratory. The results indicate a significantly higher survival rate for animals derived from the mercury-polluted site, in each of six repetitive experiments. We conclude that mercury resistance in marine organisms is reinforced in mercury polluted sites, presumably by natural selection for increased resistance. The evolution of metal tolerance in marine organisms may be as fast as that of metal tolerance in plants and the evolution of industrial melanisms in moths.

Key words. Cerithium rupestre; gastropods; mercury pollution; mercury resistance; metal tolerance.

Evolutionary adaptation to a changing biotic or physical environment depends on available genetic diversity and on natural selection. We have shown earlier that allozyme genotypes are sensitive to and vary with the quality and quantity of specific thermal and heavy metal[1], as well as organic[2] pollutants. In multiple repetitive laboratory experiments we have shown significant differential viability due to diverse pollutants among allozyme genotypes of barnacles, shrimps and marine gastropods. We hypothesized that the differential viability found is associated with the different degree of heavy metal inhibition uniquely related to each specific heavy metal pollutant. Likewise, we have demonstrated in two closely related species of the marine gastropod *Monodonta* parallel gentoypic differentiation as a response to pollution.

Recently, we tested the geographic distributions of mercury tolerant allozyme genotypes of the enzyme phosphoglucomutase (Pgm) in the shrimp *Palaemon elegans* and the enzyme phosphoglucose isomerase in the marine gastropod *Monodonta turbinata* in a mercury-polluted site versus several unpolluted sites on the Israeli coast of the Mediterranean Sea. For both enzymes the level of mercury-tolerant allozyme genotypes was higher in the polluted than in the unpolluted sites[3]. The results

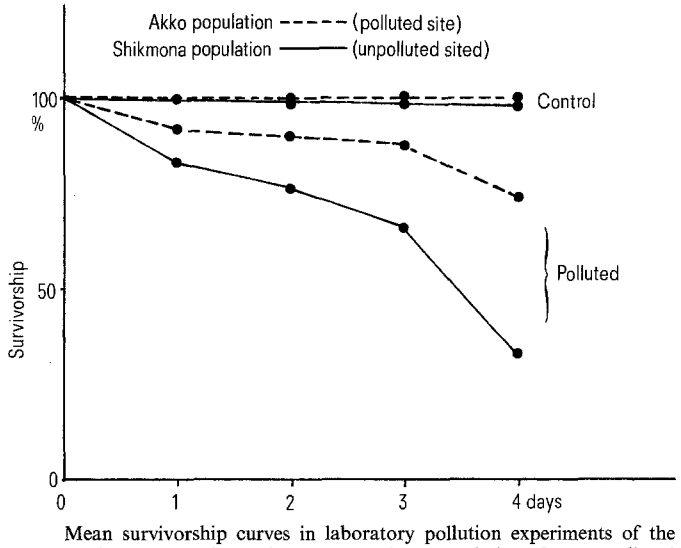
suggest that mercury selection is operating in nature on allozyme genotypes of these marine organisms along patterns comparable to those found previously in laboratory experiments. We therefore suggested that the enzymes studied display an adaptive pattern in polluted environments, and could be used for monitoring pollutants. We did not, however, test in the laboratory the mercury tolerance of the population as a whole when derived from the mercury-polluted site, as compared to a population derived from a mercury-free site.

The objective of the present paper was to compare and contrast the mercury-resistance levels of two populations derived from the mercury-polluted and nonpolluted sites. We present here evidence indicating that the population derived from the mercury-polluted site displayed higher resistance to mercury pollution than the population derived from the mercury-free site, presumably due to recent evolution for high resistance, by means of natural selection.

Materials and methods. Natural populations of the marine gastropod *Cerithium rupestre*, one from a mercury-polluted site (Akko) the other from a mercury-free site (Shikmona), in the northern and southern regions of the Mediterranean Bay of Haifa, respectively, were each tested in the laboratory on a sample of 700 animals. The sites were selected according to a previous survey along the Mediterranean coast that had shown that the highest levels of mercury pollution occur near Akko due to industrial discharge in the Haifa bay[4]. 100 animals were introduced into separate aquaria containing 25 l of fresh seawater pumped from a depth of 30 m at the Shikmona National Institute of Oceanography. Conditions in all aquaria were identical (22 °C, pH = 8.3, constant aeration). No food was provided during any of the experiments. For each population one aquarium served as control and six aquaria were mercury-polluted (0.6–1 mg $HgCl_2$/l). The experiment lasted 4 days.

Results and discussion. The figure presents the survivorship curves for both the polluted (Akko) and the unpolluted (Shikmona) site. Each point in the graph represents the mean of the six experiments with various pollutant concentrations. In each of the six experiments survivorship of animals derived from the polluted site was higher (sign test)[5], $p < 0.05$ than that of animals derived from the nonpolluted site.

The results show that in the laboratory, survival in mercury-polluted conditions is significantly higher in a population that was exposed previously to mercury pollution in nature. Evidence for the relatively high mercury pollution on the Akko site could also be gained from a survey on *Monodonta turbinata* (also a gastropod) collected at Akko, which concentrated mercury at levels about 20 times higher than animals from the unpolluted Shikmona site[6].



Mean survivorship curves in laboratory pollution experiments of the marine gastropod *Cerithium rupestre* in 2 populations from a polluted (Akko) and from an unpolluted (Shikmona) site.

Direct evidence of adaptive genetic changes in populations of marine organisms exposed to man-made changes in the environment is provided by the study of Russel and Morris[7], who demonstrated copper tolerance in the marine-fouling alga *Ectocarpus siliculosus* exposed to copper containing antifouling agents. Likewise, the work of Bryan and Hummerstone[8] on *Nereis* showed that populations exposed to high levels of heavy metals were more resistant to the toxic effects of the metals than animals from a locality with a low heavy metal content. Physiological studies showed that the zinc resistance probably depends upon both a decrease in permeability and an increase in excretory ability. The copper resistance appears to involve a detoxifying mechanism which allows concentration of copper in resistant animals which is two orders of magnitude higher than those in the sensitive animals. These results are in line with the now classical demonstration of pollution and plant evolution by Bradshaw[9]. He has clearly demonstrated that old mine workings, badly polluted by heavy metals, are often occupied by resistant forms of a few plant species. Tolerant populations evolve rapidly and exist in a balance between gene flow from outside and selection pressure from within the toxic area.

We cannot yet propose an overall physiological mechanism for the higher mercury tolerance of the Akko population of *Cerithium rupestre*. The potential of evolutionary adaptation to mercury depends on the existence of genetic diversity in response to pollution within the tolerant population. When this diversity is expressed in scorable allozymic polymorphisms, the results indicate that frequency distributions found in nature are consistent with the expectations from the laboratory tests for resistance to mercury[3]. (This was the case when *Palaemon elegans* polymorphism for the enzyme phosphoglucomutase (Pgm) and *Monodonta turbinata* polymorphism for phosphoglucose isomerase (Pgi) were compared between the two sites.). For *Cerithium rupestre* a test of diversification between the two population in respect to allozyme frequency distributions is not feasible because *Cerithium rupestre* in both populations does not show enough genetic variability for the scorable enzymes[10].

The present study does demonstrate unequivocally, that mercury resistance in nature is clearly associated with sites of high mercury pollution. In conclusion, natural selection as the force shaping the genetic variation of the marine populations was reflected both on the allozyme level[3] (higher frequencies of the resistant allozymes for the polluted site) and the phenotypic level (higher survival rates in mercury pollution of the animals derived from the polluted site) as demonstrated in this study. The evolution of metal tolerance in marine organisms may be as fast as that of metal tolerance in plants[9] and the evolution of industrial melanism in moths[11]. The circumstantial evidence for this hypothesis relates to the very recent mercury pollution in Haifa Bay which is probably due to recent industrialization.

Acknowledgment. This research was supported by a grant from FAO/UNEP.

1 Nevo, E., Lavie, B., and Ben-Shlomo, R., in: Isozymes: Current Topics in Biological Medical Research, vol. 10, p. 69. Eds M.C. Rattazzi, J.G. Scandalios and G.S. Whitt. Alan R. Liss Publ., New York 1983.
2 Lavie, B., Nevo, E., and Zoller, U., Envir. Res. *35* (1984) 270.
3 Nevo, E., Ben-Shlomo, R., and Lavie, B., Proc. natn. Acad. Sci. USA *81* (1984) 1258.
4 Roth, I., and Hornung, H., Oceanographic and Limnological Research Ltd, Haifa; final unpublished report (1975).
5 Siegel, S., Nonparametric Statistics for the Behavioral Sciences. McGraw Hill Co., New York 1956.

6 Hornung, H., Raviv, D., and Krumgalz, B.S., Mar. Poll. Bull. *12* (1981) 389.
7 Russel, G., and Morris, O.P., Nature *228* (1970) 288.
8 Bryan, G.W., and Hummerstone, L.G., J. mar. biol. Ass. U.K. *53* (1973) 839.
9 Bradshaw, A.D., in: Effects of air pollutants on plants. Ed. T.A. Manfield. Soc. exp. Biol. Seminar 1. Cambridge University Press (1976).
10 Lavie, B., and Nevo, E., Mar. Ecol. (submitted, 1984).
11 Kettlewell, H.B.D., The evolution of melanism. Clarendon Press, Oxford 1973.

0014-4754/85/050697-03$1.50 + 0.20/0

Antihepatoxic constituents of *Garcinia kola* seeds

M.M. Iwu

Phytotherapy Research Laboratory, Department of Pharmacognosy, University of Nigeria, Nsukka (Nigeria), 5 March 1984

Summary. Kolaviron, a fraction of defatted methanolic extract and biflavanones of *Garcinia kola* seeds significantly antagonized the lethal poisoning of mice with phalloidin. Garcinia biflavanones GB1, GB2 and kolaflavanone were isolated as the active constituents.
Key words. Kolaviron; antiphalloidin; biflavonoids; *Garcinia kola;* antihepatotoxic.

Extracts and whole seeds of *Garcinia kola* Heckel gave remarkable improvement of liver function in patients with chronic hepatitis and cholangitis after treatment for 14 days at a Nigerian herbal home[1]. Since there are no pharmacological studies to support the use of this plant in African ethnomedicine, the extract and isolates of *G.kola* have been subjected to various tests to determine any possible protective role on the liver.

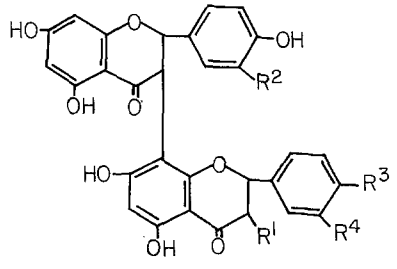
Previous phytochemical investigation of *G.kola* resulted in the isolation and characterization of cycloartenol, and 24-methylene cycloartenol from the light petroleum extract[2]; C-3/8″-link biflavanones GB1, GB2, GB1a and kolaflavanone from the ethylacetate extract of the seeds[3]. These biflavanones and their glycosides have also been isolated from the stem bark[4]. The ether soluble fraction of the alcoholic extract yielded apigenin-5,7,4′-trimethyl ether, apigenin-4′-methylether, fisetin, amentoflavone, kolaflavanone and GB1[5]. Waterman and his colleagues have also reported the antimicrobial properties of a benzophenone, kolanone, isolated from the light petroleum extract of *G.kola* seeds[6].

This communication describes the antihepatotoxic properties of kolaviron (a fraction of the defatted alcoholic extract) and isolates of *G.kola* seeds as evidenced by liver protection of laboratory animals challenged with phalloidin, a known liver toxin.

Materials and methods. Seeds of *Garcinia kola* Heckel (Fam. Guttiferae) were collected from a cultivated tree at Umukabia (Mbano, Nigeria) in November 1980. A voucher specimen has been deposited at the Pharmacy Herbarium at the University of Nigeria, Nsukka.

20 kg of powdered seeds of *G.kola* were extracted with light petroleum spirit (b.p. 40–60 °C) and then methanol in a soxhlet. The petrol extract, which showed no antihepatotoxic activity, was discarded. The methanolic extract was concentrated under reduced pressure and extracted with petrol. The defatted alcoholic extract was partitioned between chloroform and water. The chloroform extract on evaporation of the solvent gave a golden-yellow powder, kolaviron. Thin layer chromatography of this substance revealed the presence of three main compounds. Kolaviron was separated by Droplet Counter Current Chromatography using chloroform-methanol-water (7:13:8) as the solvent system (the more polar upper layer as the mobile phase). The mobile phase was delivered at a regulated pressure of 8 psi and the eluates collected in 15 ml fractions. Biflavanones GB1 (II-3-I-4′-II-4′-I-5-II-5-I-7-II-7-heptahydroxy-3/8″-biflavanone), GB2(II-3-II-3′-1-4′-II-4′-1-5-II-5-1-7-II-7-octahydroxy-3/8″-biflavanone) and kolaflavanone (I-3′-II-3-I-4′-II-4′-I-5-II-5-I-7-II-7-octahydroxy-II-3′-methoxy-3/8″-biflavanone) were identified from the analysis of their spectral data (UV, IR, MS, H and ^{13}C-NMR) and direct chromatographic comparison with reference compounds. The biflavanone mixture, kolaviron and the isolates were suspended in normal saline with Tween 20.

Biological methods. 240 female Swiss mice with an average weight of 24 g were maintained on laboratory chow and tap water ad libitum. Test substances in 10 ml/kg at stated doses (table) were given by intraperitoneal injection and followed 1 h later with 3 mg/kg of phalloidin (Sigma Chemical Company,



	R1	R2	R3	R4
GB 1	OH	H	OH	H
GB 2	OH	H	OH	OH
GB 1a	H	H	OH	H
Kolaflavanone	OH	H	OMe	OH

Antiphalloidin action of extract and isolates of *G.kola* in female mice

Substance	Dose mg/kg i.p	Number of animals	Survival[1] rate (%)
Control (phalloidin) only	3.0	20	5
Vehicle control	3.0 phalloidin and 10 ml Tween 20 in normal saline	20	10
Kolaviron	10	20	30
Kolaviron	50	20	50
Kolaviron	100	20	100
GB1	10	20	50
GB1	50	20	85
GB1	100	20	100
GB2	10	20	50
GB2	50	20	100
Kolaflavanone	10	20	35
Kolaflavanone	50	20	100

[1] $p = 0.05$ in all treated groups against toxin and vehicle control group.