

**Results and discussion.** Characteristic esterase patterns of mites of several local populations and strains can be seen in figure 1. These patterns are typical for each population, i.e. they are the same for groups of females of a population living on various parts of the plant. The influence of the plant on the esterase pattern of mites seems to be unimportant. The data presented give evidence of different esterase patterns in various populations of spider mites<sup>12</sup>. They also confirm the assumption by other authors that interpopulation variability is high<sup>3,5,6</sup>. In order to verify their second assumption – low intrapopulation variability – single females of the following populations and strains were examined (number of females in brackets): CB1 (90), CB2 (34), MH (21) and ST (45). 14 esterase zones of different mobilities were found. They were marked E<sub>1</sub>–E<sub>14</sub> (E<sub>1</sub> possessed the highest mobility). The relative mobilities of esterase zones were related to E<sub>1</sub>. The maximum and minimum number of esterases that we observed was 7 and 3, respectively. All females of the strain MH had only 3 esterase zones. In CB1, females with 3 esterase zones were found in 51% and in CB2 and ST in 15% and 18%, respectively. Females with 4 esterase zones were the most numerous in ST (47%), then in CB2 (24%) and CB1 (20%). Females with 5 esterase zones were present in CB2, ST and CB1 by 50%, 36% and 23%, respectively. The gels with esterases from single females of MH, ST and CB1 are shown in figure 2. The populations CB1 and CB2 were very heterogeneous – only a few females had identical esterase patterns. The strain ST was far less heterogeneous and the strain MH was almost homogeneous in this respect. The esterase heterogeneity of spider mite populations was examined in September and October when the mites were present in large numbers on plants and the highest variability was supposed to occur<sup>13</sup>. The results presented here argue against conceptions of intrapopulation uniformity of spider mites. The importance of genetic heterogeneity is clear – it guarantees high adaptability to changing

conditions of the environment<sup>14</sup>. And actual heterogeneity, as here observed, correlates well with practical experience as regards the ability of spider mites to develop resistance to almost any pesticide. In accordance with the data of some authors<sup>10</sup> we found by contrast a low variability in our laboratory strains of spider mites. But on the whole our findings suggest that even in the presence of a restrictive mechanism such as arrhenotoky, a high level of genetic heterogeneity is somehow maintained, as exemplified by the case of two-spotted spider mites. While esterase polymorphism is possibly related to insecticide resistance, this model may nevertheless become informative as to the maintenance of genetic variability under restrictive conditions of reproduction, like parthenogenesis.

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## Effect of reserpine, 5-hydroxytryptamine and endocrinological manipulations on ovarian maturation of a marine crab

U.M. Farooqui and R. Nagabhushanam

Department of Zoology, Marathwada University, University Campus, Aurangabad-431 004 (India), February 24, 1982

**Summary.** An increase was observed in the ovarian index in *Scylla serrata* following administration of reserpine (a neuroleptic), but there was no change after 5-hydroxytryptamine administration. The releasing and inhibiting mechanisms for reproductive hormones of *S. serrata* are discussed.

The presence of catecholaminergic and serotonergic neurons has been reported in the eyestalk and other ganglia of crustaceans<sup>1,2</sup>. The biogenic amines are known to trigger the release of neurohormones in the vertebrate hypothalamo-hypophysial system<sup>3</sup> in insects<sup>4</sup> and in the crustaceans<sup>1,5</sup>. Fingerman et al.<sup>1</sup> have shown that in the crab, *Uca pugilator* 5-hydroxytryptamine (5-HT) releases red-pigment-dispersing hormone from the eyestalk. Recently Farooqui<sup>6</sup> reported that reserpine (RSP) (a monoamine depletor) brings about ovarian maturation in the sexually quiescent crab, *Scylla serrata* Forskal (Crustacea, Decapoda) probably by either inhibiting and/or stimulating the release of hormones via depletion of monoamines from their storage sites. The present study describes the effect of RSP (a monoamine depletor) and 5-HT on female *S. serrata* subjected to different endocrinological manipulations,

with the object of understanding the stimulatory/inhibitory mechanisms of a gonad-inhibiting hormone (GIH) from the eyestalks and a gonad-stimulatory hormone (GSH) from the brain and the thoracic ganglion.

**Material and methods.** Female crabs of the species *Scylla serrata* were collected from the Karla backwaters, Ratnagiri, West coast of India. They were acclimated to the laboratory conditions for about 1 week before the start of the experiment. Crabs of approximately equal weight (110–115 g) and size (70–72 mm carapace width) were selected for the study. Besides weight and size, the ovarian color was recorded by making a hole in the carapace at the start of the experiment. The holes were sealed after applying antibiotics with araldite adhesive (Ciba, India) as described earlier<sup>6</sup>. In *S. serrata* the immature ovary is white colored, whereas the mature ovary is orange-red in color<sup>6</sup>.

In the present study only crabs having a white colored (immature) ovary were selected and grouped into 13 groups of 10 each as follows:

Group 1: Initial control, sacrificed at the start of the experiment. Group 2: Control, sacrificed at the end of the experiment. Group 3: Intact, RSP (0.05 mg/crab) injected. Group 4: Intact, 5-HT (0.05 mg/crab) injected. Group 5: Intact, RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected. Group 6: Destalked crabs. Group 7: Destalked, eyestalk extracts (3 eyestalks in 0.45 ml of sea-water) injected. Group 8: Destalked, RSP (0.05 mg/crab) injected. Group 9: Destalked, 5-HT (0.05 mg/crab) injected. Group 10: Destalked, RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected. Group 11: Destalked, eyestalk extract (3 eyestalks in 0.45 ml of sea-water) + RSP (0.05 mg/crab) injected. Group 12: Destalked, eyestalk extract (3 eyestalk in 0.45 ml of sea-water) + 5-HT (0.05 mg/crab) injected. Group 13: Destalked, eyestalk extract (3 eyestalks in 0.45 ml of sea-water) + RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected.

The eyestalks of the crabs, which were anesthetized by cooling them in ice for 3 min, were excised with the help of scissors and the stubs were cauterized with a hot needle. Eyestalk extracts, RSP and 5-HT were injected every alternate day in the coxa of the last walking leg. Initial control crabs were sacrificed at the start of the experiment and remaining groups of crabs on the 13th day of the experiment as shown above. The ovaries were dissected out and the ovarian index was calculated with the formula,

$$\frac{\text{wet weight of the ovary}}{\text{wet weight of the crab}} \times 100$$

as described earlier<sup>7</sup>.

Ovarian color of the crab, *S. serrata* at the start of the experiment and after 12 days of different experimental conditions

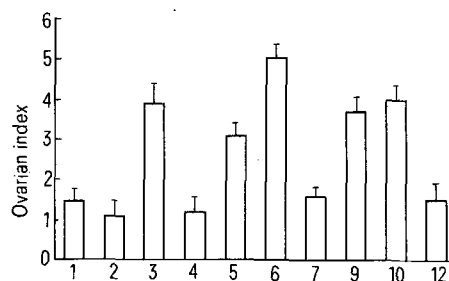
No. Treatment	Color of the ovary At the start of the experiment	At the end of the experiment
1 Initial control	White	-
2 Control	White	White
3 RSP (0.05 mg/crab) injected	White	Yellow
4 5-HT (0.05 mg/crab) injected	White	White
5 RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected	White	Yellow
6 Destalked	White	Orange red
7 Destalked + ES extract (3 ES in 0.45 ml of seawater) injected	White	White
8 Destalked + RSP (0.05 mg/crab) injected	White	-Died
9 Destalked + 5-HT (0.05 mg/crab) injected	White	Yellow
10 Destalked + RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected	White	Yellow
11 Destalked + ES extract + RSP (0.05 mg/crab) injected	White	-Died
12 Destalked + ES extract + 5-HT (0.05 mg/crab) injected	White	White
13 Destalked + ES extract + RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected	White	White
	White	-Died

RSP, reserpine; 5-HT, 5-hydroxytryptamine = serotonin; ES, eyestalk.

**Observations.** The figure and the table reveal that a significant ( $p < 0.05$ ) increase of the ovarian index takes place in a) intact, RSP injected, b) intact, RSP + 5-HT injected, c) destalked and d) destalked and 5-HT injected and e) destalked and RSP + 5-HT injected crabs, which also show changes in the color of the ovary from white (immature) to orange red (mature) in destalked crabs and yellow (maturing) in the remaining groups of crabs. On the other hand, 5-HT injected intact and destalked crabs after eyestalk extract administration and eyestalk extract + 5-HT and eyestalk extract + RSP + 5-HT administration did not show any significant change over the control ( $p > 0.05$ ). However, the destalked crabs could not survive after RSP administration.

In *S. serrata*, Farooqui<sup>6</sup> found an ovarian enlargement after RSP administration. The hormones which take part in the reproductive process in *S. serrata* are a gonad stimulating hormone (GSH) from the brain and the thoracic ganglion and a gonad inhibiting hormone (GIH) from the eyestalk<sup>6</sup>. According to Adiyodi and Adiyodi<sup>8</sup> GSH and GIH are antagonistic to each other. Enhancement in the ovarian index after RSP administration in *S. serrata* (present data) is probably either due to a rise in the level of GSH or a lowering of the level of GIH.

The existence of catecholaminergic neurons<sup>2</sup> and serotonergic neurons<sup>7</sup> have been reported in the eyestalks and other ganglia of crustaceans. In vertebrates Giachetti and Shore<sup>9</sup> have shown that RSP depletes monoamines from their storage sites, while Karki and Paasonen<sup>10</sup> have reported that RSP depletes both 5-HT and noradrenaline. Biogenic amines are shown to be responsible for the release of adipokinetic hormone in insects<sup>4</sup> and red-pigment-dispersing hormone in crustaceans<sup>1,5</sup>. However Berlind and Cook<sup>11</sup> and Berlind et al.<sup>12</sup> gave no indication that monoaminergic neurons played any role in neurosecretory release in the crab pericardial organ. The present data (table, fig.) show that 5-HT does not have any effect on the ovary of either intact or destalked *S. serrata*. This reveals that 5-HT is not having any stimulatory/inhibitory effect on the hormones involved in reproduction in *S. serrata*. It is possible that the increased ovarian index after RSP administration is via catecholamines instead of 5-HT. Noradrenaline, which is released in a larger amount after RSP administration<sup>10</sup> is probably stimulating the release of GSH



Ovarian index of the crab, *Scylla serrata* after 12 days of different experimental conditions. 1, Initial control, sacrificed at the start of the experiment. 2, Control, sacrificed at the end of the experiment. 3, Intact, RSP (0.05 mg/crab) injected. 4, Intact, 5-HT (0.05 mg/crab) injected. 5, Intact, RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected. 6, Destalked crabs. 7, Destalked, ES extract (3 eyestalks in 0.45 ml of sea-water) injected. 8, Destalked, RSP (0.05 mg/crab) injected-died. 9, Destalked, 5-HT (0.05 mg/crab) injected. 10, Destalked RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected. 11, Destalked, ES extract (3 eyestalks in 0.45 ml of sea-water) + RSP (0.05 mg/crab) injected-died. 12, Destalked + ES extract (3 eyestalks in 0.45 ml of sea water) + 5-HT (0.05 mg/crab) injected. 13, Destalked ES extract (3 eyestalks in 0.45 ml of sea-water) + RSP (0.05 mg/crab) + 5-HT (0.05 mg/crab) injected. - died, RSP, reserpine; 5-HT, 5-hydroxytryptamine = serotonin; ES, eyestalk.

and/or inhibiting GIH and is responsible for the ovarian enlargement.

The data clearly indicate that RSP can bring about an ovarian enlargement only in intact crabs (table, fig.). The ovarian index in the destalked crabs after RSP and 5-HT administration does not differ much from that in untreated destalked crabs. So, the changes obtained after RSP administration are mediated through the eyestalk, which is a source of moult inhibiting hormone (MIH) and GIH. It is possible that RSP is inhibiting the activity of GIH and thus upsetting the balance between GSH and GIH in the blood. The increased level of GSH is probably responsible for ovarian enlargement.

The problem as to why destalked crabs died after RSP administration can be explained with the help of our earlier studies on *S. serrata*<sup>12</sup>. We have shown that the eyestalk possesses a hormone(s) which act against stress<sup>12</sup>. In the destalked crabs, as the hormone which acts against stress is lacking, the death of the crabs injected with RSP may be owing to its pharmacological action. It has already been demonstrated that RSP could produce toxic effects by its pharmacological actions<sup>4</sup>, since the same dose of RSP is effective in the intact crabs and brings about changes in the ovary, and it has a toxic effect only in the destalked crabs. Perhaps some factors from the eyestalk may be preventing the toxic action of RSP.

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## Nematicidal activity of dimethyl 1-dodecanephosphonate<sup>1</sup>

J. Feldmesser<sup>2</sup>, J. Kochansky and W.E. Robbins

*Nematology Laboratory and Insect Physiology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville (Maryland 20705, USA), July 20, 1982*

**Summary.** Dimethyl 1-dodecanephosphonate has been shown in laboratory bioassays and greenhouse tests to be highly active against 2 species of nematodes. Other phosphonate esters showed little or no activity.

Plant parasitic nematodes cause damage in the United States estimated at about approximately 7.5% of crop value. Adjusted to current values these damages are estimated to total US\$  $6 \times 10^9$ /year<sup>3</sup>.

Control of plant parasitic nematodes is difficult for several reasons. They are intrinsically resistant to chemical control because nematode cuticle withstands penetration by most pesticides. Many target nematodes live deep within the soil mass, or within roots in the soil. The soil itself serves as a formidable barrier, preventing many chemicals from penetrating target sites efficiently, or degrading or inactivating them before they can reach sites of action in concentrations sufficient to exert control.

Consequently, nematicides must be applied at relatively high, costly, and frequently hazardous concentrations to be even minimally effective.

As a result, the number of currently registered nematicides (approximately 25) is small and inadequate. Many of these are specialty materials which cannot be considered for wide spectrum use. For example, phorate<sup>1</sup> is labeled only for use on easterlilies in the Pacific Northwest. Several are dangerous and may pose environmental risks and may consequently be removed from use by regulatory pressures.

Because of this dearth of relatively safe, effective nematicides, we have been engaged in evaluating various compounds found to be biologically active in other systems, for

Table 1. Range of concentrations (in ppm) of alkanephosphonate esters required to kill 95% of exposed *Panagrellus redivivus* populations in direct contact tests

Compound RPO(OR') <sub>2</sub>	R' = -CH <sub>3</sub>	R' = -CH <sub>2</sub> CH <sub>3</sub>	R' = -(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>
R = CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub>	> 100		> 100
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub>	> 100		
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>9</sub>	20-40		> 100
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>10</sub>	20-40	> 80	
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> (I)	0.5-1	> 80	
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>12</sub>	20-40	> 80	
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>13</sub>	80-100		> 100
CH <sub>3</sub> (CH <sub>2</sub> ) <sub>15</sub>	> 100		> 100