Table 2. Results of skin tests against the antigen of D. metel

Antigen tested	Total number of tests	Patients (%)				Normal persons (%)			
		Negative	1+	2 +	3 +	4 +	Negative	1+	2 +
Datura metel	98	71.0	16.3	10.6	2.1	0.0	90	10	0

Table 3. Age-wise distribution of patients who showed positive skin response to D, metel pollen antigen

Age group	Male	Female	Total	
1-10	}	<u> </u>	i	
11-20	2	2	4	
21-30	4	1	5 9 4 5	
31-40	5	4		
41-50	2	2		
51-60	3	2		
Total	17 (60.7%)	11 (39.3%)	28 (100%)	

tested were between the age group of 9 and 56 years. Tables 2 and 3 give the results of the skin tests. Of the 29% patients showing positive response, 16.3% gave 1+ reaction, 10.6% gave 2+ reaction and 2.1% gave 3+ reaction. 4+ reaction was observed in none of the patients.

The pollen of *D. metel* thus reveals a high level of proteins (22%) and high level of proline, amino-n-butyric acid and arginine. SDS-PAGE of protein of the pollen showed 12 bands between molecular weight range of 18 kD and 127.2 kD. Results of skin tests showed positive response in 29% patients with 16.3% patients giving 1+ reaction, 10.6% giving 2+ reaction and 2.1% showing 3+ reaction. Although there are earlier reports of allergy of *D. metel* pollen, the present observation concerning perceptions of cause of respiratory allergy due to *D. metel* pollen supports the notion that *D. metel* should not be dismissed as a serious allergen.

- 1. Vishwanathan, R., Indian J. Chest Dis., 1964, 6, 108-124.
- 2. Agashe, S. N., in Recent Researches in Ecology, Environment and Pollution (eds Tilak, S. T.), Today and Tomorrow's Printers and Publishers, New Delhi, 1989, vol. 3, pp. 153-157.
- 3. Agashe, S. N., Anand, P., Manjunath K. and Jacob, N. Abraham, Aspects Allergy Appl. Immunol., 1983, 16, 53-57.
- 4. Atluri, J. B., Nurayana Rao, K. V. V. and Ramachandraiah, M., Indian J. Aerobiol., 1992, Special vol., 29-36.
- 5. Chen, S. H. and Huang, Ts-Cu, Grana, 1980, 19, 147-155.
- Cua-Lim, F., Palywal, P. C. and Laserna, G., Ann. Allergy, 1978, 40, 117-123.
- 7. Deshpande, S. V. and Chitaley, S. D., Indian Rev. Palaeobot. Palynol., 1976, 21, 253-262.
- 8. Guar, R. D., J. Indian Bot. Soc., 1978, 57, 353-365.
- 9. Hyde, H. A., New Phytol., 1950, 49, 407-420.
- 10. Hyde, H. A., Acta Allergy, 1959, 13, 186-209.
- 11. Santra, S. C., Gupta, S. and Chanda, S., Grana, 1991, 30, 63-66.
- 12. Satheesh Kumar, S. and Vittal, B. P. R., National Conference on Impact of Airborne Microbes, Pune, 1996, Abst., 2.5, p. 20.
- 13. Singh, A. B. and Babu, C. R., Ann. Allergy, 1982, 48, 115-122.

- 14. Singh, N. I. and Devi, K. K., *Indian J. Aerobiol.*, 1992, Spl. vol., 37-42.
- 15. Gregory, P. H., Int. Aerobiol. Newsl., 1983, 17, 9-16.
- 16. Durham, D., J. Allergy, 1947, 18, 231-238.
- 17. Jain, A. K., Patel, P. and Datta, T. R., *Indian J. Aerobiol.*, 1992, Spl. vol., 95-98.
- 18. Singh, N. I., Botonical Aspects, Proceedings of the National Conference on Env. Biopoll., 1981, 151-157.
- 19. Singh, N. I., J. Econ. Tax. Bot., 1983, 4, 191-199.
- 20. Stanley, R. G. and Linskens, H. F., Pollen-Biology Biochemistry Management, Springer, Berlin, 1974.
- 21. Sadasivam, S. and Manickam, A., Biochemical Methods for Agricultural Sciences, Wiley Eastern Limited and Tamil Nadu Agricultural University, Coimbatore, 1992.
- 22. Morris, D. L., Science, 1948, 107, 254-255.
- 23. Folch, J., Lees, M. and Sloane-Stanley, G. H., J. Biol. Chem., 1957, 226, 497-509.
- 24. Itoh, T. and Kaneko, H., Yakugaku, 1974, 23, 350-354.
- 25. Lowry, C. H., Rosebrough, N. J., Farr, A. L. and Randall, R. I., J. Biol. Chem., 1951, 193, 265-275.
- 26. Laemmli, U. K., Nature, 1970, 227, 680-685.
- 27. Sheldon, J. M., Lovell, R. G. and Mathews, K. P. (eds), A Manual of Clinical Allergy, W.B. Saunders Co., Philadelphia, 1967.
- 28. Britikov, E. A., Musatova, N. A., Vladimirtseva, S. V. and Protsenko, M. A., in *Pollen Physiology and Fertilization* (eds Linskens, H. F.), North Holland, Amsterdam, 1964, p. 77.

ACKNOWLEDGEMENTS. We thank the Council of Scientific and Industrial Research, New Delhi for financial support and Calcutta Medical Research Institute, Calcutta for carrying out the skin tests.

Received 21 July 1997; revised accepted 31 October 1997

## Methyl farnesoate stimulates ovarian maturation in the freshwater crab Oziotelphusa senex senex Fabricius

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Methyl farnesoate stimulation of ovarian maturation in the crab, Oziotelphusa senex senex is demonstrated. Greater mean oocyte diameter and mean ovarian indices of the crabs that received methyl farnesoate provide evidence that methyl farnesoate acts as a reproductive hormone in crustacea.

Since the discovery of the mandibular organs (MO) in CURRENT SCIENCE, VOL. 74, NO. 1, 10 JANUARY 1998

decapod crustacea by Le Roux<sup>1</sup>, attempts to determine the function of these glands have been made by several authors<sup>2</sup>, but the results were not conclusive. Methyl farnesoate (MF) is a terpenoid compound synthesized by the mandibular organs<sup>3-6</sup> and secreted into the hemolymph. MF is an unepoxidated juvenile hormone (JH III) and is found in the blood of several insects and crustaceans<sup>4</sup>. In insects JH is a gonadotropin in the adult stage. The target tissues for MF in crustacea have remained elusive, although it has a role in molting. Implantation of MO leads to a shortening of molt cycle in *Penaeus setiferus*<sup>7</sup> and *Caridina denticulata*<sup>2</sup>. Tamone and Chang<sup>8</sup> demonstrated stimulation of ecdysteroid secretion by isolated Y-organs of the crab, *Cancer magister*, when incubated with a MO.

The evidence supporting a role for MF in the regulation of crustacean reproduction is increasing. Implantation of MO into female juvenile *Libinia emarginata* causes premature vitellogenesis<sup>9</sup>. Secretory output of MF from MO is high in animals undergoing oocyte development<sup>3,10</sup>. In the present study, we demonstrate the stimulation of ovarian maturation by MF in the freshwater crab, *Oziotelphusa senex senex*.

Indian rice field crabs, Oziotelphusa senex senex Fabricius, were collected from paddy fields in and around Tirupati (Andhra Pradesh, India) and maintained in the laboratory at  $25 \pm 1$ °C in freshwater tanks. They were acclimatized to laboratory conditions (12:12 L:D) for at least 10 days before being used in experiments. Only intermolt (stage  $C_4$ )<sup>11</sup> crabs with a 30–32 mm carapace width and weighing 30–32 g were used. During the experimental period, the crabs were fed on sheep meat ad libitum.

The crabs were divided into three groups of thirty animals each. The first group, which served as initial control and did not receive any treatment, was sacrificed on the first day of the experiment. The second group, which served as a concurrent control, was treated the same as the experimental group, but received injections of physiological saline<sup>12</sup> through the arthrodial membrane of the coxa of the third pair of walking legs. The third group received MF at a dose of 16 ng/crab in 100 µl volume. Reddy<sup>13</sup> found that the hemolymph volume (ml) of the crab *Oziotelphusa* is 27% of body mass. The calculated hemolymph volume for 30 g crab used was

8.1 ml. The circulating concentration of MF in Scylla serrate was 2 ng/ml hemolymph<sup>6</sup>. To mimic the physiological concentration of MF, the crabs were injected with 16 ng of MF/30 g crab. Injections were given on the first, seventh and fourteenth day and were sacrificed on day 21. No deaths occurred in experimental or in control groups.

After sacrifice, the crabs were weighed and their ovaries removed. The ovaries were weighed and fixed in Bouins fluid. After 24 h of fixation, the ovaries were dehydrated through an alcohol series and then embedded in paraffin. Sections of 7 µm thickness were cut and stained with hematoxylin and counter-stained with eosin<sup>14</sup>. Ovarian indices were determined using the standard formula:

Ovarian index = 
$$\frac{\text{Wet weight of the ovary}}{\text{Wet weight of the crab}} \times 100$$
.

The diameters of fifty oocytes (µm) in each ovary were determined with the aid of a compound microscope and ocular micrometer.

In Oziotelphusa the immature ovary is white, whereas the mature ovary is orange red. The oocytes of the initial control crabs were in the immature stage (white in colour). The ovaries of the crabs that received MF had entered the late vitellogenic stage (orange red in colour). MF-injected crabs had a much larger mean ovarian index than that of the simultaneous control (Table 1). The mean oocyte diameters of the crabs that received MF were significantly greater than the corresponding value for the initial control value and concurrent control value. The mean oocyte diameter and mean ovarian index of the concurrent control crabs were slightly, but not significantly, larger than the corresponding values for the initial control crabs, showing that only a small amount of ovarian growth had occurred during the experimental period in the crabs injected with saline alone. The present results demonstrate that MF accelerates ovarian maturation in crabs. Previous studies have only correlated increased MF synthesis rates by the MO with developing ovaries<sup>3-5,10,15</sup>.

Ovarian development in crustaceans is regulated by two neurohormones, the gonad inhibiting hormone (GIH) from the X-organ-sinus gland complex in the eyestalks and gonad stimulating hormone (GSH) from the brain

Table 1. Ovarian colour, ovarian index and oocyte diameter of the crab, Oziotelphusa senex senex after 21 days of different experimental conditions

Group	Ovarian index	Oocyte diameter (µm)	Colour of the ovary White	
Initial control	1.7 ± 0.09	200 ± 20		
Concurrent control	$2.0 \pm 0.11$	$230 \pm 27$	White	
MF-injected	$3.9 \pm 0.18 *$	950 ± 27*	Orange red	

Values are mean  $\pm$  SD of 30 individual crabs. \*P < 0.001.

and thoracic ganglia<sup>16-19</sup>. GIH has been the primary regulator of ovarian growth in Oziotelphusa<sup>20</sup>. Eyestalkablated crustaceans show accelerated ovarian growth 11.20 and increased MF levels in hemolymph<sup>21,22</sup>. The stimulatory action of MF on ovarian maturation could have been due to stimulation of GSH synthesis and release or inhibition of release of the GSH antagonist (GIH) or direct action of MF on ovary. It is not yet known how hemolymph levels of GIH and GSH change during the reproductive cycle of crustaceans, since a sensitive assay for GIH and GSH does not exist. It could be that all the three hormones (GIH, GSH and MF) are very important in regulating the timing of onset of reproduction in crustaceans. Studies are underway in this laboratory to test the effectiveness of MF in inducing ovarian development and spawning in broodstock females of aquaculturally important crustacean species.

1. Le Roux, A., C. R. Hebd. Acad. Sci. Ser. D. Sci. Nat., 1968, 266, 1414-1417.

- 2. Taketomi, Y., Motono, M. and Miyawaki, M., Cell Biol. Int. Rep., 1989, 13, 463-469.
- 3. Laufer, H., Landau, M. and Homola, E., Adv. Invertebr. Reprod., 1986, 4, 135-143.
- Laufer, H., Borst, D., Baker, F. C., Carrasco, C., Sinkus, M., Reuter, C. C., Tsai, L. W. and Schooley, D. A., Science, 1987, 235, 202-205.
- 5. Laufer, H., Landau, M., Homola, E. and Borst, D. W., *Insect Biochem.*, 1987, 17, 1129-1131.
- 6. Tobe, S. S., Young, D. A. and Khoo, H. W., Gen. Comp. Endocrinol., 1989, 73, 342-353.
- 7. Yudin, A. I., Diener, R. A., Clark, W. H. and Chang, E. S., *Biol. Bull.*, 1980, 159, 760–772.
- 8. Tamone, S. L. and Chang, E. S., Gen. Comp. Endocrinol., 1993, 89, 425-432.
- 9. Hinsch, G. W., Trans. Am. Microsc. Soc., 1980, 99, 317-322.
- 10. Borst, D. W., Laufer, H., Landau, M., Chang, E. S., Hertz, W. A., Baker, F. C. and Schooley, D. A., Insect Biochem., 1987, 17, 1123-1127.
- 11. Reddy, P. S., Trop. Freshwater Biol., 1990, 2, 213-222.
- 12. Van Harreveld, A., Proc. Soc. Exp. Biol. Med., 1936, 34, 428-432.
- 13. Reddy, V. V., Doctoral dissertation, S. V. University, Tirupati, India, 1980, pp. 1-140.
- 14. Bancrost, J. D. and Stevens, A., Theory and Practice of Histological Techniques, Churchill Livingstone, New York, 1982, 2nd edn.
- 15. Tsukimura, B. and Kamemoto, F. I., Aquaculture, 1991, 92, 59-66.
- Panouse, J. B., C. R. Hebd. Seanc. Acad. Sci. Paris, 1943, 217, 553-555.
- 17. Panouse, J. B., C. R. Hebd. Seanc. Acad. Sci. Paris, 1944, 218, 293-294.
- 18. Otsu, T., Embryologia, 1963, 8, 1-20.
- 19. Gomez, R., Naturwissenschaften, 1965, 52, 216-217.
- 20. Reddy, P. S., Bhagyalakshmi, A. and Ramamurthi, R., *Toxicol. Lett.*, 1983, 18, 273–276.
- 21. Landau, M., Laufer, H. and Homola, E., Invertebr. Reprod. Dev., 1989, 16, 165-168.
- 22. Tsukimura, B. and Borst, D. W., Gen. Comp. Endocrinol., 1992, 86, 297-303.

ACKNOWLEDGEMENTS. P.S.R. thanks the Management, Pondicherry University, Pondicherry for granting sabbatical leave during which the present work was carried out and the Staff of Zoology

Department, Sri Venkateswara University, Tirupati for critical support. We are grateful to Prof. Ernest S. Chang, Bodega Marine Laboratory, University of California at Davis (USA) for his encouragement and advice and to Prof. Hans Laufer, University of Connecticut for his critical comments, Portions of this work are a result of research sponsored by the Rockefeller Foundation Biotechnology Carrier Fellowship to P.S.R.

Received 12 September 1997; revised accepted 28 October 1997

## Ion microprobe <sup>207</sup>Pb/<sup>206</sup>Pb zircon ages for gneiss-granitoid rocks from Bundelkhand massif: Evidence for Archaean components

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Radiometric <sup>207</sup>Pb/<sup>206</sup>Pb ages of individual zircons from the Bundelkhand massif, central India, have been determined by an ion microprobe. Samples from various major litho-units, viz. the granite gneiss, hornblende-, biotite- and leuco-granitoid are included in this study. The results provide evidence for the presence of Archaean crustal components within this massif. Zircons with ages of 3.0 to 3.2 Ga occur as xenocrysts in a sample of granite gneiss from Lalitpur having a minimum formation age of 2.5 Ga. Another sample of a highly deformed gneiss from Babina has a well-defined age of 2.7 Ga. Emplacement of hornblende-granitoid and biotite-granitoid took place in quick succession at ~2.5 Ga. No firm age for the youngest plutonic component, the leuco-granitoid, could be obtained due to poor recovery of zircons suitable for analysis. The geochronological data confirm the suggestion, based on geochemical studies, that the gneisses and the granitoids in this region represent Archaean and post-Archaean components, respectively.

The Bundelkhand 'massif' is a composite granite-gneiss province occupying an area of about 26,000 km² in the central portion of the Indian shield covering the southern parts of Uttar Pradesh and the north-eastern parts of Madhya Pradesh (Figure 1). The massif is unconformably overlain by the meta-sedimentary and associated meta-volcanic rocks of the Bijawar Group in the south and south-east, and by the still younger Vindhyan Supergroup of sedimentary rocks in the south-east, south and west. The Indo-Gangetic alluvium covers much of the northern portion of the massif. The geology of the region has