TECHNICAL MONOGRAPH No. 13

MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRA

NEELKAMAL PRASAD
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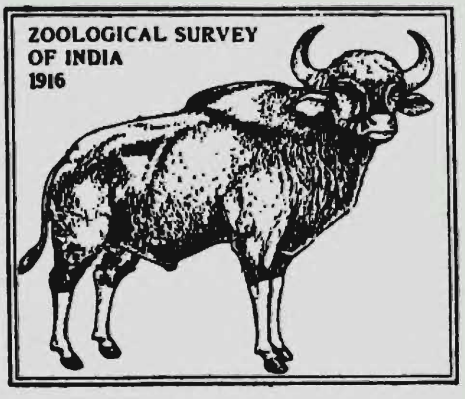

ZOOLOGICAL SURVEY OF INDIA

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By

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Edited by the Director, Zoological Survey of India

1986

Published in October, 1986

PRICE : Inland : Rs. 60·00
Foreign : £ 8·00, $ 10·00

PRINTED IN INDIA BY SRI AUROBINDO PRESS, 16, HEMENDRA SEN STREET, CALCUTTA-700 006, PRODUCED BY THE PUBLICATION DIVISION AND PUBLISHED BY THE DIRECTOR, ZOOLOGICAL SURVEY OF INDIA, CALCUTTA 700 012

ZOOLOGICAL SURVEY OF INDIA

TECHNICAL MONOGRAPH

Vol. 13 **1986** **Pages 1—118**

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MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRA

Part I : Review of Species of Hydra Reported from Various Parts of the World

Linnaeus (1758) placed all hydras under one specific name *Hydra polyps*. Attempts to classify hydra into species have been made since 1766 ; when Pallas gave specific names to the species of hydras whose characteristics had already been defined by Trembley (1744). These species were *viridissima*, *vulgaris*, and *oligactis* and added a fourth species *attenuata* from Rosel's description (1755). Linnaeus (1767) recognized Pallas' description, but without any justification changed the names to *viridis, grisia, fusca* and *pallens* respectively. Brauer (1908) pointed out that Linnaeus' names were wholly invalid and that Pallas' names had indisputable priority.

Annandale (1905, 1906) was the first to study the hydras in India and located *H. vulgaris* in Calcutta, Bihar, Orissa, Bombay, Allahabad and Madras and *H. oligactis* Pallas (1906) in Lahore. He also identified *H. orientalis* as a distinct species in 1905, but later (1911) concluded that it was only a variety of *H. vulgaris*.

Schulze (1914, 1917) selected some features of hydra as diagnostic character and erected the sub genus *Chlorohydra* in 1909, of those hydras which had symbiotic algae and embryotheca with polygonal plates. This subgenus was later (Schulze, 1917) converted into the genus *Chlorohydra*, and *C. viridissima* (Pallas) was recognized as the correct name of the green hydra. He put all those hydras which were characterized by the differentiation of the column into a slender basal stalk region into the genus *Pelmatohydra* ; and all these species with neither symbiotic algae nor stalk under the genus *Hydra*.

Hyman (1929, 1930, 1931a, b ; 1938) carried out extensive taxonomic studies of the hydras of North America. She questioned the generic distinction suggested by Schulze (1917) since the presence of symbiotic algae in other groups had not generally been regarded as constituting a generic character. She felt that it was almost impossible to decide in many cases whether or not the stalk was sufficiently distinct from the body to assign a species status according to Schulzes' distinction, since intermediate forms exist between fully stalked and stalkless forms (Hyman, 1929, 1930). The decision in such cases became purely arbitrary. Hyman also classified *H. cauliculata* under the genus *Hydra* although it was described as possessing a slender stalk (Hyman, 1938).

The characters considered to be reliable for the identification and description of species by Hyman (1929) were : the general shape and form of the column both during contracted and expanded states, the lengths of the tentacles and column, the shape, size and internal structure of nematocysts, the shape of the testes, the forms of embryonic theca, the separation or non-separation of sexes and the manner of origin of tentacles on buds. Hyman (1929) made attempts to resolve the synonymy which had crept in the identification and naming of hydra species, and also put forward a key to the known species of *Hydra* (Hyman, 1931b).

Ewer (1948) criticised the use of symbiotic algae as a generic character, but felt that its retention was justified on the basis of the nature of embryotheca alone. She also questioned the validity of using the presence or absence of stalk as a generic character. Of the two Natal (Africa) species described by Ewer (1948), *H. intaba* was observed to possess a stalk only when fully grown, while in *H. umfula* the distinction between stalk and body was not clearly marked. This compelled Ewer (1948) to recombine the genera *Pelmatohydra* and *Hydra* under *Hydra* Linnaeus (1758) sensu Ewer 1948. Ewer also questioned the idea of uniting different forms as subspecies of a single species, since very few breeding experiments have been done. For instance, Hadzi (1906) made unsuccessful attempts to fertilize eggs of *Hydra oligactis Pallas* with sperms of *H. viridis* pallas. Schulze (1917) also failed to effect the fertilization of the eggs of *H. attenuata* Pallas with sperms of *H. oligactis* Pallas. Ewer (1948) therefore suggested that the species be regarded as the ultimate taxonomic unit and raised all described subspecies and geographical races to the rank of species, till their correct status could be thoroughly elucidated. For instance, Cordero (1941) had described an animal which he considered to be a subspecies of *H. attenuata* and named it *H. a. thomseni*, although it differed from *H. attenuata* in the mode of coiling of the thread of the holotrichous isorhizas, in the way in which the buds were borne and in the mode of origin of the tentacles on the buds. Moreover, no cross fertilization experiments between these two hydra types were done. Ewer (1948) therefore felt that until full description of all species of hydra became available, it should be regarded as a distinct species *H. thomseni* Cordero.

In 1956 Caleb described a new species of hydra, *H. gangetica* from Allahabad (India). The characters utilized for identification purposes were : shape and size of the column, number of tentacles and length of the column, number of buds produced by the polyp, position of the bud zone, hermaphroditic nature of the organism, number and shape of gonad and the nature of the embryotheca. On the basis of these criteria however, this hydra type

does not appear to be distinctly different from the hydra described as *H. vulgaris* by Annandale (1905).

In 1959, Forrest described a new species of hydra, *H. hadleyi* from North America, which she assigned to the genus *Chlorohydra* Schulze (1917). *Chlorohydra hadleyi* had a unique two chambered embryotheca, but resembled *C. viridissima* in all other characters. Forrest (1959) opted for the retention of the genus *Chlorohydra* to accommodate the two species *C. hadleyi* and *C. viridissima* pending further investigation.

Muscatine (1961a, 1965) and Oschman (1967) employed various strain designations depending upon the geographic location of collection (e. g. Carolina, Florida, California, European) to distinguish between apparently different clones of green hydra. Oschman (1967) found differences between algae from different strains of hydra and interpreted them as either ndicative of differences in symbiotic species or simply adaptive changes resulting from a symbiotic habit with different strains of hydra.

Ball in 1967 studied the hydras occurring in Britain. He supported the view of Forrest (1959) regarding the retention of the genus *Chlorohydra* pending further investigation.

Grayson (1971) abandoned the genera *Chlorohydra* Schulze (1917) and *Pelmatohydra* Schulze (1917) and transferred their species to the genus *Hydra* Linnaeus 1758. He argued that the usage of symbiotic algae as important taxonomic character may be misleading. He suggested that *Hydra hadleyi* and *Hydra viridissima* may be conspecific. Since the taxonomic merit of most of the characters of hydra have not been adequately assessed, he considered it prudent to group all hydras under one genus *Hydra Linnaeus* 1758.

Cox and Young (1973) studied specimens from four separate localities in Kenya (East Africa) and suggested a new species *H. mariana* on the basis of nematocyst characteristics and structures associated with sexual reproduction. It was also found that characters such as body length, tentacle number, tentacle length, nature of peduncle, and maximum number of buds produced per polyp varied in the four populations.

Muscatine (1974) defended the retention of *Chlorohydra* as a genus on the basis of two facts (1) Green hydra (Carolina strain are dependent on the algae for survival when food is limiting (Muscatine and Lenhoff, 1965). (2) Symbiotic algae are recognized by green hydra, and this association exhibits a very high degree of specificity in terms of both the algae and the host type (Pardy and Muscatine, 1973).

On the basis of above, possession of symbiotic algae emerges as a genetically based character (Muscatine, 1974).

The most recent study on the identification of hydras (Campbell, 1983) utilizes three main characteristics : presence or absence of symbiotic algal cells, manner of origin of the tentacles on the bud, and shape of the holotrichous isorhiza.

Ewer (1948) and Grayson (1971) presented a list of the world species of hydra, which reveals that twenty-eight species of hydra have been well identified, seven from Europe, sixteen from America, five from Japan, three from Africa, five from Britain and one from India. Apart from these, thirty-two other species have been incompletely described.

It is thus clear that the twenty eight Hydras have been recognized as separate species mainly on the basis of morphological criteria. So far, no attempt has been made to study the problem of species in hydra in details at physiological, ecological or cellular levels. Very little is known yet about the geographical and phenotypic variations within ecological types of hydra. Also almost no knowledge is available about their molecular differences. A meticulous classification, therefore, becomes extremely necessary, since hydra has emerged as an organism of choice for experiments in ecology, neurophysiology, development and differentiation. Moreover since a number of new facts have emerged in the biology of hydra.

The present study has been undertaken to look at the species problem in hydra from more than one criteria. The primary basis of the present investigation centres round the various facets of morphogenetic differences such as growth, regeneration and differentiation. In this study hydras collected from fifteen different geographical localities in India were studied under stable laboratory conditions. The Part I constitutes a general introduction to the species problem in hydra citing almost all references on systematics of this organism. It would serve as basic perception of the whole theme of the study. In part II of this series, the morphological variations in 16 ecotypes of hydra were considered. In part III, the differences at the cellular level was considered. In part IV behavioural patterns of Hydras were analysed. In part V physiological responses of the different ecotypes were studied. In part VI a theoretical paradign had been constructed on the basis of the magnitude of differences observed in this investigation, within the ecological races of Indian hydra. Though each part had been written in a self contained manner but more each paper seemed to contribute in a certain order of chronology in unfolding the extent of difference of hydra races found in India thereby exerting a common insight into the total thematic pattern of understanding species problem in hydra.

SUMMARY

The review of literature shows that the characters employed for identification purposes are often arbitrary and contradictory such as presence or absence of symbiotic algae and presence or absence of stalk. No single character so far employed could be used as a key for identification purposes in *Hydra*. The coverage of literature has convinced the authors that the riddle of biosystematics of *Hydra* could be better analysed if multidirectional analysis of the biology of *Hydra* is undertaken at different levels from morphological, cellular, behavioural to physiological. It might yield more reliable clues for establishing classificatory orders in *Hydra*.

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MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRAS

Part II : Understanding The Extent of Morphological Differences

Introduction

In this study hydras collected from 15 distantly located places in India often separated by hundreds of miles were studied. The various ecotypes were examined carefully to detect differences in their form, shape and size of the column, length of the tentacles, density and arrangement of nematocysts on the tentacles, position of the bud zone and the order of emergence of tentacles on a new bud. Total protein content per hydra belonging to different ecotypes were also measured. Various morphogenetic criteria used in this study would reveal how reliable are these in understanding the species problem in hydra.

Materials and Methods

Different ecotypes of hydra collected from ponds of far and distant places of India constitute the experimental material of this study. From Hyderabad, green and white hydras were collected from the same locality. Abbreviations used whereever necessary for the different ecotypes collected from 15 different locations are given within brackets.

(a) Green hydra from Hyderabad (GHy)
(b) Hydra from Imphal (Imp)
(c) Hydra from Trivandrum (Triv)
(d) Hydra from Bolpur (Bol)
(e) Hydra from Srinagar (Sri)
(f) Hydra from Pune (Pun)
(g) Hydra from Chandan Nagar (CN)
(h) Hydra from Lucknow (Luc).
(i) Hydra from Delhi (Del)
(j) Hydra from Calcutta (Cal)
(k) Hydra from Shillong (Shil)
(l) Hydra from Madurai (Mad)
(m) Hydra from Santiniketan (San)
(n) Hydra from Jammu (Jam)
(o) White hydra from Hyderabad (WHy)
(p) Hydra from Tirupati (Tiru)

1. *Culture Technique* : A steady culture of different hydras was maintained in the laboratory following the method of Loomis and Lenhoff (1956).

Hydras were fed on alternate days with freshly hatched nauplii of brine shrimp (*Artemia* sp), Culture medium was changed 1 h and again 6 h following feeding. The temperature of the culture room was kept at 23 ± 1°C. For the Green hydras from Hyderabad, a constant photoperiod of 10 h light and 14 h dark was maintained.

2. *Whole Mount Preparation* : For whole mount preparations, hydras were fixed in Bouins's fixative for 4 h, stained with aqueous solution of Borax Carmine, dehydrated in upgrades of alcohol, cleared in xylene and mounted in DPX, photographs of whole mount preparations were taken in Carl Zeiss Photomicroscope. Living hydras were photographed with Nicon F2 photomic along with Bellows focusing attachment system.

3. *Measurements of column and tentacle length* : To measure the normal average length of the hydra column and the length of tentacles, living hydras were selected 6 h after feeding (i.e. after the total egestion of the residual Artemia) when their stretching and locomotion were minimum. A paper scale (in mm) was placed below the petri dishes containing 20 hydras. The normal average body length was measured by directly taking the measurement of the distance between the tip of the hypostome and the tip of the basal disc. The length of the tentacle was also recorded at the same time.

To measure the maximum length of the hydra, and the maximum length of the tentacles 24 h starved hydras were selected, and in a state of maximum extended condition the length obtained by each hydra type was measured as described above.

4. *Distance between the bud zone and the basal disc* : To measure the distance between bud zone and the basal disc hydras were selected 6 h after feeding. Hydras were taken in cavity slides with some culture medium and the distance was measured by means of a micrometer (1 division = 3.8 μm) placed in the eyepiece of a binocular microscope (Carl Zeiss). This distance was rechecked by taking once again measurements in the whole mount preparations of hydra.

Three groups of such measurements were made on different days. These were then averaged together to yield a single value for each polyp. In this way, the influence of periodic variations from day to day, and the possibility of inaccurate measurement due to temporary lack of extension was minimized. These estimates were then combined to give average data for each type.

5. *Protein content* : Ten hydras per type were homogenized in 2% sodium lauryl sulphate (0.5 ml). Total protein content per hydra was estimated by the Lowry's method (1951). Average value of 60 hydras was taken in each case for all the 16 hydra types.

RESULTS

Morphological Variations of Ecotypes

(a) *Green Hydra from Hyderabad*

(1) Colour — Grassy green

(2) Column — Small and cylindrical (Fig. 1a)
Average length in normal and stretched condition 3·88 mm and 5·03 mm respectively (Table 1).

(3) Hypostome — Small and conical, subhypostome distinct (Fig. 2a)

(4) Bud Zone — 1.285 ± 0.06 mm from the basal disc (length of penduncle ; Table 2, Fig. 5).

(5) Order of emergence of tentacle on buds — the tentacle appear simultaneously on the bud.

(6) Basal disc — medium size (Fig. 3a)

(7) Tentacle —

I. Number — varies between 6 and 7 (generally 6) (Fig. 2a)

II. Average length — about $\frac{1}{3}$ of column but becomes $\frac{3}{5}$ its length on stretching (Table 1).

III. Nematocyst density and arrangement :

(i) Base of the tentacle — High density (Table 3) scattered arrangement (Fig. 4A, C)

TABLE 1. Length of the body column (mm) and length of the tentacles relative to the column length (%), in normal and fully stretched conditions of hydra.

Sl. No.	Hydra type	*Length of the body column (mm) ± S.E.M.*		*Relative length of the tentacles (%) ± S.E.M.*	
		Normal	Fully Stretched	Normal	Fully Stretched
1.	Green Hyderabad	3.88±0.05	5.03±0.08	37.16± 6.80	62.5±4.16
2.	Imphal	4.70±0.07	5.83±0.11	81.0 ± 8.09	172.5±6.91
3.	Trivandrum	4.83±0.08	6.1 ±0.05	94.0 ± 6.99	222.5±6.91
4.	Bolpur	4.53±0.08	5.63±0.08	85.0 ±10.27	172.5±6.60
5.	Srinagar	4.73±0.06	5.96±0.15	112.0 ±12.73	227.5±5.83
6.	Pune	4.56±0 02	5.83±0.11	87.0 ±12.06	180.5±8.37
7.	Chandan Nagar	4.86±0.06	5.86±0.11	92.0 ±06.82	250.0±4.07
8.	Lucknow	4.80±0.11	6.10±0.17	81.0 ±10.01	212.5±3.81
9.	Delhi	4.80±0.15	6.06±0.26	110.0 ±11.57	177.5±6.66
10.	Calcutta	6.05±0.11	7.65±0.10	67.0 ±11.83	92.0±3.18
11.	Shillong	4.5 ±0.02	5.62±0.08	113.0 ±13.78	177.5±5.18
12.	Madurai	4.95±0.02	6.26±0.05	85.0 ±10.27	215.0±5.52
13.	Santiniketan	5.03±0.08	6.30±0.08	85.0 ±10.27	210.0±5.49
14.	Jammu	4.96±0.08	6.2 ±0.14	85.0 ±11.67	187.5±4.16
15.	White Hyderabad	4.8 ±0.06	5.96±0.14	87.0 ± 9.50	200.5±3.87
16.	Tirupati	4.53±0.02	5.8 ±0.15	98.0 ± 4.21	230.0±7.26

TABLE 2. Distance between the basal disc and budding region (mm)

Sl. No.	Hydra type	Distance (mm) ± S.E.M.
1.	Green Hyderabad	1.285±0.06
2.	Santiniketan	0.909±0.07
3.	White Hyderabad	0.873±0.06
4.	Shillong	0.826±0.08
5.	Calcutta	0.855±0.04
6.	Lucknow	0.814±0.05
7.	Delhi	0.800±0·05
8.	Tirupati	0.798±0.03
9.	Pune	0.798±0.06
10.	Bolpur	0.788±0.07
11.	Jammu	0.778±0.05
12.	Madurai	0.776±0.04
13.	Imphal	0.741±0.09
14.	Chandan Nagar	0.732±0.03
15.	Trivandrum	0.710±0.08
16.	Srinagar	0.623±0.02

TABLE 3. Relative density of nematocysts at the basal, middle and tip regions of the tentacles of whole mount preparations, +++++ – very high ; ++++ – high ; +++ – moderate ; ++– low ; + – very low.

Sl. No.	Hydra type	*Relative density of Nematocysts in tentacles*		
		Base	Middle region	Tip
1.	Green Hyderabad	++++	++++	++++
2.	Imphal	++	++	+++
3.	Trivandrum	+++	+++	++++
4.	Bolpur	+	+	+
5.	Srinagar	++++	++++	+++++
6.	Pune	+++	++	+++
7.	Chandan Nagar	+++++	++++	++++
8.	Lucknow	+++++	++++	+++++
9.	Delhi	+	+	++
10.	Calcutta	+++	+++	+++
11.	Shillong	+++	++++	+++++
12.	Madurai	+++	++	+
13.	Santiniketan	+++++	++++	+++++
14.	Jammu	+++++	++	++
15.	White Hyderabad	+++++	+++++	+++
16.	Tirupati	++++	+++	+++

(ii) Middle region of the tentacle — High density (Table 3) minute space between successive rows (Fig. 4B, a)

(iii) Tip region of the tentacle — High density (Table 3) minute space between the successive rows ; dense clusters forming an apical knob (Fig. 4C, a)

(8) Protein content per hydra — 5.56 ± 0.26 μg (Table 4)

TABLE 4. Total protein content per hydra (μg)

Sl. No.	Hydra types	Protein content (μg) ±S.E.M.
1.	Green Hyderabad	5.56±0.26
2.	Bolpur	6.08±0.15
3.	Pune	6.22±0.45
4.	Tirupati	6.35±0.23
5.	Shillong	6.43±0.66
6.	Santiniketan	6.76±0.29
7.	Lucknow	6.78±0.32
8.	Srinagar	6.83±0.31
9.	Chandan Nagar	6.84±0.41
10.	White Hyderabad	6.95±0.41
11.	Jammu	7.26±0.14
12.	Madurai	7.26±0.24
13.	Delhi	7.56±0.24
14.	Imphal	7.36±0.55
15.	Trivandrum	7.41±0.28
16.	Calcutta	9.84±0.99

(b) *Hydra from Imphal*

(1) Colour — Translucent white

(2) Column — either cylindrical throughout or with a slightly stalked peduncle (Fig. 1b). Average length in normal and stretched condition 4.7 mm and 5.38 mm respectively (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2b).

(4) Bud zone — 0.741 ± 0.09 mm from the basal disc (length of the peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on buds — Tentacle rudiments do not appear simultaneously on the bud. (generally 2 opposing tentacles form first and then the next two).

(6) Basal disc — large and broad generally (Fig. 3b)

(7) Tentacle :

I. Number — Varies between 4 and 6 (generally 6 ; Fig. 2b)

II. Average length — equal to the body column but in stretched condition becomes $1\frac{1}{3}$ times as long (Table 1)

III. Nematocyst density and arrangement :

(i) Base of the tentacles — low density (Table 3) scattered arrangement (Fig. 4A, b)

(ii) Middle region of tentacle — low density (Table 3) small clusters present in close successions (Fig. 4B, b)

(iii) Tip of the tentacle — moderate density (Table 3) annular ring arrangement at the extreme tip close clusters giving the tip a club shaped appearance (Fig. 4C, b)

(8) Protein content per hydra — 7.36 ± 0.55 μg (Table 4)

(c) *Hydra from Trivandrum*

(1) Colour — Translucent white

(2) Column — either cylindrical or showing a slightly stalked condition (Fig. 1c) column length is 4.83 mm and 6.1 mm in *normal* and stretched condition respectively (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2c).

(4) Bud zone — 0.710 ± 0.08 mm from the basal disc (length of the peduncle) (Table 2, Fig. 5)

(5) Order of emergence of tentacles on buds — Tentacle rudiments do not appear simultaneously

(6) Basal disc — small (Fig. 3c)

(7) Tentacles

I. Number — varies between 4 and 5 (generally 4 ; Fig. 2c)

II. Average length — equal to the column but in stretched condition becomes 2 times the column length (Table 1)

III. Nematocyst density and arrangement

i. Base of the tentacle — moderate density (Table 3), nematocyst randomly scattered (Fig. 4A, c)

ii. Middle region of the tentacle — moderate density (Table 3) arranged in succession with space in between (Fig. 4B, c)

iii. Tip of the tentacle — High density (Table 3) annular arrangement, extreme tip knob shaped (Fig. 4C, c)

8. Protein content per hydra — 7.41 ± 0.28 μg (Table 4)

(d) *Hydra from Bolpur*

(1) Colour — Translucent white

(2) Column — Medium size, either cylindrical or with a slightly narrower peduncle region (Fig. 1, d). Average length 4. 5 mm but becomes 5.63 mm when stretched fully (Table 1)

(3) Hypostome — Rounded, subhypostome indistinct (Fig. 2d)

(4) Bud zone — 0.788 ± 0.07 mm from the basal disc (length of the peduncle ; Table 2 ; Fig. 5).

(5) Order of emergence of tentacles on buds — Tentacle rudiments do not appear simultaneously on buds

(6) Basal disc — Medium size (Fig. 3d)

(7) Tentacles

I. Number — varies between 4 and 6 (generally 4, very rarely 6 ; Fig. 2d)

II. Average length — equal to the column but becomes 2-times the column length in stretched condition (Table 1)

III. Nematocyst density and arrangement

i. Base of the tentacles — very low density (Table 3), randomly scattered arrangement (Fig. 4A, d)

ii. Middle and tip region — very low density (Table 3), small clusters arranged in incomplete rings. Apical knob small and insignificant (Fig. 4B, d and 4C, d)

(8) Protein content per hydra — 6.08 ± 0.15 μg (Table 4)

(e) *Hydra from Srinagar*

(1) Colour — Translucent white

(2) Column — medium size, either cylindrical or with a slight stalked condition (Fig. 1e). Average length 4.73 mm but becomes 5.96 mm when fully stretched (Table 1)

(3) Hypostome — Rounded, subhypostome indistinct (Fig. 2c)

(4) Bud zone — 0.623 ± 0.02 mm from the basal disc (length of the peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — medium size (Fig. 3c)

(7) Tentacles

I. Number — varies between 4 and 6 (generally 4, very rarely 6 ; Fig. 2c)

II. Average length — equal or slightly longer than the column but become 2½ times as long when stretched (Table 1)

III. Nematocyst density and arrangement

i. Base of the tentacle — High density (Table 3) annular arrangement (Fig. 4A, c)

ii. Middle of the tentacle — High density (Table 3) annular arrangement, with very small spaces between the circles (Fig. 4B, c)

iii. Tip of the tentacle— very high density (Table 3) closely packed annular rings, a small knob at the apex (Fig. 4C, e)

(8) Protein content per hydra— 6.83 ± 0.31 μg (Table 4)

(f) *Hydra from Pune*

(1) Colour — Translucent white

(2) Column — either cylindrical or with a slightly narrower peduncle (Fig. 1f) Average length 4.56 mm but besomes 5.83 mm when stretched fully (Table 1)

(3) Hypostome — Rounded, subhypostome indistinct (Fig. 2f)

(4) Bud Zone — 0.798 ± 0.06 mm from the basal disc (length of the peduncle ; Table 2, Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — large and elongated (Fig. 3f)

(7) Tentacles

I. Number — varies between 4 and 5 (generally 4 ; Fig. 2f)

II. Average length — slightly smaller than the column but becomes almost 2 times the column length when stretched. (Table 1)

III. Nematocyst density and arrangement in the tentacles

(i) Base of the tentacles — Moderate density (Table 3), randomly scattered (Fig. 4A, f)

(ii) Middle region of the tentacle — Low density (Table 3), undefined annular rings (Fig. 4B, f)

(iii) Tip of the tentacle — Moderate density (Table 3) overlapping annular arrangement, an apical knob present (Fig. 4C, f)

(8) Protein content per hydra — 6.22 ± 0.45 μg (Table 4)

(g) *Hydra from Chandan Nagar*

(1) Colour — Translucent white

(2) Column — either cylindrical in shape or shows a slightly stalked condition (Fig. 1g) Average length is 4.86 mm but becomes 5.86 mm when stretched (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2g)

(4) Bud zone — 0.732 ± 0,03 mm from the basal disc (length of peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — Small (Fig. 3g)

(7) Tentacle

I. Number — varies between 4 and 6 (generally 4 ; Fig. 2g)

II. Average length — equal to the column but becomes $2\frac{1}{2}$ times as long when stretched (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — very high density (Table 3), randomly scattered (Fig. 4A, g)

(ii) Middle region of the tentacle — High density (Table 3), ill-defined annular arrangement (Fig. 4B, g)

(iii) Tip of the tentacle — High density (Table 3) closely packed nematocyte clusters, cylindrical apical tip (Fig, 4C, g)

(8) Protein content per hydra — 6.84 ± 0.41 μg (Table 4)

(h) *Hydra from Lucknow*

(1) Colour — Translucent white

(2) Colomn — either cylindrical or shows a slight stalked condition (Fig. 1, h). Average length 4.8 mm but becomes 6.1 mm when stretched (Table 1)

(3) Hypostome — Bluntly conical, subhypostome indistinct (Fig. 2h)

(4) Bud zone — 0.814 ± 0.05 mm from the basal disc (length of the peduncle ; Table 2, Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — small (Fig. 3, h)

(7) Tentacles

I. Number — varies between 4 and 6 (generally 4 ; Fig. 2h)

II. Average length — equal to the column but becomes almost 2 times its length when stretched (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — very high density (Table 3) randomly scattered (Fig. 4A, h)

(ii) Middle region of the tentacle — High density (Table 3) annular arrangement (Fig. 4B, h)

(iii) Tip of the tentacle — very high density (Table 3), clusters in close succession, extreme tip cylindrical shaped (Fig. 4C, h)

(8) Protein content per hydra — 6.78 ± 0.32 μg (Table 1)

(i) *Hydra from Delhi*

(1) Colour — Translucent white

(2) Column — Almost uniformly cylindrical, Average length 4.8 mm but when fully stretched becomes 6,06 mm

(3) Hypostome — small, conical, subhypostome never distinct (Fig. 2i)

(4) Bud zone — 0.800 ± 0.05 mm from the basal disc (length of penduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — Small (Fig, 3, i)

(7) Tentacles

I. Number — varies between 4 and 5 (generally 4 ; Fig. 2i)

II. Average length — equal to the column but becomes 2 times its length on stretching (Table 1)

III. Nematocyst density and arrangement

(i) Base and middle region of tentacle — very low density (Table 3) randomly scattered (Fig. 4A, i and 4B, i)

(ii) Tip of the tentacle — low density (Table 3) small clusters separated by large spaces. Apex slender with a small clump of nematocysts (Fig. 4C, i)

(8) Protein content per hydra — 7.56 ± 0.24 μg (Table 4)

(j) *Hydra from Calcutta*

(1) Colour — Translucent white

(2) Column — either cylindrical, or shows a slightly stalked condition (Fig. 1 j). Average length 6.05 mm long but becomes 7.65 mm in length when stretched (Table 1)

(3) Hypostome — Slightly flattened, subhypostome indistinct (Fig. 2j)

(4) Bud zone — 0.855 ± 0.04 mm from the basal disc (length of peduncle) (Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — Medium size (Fig. 3, j)

(7) Tentacles

I. Number — varies between 4 and 6 (Hydras with 4, 5 or 6 tentacles occurring with almost equal frequencies in the cultures)

II. Average length — always shorter than the column even when stretched (Table 1)

III. Nematocyst density and arrangement

(i) Base and middle of tentacle — Moderate density (Table 3), randomly scattered (Fig. 4A, j ; Fig. 4B, j)

(ii) Tip of the tentacle — moderate density (Table 3) large clusters, apex with closely packed clusters, forming a cylindrical apical club (empty area devoid of nematocyst seen in succession (Fig. 4C, j)

(8) Protein content per hydra — 9.84 ± 0.99 μg (Table 4)

(k) *Hydra from Shillong*

(1) Colour — Translucent white

(2) Column — Either cylindrical or slightly stalked aborally (Fig. 1-k). Average length 4.5 mm long but becomes 5.62 mm in length when stretched (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2k).

(4) Bud zone — 0.862 ± 0.08 mm from the basal disc (length of peduncle ; Table 2, Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — medium size (Fig. 3-k)

(7) Tentacle

I. Number — varies between 4 and 5 (generally 4, Fig. 2h)

II. Average length — equal to the column but becomes almost 2 times as long when stretched (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — Moderate density (Table 3) randomly scattered (Fig. 4A, h)

(ii) Middle region of the tentacle — High density (Table 3), clusters with small intercongregational spaces (Fig. 4B, h)

(iii) Tip of the tentacle— Very high density (Table 3), clusters very closely packed and intercongregational spaces almost absent, cylindrical apex (Fig. 4C, h)

(8) Protein content per hydra — 6.43 ± 0.66 μg (Table 4).

(l) *Hydra from Madurai*

(1) Colour — Translucent white

(2) Column — Either cylindrical or slightly stalked near the aboral end (Fig. 1, 1). Average length 4.95 mm but becomes 6.26 mm on stretching (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2,1)

(4) Bud zone — 0.776 ± 0.04 mm from the basal disc (length of peduncle) (Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basal disc — Small (Fig. 3, 1)

(7) Tentacle

I. Number — Varies between 4 and 5 (generally 4, Fig. 2, 1)

II. Average length — Almost equal to the column but becomes 2 times the column length when stretched fully (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — Moderate density (Table 3) randomly scattered (Flg. 4A, 1)

(ii) Middle region of the tentacle — Low density (Table 3), small clusters with large spaces in between (Fig. 4B, 1)

(iii) Tip of the tentacle — Very low density (Table 3) large clusters with small spaces in between. A small apical knob present (Fig. 4C, 1)

(8) Protein content per hydra — 7.26 ± 0.24 μg (Table 4)

(m) *Hydra from Santiniketan*

(1) Colour — Translucent white

(2) Column — Either cylindrical or slightly narrower near the aboral end (Fig. 1m). Average length 5.03 mm, but becomes 6.3 mm long when completely stretched (Table 1)

(3) Hypostome — Slightly flattened, subhypostome indistinct (Fig. 2m)

(4) Bud zone — 0.909 ± 0.07 mm from the basal disc (length of peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously on the bud

(6) Basat disc — Medium size (Fig, 3 m)

(7) Tentacle

I. Number — Varies between 4 and 5 (generally 4 ; Fig. 2 m)

II. Average length — Almost equal to the column but when fully stretched becomes almost 2 times its length (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — Very high density (Table 3) randomly scattered (Fig. 4A, m)

(ii) Middle region of the tentacle — High density (Table 3) ill-defined annular arrangement (Fig. 4B, m)

(iii) Tip of the tentacle — Very high density (Table 3) closely packed clusters forming a stout apical club (Fig. 4C, m)

(8) Protein content per hydra — 6.76±0.29 μg (Table 4)

(n) *Hydra from Jammu*

(1) Colour — Translucent white

(2) Column — Either uniformly cylindrical or slightly narrow near the aboral end (Fig. 1 n) Average lengrh 4.96 mm, but becomes 6.2 mm when completely stretched (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2n).

(4) Bud zone — 0.778 ± 0.05 mm from the basal disc (length of peduncle ; Table 2, Fig. 5)

(5) Order of emergence of tentacle on bud — Tentacle rudiments do not appear simultaneously

(6) Basal disc — small (Fig. 3 n)

(7) Tentacle

I. Number — Varies between 4 and 6 (generally 4 ; very rarely 6 ; Fig. 2, n)

II. Average length — Almost equal to the body column but becomes 2 times its length when stretched fully (Table 1)

III. Nematocyst density and arrangement :

(i) Base of the tentacle — High density (Table 3), randomly scattered (Fig. 4A, n)

(ii) Middle region of the tentacle — Low density (Table 3) ill-defined annular arrangement (Fig. 4B, n) large empty spaces present between the clusters

(iii) Tip of the tentacle — Low density (Table 3) annular arrangement with large spaces in-between successive circlets. A well defined apical knob present (Fig. 4C, n)

(8) Protein content per hydra — 7·26 ± 0.24 μg (Table 4)

(o) *White Hydra from Hyderabad*

(1) Colour — Translucent white

(2) Column — Either uniformly cylindrical or with a slightly narrower aboral region (Fig. 1, o). Average length 4.8 mm but becomes 5.96 mm long when fully stretched (Table 1)

(3) Hypostome — Rounded, subhypostome indistinct (Fig. 2, o)

(4) Bud zone — 0.873 ± 0.06 mm from the basal disc (length of peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudiments do not appear simultaneously.

(6) Basal disc — Elongated in shape (Fig. 3, o)

(7) Tentacle

I. Number — Varies between 4 and 5 (generally 4 ; Fig. 2, o)

II. Average length — Almost equal to the column but becomes 2 times as long when stretched fully (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — Very high density (Table 3), randomly scattered (Fig. 4A, o)

(ii) Middle region of the tentacle — Very high density (Table 3) annular arrangement (Fig. 4B, o)

(iii) Tip of the tentacle — Moderate density (Table 3) ill-defined annular arrangement. An apical knob present ; space between successive rows of nematocysts (Fig. 4C, o)

(8) Protein content per Hydra — 6.95 ± 0.41 μg (Table 4)

(p) *Hydra from Tirupati*

(1) Colour — Translucent white

(2) Column — Either cylindrical throughout or slightly narrower near the aboral end (Fig. 1p) Average length 4.53 mm long but becomes 5.8 mm when stretched fully (Table 1)

(3) Hypostome — Conical, subhypostome indistinct (Fig. 2, p)

(4) Bud zone — 0.798 ± 0.03 mm from the basal disc (length of peduncle ; Table 2 ; Fig. 5)

(5) Order of emergence of tentacles on bud — Tentacle rudimentsdo not appear simultaneously on the bud

(6) Basal disc — Small (Fig. 3, p)

(7) Tentacles

I. Number — Varies betweed 4 and 5 (generally 4 ; Fig. 2, p)

II. Average length — Is equal to the column but becomes almost $2\frac{1}{2}$ times as long when fully stretched (Table 1)

III. Nematocyst density and arrangement

(i) Base of the tentacle — High density (Table 3), randomly scattered (Fig. 4A, p)

(ii) Middle region of the tentacle — Moderate density (Table 3) annular arrangement (Fig. 4B, p)

(iii) Tip of the tentacle — Moderate density (Table 3), well demarcated rings with spaces in between successive rings. Apical knob is slender (Fig. 4C, p)

(8) Protein content per hydra — 6.35 ± 0.23 μg (Table 4)

DISCUSSION

In this study several characteristics of hydra at different levels of structural organisation have been taken into account to ascertain their reliability for taxonomic purposes. For the sake of comparison, values of different characteristic of Calcutta hydra have been considered as the starting point (0 value) and all other ecotypes are considered relative to this type. One of

the main reasons for selecting the Calcutta ecotype as the standard is the fact that it was this ecotype which was first classified as *Hydra vulgaris* phase orientales by Annandale (1911). Calcutta hydras used in the present study were also collected from the same locality as in the case of *H. vulgaris* after 71 years of its first collection. Ecotypes differing from each other by a difference of 20 per cent or more will be arbitrarily put into distinct ecological clusters. The Calcutta ecotype will always be considered as ecological cluster I, while the other groups will be in the descending order from +100 to −100 per cent. This will give an indication of the affinities between the different ecotypes on the basis of each of the characteristics analysed in this study. Various parameters studied in this investigation are discussed as follows :

1. *Size*—Hydra size has been estimated in terms of the length (Table 1) and the total protein content (Table 5). Calcutta polyps are significantly large and the Green hydras from Hyderabad are the smallest while all other types fall within a narrow range. It is important to note that the polyp size varies depending particularly on the feeding rate and the ambient temperature (Stiven, 1962 ; Schulze and Lesh, 1970 ; Park and Ortmeyer, 1972 ; Bisbee, 1973). In the present study although the number of *Artemia* nauplii caught was found to vary in the different hydra types, the number finally ingested did not vary significantly. Therefore, it is clear that the differences in size, exhibited by the hydra types is not due to differences in feeding rate. Moreover, it has also been observed that under standard conditions specified by Loomis and Lenhoff (1956) hydra body size and cell number remain relatively constant. Earlier investigators (Hyman, 1929, 1930 ; Caleb, 1956) considered body size of freshly collected hydras as a diagnostic character. Size differences observed in these cases could be the result of differences in feeding and climatic conditions. Comparison of the total protein content (Table 4) indicates that the ecotypes fall into 4 clusters :

Ecological clusters	*Ecotypes*
I	Calcutta
II	Delhi, Trivandrum, Imphal, Madurai, Jammu, White hydra from Hyderabad
III	Srinagar, Chandan Nagar, Santiniketan, Lucknow, Shillong, Tirupati, Pune, Bolpur
IV	Green hydra from Hyderabad

2. *Number of tentacles*—The tentacle number varied widely within an ecotype. In the Green hydra from Hyderabad, tentacle number varied between 6 and 7, although, generally 6 tentacled polyps were found. In all other hydra types tentacles number varied between 4 and 6. However, polyps with 4 tentacles were most frequent in all hydra types except the Calcutta hydra in which hydras with 4, 5 or 6 tentacles were observed to occur with almost equal frequency. Earlier work has shown the number of tentacles is influenced by factors such as the rate of feeding (Otto and Campbell, 1977), and certain chemicals like lithium chloride, chloretone (Ham and Eaken, 1958) and colchicine (Corff and Burnett, 1969). The influence of environmental factors on the number of tentacles has been shown by Park (1900) who observed that the tentacle numbers of *H. fusca* differed from those of *H. viridis* and also different populations of the same species taken from different locations had different mean tentacle number. Shostak *et al.* (1978) found that under laboratory conditions variations in tentacle number found in the population could not be attributed to changes in individual tentacle numbers but were due to variations resulting from the production of buds with different tentacle numbers. Hyman (1929, 1930, 1938), Ewer (1948), Forrest (1963), Caleb (1956) used the number of tentacles in freshly collected hydras as a taxonomic character. However, it is obvious now that the tentacle number is not only influenced by a number of factors but its genetic control is also rather complex. Hence the number of tentacles in a polyp may not provide us reliable information as to the species status of an ecotype.

3. *Length of the tentacle*—Average tentacle length was found to be 37% of the body length in the Green hydra for Hyderabad, 67% in the Calcutta and between 81-113% in all other hydra types (Table 1).

Thus, the length of the tentacle has been found to be specific for different ecotypes. Earlier investigators (Hyman, 1929, 1930 ; Caleb, 1956) have used this parameter as a diagnostic character. The present results show that this character is consistent within each ecotype and therefore, confirms that this is a reliable character and can be used for taxonomic purposes.

Comparison of the relative tentacle length (Table 1) between the different ecotypes shows that there are 4 ecological clusters. Green hydra from Hyderabad has smaller tentacles than Calcutta hydra, while all other have longer tentacles.

Ecological clusters	*Ecotypes*
I	Calcutta
II	Tirupati, Trivandrum, Srinagar, Chandan Nagar, Shillong

Ecological clusters	*Ecotypes*
III	Bolpur, Delhi, Madurai, Santiniketan, Jammu, Imphal, Lucknow
IV	Green hydra from Hyderabad

Pune and White hydra from Hyderabad fall between clusters II and III

4. *Nematocyst density and their arrangement in the tentacles*—Microscopic examination of the density of nematocysts on the tentacles showed distinct differences in the various ecotypes (Table 3). In studying the nematocyst distribution across the tentacles it was observed that more discrete arrangement is found in the tip region of each tentacle whereas middle and base regions appear to have more uniform distribution. While the density may vary at the base and middle regions, the arrangement generally confirms to a pattern of homogenous distribution. Another significant point is the presence or absence of space between the successive clusters of nematocysts. Lastly apart from the arrangement of nematocysts the shape of tentacles particularly at the anterior region seems to bear some ecotypic diagnostic feature. On comparison of the relative density of nematocysts (established by visual estimation ; Table 3), 5 ecological clusters are found to be present.

Ecological clusters	*Ecotypes*
I	Calcutta, Tirupati, Trivandrum, Jammu.
II	Lucknow, Santiniketan
III	Chandan Nagar, Shillong
IV	Pune, Delhi, Imphal
V	Madurai and Bolpur

Green and white hydras from Hyderabad, and hydras from Srinagar fall between clusters II and III

5. *Position of the Budding region and the order of emergence of tentacles in the bud*—The most significant phenotypic character of hydra revealed in this study and which may be a reliable taxonomic character is the position of the budding region. The bud zone in each hydra type was found to be constant and distinctly located. While it was almost in the middle of the column in the Green hydra from Hyderabad, in the hydra from Srinagar it was very near to the basal disc (Fig. 5). In all other types, the position was between 0.673 and 0.9 mm from the basal disc.

Comparison of the distance between the basal disc and the bud zone (Table 3) relative to the hydra from Calcutta indicates that the ecotypes fall into 5 clusters.

Ecological clusters	*Ecotypes*
I	Calcutta, Lucknow, Delhi, Madurai, Jammu
II	Green Hydra from Hyderabad
III	Santiniketan
IV	Imphal, Chandan Nagar, Pune, Trivandrum, Bolpur, Tirupati
V	Srinagar

Hydras from Shillong and White hydra from Hyderabad fall between group I and III.

Although it has been noted that a new bud is formed at a certain distance from the head and foot in tissue of rather uniform composition (Mookerjee and Sinha, 1967 ; Bode *et al.*, 1973), and the location of the bud region in some species of hydras has been recorded (Berking and Gierer, 1977), the significance of bud zone in species identification has not been previously used as a parameter. The occurrence of distinct bud zones in different ecotypes assumes significance in view of the possibility long recognized that budding occurred at a specific position in the column because everywhere else the inhibition of either head or foot formation was too great (Child and Hyman, 1919 ; Hyman, 1928). According to this view the gradient of inhibition provided the mechanism through which the budding region became physiologically localised from the dominent region of the animals. More recently evidence supporting the inhibitory power of the head and foot with regard to the budding region, has been provided by Burnett (1961), Shostak (1970), Kass-Simon and Potter (1971).

Recently, hydra tissue was found to contain a substance which has an inhibitory action on budding (Berking, 1977). When added to the culture medium it prevented bud formation. This substance occurs naturally in the animal and exerts its effect in very low concentrations. It is, therefore, likely to be involved in the regulation of bud development, possibly in the spatial determination of the bud position. Since both the stimulatory (Shostak, 1974) as well as inhibitory (Berking, 1977) activities have been implicated of the regulation of budding and possibly the position of bud zone, the occurrence of a characteristic budding region in the polyp may provide a valuable diagnostic clue in the identification of different species.

Depending on the order of emergence of tentacles on the bud only two groups were obtained. In Green hydra from Hyderabad tentacle rudiments appeared simultaneously on the bud. In all other ecotypes the tentacle rudiments arrived unevenly on the buds. A number of investigators (Hyman,

1929, 1930, 1931 ; Campbell, 1983) have used this character in the classification of hydra.

6. *Shape and size of the basal disc*—It is long and broad in the hydra from Imphal and Pune, elongated in the White hydra from Hyderabad, of medium size in the hydras from Shillong, Calcutta, Srinagar, Trivandrum and Green hydra from Hyderabad and small in the hydras from Delhi, Jammu, Tirupati, Madurai, Lucknow, Chandan Nagar and Bolpur. Basal disc appears to have a characteristic shape and size in different ecotypes. It has previously been found to be an important feature in *H. pseudolegactis* where the basal disc is of an elongated shape and clearly demarcated from the peduncle (Burnett and Lambrushi, 1973). The consistency of the shape and size of the basal disc within this species was also noted. Since the 16 ecotypes fell into 3 groups on the basis of the typical shape and size of the basal disc ; this character may be a reliable taxonomic character.

TABLE 5. Projection of major morphological characters of the various ecotypes

Hydra type	Tentacle length	Nematocyst density	Bud position	Origin of tentacle rudiments	Basal disc size	Protein content
Ghy	IV	II & III	II	SO	M	IV
Imp	III	IV	IV	UO	L	II
Triv	II	I	IV	UO	S	II
Bol	III	V	IV	UO	M	III
Sri	II	II & III	V	UO	M	III
Pun	II & III	IV	IV	UO	L	III
CN	II	III	IV	UO	S	III
Luc	III	II	IV	UO	S	III
Del	III	IV	I	UO	S	II
Cal	I	I	I	UO	M	I
Shil	II	III	I & III	UO	M	III
Mad	II	V	I	UO	S	II
San	III	II	III	UO	M	III
Jam	III	I	I	UO	S	II
Why	II & III	II & III	I & III	UO	L	II
Tiru	II	I	IV	UO	S	III

Abbreviations :

I to V — Ecological cluster number of each character (see p. 26-30)

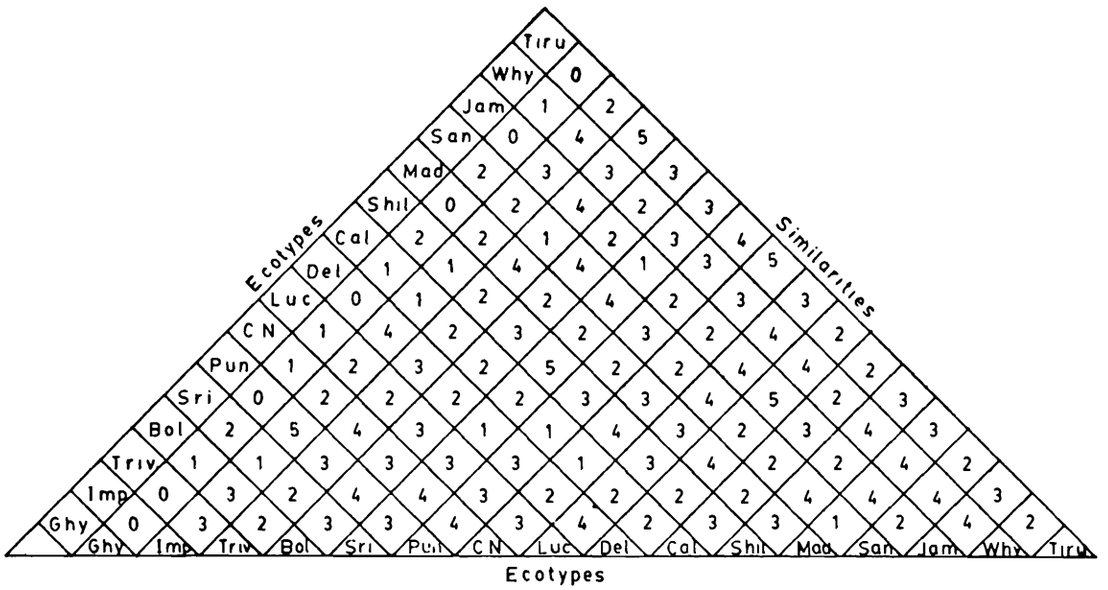
SO — simultaneous origin of tentacle rudiments

UO — uneven origin of tentacle rudiments

S — small ; M — Middle ; L — Large

An interecotypic reckoner based on similarities in six diagnostic morphological characteristics (Table 5) among the 16 ecotypes has been put forward to understand their magnitude of semblances (Table 6).

Table 6
Magnitude of inter-ecotypic affinities at morphological level



SUMMARY

In this paper morphological characteristics of 15 ecotypes of hydra have been analysed. Certain structural features remain fairly consistent in this organism and can therefore be used for identification of an ecotype. With two exceptions (hydra from Delhi and green hydra from Hyderabad) the shape of the colomn was found to be inconsistent within an ecotype. Characters found to be consistent within an ecotype were : length of the tentacles, density of nematocysts, arrangement of nematocysts in the tip region of the tentacles, position of the budding region, order of emergence of tentacle rudiments and shape and size of the basal disc.

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FIGURES

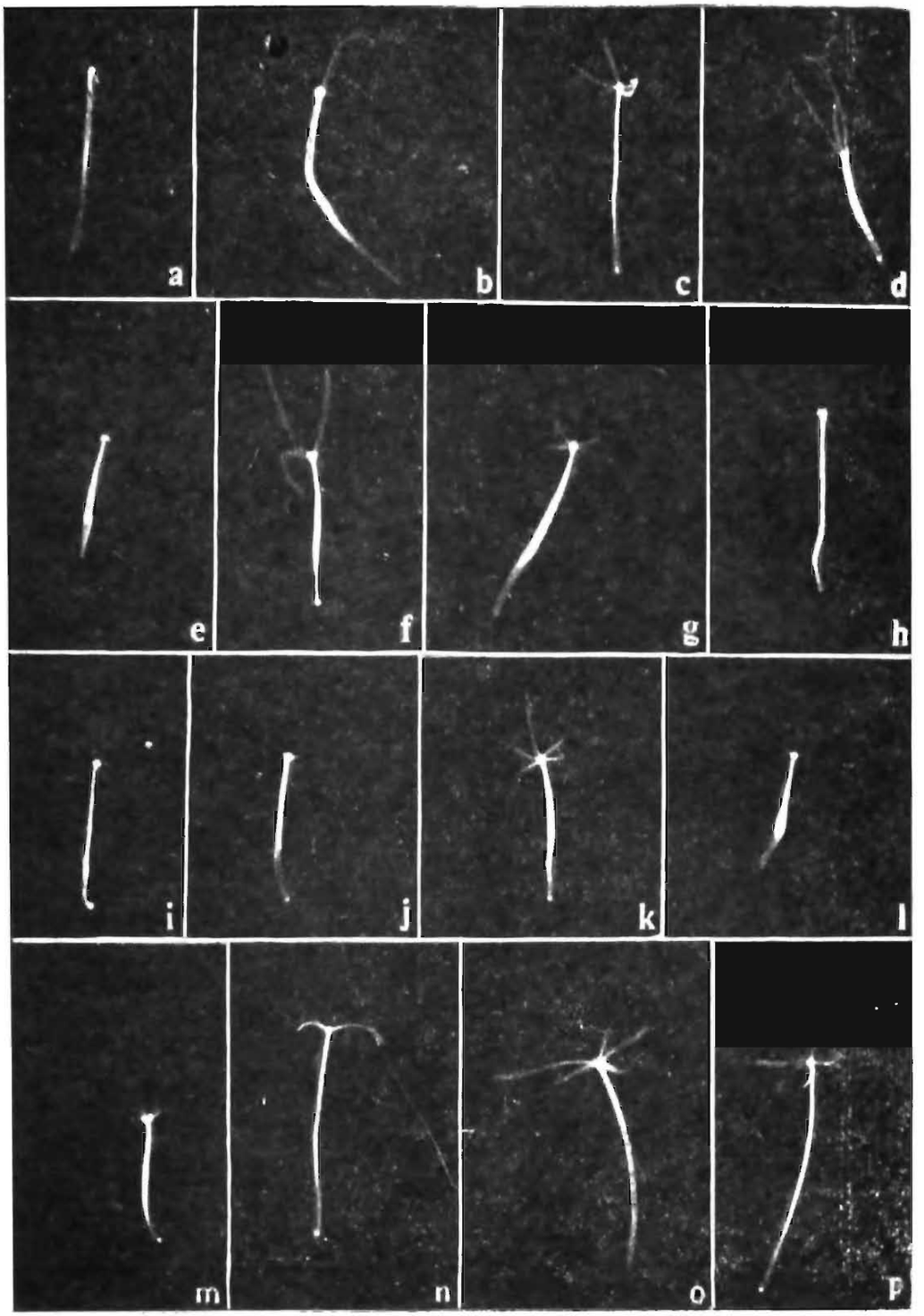


Fig. 1 : Photographs of 16 ecotypes in living condition (X 5.5)

a— Green hydra from Hyderabad ; b— Imphal ; c— Trivandrum ; d— Bolpur ; e— Srinagar ; f— Pune ; g— Chandan Nagar ; k— Shillong ; l— Madurai ; m— Santiniketan ; n— Jammu ; o— White hydra from Hyderabad ; p— Tirupati.

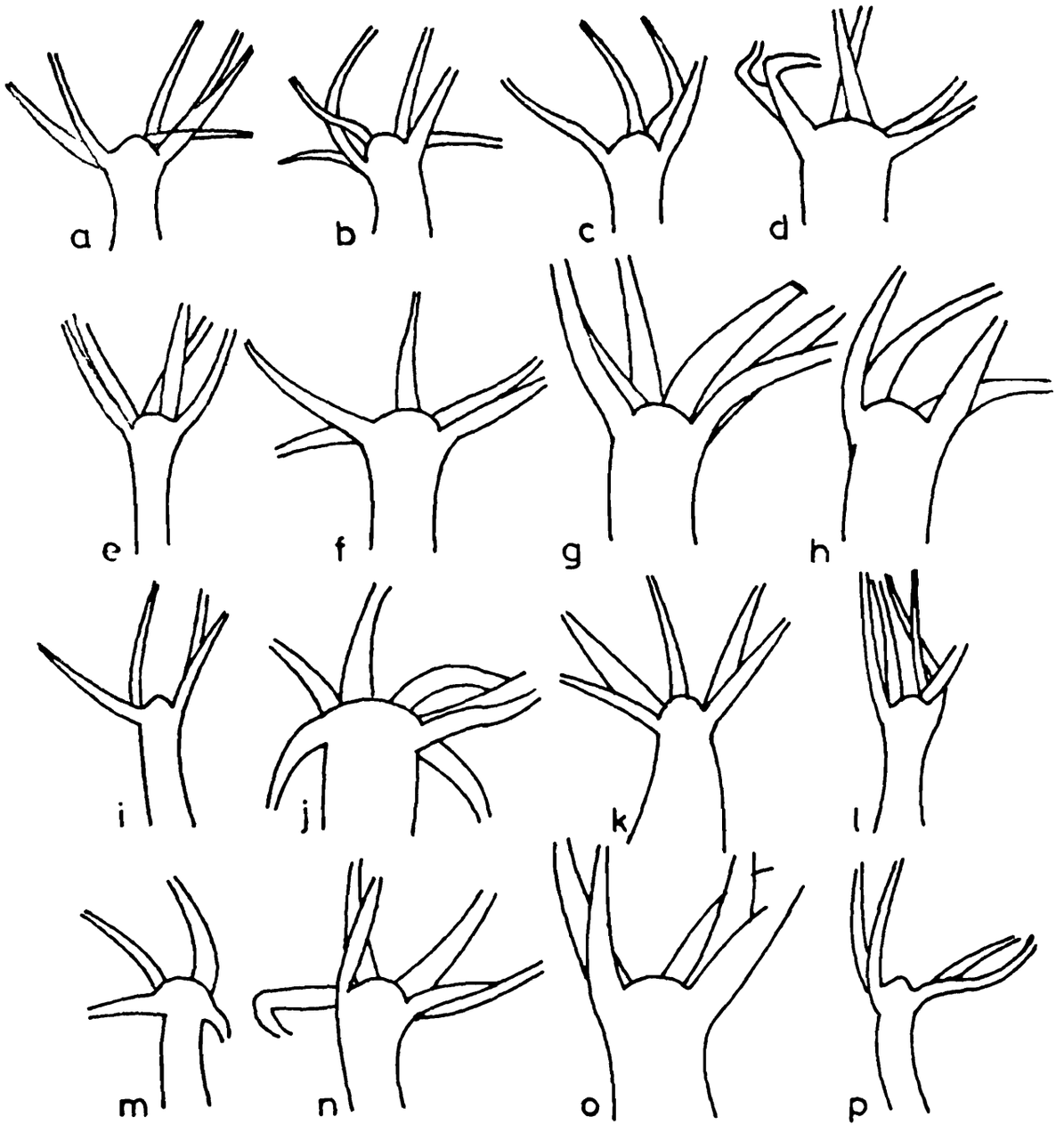


Fig 2 : Configuration of the hypostome (traced from photographs of the whole mount preparation) of the 16 hydra ecotypes showing the number of tentacles ; shape of the hypostome and sub-hypostome region (X 24)

a— Ghy ; b— Imp ; C— Triv ; d— Bol ; e— Sri ; f— Pun ; g— C. N. ; k — Shil ; l— Mad ; m— San ; n— Jam ; o— Why ; p— Tiru.

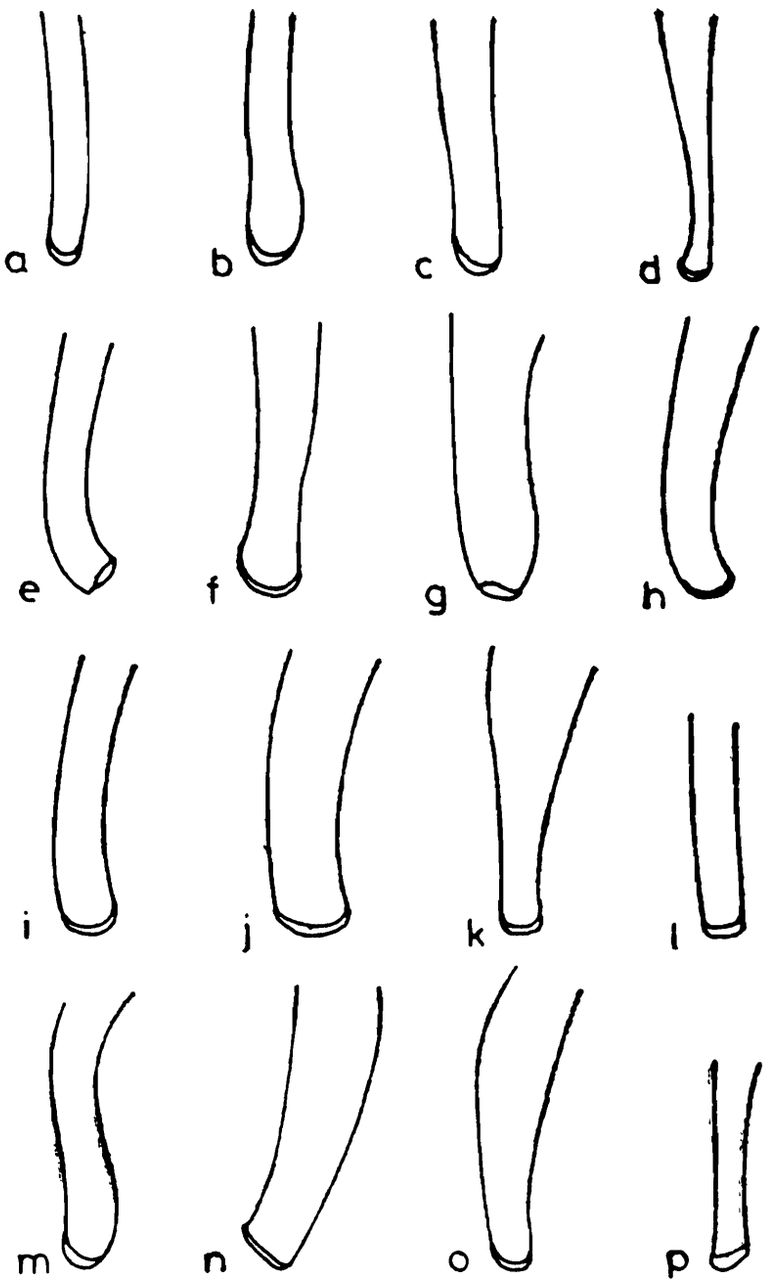


Fig. 3 : Configuration of the basal discs (traced from photographs of the whole mount preparation) of the 16 hydra ecotypes, showing the shape of the peduncle and basal disc (X 24)

a— Ghy ; b— Imp ; c— Triv ; d— Bol ; e— Sri ; f— Pun ; g— C. N. ; h— Luc ; i— Del ; j— Cal ; k— Shil ; l— Mad ; m— San ; n— Jam ; o— Why ; p— Tiru.

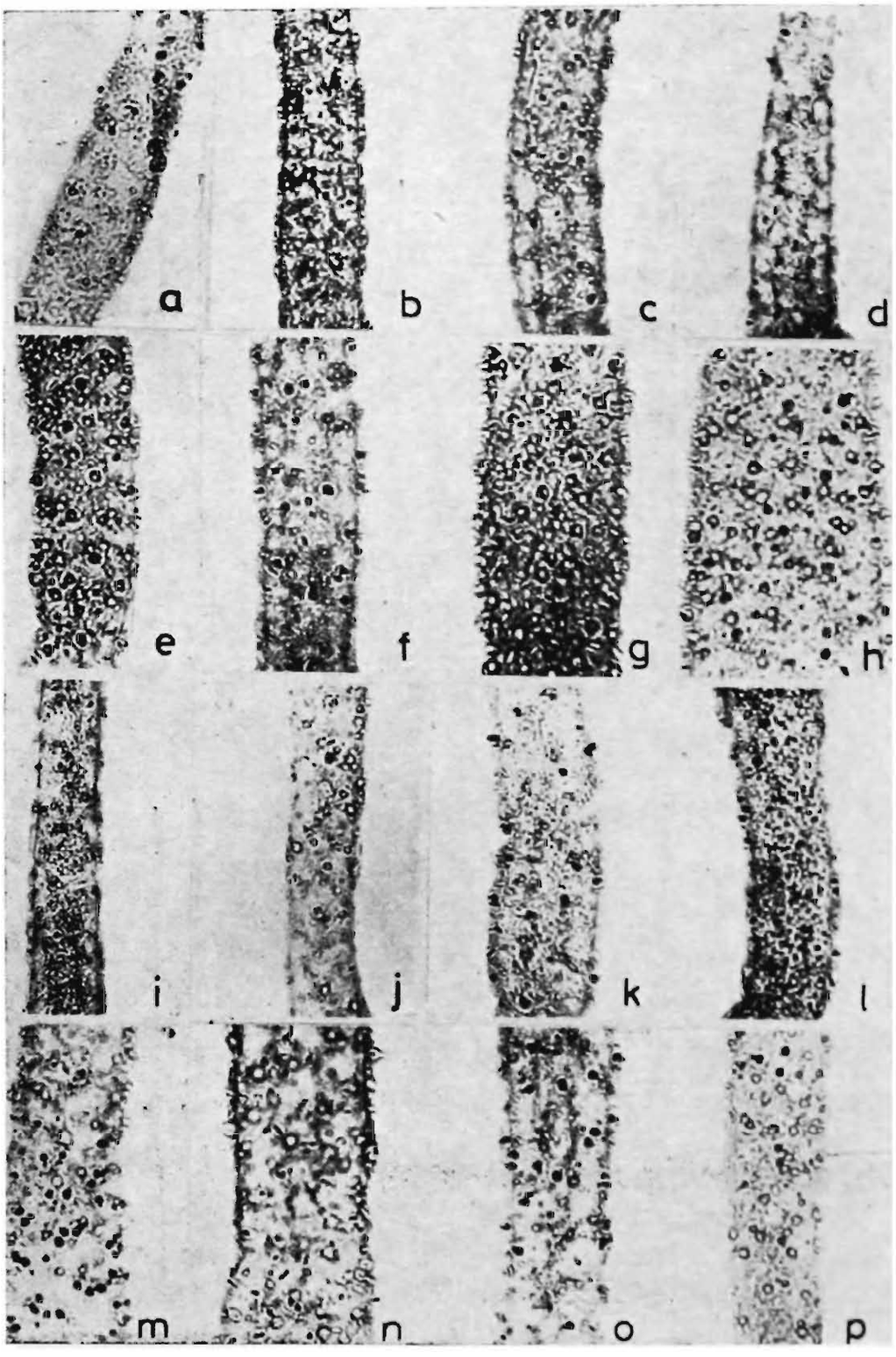
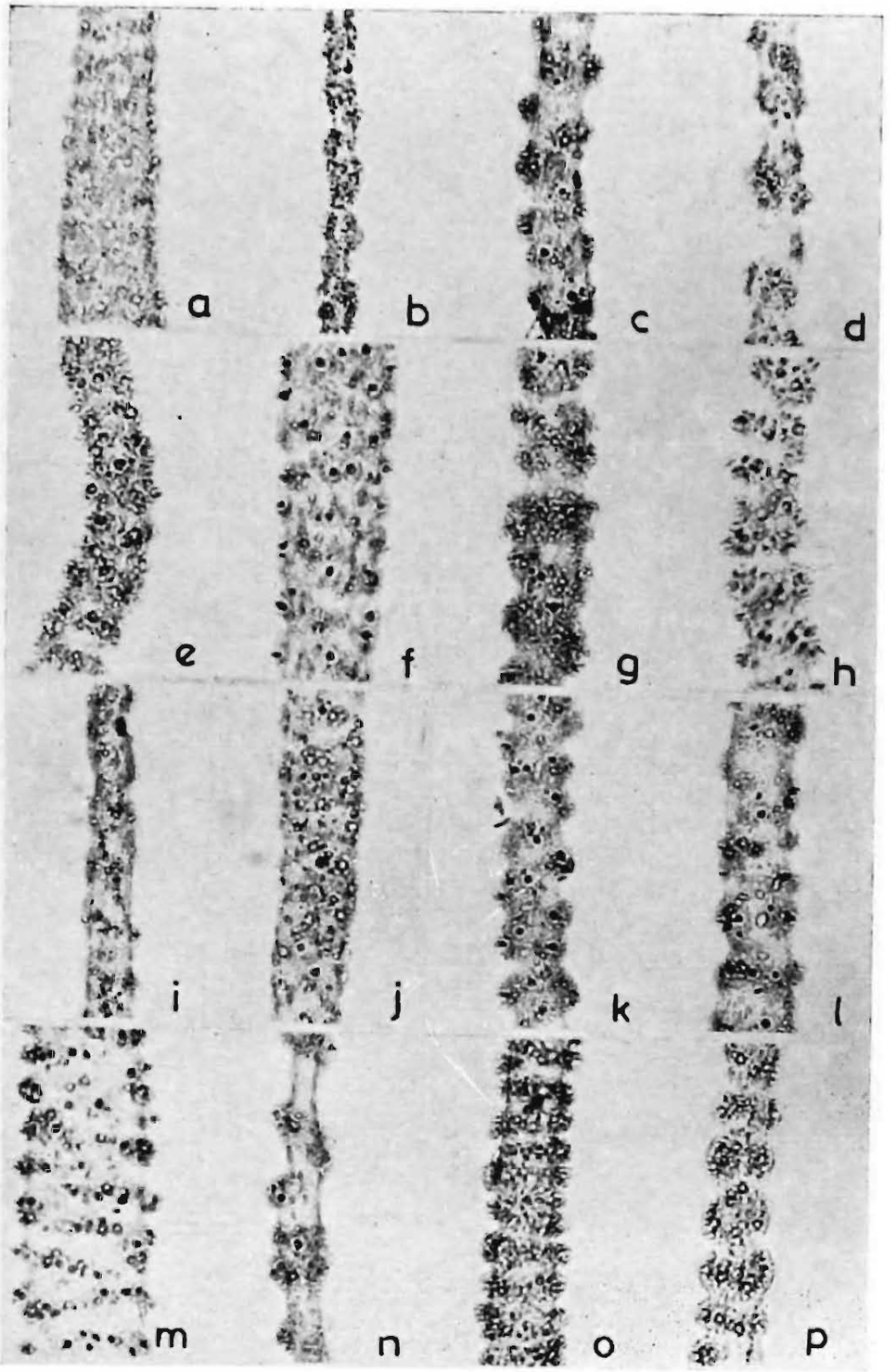


Fig. 4 : Photographs of the portions of tentacles to show the nematocyst density and arrangement in tentacles of 16 hydra ecotypes (whole mount preparation X 167)

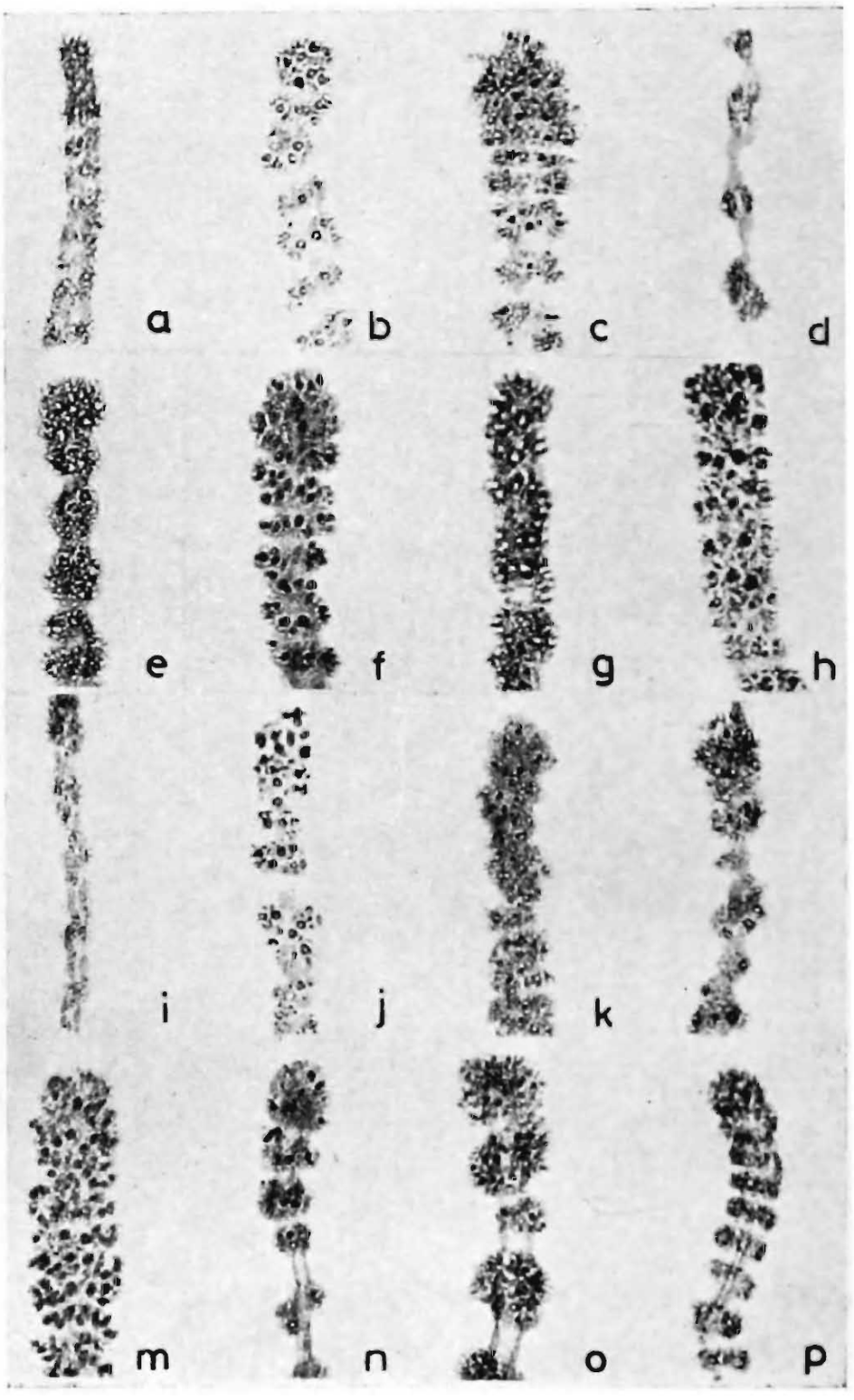
4A— Basal part

a— Ghy ; b— Imp ; c— Triv ; d— Bol ; e— Sri ; f— Pun ; g— C. N. ; h— Luc ; i— Del ; j— Cal ; k— Shil ; l— Mad ; m— San ; n— Jam ; o— Why ; p— Tiru.



4B— Middle part (X 167)

a—Ghy ; b—Imp ; c—Triv ; d—Bol ; e—Sri ; f—Pun ; g—C. N ; h—Luc ; i—Del ; j—Cal ; k—Shil ; l—Mad ; m—San ; n—Jam; o—Why ; p—Tiru.



4C— Tip part (X 167)

a—Ghy ; b—Imp ; c—Triv ; d—Bol ; e—Sri ; f—Pun ; g—C. N ; h—Luc ; i—Del ; j—Cal ; k—Shil ; l—Mad ; m—San ; n—Jam ; o—Why ; p—Tiru.

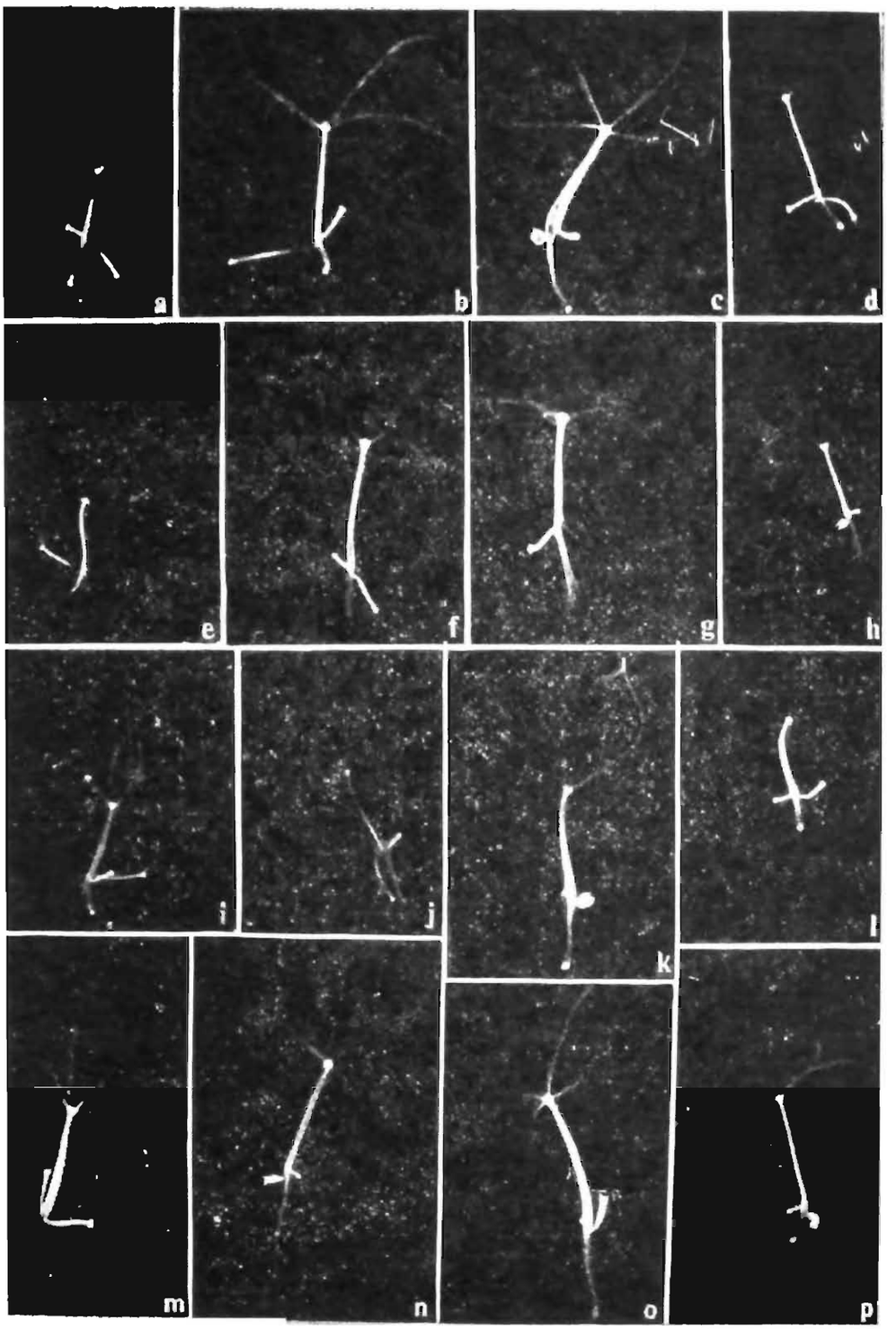


Fig. 5 : Photographs to show the position of the budding zone along the body column of living hydra ecotypes (X 4.5)

a— Ghy ; b— Imp ; c— Triv ; d— Bol ; e— Sri ; f— Pun ; g— C. N. ; h— Luc ; i— Del ; j— Cal ; k— Shil ; l— Mad ; m— San ; n— Jam ; o— Why ; p— Tiru.

MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRA

Part III : Understanding the ecotypic differences at cell level

Introduction

Hydra is characterised by the participation of a few distinct cell types unlike a multicellular organism where there are large number of cell types knitted together in the make up of the organism. To take advantage of the so-called simplistic organisation in hydra attributed only by a few types of cells, in building its form, the recent maceration technique of David (1973) was employed in unearthing the hidden mystery of the density of the different cell types, cell size and other cellular characteristic found in the different ecotypes. The idea has been to elicit as many morphogenetic parameters based on cell differences that can be warranted by demarcating the ecotypes and be used for species-making criteria in hydra. Important cell types like epithelial cells, and mucous cells, interstitial cells, nerve cells, nematocytes and nematocyte precursors were taken into consideration. Five ecotypes were analysed for estimation of density of different cell types. They were : (I) Green hydra from Hyderabad ; (II) hydra from Bolpur ; (III) hydra from Srinagar ; (IV) hydra from Madurai, and (V) hydra from Calcutta. Such basic information is expected to provide clues in unravelling ecotypic differences in hydra at the cell level.

Material and Methods

For analysis of cellular characteristics, 5 ecological types of hydra—Green hydra from Hyderabad, and hydras from Bolpur, Srinagar, Calcutta and Madurai were selected.

The cellular composition of hydras was analysed using the maceration technique of David (1973). Since tentacles were more resistant to dissociation, hydra column and hypostome were characterized separately. The hypostome region (including hypostome and tentacles) was amputed just proximal to the base of the tentacle.

Maceration Procedure

(i) *Tissue dissociation*—The tissue was macerated in a maceration solution containing glycerine : glacial acetic acid : water (1 : 1 : 13). Hydras without hypostomes were placed in a small glass tube. Since the hydra size varies the number of hydra columns taken per tube was 3 in case of hydra from Calcutta, 4 in case of Bolpur, Srinagar and Madurai types and 6 in case of Green hydra from Hyderabad. Excess medium was drawn off with a very

fine pasture pipette and 0.1 ml of maceration solution added. After 2-3 minutes, the tubes were gently tapped to dissociate the tissue. The sample was checked for complete dissociation under a dissecting microscope.

In case of hypostome tissue, 20 hypostomes were taken in 0.1 ml of maceration solution and soaked for about ten minutes before tapping.

(ii) *Cell fixation and preparation of cells spreads for counting*—After dissociation, 10% formalin (0.1 ml) was added to the tube. On a subbed microscope slide, 1% solution of the detergent Tween 20 (10 μl) was put and cell suspension (50 μl) was added to it. The mixture was uniformly spread over a marked area of 300 mm^2 (20 × 15 mm), with the help of a fine needle. After spreading the slides were placed on a level wooden platform (level checked by means of a spirit level) so that the suspension dried evenly and that drying was not assymetric.

(iii) *Measurement of the cell size*—After drying of the cells spreads at room temperature, a drop of 10% glycerine and a coverslip was placed on the slide and the preparation examined using 25 × objective lens in the phase contrast microscope (Leitz Orthoplan Microscope). Cell size was measured using a micrometer (1 division = 3.8 μm) placed in the eyepiece of the microscope. Cell dimension thus measured in maceration preparation are about 30% larger than cell dimensions observed in histological sections of formaldehyde fixed hydra. This is due to swelling effect of of acetic acid (David, 1973). Photographs (at 25x, 40x and 100x (oil immersion) were taken in the phase contrast microscope.

(iv) *Data Analysis*—Cell composition in the macerated samples of columns of hydras was analysed in 6-8 cell preparations per hydra type. Different cell types were counted in at least 20 fields at 25x. In case of hypostome 20 pieces were macerated and at least 2-4 such preparations were observed. During each observation, the boundaries of the cell spread were confirmed using the stage micrometer.

The number of cells of each type per hydra was calculated as follows :

$$\text{Total number of cells (one type) per piece} = \frac{Y \times a \times V}{n \times v \times \pi r^2}$$

where,

Y = Average number of cells per field
a = Area of cell spread = 300 mm^2
V = Total volume = 200 μl
n = Total number of tissue pieces used
v = volume per spread = 50 μl
r = radius of the circular field = 0.35 mm.

RESULTS

In the dissociated conditions, the characteristic morphology of all basic cell types could be easily identified and compared. Except for the nematocysts, the dimensions of the different cell types was found to be same in all hydra types. For easy identification of the cell types a description of the various cell types in hydra is as follows :

(a) Epithelial cells included epithelio-muscular and epithelio-digestive cells as well as battery cells in the tentacle (Fig. 1a-d). In ectoderm were present large cuboidal or columnar cells length in the range of 42-83μm and width 13-38 μm with two long muscle processes at their proximal end and a large nucleus with 1 or 2 nucleolus at the centre or to the proximal end.

In endoderm were present tall, columnar (57-102 μm long, 15-30 μm wide) epithelio-digestive cells with short muscle processes at the proximal 1 end, a large number of vacuoles and granules in the cytoplasm and a large nucleus was present usually near the centre (Fig. 1a, b).

Battery cells present in the tentacle were short (20-31 μm) and wide 41-60 μm) containing 10-20 nematocytes (Fig. 1d).

In the Green hydras from Hyderabad, a number of algal cells (11-22) were seen in the epithelio-digestive cells (Fig. 1b).

(b) Gland and mucous cells : Gland cells were oval or tear-drop in form (19-42μm long ; 11.4-19μm wide) with cytoplasmic granules, presence of nucleus usually with a nucleolus in the proximal end of the cell (Fig. 1p).

Mucous cells found mainly in the hypostome were long (15.3-31 μm) and narrow (7-9 μm wide) with cytoplasmic granules, and a small nucleus present at the proximal end.

(c) Interstitial cells : Big interstial cells were 15.2-19μm long and 7·6-13.3μm wide, with a large nucleus with conspicous nucleolus (Fig. 1f). Little interstitial cells were 7.6-13.3 μm long and 7.6-11.4 μm wide with a small nucleus without a nucleolus (Fig. 1e).

In maceration preparation, interstitial cells occurred frequently in clusters of 2, 4, 8, 16 and 32 (Fig. 1i).

(d) Nematocyte precursors included nests of 4, 8 and 16 cells (nematoblasts) and nests of post-mitotic cells in which nematocyte capsules were differentiating (differentiating nematocytes) (Fig. 1m, n, o).

(e) Nerve cells : In phase contrast microscopy neurosecretory and ganglionic cells are indistinguishable. Ganglionic cells had a small cell body (7.6-15.2 μm) and a long process (15.2-45.6 μm) were bipolar or multipolar (Fig. 1r, s). Sensory cells had a small cell body (7.6-9.5um)

with a long process at one end (64.6-95 μm) and a short cytoplasmic extension ending in a bulb-like thickening at the other end (Fig. 1q).

(f) Nematocyst : They were of 4 types—stenoteles, desmonemes, holotrichous isorhizas and atrichous isorhizas. Stenoteles varied in size in each hydra ecotype depending on the stage of maturity. The range of stenotele size found in Green hydras from Hyderabad (11.4-15.2 μm capsule length) was smaller as compared to the other types (15.3-19 μm capule length). The length of the thread was in the range of 13.3-26.6 μm in all ecotypes.

(ii) Desmoneme : It was a small nematocyst, many times smaller than stenotele (Fig. 1c). It did not appear to vary significantly in different ecotypes.

(iii) Isorhizas :

I. Atrichous isorhizas was found to be a small nematocyst (Fig. 2d) and did not vary significantly in the different ecotypes.

II. Holotrichous isorhiza were of four main types : slipper shaped, cylindrical, pyriform and bean shaped. They were studied in all the 16 ecotypes.

In Green and White hydras from Hyderabad, hydras from Lucknow Santiniketan and Jammu the holotrichous isorhizas were slipper shaped. In hydras from Imphal, Trivandram, Bolpur, Srinagar, Pune, Chandan Nagar, Calcutta, Shillong, and Madurai the capsule was cylindrical in shape. In the hydras from Delhi, the capsule was pyriform in shape while in hydras from Tirupati it was bean shaped (Fig. 3).

III. Cell composition of Hydra : Classification of cells which differentiate from one type to another and were in an in-between stage becomes ambiguous. Hence dividing nemotoblasts and post-mitotic nematoblasts have been considered together under the category of nematocyte precursors. All big and little interstitial cells occurring singly have been put in IS category and all big and little interstitial cells occurring in pairs 2S category.

Epithelio-muscular, epithelio-digestive and the battery cells have been put in the category of epithelial cells. Similarly gland and mucous cells and ganglionic and neurosecretry nerve cells have been considered under the categories of gland cells and ganglionic cells, respectively.

Cell composition analysis has been taken up separately for the body column and hypostome of five hydra types.

(a) *Counts of Total Number of Cells per Hydra* :

The total number of cells per hydra was obtained by adding the average value obtained for the total number of cells in the hypostome and column

of each hydra type. It was found to be maximum for the Calcutta hydra (31501) followed by the hydra from Madurai (29016) and Srinagar (28780). The number of cells was slightly low in the hydra from Bolpur (23439) and found to be lowest in the Green Hyderabad hydra (20630) (Fig. 4).

(i) Total number of cells per hypostome—It was maximum in the hydra from Calcutta (5841-6343). This was followed by the hydra from Madurai (3608-4603), Srinagar (3570-4033), Green Hyderabad (3069-4202) and Bolpur (3091-3462). (Fig. 4)

(ii) Total number of cells per column—The values for the hydras from Calcutta, Madurai and Srinagar, fall in a close range being 21467-26951 : 23964-26442 and 22680-27573 respectively. The hydra from Bolpur had 16284-20960 cells and the Green hydra from Hyderabad polyps had the minimum number of cells in the column (14792-19295) (Fig. 4).

(b) *Total Number of Epithelial Cells in the Hypostome and Column of each Hydra type*

(i) *Hypostome* :

Total number of epithelial cells was maximum in the Calcutta hydra (2662-4102) and less in the Madurai (1835-2267) hydra. The Bolpur hydra had 1441-1565 cells, the Srinagar hydra had 1241-1357 cells and the Green Hyderabad polyp showed the minimum number of cells (940-1011) (Fig. 5).

(ii) *Column* : Here the epithelial cell composition in the five hydra types was 8882-11038 , 8234-9423 ; 5805-7046 ; 5181-7012 and 2986-3791 in the Calcutta, Madurai, Srinagar, Bolpur and Green Hyderabad polyps respectively. (Fig. 5)

(c) *Density of Gland plus Mucous cells, Nerve cells, Stem Cells plus Nematocyte Precursors and Nematocysts in the Hypostome and Column of each Hydra type* :

(i) *Hypostome* :

Gland plus mucous cells : The densities of these cells were in a very close range for the Srinagar, Green Hyderabad, Madurai, and Bolpur hydras, being hetween 41.65 ± 2·47 and 49.063 ± 3.39. The Calcutta hydra displayed the least number of gland and mucous cells (18.24 ± 1.65) (Fig. 6).

Nerve cells : Hypostomes of Srinagar, Bolpur and Madurai hydras had nerve cell densities close to each other (53.908 ± 5.89 – 58.96 ± 4.13). Green Hyderabad hydra had less (37.63 ± 5.9) and Calcutta hydra had least number of nerve cells (16.01 ± 1.91) (Fig. 6).

Stem cells and nematocyte precursors : These cells had a low density in the range of 5.3-10.1 in the hypostomes of all hydra types.

Nematocysts : Maximum density of nematocysts was found in Green Hyderabad hydra (190.8 ± 26.78). It was less in Srinagar (90.71 ± 9.23) and quite low in the Madurai and Calcutta hydras (39.59 ± 3.45 to 41.88 ± 3.50). In Bolpur hydra nematocyst density was quite low being only 17.35 ± 0.72 (Fig. 6)

(ii) *Column* :

Gland and mucous cells : The density of these cell types was almost similar being in the range of 12.41-14.0 in all the five ecotypes (Fig. 7).

Nerve cells : The density of nerve cells was low in the column of all the five hydra types. The Srinagar hydra had the maximum (13.09 ± 1.03) and the Bolpur hydra had minimum density (4.67 ± 0.29). In the remaining hydras, the density of nerve cells was between 6.13 and 9.99. (Fig. 7)

Stem cells and nematocyte precursor : The density of stem cells and nematocyte precursors was high in the columns of all the five hydra types. The density was maximum in the Green Hyderabad (292.42 ± 14.51) and minimum in the Calcutta hydra (129 ± 10.5). In other three hydra types the range was between 177 and 280 (Fig. 7).

Nematocysts : The density was low in the column, being maximum in the Green Hyderabad hydra (40.98 ± 1.64), less in Srinagar hydra (20.39 ± 0.809) and very low in the Madurai (13.8 ± 0.05), Bolpur (11.11 ± 0.443) and the Calcutta hydra (5.125 ± 0.29) (Fig. 7).

(d) *Density of the Sensory and Ganglionic Nerve Cells in the Hypostome and Column* :

Density of ganglionic nerve cells was almost fifty times that of sensory nerve cells in the hypostome, in all the five hydra types. The values for ganglionic cells were 57.5, 54.9, 51.5, 35.79 and 15.48 in the Srinagar, Bolpur, Madurai, Green Hyderabed and Calcutta hydras repectively. The density of sensory nerve cells was between 0.6-2 in these five hydra types. In the column, ganglionic cells were present in maximum density in the Srinagar hydra (11.64 ± 0.67) and were minimum in the Bolpur hydra (4.043 ± 0.29). In the Calcutta, Green Hyderabad and Madurai hydras, the density was between 6.34-8.27 (Fig. 8).

(e) *Density of each Nematocyst type in the Hypostome and Column of the Various Hydra Types* :

(i) *Hypostome* : The density of stenotele nematocysts was maximum in the Green Hyderabad hydra (45.63 ± 5.09) and minimum in the Bolpur hydra (8.31 ± 0.76).

Density of desmoneme was always higher than stenotele in the Green

TABLE 1. Total number and relative density of stem cells and nematocyte precursors in the column of the five hydra types

Sl. No.	Hydra type	1S (±S.E.M.)		2S (±S.E.M.)		Nematocyte precursors (±S E.M.)	
		Total number	Relative density	Total number	Relative density	Total number	Relative density
1.	Green Hyderabad	950.50± 59.56	25.97± 1.84	1734.29± 110	46.99± 2.41	6064.7± 507.9	164.15± 10.29
2.	Bolpur	872.70± 123.66	14.11± 1.05	1799.99± 156.23	24.46± 1.22	7792.0± 528.47	109.67± 4.21
3.	Srinagar	662.30± 127.26	12.17± 1.35	4623.25± 393.05	70 01± 1.219	10637.6± 1427.74	181.1 ± 12.73
4.	Calcutta	454.53± 37.18	5.28± 0·27	948.02± 53.85	10.23± 0.73	7999.78± 550.42	87.03± 6.46
5.	Madurai	808.26± 93.61	7.86± 0.7	2162.28± 111.90	12.00± 0.61	11045.16± 1051.42	107.9 ± 5.3

Hyderabad, Srinagar and Calcutta hydras. In the Bolpur and Madurai hydras, desmoneme density was slightly lower than the stenotele density (Fig. 9).

The density of isorhizas was very low in all cases and was between 2 and 7 for the five hydra types (Fig. 9).

In the column the density of stenotele was higher than desmoneme in all cases, except Bolpur hydra.

In the Bolpur polyps stenotele and desmonemes were of equal density. The density of desmonenes was very close to the value of isorhiza density in each hydra type (Fig. 9).

(f) *Stem cells and Nematocyte Precursors* :

The total density of 1S and 2S stem cells and nematocyte precursors was too low in the hypostome to be considered separately. The total number of cells and density of 1S and 2S stem cells and the nematocyte precursors (occurring in clusters of 4, 8, 16 and 32 cells) in the column are given in Table 1. The density of 1S and 2S stem cells was high in the Green Hyderabad and Srinagar hydras and in the Bolpur, Calcutta and Madurai hydras. The density of nematocyte precursors was also high in the Green Hyderabad and Srinagar hydras and low in the remaining three hydra types (Table 1).

Discussion

I. *Shape of holotrichous isorhiza*

In the present study, holotrichous isorhiza of characteristic shape have been observed in different ecotypes. While the Green hydra from Hyderabad and hydras from Lucknow and Santiniketan had slipper shaped isorhiza, in Delhi and Shillong polyps it was pyriform in shape. In hydras from Trivandrum, Bolpur Srinagar, White hydra from Hyderabad, Calcutta, Pune, Chandan Nagar, Imphal and Madurai, the holotrichous isorhiza was cylindrical in form, but in hydras from Tirupati, it exhibited a bean shape. The consistency in the shape of the holotrichous isorhizae in different ecotypes is in agreement with the view of other investigators (Hyman, 1929 ; 1930, 1931a, b ; 1938 ; Ewer, 1948 : Forrest, 1963 ; Campbell, 1983) who have considered this parameter as an important diagnostic character in the identification of different species. This feature assumes additional significance in view of the suggestion by Sugiyama and Fujisawa (1978a) that interstitial cells have a predominant role in some aspects of development, such as the shape of nematocysts.

II. *Cell Composition*

In the five ecotypes (Calcutta, Madurai, Srinagar, Bolpur and Green Hyderabad), analysis of cell composition revealed that the total number of

epithelial cells per hydra was characteristic of each ecotype. This analysis assumes tremendous importance in view of the work done by Sugiyama and Fujisawa (1978b). Based on the studies of chimeric hydras they have suggested that characters such as growth rate, bud development rate, budding rate, tentacle number and polyp size are all controlled primarily by epithelial cells.

In the light of experiments, conducted by Marcum *et al.* (1977), Marcum and Campbell (1978a, b,) Sugiyama and Fujisawa (1977a, b ; 1978a, b,) these resultss give clues to the differences observed at morphological and physiological level. They have suggested that although many traits like growth and regeneration capacity are influenced mainly by epithelial cells, some characteristics such as nervous system development, nematocyst shape are controlled by the interstitial cells. It has also been demonstrated that the epithelial cell content in the hydra tissue varies between different mutant strains of hydra (Sugiyama and Fujisawa, 1977b ; Rubin and Bode, 1982). Examination of the epithelial cells ftom the five hydra types in the present study revealed the presence of photosynthetically competent algal cells of the genus *Chlorella* within each of the endodermal digestive cells in Green hydras from Hyderabad. The algal cells were completely absent in all other ecotypes studied in the present investigation, indicating that the Green hydra from Hyderabad were symbiotic green hydra and others were nonsymbiotic hydras.

Differences in the density of nerve cells in the hydras of dfferent ecotypes gives an indication of the differences in the behavioural repertoire found in different types. The similarity in the density of gland cells between the different ecotypes studied (except Calcutta, where the density was low in the hypostome) indicates that gland cells is the basic cell type which may not be significant for taxonomic purpose.

The total cell number per hydra, the number of epithelial cells and the density of interstitial cells and nerve cells give indications of distinct differences in the various ecotypes at the cellular level. They also pinpoint to significant differences at physiological and morphological level, and have been found to be consistent within an ecotype. They can be considered as reliable parameters for identification of different species in hydra.

In the hydra from Calcutta, the density of interstitial cells has been found to be very low. The correspondingly low density of nematocyte precursors, and nerve cells suggests that density of interstitial cell and its derivatives are correlated. Previouly, it has been observed that in nerve free hydra a low level of interstitial cells remain stable (Marcum and Campbell, 1978b). The low level is found to persist and these few inter-

stitial cells do not proliferate to repopulate the hydra, nor are there any derivative cells found. There is apparently a threshold interstitial cell density below which they do not differentiate into derivative cells.

In the Green hydras from Hyderabad and hydras from Srinagar, the density of interstitial cells as well as nematocysts was observed to be high. In the Bolpur and Madurai ecotypes a low density of interstitial cells, nematocyte precursors, and nematocysts was found. These results agree with the study of Yaross and Bode (1978) who found that interstitial cell density and nematocyte commitment are tightly coupled in hydra. It appears that interstitial cell commitment to nematocyte differentiation may be regulated by feedback from the interstitial cell population.

Rubin and Bode (1982) have described a mutant of *H. attenueta* which has an abnormally high number and an altered mounting pattern of nematocytes. They have found that the commitment and differentiating behaviour of the interstitial cells and subsequent migration and mounting pattern of the nematocytes is dependent on both the interstitial cells and cells of the interstitial cell lineage. Another line of evidence regarding nematocyte arrangement comes from Wood and Novak (1982) who demonstrated that nematocyte distribution in hydra is not random. It can therefore, be speculated that the specific arrangement and density of nematocysts in the tentacles of hydra of each ecotype is dependent on the interstitial cells and its precursors, the density of which in turn appears to be specific for each ecotype. Since nematocyst density has been found to be consistent within an ecotype and also characteristic for each ecotype, it emerges as a reliable taxonomic character of significance.

Apart from characterisation of the various ecotypes at the cellular level, our own data on the extent of similarities or differences at the molecular level needs to be mentioned. Estimation of the DNA content (Prasad *et al.*, under preparation) in interstitial, nematocyte and gland cells of six nonsymbiotic hydra types (Madurai, Bolpur, Imphal, Chandannagar, Santiniketan and Pune) have demonstrated the conservation of the genomic entity in the cell types in hydra. Further, our study of the pattern of RNA synthesis (Prasad and Mookerjee, 1985) during hypostome and basal disc regeneration revealed significant differences between the symbiotic green hydra from Hyderabad and nonsymbiotic hydra ecotypes from Bolpur, Madurai, Srinagar and Calcutta. The overall RNA synthetic rate during regeneration was much faster in the Green hydra in comparison with nonsymbiotic ecotypes in which the pattern was almost similar. During growth, however the pattern of RNA synthesis did not vary appreciably between the symbiotic and the nonsymbiotic ecotypes.

SUMMARY

Cellular characteristics revealed significant differences in number of epithelial cells, density of interstitial, nerve cells and nematocyte precursors in the five ecotypes of hydra. The low density of nematocysts found in hydra from Calcutta, Bolpur and Madurai showed correlation with low density of interstitial cells and nematocyte precursors. In Green hydra from Hyderabad and the hydra from Srinagar a high density of interstitial cells, and nematocyte precursors was found. Significant differences in the shape of holotrichous isorhiza was found in different hydra types. The similarity in the density of gland cells in all these ecotypes (except the hydra from Calcutta when the density was low in the hypostome) indicated that it might not be significant for diagnostic purpose.

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FIGURES

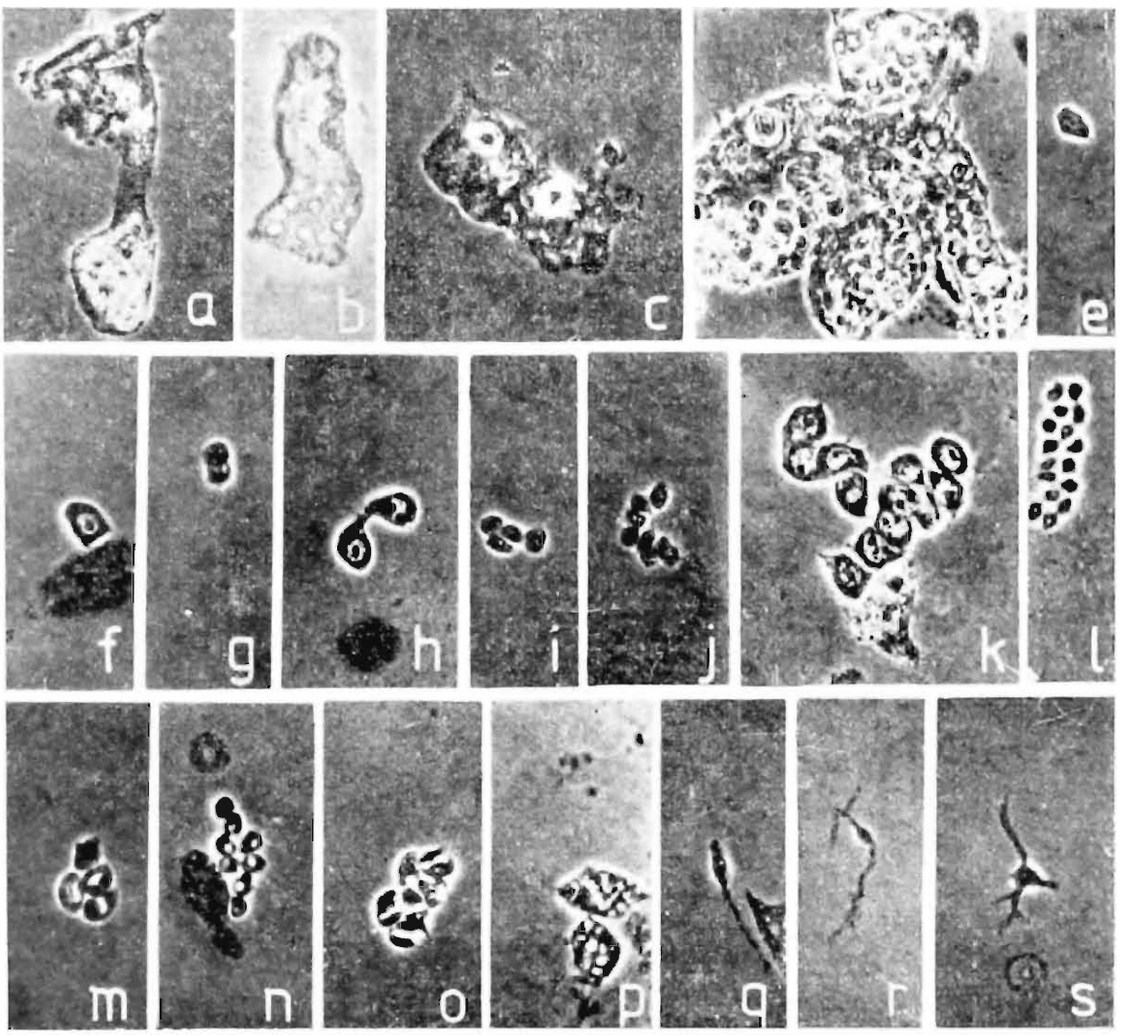


Fig. 1 : Phase contrast photographs of the various cell types (maceration preparation X 303) : (a) Epithelio-digestive cell (Srinagar hydra), (b) Epithelio-digestive cell (Green hydra from Hyderabad), (c) Epithelio-muscular cell ; (d) Battery cell ; (e) Single small interstitial cell ; (f) Single big interstitial cell ; (g) Small interstitial cell in pair ; (h) Big interstitial cell in cluster of (i) 4, (j) 8 ; (k) 4, 8 ; (l) 16. Differentiating nematocytes destined to form (m) stenotele, (n) desmoneme, and (o) isorhiza, (p) gland cells ; (q) sensory nerve cell (r) Bipolar and (s) multipolar ganglionic nerve cells

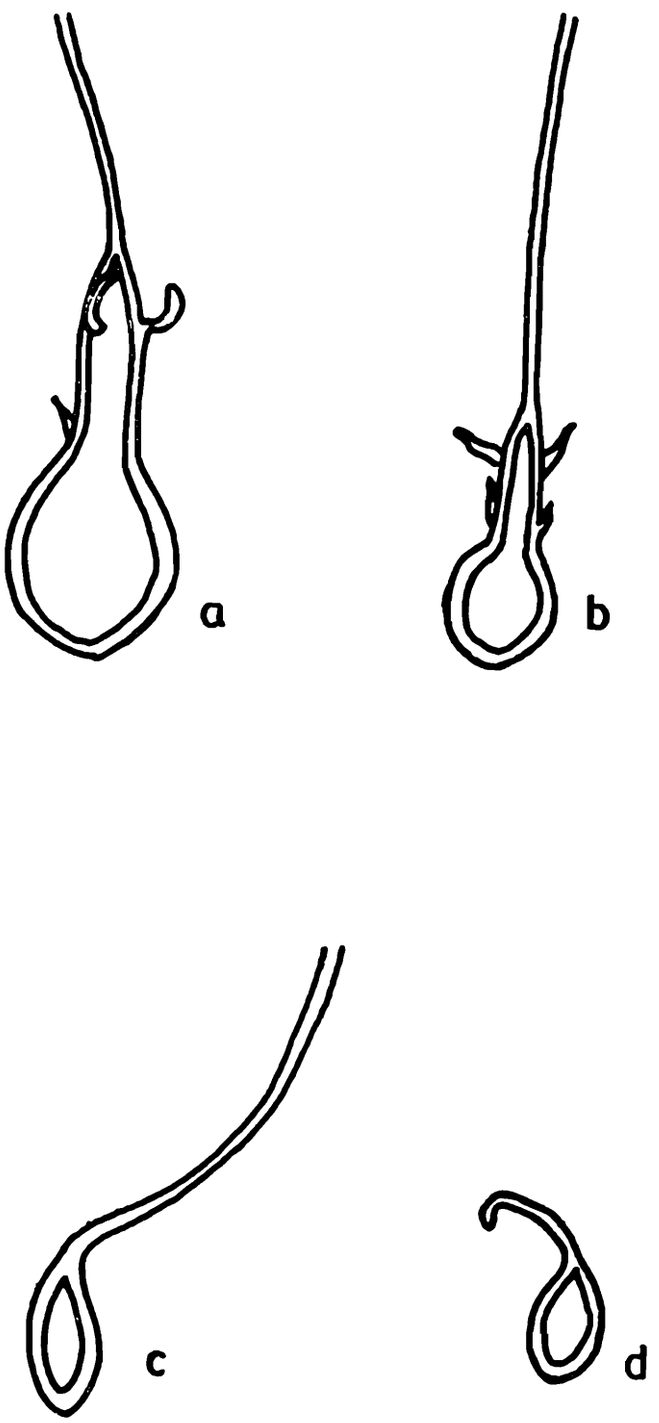


Fig. 2 : Illustrations traced from the phase contrast photographs of nematocysts (maceration preparation X 1205) :
(a) Stenotele (Srinagar hydra) ; (b) Stenotele (Green hydra from Hyderaba) ;
(c) Desmoneme ; (d) Atrichous isorhiza

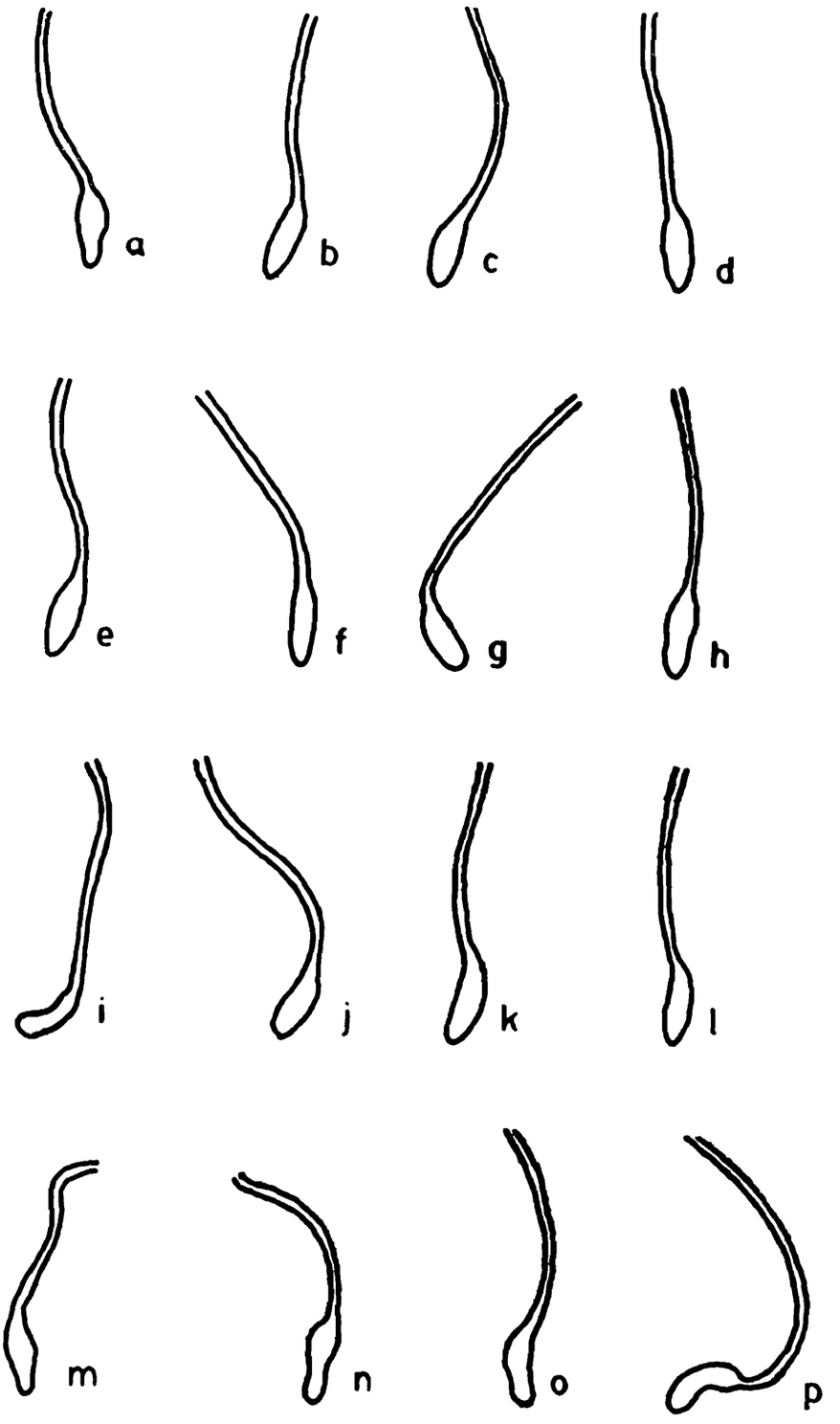


Fig. 3 : Illustrations traced from the phase contrast photographs of holotrichous isorhiza in the 16 hydra ecotypes (maceration preparation X 656) :
(a) Green Hyderabad ; (b) Imphal ; (c) Trivandrum ; (d) Bolpur ; (e) Srinagar ; (f) Pune, (g) Chandan Nagar ; (h) Lucknow ; (i) Delhi ; (j) Calcutta ; (k) Shillong ; (l) Madurai ; (m) Santiniketan ; (n) Jammu ; (o) White Hyderabad ; and (p) Tirupati

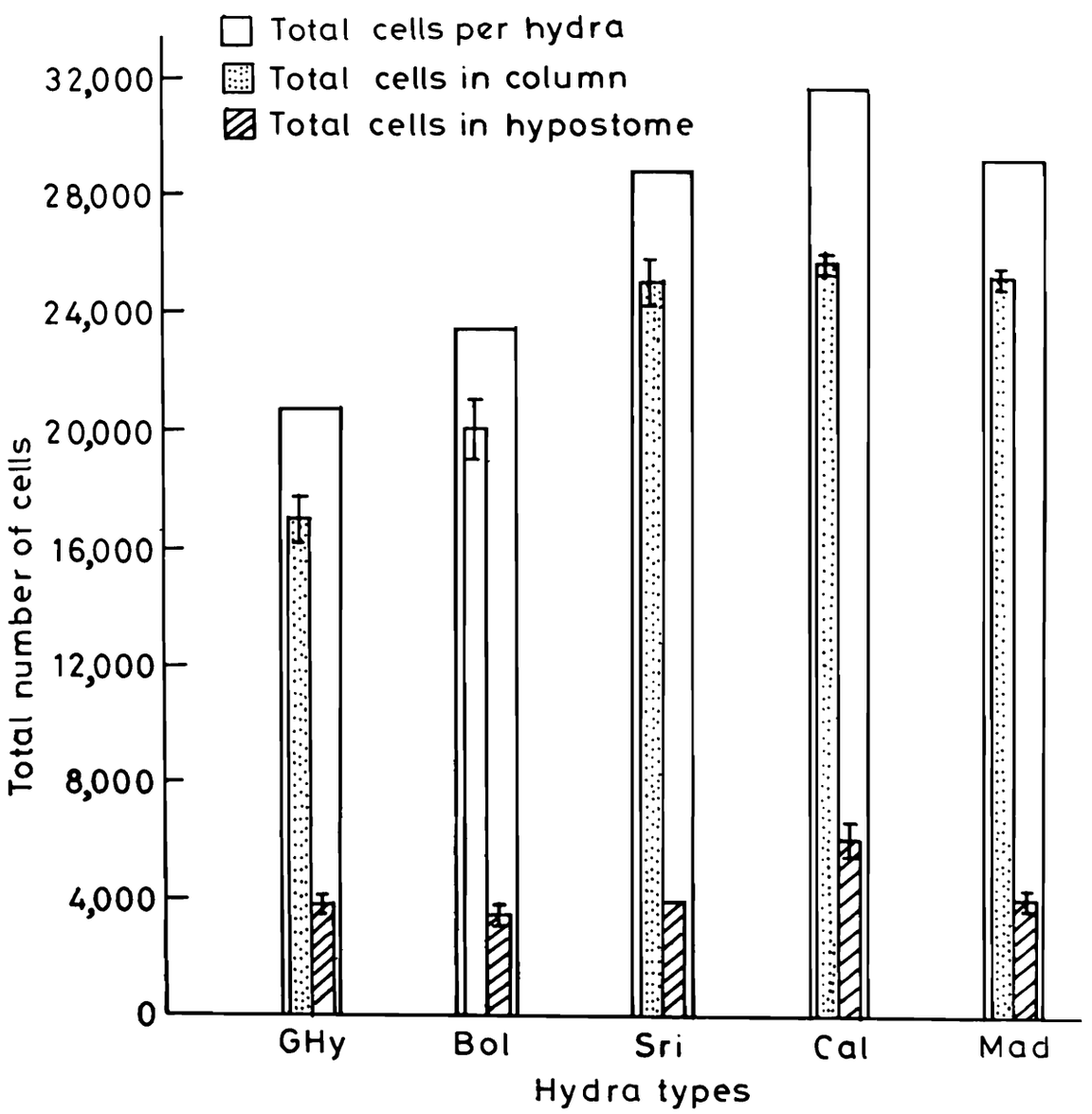


Fig. 4: Average number of cells in the column and hypostome of Green Hyderabad, Bolpur, Srinagar, Calcutta and Madurai hydras. Bars: ± S. E. M. Total number of cells per hydra is the sum of the mean value of the number of cells in the column and hypostome

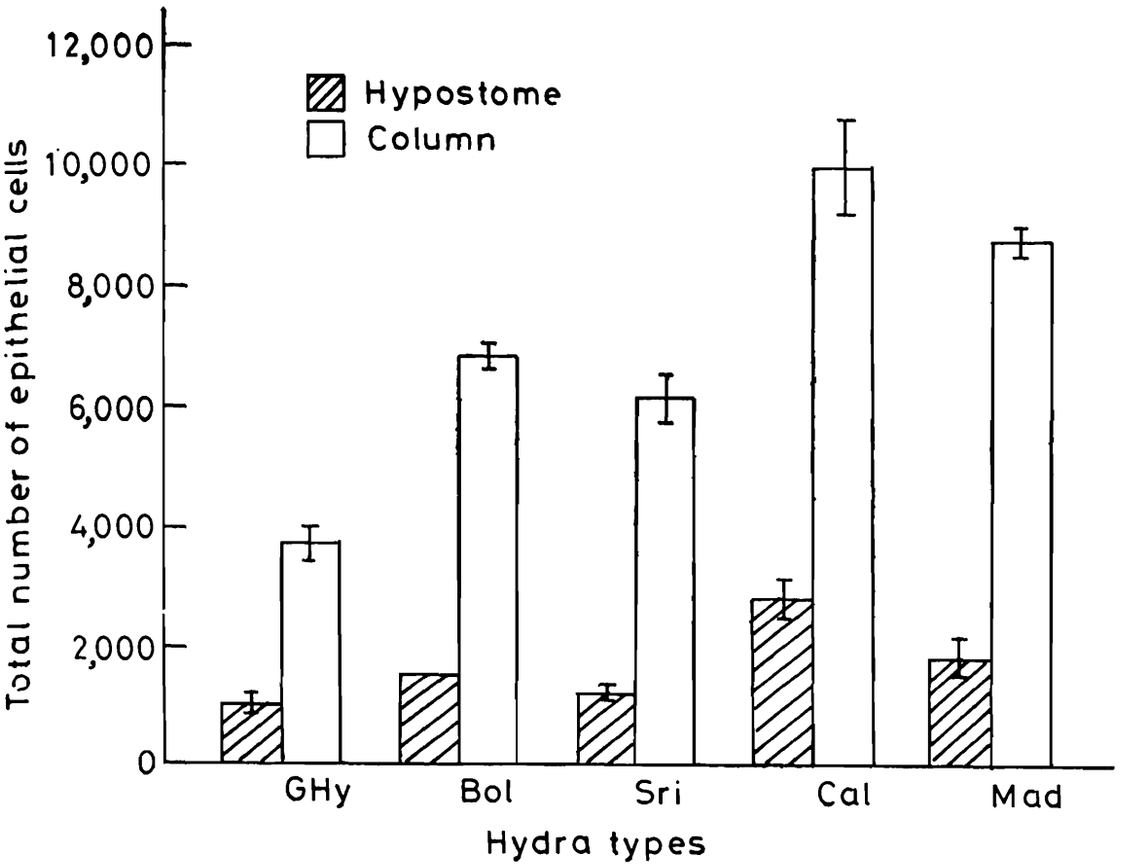


Fig. 5 : Total number of epithelial cells per hydra in Green Hyderabad, Bolpur Srinagar, Calcutta and Madurai hydras. Bars : ± S. E. M.

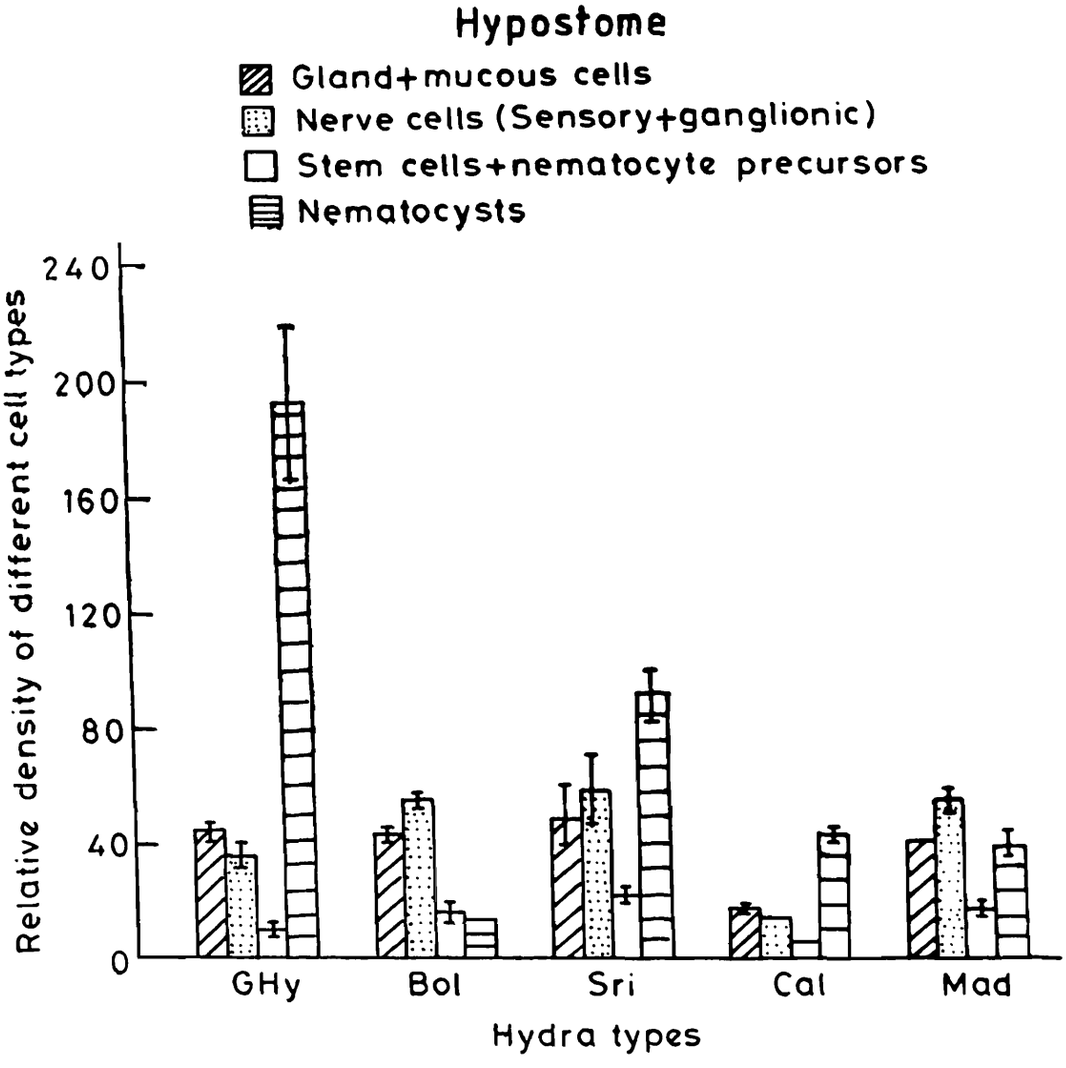


Fig. 6 : Density of the various cell types (in relation to 100 epithelial cells) gland + mucous cells, nerve cells, stem cells + nematocyte precursors and nematocysts in the hypostome of Green Hyderabad, Bolpur, Srinagar, Calcutta and Madurai hydras. Bars : ± S.E. M.

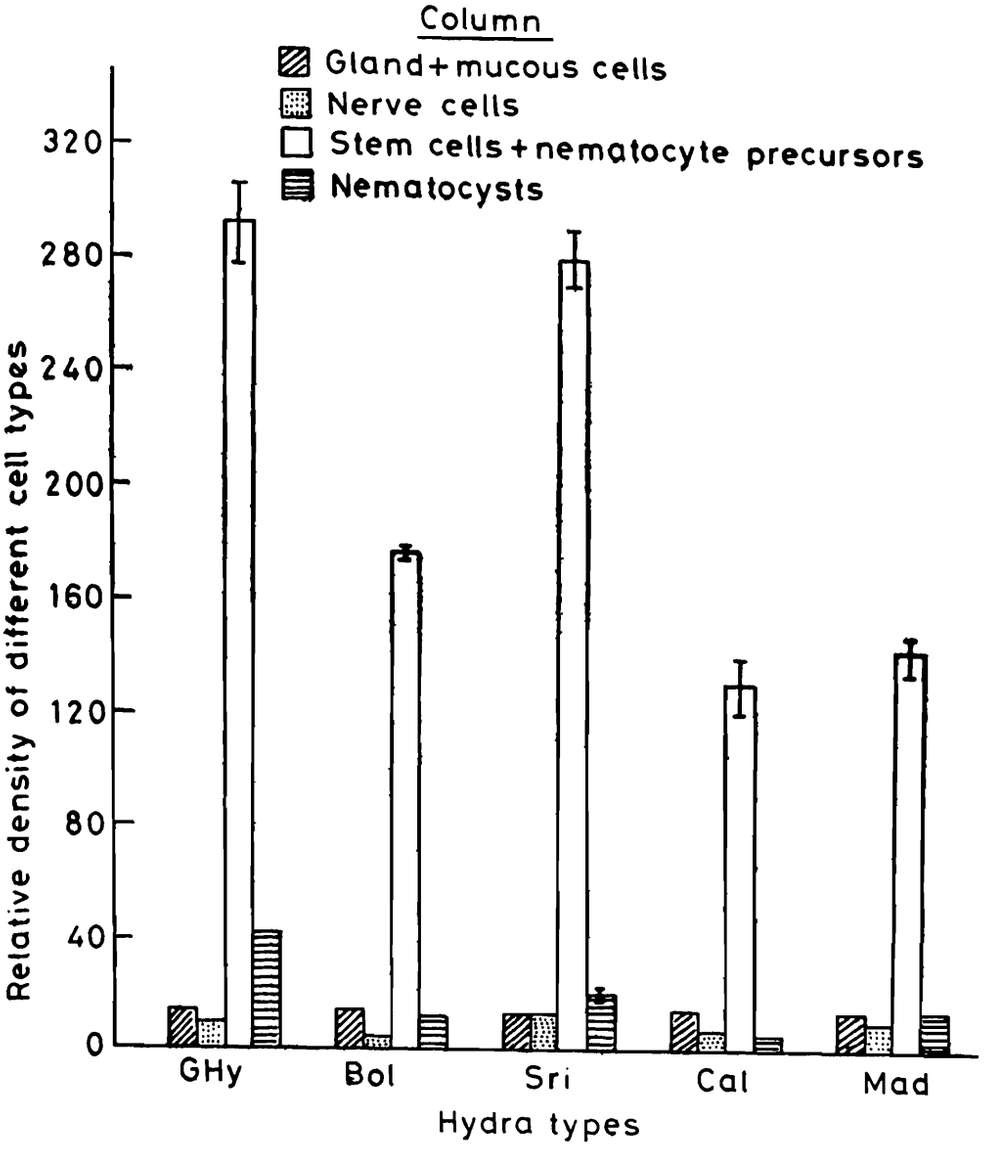
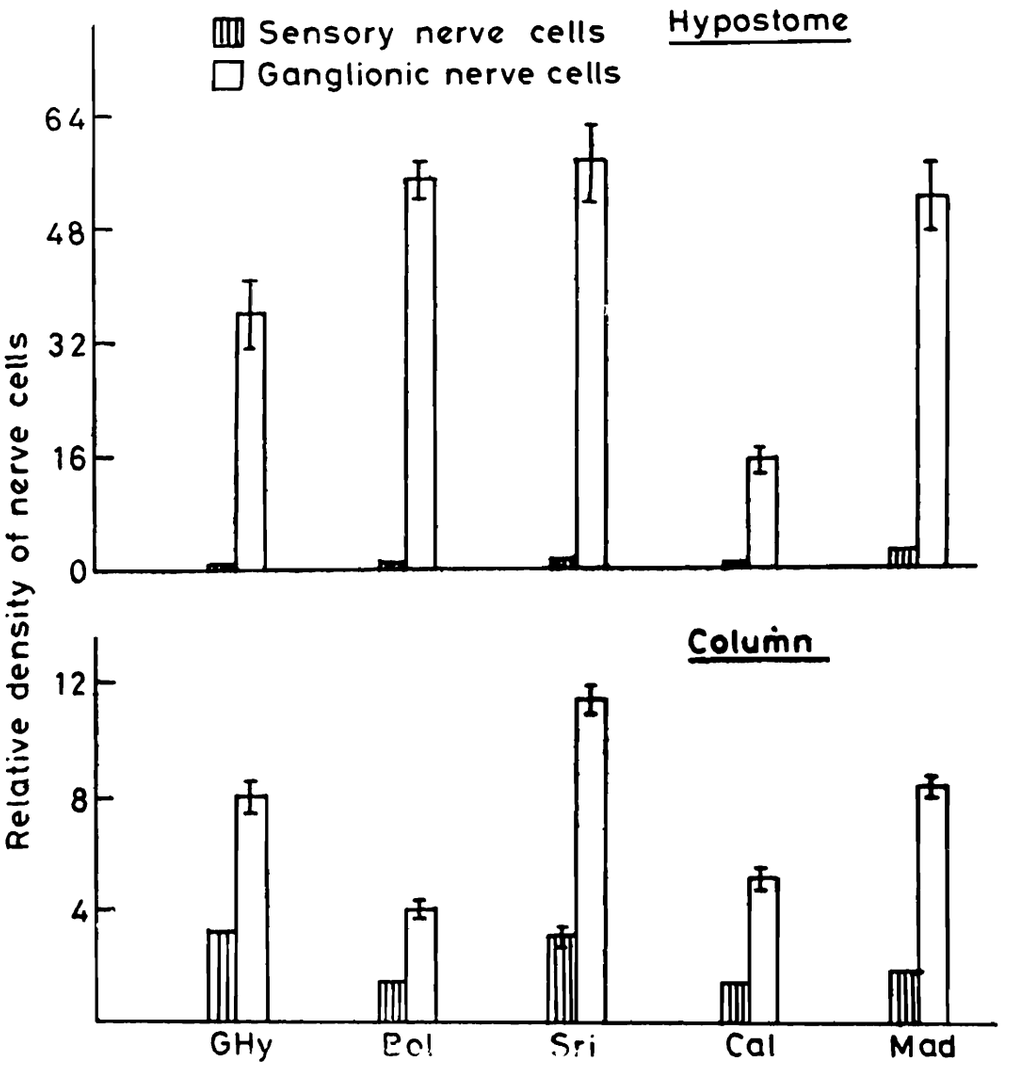


Fig. 7 : Density of the various cell types (in relation to 100 epithelial cells) gland + mucous cells, nerve cells, stem cells+nematocyte precursors, and nematocysts in column of Green Hyderabad Bolpur, Srinagar, Calcutta and Madurai hydras. Bars : ±S. E. M.



ig. 8 : Density of the two nerve cell types (in relation to 100 epithelial cells), sensory and ganglionic in hypostome and column of Green Hyderabad, Srinagar. Calcutta and Madurai hydras. Bars : ±S. E. M.

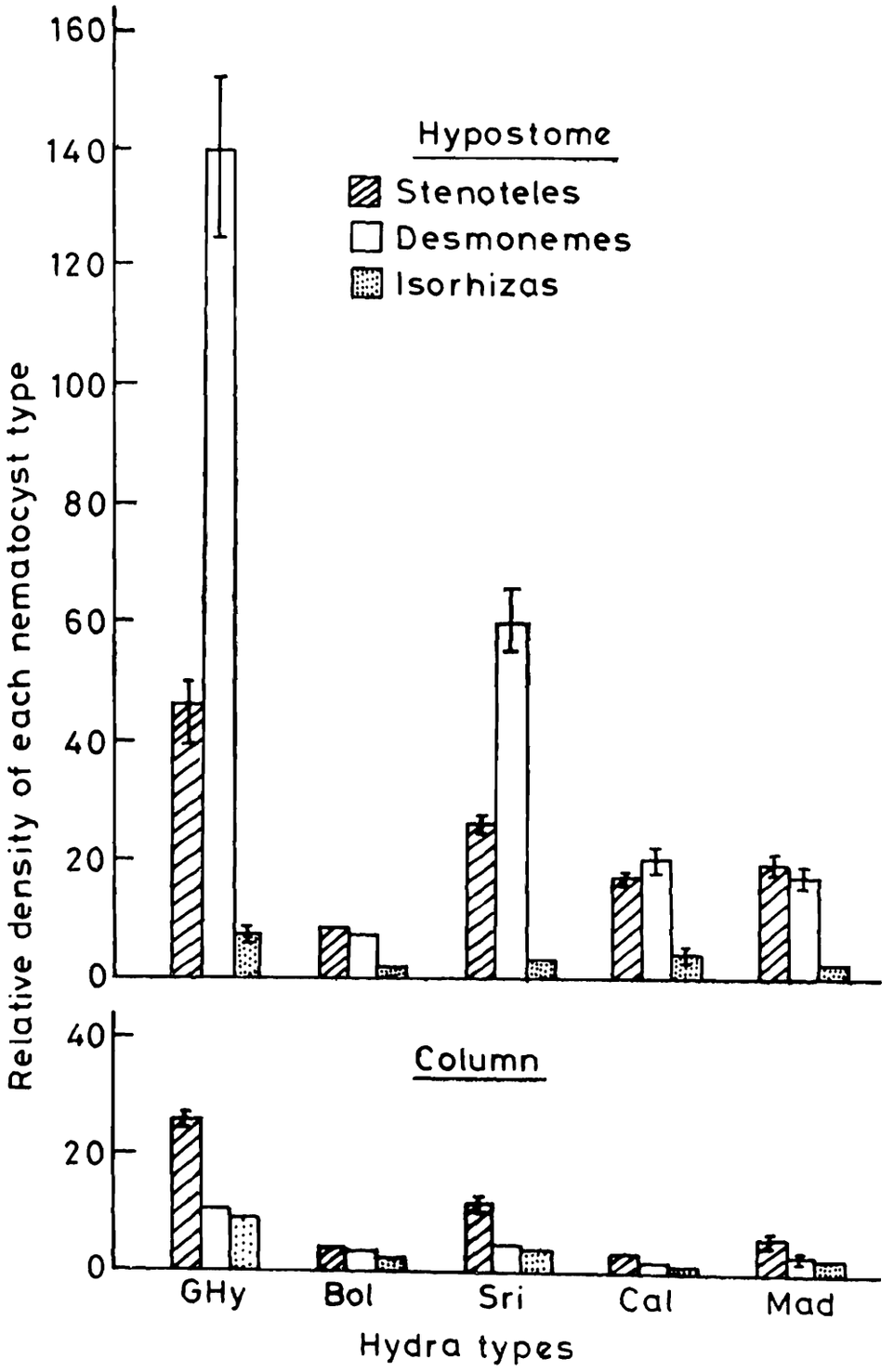


Fig. 9 : Density of the various nematocyst types (in relation to 100 epithelial cells) stenoteles, desmonemes and isorhizas in hypostome and column of Green Hyderabad, Srinagar, Calcutta and Madurai hydras. Bars : ± S. E. M.

MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRA

Part IV : Understanding the Behavioural Differences

Introduction

In this part, some information concerning the behavioural pattern of the Indian ecotypes of hydra will be presented. The behavioural pattern of different hydra types will concern some aspects of (i) nature of locomotory activity under different conditions ; (ii) feeding behaviour and (iii) response to external stimuli. The feeding behaviour will unfold basic facts about the number of nauplii captured and time interval between ingestion and egestion. In response to external stimuli, tactile response, temperature sensitivity, stimulation to light will be examined.

The impelling idea of the present study is to weigh the possibility of taking behavioural pattern as taxonomic criteria in deciphering the status of the different ecotypes.

Material and Methods

The experimental material used in this study were hydras collected from 15 distantly located places in India. From Hyderabad, Green and White hydras were collected from the same pond. Abbreviations used wherever necessary are given below in brackets :

(a) Green hydra from Hyderabad (GHy)
(b) Hydra from Imphal (Imp)
(c) Hydra from Trivandrum (Triv)
(d) Hydra from Bolpur (Bol)
(e) Hydra from Srinagar (Sri)
(f) Hydra from Pune (Pun)
(g) Hydra from Chandan Nagar (C. N.)
(h) Hydra from Lucknow (Luc)
(i) Hydra from Delhi (Del)
(j) Hydra from Calcutta (Cal)
(k) Hydra from Shillong (Shi)
(l) Hydra from Madurai (Mad)
(m) Hydra from Santiniketan (San)
(n) Hydra from Jammu (Jam)
(o) White hydra from Hyderabad (WHy)
(p) Hydra from Tirupati (Tiru)

Culture technique : Hydras were cultured at 23 ± 1°C by the method of Loomis and Lenhoff (1956). They were fed nauplii of brine shrimp (*Artemia* sp.) on alternate days.

(a) *Locomotory Activity* : Nature of locomotory activity was observed in 72 h starved cultures of hydra. Number of hydras floating or attached to the bottom of the petri plates in each ecotype was recorded to ascertain their innate behavioural pattern without disturbing the hydras in the culture plate.

(b) *Feeding Behaviour* : Feeding behaviour was observed in 3.1cm diameter petri plates containing a single hydra (so that it faced no competition in capturing the prey) in 3ml of the culture medium. Feeding rates were determined by exposing individual hydras to large number of *Artemia* nauplii, and by recording the number of nauplii hooked and the number finally ingested. The number of nauplii ingested was double checked by longitudinally opening the enteron by means of needles immediately after the hydras ceased capturing the prey. Time taken for the commencement of tentacles movement after introduction of the nauplii in the culture dish and for the replenishing the hydra culture was also kept at the above temperature.

RESULTS

Behaviour during locomotory activity

Two types of locomotory activities were observed

(i) rhythmic contraction and relaxation of the body column, displayed by hydras attached to the bottom of the culture dish

(ii) dextrous up and down movement from the bottom to the water surface and vice-versa

Locomotory activity was minimum just after feeding. In 72 h. starved cultures (Table 1) the percentage of hydras adhering to the bottom was few (0-30 per cent) except in Green hydra from Hyderahad (66 per cent). These hydras showed exploratory activity by contracting and bending in one direction than re-extending and sweeping the bottom with the tentacle stretched many times, their normal length. After sometime (0.5-3 minutes) the attached hydras contracted and bending in another direction repeated the sweeping movements. After repeating such type of movement several times, the hydras detached and moved towards the surface of the medium by producing a gas bubble clearly visible as a bright spot at their basal disc. The floating hydras either remained vertically suspended from the surface with tentacles stretched downwards, or moved horizontally on

TABLE 1 Number of hydras attached/floating in 72 h starved hydra culture

S. No.	Hydra type	No. of hydras attached to the bottom %	No. of hydras floating%	
			Horizontally	Vertically
1.	Green Hyderabad	66.5	8.9	24.6
2.	Imphal	16.66	5.5	8.3
3.	Trivandrum	20.5	11.7	67.64
4.	Bolpur	—	—	100
5.	Srinagar	25.0	16.6	58.3
6.	Pune	20.0	10	70
7.	Chandan Nagar	22.5	37.5	40
8.	Lucknow	—	—	100
9.	Delhi	25	33	41.6
10.	Calcutta	26.6	20	53.3
11.	Shillong	14.2	—	85
12.	Madurai	8.3	8.3	83.3
13.	Santiniketan	10.5	9.0	80.5
14.	Jammu	30.7	30.7	38.46
15.	White Hyderabad	—	33.3	66.6
16.	Tirupati	16.12	22.5	61.29

the surface in a circular manner. The horizontally moving hydras used both the column and the tentacles in the locomotory movement. In a situation when 72 h starved hydras were kept in a 19.1 cm diameter dish containing 23 ml of culture medium, 90% of the hydras were found to be floating. They suspended themselves from the surface of the medium and with tentacles stretched to the maximum extent swept the bottom in an explanatory manner. On increasing the volume to 27 ml, the tentacle could no longer reach the botton of the dish and the up and down movement from the bottom to the surface increased significantly.

In a starved condition, 0-30% hydras were found attached to the bottom of the dish in all hydra types except the Green hydra (Table 1). The Green Hyderabad hydra showed maximum percentage of polyps attached to the bottom (66%).

II. *Response to external stimuli*

(a) *Tactile Responses* ; Green Hyderabad polyps were found to be most sensitive to mechanical disturbance. When a steel needle was brought near their body without even touching them they showed immediate contraction. However, all other types of hydra would contract only when repeatedly their bodies were tapped with the needle. Recovery in all cases occurred within 1-2 minutes.

(b) *Temperature Sensitivity* : The high temperature influenced the locomotory activity in a distinct way. Hydras of all ecotypes (starved previously for 24 h) when transferred from 23 ± 1°C to 35° ± 1°C showed invariably a tendency to detach their basal disc from the bottom and either restored to a floating position on the water surface or made itself attached to the side wall of the petti dish. Even when well fed, the number of hydras floating or attached to the side wall of the dish varied from 10-100% at the high temperature. This effect was observed in all hydra types (fed daily) until the 6th day at the stress temperature, after which most of them seemed acclimatised and were found to be attached to the bottom of the dish.

(c) *Stimulation by light* : Maximum locomotory activity was observed in the Green Hyderabad hydra all of which were observed to move towards the light source and arranged themselves in an extremely overcrowded semi-circle on the side of the petri dish. Other hydra types exhibited this effect but to a reduced extent.

III. *Feeding behaviour*

When *Artemia nauplii* were dropped in the culture medium near the hydras, they were pursued and paralysed by the nematocysts in the tentacles, of the polyps. Within 1-2 seconds after exposure to the prey, polyps in all hydra types presented a "captive posture", with most of the tentacles exhibiting writhing movement. In all types the first *Artemia* naupleus was ingested

TABLE 2. Number of *Artemia* nauplii captured and ingested in 72 h starved hydras culture

S. No.	Hydra type	Average number of *Artemia* nauplii captured ± SEM	Average number of *Artemia* nauplii ingested ± SEM
1.	Green Hyderabad	10.4 ±1.28	6.8±0.42
2.	Imphal	10.4 ±0.42	8.5±0.34
3.	Trivandrum	10.7 ±0.41	8.6±0.37
4.	Bolpur	12.8 ±0.86	6.8±0.2
5.	Srinagar	11.9 ±0.54	7.4±0.4
6.	Pune	11.3 ±0.91	7.4±0.26
7.	Chandan Nagar	11.6 ±0.58	7.8±0.38
8.	Lucknow	16.2 ±1.42	8.7±0.26
9.	Delhi	10.7 ±0.89	7.9±0.58
10.	Calcutta	11.27±0.67	9.1±0.29
11.	Shillong	10.1 ±0.52	7.5±0.30
12.	Madurai	12.9 ±1.08	7.8±0.38
13.	Santiniketan	12.72±0.86	7.9±0.37
14.	Jammu	11.2 ±0.81	8.3±0.26
15.	White Hyderabad	20.45±1.09	9.1±0.48
16.	Tirupati	13.3 ±0.88	7.4±0 30

TABLE 3. Time interval between ingestion and egestion (h)

Sl. No.	Hydra type	Time interval (h±S.E.M.)
1.	Green Hyderabad	3.82 ± 0.11
2.	Imphal	3.87 ± 0.12
3.	Trivandrum	4.12 ± 0.17
4.	Bolpur	3.46 ± 0.15
5.	Srinagar	4.96 ± 0.08
6.	Pune	3.56 ± 0.12
7.	Chandan Nagar	5.13 ± 0.06
8.	Lucknow	4.67 ± 0.26
9.	Delhi	3.73 ± 0.17
10.	Calcutta	4.1 ± 0.09
11.	Shillong	3.53 ± 0.17
12.	Madurai	3.8 ± 0.19
13.	Santiniketan	3.7 ± 0·15
14.	Jammu	3.8 ± 0.2
15.	White Hyderabad	4.82 ± 0.11
16.	Tirupati	4.73 ± 0.32

within 1-2 minutes after being captured. The captured prey was maneuvoured into the enteron by the tentacles.

After 25-30 minutes of ingestion of the first *Artemia*, 90-98% hydras stopped further ingesting. Those *Artemia* caught but not ingested were released back into the medium. The number of *Artemia* caught and the number finally ingested were recorded in cases of all hydra types. Table 2 shows that although the number of *Artemia* caught (10-20) by the various ecotypes may vary, the number finally ingested did not vary significantly (7-9). Table 3 shows the time interval between ingestion and egestion for the different hydra types at 23±1°C. The time interval varies from 3.46 to 5.13 h.

DISCUSSION

The present investigation demonstrated that in 72 h starved polyps, the Green Hyderabad hydras showed reduced planktonic behaviour as compared to the nonsymbiotic ecotypes. The occurrence of large number of vertically and horizontally floating hydras among the nonsymbiotic hydras indicates that the search for prey occurs earlier in these polyps. In other words the Green Hyderabad hydra appears to show greater resistance to starvation.

Kelty and Cook (1976) have studied the survival during starvation of symbiotic, aposymbiotic and nonsymbiotic hydras. They found that aposymbiotic show much lower survival during starvation, than either symbiotic green hydra or nonsymbiotic hydra. It has also been shown that the photosynthetically active algae present in the cells of *H. viridis* provide

the host with photosynthetically fixed carbon mainly in the form of maltose (Muscatine and Lenhoff, 1963 ; Muscatine, 1965) which augments the nutrition of the hydra under starvation conditions (Muscatine and Lenhoff, 1965). Apart from locomotory behaviour, the Green Hyderabad hydras also showed enhanced response to mechanical and photic stimuli compared to other hydra types. Pardy (1976) demonstrated that aposymbiotic hydra exhibit a positive phototaxis that appears equal to that of algal bearing polyps. It appears that while locomotory activity in the green Hyderabad is dependent on the presence of algal cells, the response to photic stimuli is independent of it. Thus, the positive response to light is an intrinsic feature of the green hydras and the presence of algal symbionts does not play a role in the host behaviour. This behavioural feature can therefore, be used in the indentification of species.

The effect of high temperature on the locomotory activity of hydra, though not noticably different in the different ecotypes is of interest since it has not been studied before. Interestingly Annandale (1911) rcports that Indian hydras (classified by him as *H. vulgaris*) are strongly repelled by heat.

Summary

Some aspect of hydra behaviour revealed that the green hydra from Hyderabad behaved significantly from all other ecotypes. It showed reduced planktonic behaviour after 72 h of starvation and enhanced response to mechanical and photic stimuli.

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MORPHOGENETIC ANALYSES OF ECOTYPES OF INDIAN HYDRAS

Part V. Understanding the physiological responses

Introduction

In this paper certain physiological responses of the different ecotypes have been taken to unearth their ecological importance. Experimental set-up was made to expose hydras to opposite temperature gradients-one in high temperature (29°C) and another in low temperature (15°C). The idea has been to study their physiological responses over certain lengths of time to analyse their innate properties. Three criteria have been considered at the imposed temperature level (i) their ability of growth (ii) formation of gonads, and (iii) their ability for regeneration.

The purpose of the work is to measure the physiological responses as shown by the action of temperature on asexual as well as sexual reproduction of the various ecotypes to see if any physiological criteria can be used as a second criteria for affixing any taxonomic value of certain orders for hydra.

Materials and Methods

Ecotypes of hydra were collected from 15 different places in India and used as the experimental material in this study. Abbreviations used whereever necessary for the different ecotypes are given within brackets.

(*a*) Green hydra from Hyderabad (GHy)
(*b*) Hydra from Imphal (Imp)
(*c*) Hydra from Trivandrum (Triv)
(*d*) Hydra from Bolpur (Bol)
(*e*) Hydra from Srinagar (Sri)
(*f*) Hydra from Pune (Pun)
(*g*) Hydra from Chandan Nagar (CN)
(*h*) Hydra from Lucknow (Luc)
(*i*) Hydra from Delhi (Del)
(*j*) Hydra from Calcutta (Cal)
(*k*) Hydra from Shillong (Shil)
(*l*) Hydra from Madurai (Mad)
(*m*) Hydra from Santiniketan (San)
(*n*) Hydra from Jammu (Jam)
(*o*) White hydra from Hyderabad (WHy)
(*p*) Hydra from Tirupati (Tiru)

1. *Culture Techniques* : Hydras of each type were maintained at 23 ± 1°C by the method of Loomis and Lenhoff (1956) and were fed daily. Twenty four hours after feeding they were transferred to 15°C and 29°C and were cultured regularly at these temperatures. The medium used for replenshing the hydra culture was also kept at the above temperatures.

2. *Growth* : Growth was studied in ten hydras per ecotype at each temperature for 10 days. Everyday, the following observations were recorded : (i) number of hydras ; (ii) number of buds detached, and (iii) number of hydras with 1, 2, 3 or 4 buds.

The time taken for bud development and detachment, but maturation and time interval between successive bud initiation was recorded only after a stable budding rate was reached (about one month).

3. *Regeneration* : The regenerative time of organisms were recorded, after 1 month of maintenance at each stress temperature (i. e., a shift from the optimum temperature 23° ± 1°C).

At 23° ± 1°C regeneration timings were recorded for (a) the midgastric annulus ; (b) hypostome regeneration and (c) basal disc regeneration in *in situ* hydras where as at the stress temperatures, only the hypostome and basal disc regeneration was studied.

For all regeneration experiments 24 hs starved mature animals without buds and gonads were used. In each case, amputation was done, when the animal was in a state of maximum stretching, by a single straight cut, by means of a pair of sharp steel needles.

4. *Gonad production* : The percentage of hydras with gonads (testis or ovaries or both) at 23 ± 1°C, was recorded in normal hydra culture at the end of every month from August 1981 to March 1983, for all hydra ecotypes except the green and white hydras and the Calcutta hydras. These two types were obtained on 18.12.81 and 30.12.81 respectively, so observations were carried on from these dates onwards to March 1983.

The effect of temperature on the appearance of gonads was studied by transferring hydras with and without gonads to 29 ± 1°C and 15 ± 1°C. Hydras were kept at the high and low temperature for 45-60 days.

Results

1. *Selection of high and low stress temperature*

To select the highest temperature at which hydras continue to feed and to bud without showing any kind of morphological or developmental abnormality, hydras were cultured at 36°C, 34°C, 32°C and 29°C. temperatures and the effects studied as follows :

Temperature	Time interval (in hours)	Ecotypes	Number of Hydras (%)	Effect
1	2	3	4	5
36±1°C	12	All	100	Stunted tentacle
	24	All	100	Completely disintegrated
34±1°C	24	Pune, Imphal	100	Stunted tentacles
		Delhi	25	stunted tentacles
		Other typcs	100	normal morphological appearance and feeding behaviour
	36	Chandan Nagar	100	stunted tentacles
		Trivandrum, Green Hyderabad, Delhi, Calcutta		
		White hydra Shillong, Lucknow, Jammu, Bolpur	40	stunted tentacls
		Jammu, Srinagar, Tirupati, Santiniketan, Madurai	100	normal morphological appearance and feeding behaviour.
	96	Pune	100	Body column smalland
		Delhi	20	barrel shaped, development
		Trivandrum	10	of new tentacles (10-15)
		Chandan Nagar	10	inner to the original
		Imphal	10	tentacle ring as well as inbetween the original tentacles (Fig. 1).
		Shillong	100	Body column small and barrel shaped with vertical constrictions dividing the body into 4 incomplete segments. Development of new tentacles (Fig. 1).
		Green hydra	100	Very small knob like tentacles
		Madurai	40	Hydras could capture *Artemia* nauplii but
		White hydra	20	could not ingest them.
		Santiniketan	30	30% hydras could ingest
		Triupati	70	70% hydras could ingest
		Srinagar	40	40% hydras could ingest
		Bolpur	30	30% hydras could ingest
		Lucknow	30	30% hydras could ingest
		Jammu	20	20% hydras could ingest
		Calcutta	10	10% hydras could ingest the prey
32±1°C	48	All	100	normal morphological appearance and feeding behaviour.
	72	All	100	normal morphological appearance but number of artemia ingested was reduced to 1-2 per hydra in all cases.
29±1°C	96	All	100	normal morphological appearance and feeding behaviour.

Since at temperature above 29 ± 1°C deleterious effects were observed, further study of the effect of high stress temperature on growth and regeneration was carred out at this temperature.

To select the low stress temperature hydra were cultured at 15 ± 1°C. Since the polyps continued to feed and bud and remained healthy at this temperature 15 ± 1°C was selected for study of low stress temperature.

2. *Temperature Effects on Growth*

The growth of hydras was observed to be significantly influenced by the temperature. Since the bud development time and time interval between successive bud initiation were also observed to vary (Section II, c, e) with temperature, in this study growth will be expressed in terms of an increase in the number of polyps in the culture and increase in the total number of hypostomes (total number of polyps plus total number of attached buds) after 10 days.

(a) Total number of polyps and the total number of hypostomes on the 10th day, for different temperatures :

I. At 23 ± 1°C : The total number of polyps and the total number of hypostomes on the 10th day for each type is shown in Fig. 2. The data can be further arranged in 3 categories in the decreasing order as follows :

Category	Total number of polyps	Total number of hypostomes	Hydra types
I	More than 300	More than 770	Green Hyderabad
II	Between 140-185	Between 268-280	Imphal, Shillong, Bolpur, Srinagar, Chandan Nagar, Lucknow, Santiniketan, Tirupati, Calcutta, Pune, Madurai.
III	Between 100-183	Between 126-205	White Hyderabad, Jammu, Trivandrum, Delhi.

II. 29 ± 1°C : The total number of polyps and the total number of hypostomes on the 10th day at 29 ± 1°C is shown in Fig. 2. Here, the various hydra types could be placed in 4 categories, arranged in the descending order as follows :

Category	Total number of polyps	Total number of hypostomes	Hydra type
I	Between 280-300	Between 330-435	Santiniketan, Srinagar, Bolpur, Lucknow, Green Hyderabad
II	Between 169-216	Between 216-300	Madurai, Chandan Nagar, Shillong, Tirupati.
III	Between 126-134	Between 164-180	Calcutta, White Hyderabad, Pune, Jammu.
IV	Between 80-103	Between 93-120	Imphal, Trivandrum, Delhi.

III. At 15 ± 1°C : The total number of polyps and the total number of hypostomes on the 10th day, in the various hydra types is as shown in Fig. 2. Here, the well defined categories formed are as follows :

Category	Total number of polyps	Total number of hypostomes	Hydra types
I	More than 27	More than 50	Green Hyderabad.
II	Between 14-17	Between 31-41	Santiniketan, White Hyderabad, Pune, Tirupati, Lucknow.
IV	Between 10-12.5	Between 18-28	Imphal, Trivandrum, Delhi, Madurai, Calcutta, Srinagar, Chandan Nagar, Bolpur, Jammu.

(b) *Population doubling time*

Hydras shifted from the optimum (23 ± 1°C) to the stress temperature (29 ± 1°C) showed a decrease or increase in doubling time the degree of change showed distinct differences in the various ecotypes (Table 1).

TABLE 1. Population doubling time of various hydra types at different temperatures (days)

S. No.	Hydra type	Time (days) 23°±1°C ± S. E. M.	29±1°C
1.	Green Hyderabad	1.35±0.13	2.31±0.16
2.	Imphal	2.41±0.14	3.86±0.23
3.	Trivandrum	2.46±0.57	3.43±0.16
4.	Bolpur	2.68±0.16	1.85±0.13
5.	Srinagar	2.03±0.15	1.81±0.07
6.	Pune	1.91±0.14	2.7 ±0.18
7.	Chandan Nagar	2.13±0.11	2.45±0.05
8.	Lucknow	2.55±0.08	2.2 ±0.2
9.	Delhi	2.68±0.05	4.15±0.13
10.	Calcutta	2.2 ±0.05	2.76±0.23
11.	Shillong	2.31±0.16	2.35±0.08
12.	Madurai	2.51±0.02	2.3 ±0.08
13.	Santiniketan	2.41±0.14	2.16±0.2
14.	Jammu	3.03±0.15	2.76±0.2
15.	White Hyderabad	3.25±0.25	2.63±0.07
16.	Tirupati	2.15±0.13	2.26±0.2

(c) *Bud development time*

The length of time between the appearance of rudimentary bud and its detachment from the parent in the 16 types of hydras cultured at 23°, 29° and 15°C is given in Table 2. Except for the Green hydra from Hyderabad, the time taken for bud development was observed to vary inversely with temperature in all the cases (Table 2). Hydras maintained at 29°C were quicker in bud production, whereas at 15°C the bud development time was prolonged. The Green Hyderabad hydras took longer time at the high and low stress

temperatures (26.57 h at 29° and 68.2 h at 15°C), as compared to the bud development time (18.87 h) at the optimum temperature (23 ± 1°C).

(d) *Bud maturation time*

The time taken by a newly detached bud to produce its own first bud, i. e., bud maturation time was found to increase with a shift in temperature from the optimum to 29°C or 15°C (Table 3).

TABLE 2. Time taken for bud development at different temperatures (h)

S. No.	Hydra types	Time (h)±S. E. M.		
		23±1°C	29±1°C	15±1°C
1.	Green Hyderabad	18.17±0.826	26.57±0.29	68.2 ±1.67
2.	Imphal	50.57±1.25	31.5 ±1	104.8 ±7.89
3.	Trivandrum	53.0 ±2.54	32.5 ±1	98.66±1.44
4.	Bolpur	48.58±0.3	30.0 ±0.57	101.34±6.80
5.	Srinagar	47.65±2.57	29.95±0.52	82.53±6.12
6.	Pune	50.66±0.53	30.5 ±1.50	85.73±6.96
7.	Chandan Nagar	5.16±1.89	31.16±1.01	76.75±1.24
8.	Lucknow	50.76±0.78	30.67±0.11	90.3 ±11.73
9.	Delhi	51.5 ±1.80	30.73±0.14	109.12± 4.81
10.	Calcutta	50.36±0.75	30.66±0.66	194.4 ± 2.84
11.	Shillong	59.06±0.25	30.08±0.42	90.0 ± 8.02
12.	Madurai	49.6 ±0.60	30.55±0.45	113.75± 1.24
13.	Santiniketan	46.25±3.47	31.5 ±0.50	92.26± 5.2
14.	Jammu	50.13±0.63	30.25±0.47	103.3 ± 4.7
15.	White Hyderabad	51.13±0.75	32.23±0.39	95.76± 3.90
16.	Tirupati	50.93±0.74	30.5 ±1.25	99.81± 3.40

TABLE 3. Bud maturation time at different temperatures (h)

S. No.	Hydra types	Time (h)±S. E. M.		
		23±1°C	29±1°C	15±1°C
1.	Green Hyderabad	30.5 ±0.99	77.5 ± 2.5	68.2 ±1.68
2.	Imphal	54.0 ±1.44	105.5 ± 2.5	102.0 ±6.01
3.	Trivandrum	50.06±1.02	86.25± 4.25	94.62±7.21
4.	Bolpur	52.6 ±1.95	68.5 ± 7.49	109.25±1.24
5.	Srinagar	47.83±1.29	73.5 ± 1.5	102.0 ±6.50
6.	Pune	58.96±1.84	91.0 ±10.0	95.75±2.25
7.	Chandan Nagar	59.1 ±0.48	80.0 ± 2.0	102.83±5.00
8.	Lucknow	58.62±3.86	93.66± 6.0	108.83±8.47
9.	Delhi	64.75±0.87	102.75± 2.49	97.16±8.39
10.	Calcutta	58.62±1.87	89.66± 7.05	94.25±3.11
11.	Shillong	59.93±1.21	104.0 ± 0.57	94.25±3.75
12.	Madurai	50.93±1.21	82.5 ± 3.5	94.25±6.26
13.	Santiniketan	50.85±1.25	84.66± 6.33	89.75±6.42
14.	Jammu	50.9 ±1.01	88.36± 5.49	90.05±2.04
15.	White Hyderabad	86.25±3.24	97.1 ± 3.26	93.85±1.75
16.	Tirupati	52.93±2.69	75.66± 6.20	82.33±9.68

(e) *Time interval between successive bnd initiations*

It was found to vary inversely with the shift in temperature from the normal in all the hydra types (Table 4).

TABLE 4. Time interval between successive bud initiation at different temperature (h)

S. No.	Hydra types	Time interval (h)± S. E. M. at		
		23±1°C	29±1°C	15±1°C
1.	Green Hyderabad	19.33±0.66	6.18±0.49	44.83± 1.43
2.	Imphal	24.8 ±2.0	19.66±1.73	48.5 ± 4.49
3.	Trivandrum	26.66±0.66	12.66±1.29	54.5 ± 1.5
4.	Bolpur	26.0 ±2.0	7.5 ±0.86	61.16± 9.50
5.	Srinagar	26.5 ±2.02	6.66±0.84	51.0 ± 3.0
6.	Pune	24.5 ±0.2	7.8 ±0.84	64.0 ± 6.07
7.	Chandan Nagar	22.66±0.32	8.46±0.84	51.15± 2.35
8.	Lucknow	24.25±0.20	7.75±0.24	62.16± 9.68
9.	Delhi	26.83±0.72	19.83±2.12	68.05±12.60
10.	Calcutta	25.75±0.20	12.7 ±1.27	76.6 ± 1.39
11.	Shillong	26.65±0.35	7.25±1.24	55.5 ± 2.5
12.	Madurai	25.0 ±0.57	7.5 ±1.5	50.85± 1.49
13.	Santiniketan	23.85±0.34	7.75±0.25	51.53± 2.64
14.	Jammu	26.61±1.44	6.0 ±1.0	66.5 ± 6.37
15.	White Hyderabad	25.5 ±0.76	9.25±1.24	52.1 ± 3.10
16.	Tirupati	26.66±0.66	7.5 ±0.50	64-43± 6.28

(f) *Budding rates*

At 23°C as well as at 29°C, hydra types initiated buds between O and 1st day and detached the first buds by 2nd and 3rd day.

At the low stress temperature (15°C) most of the hydras exibited a long lag period for initiation and detachment of the first few buds. On the first day at 15°C the hydras from Santiniketan initiated the first bud. The hydras from Lucknow, Green hydra from Hyderabad and hydra from Shillong were found to initiate the first bud on 2nd, 3rd and 4th day respectively. The hydras from Pune, Calcutta, White hydra from Hyderabad and hydras from Tirupati initiated the first bud on the 5th day, and Chandan Nagar polyps on the 6th day. The hydras from Imphal, Bolpur, Srinagar, Delhi, Madurai initiated the first bud on the 7th day, and Trivandrum on the 8th day.

The first buds were detached only in the second week at 15°C. The hydras from Santiniketan and Lucknow dropped buds on the 6th day, the Green hydra from Hyderabad on the 7th day the White hydra from Hyderabad and the hydras from Calcutta detached their buds on the 8th day. In Chandan Nagar and Tirupati ecotypes hydras bud detachment

occurred only on the 9th day. Imphal, Trivandrum, Delhi, Madurai hydra types failed to detach any bud even by the 10th day.

(g) *Number of buds per hydra*

At 23°C, production of 4 buds per hydra was recorded only in the Green hydra from Hyderabad (1-2%). Polyps with 3 buds were formed in all types bud accurred in maximum numbers in the Green hydra from Hyderabad (5-10%).

At 29°C, hydras with 4 buds each were observed in the Trivandrum, Bolpur, Srinagar, Pune, Lucknow, Santiniketan and Tirupati types (10-20%) Appearance of 3 buds per hydra was maximum in the hydras from Bolpur, Srinagar, Lucknow, Santiniketan and Madurai (10-30%). The frequency was low in the Green hydra from Hyderabad, Imphal, Trivandrum, Pune, Chandan Nagar, Shillong, Jammu, White Hyderabad and Tirupati members (2-5%). Hydras with 3 buds each were never observed in Delhi and Calcutta hydras.

At 15°C, 4 buds per hydra were not observed in any type. Hydras with 3 buds each were observed in Green Hyderabad, Bolpur, Pune, Lucknow and Tirupati types.

Hydras with one and two buds were found on all sixteen ecotypes, at all the three temperatures.

3. *Sexual Reproduction*

The Green hydra from Santiniketan showed hermaphroditism under the laboratory conditions. All other types except hydras from Srinagar, Delhi and White hydra from Hyderabad showed dioecious nature. While the Bolpur, Calcutta and Tirupati ecotypes exhibited only ovary production, the Imphal, Trivandrum, Pune, Lucknow, Shillong, Madurai and Jammu showed only testes production. Hydras from Chandan Nagar produced either ovaries or testes. The Green hydras from Hyderabad and hydra from Santiniketan produced ovaries and testes on different hydras, as well as simultaneously on the same hydra. In the Srinagar, Delhi and White Hyderabad ecotypes, gonads were not produced.

(a) *Occurrence of Gonads*

The frequency of occurrence of gonads was studied from August 1981 to March 1983. The arrangement of gonads on the column and the shape of the gonads was also observed.

I. *Testes*

(i) *Frequency of occurrence of testes*: In the Green hydra from Hyderabad, the sexual activity, i. e., the pattern of testes production observed was very distinct. In June 1982, it showed maximum hydras with testes (48% of the population in the culture condition). The number reduced

very suddenly to 8% in July and dropped still further to 1% in August 1982. In January 1983, the production of testes increased to 10% and remained constant at this level till March 1983. (Fig. 3a).

In the hydras from Pune, on the other hand, single peak of testes production (16% of the population) was observed in January 1982. The number of hydras with testes was very few (2%) from August to November 1981. The number increased to 16% in January 1982 and dropped abruptly to zero in March 1982. (Fig. 3b). The hydras from Imphal and Trivandrum showed a single peak of sexual activity in November 1981, when 1—5% of hydras bore testes (Fig. 3c).

In the hydras from Lucknow and Chandan Nagar, only one peak of sexual activity (8—15%) was seen in September 1981, while a few (2%) hydras from Lucknow continued to bear testes in December 1981, hydras from Chandan Nagar reverted back to the normal sexual state. (Fig. 3d).

In the hydras from Santiniketan, Madurai, Shillong and Jammu, the percentage of hydras bearing testes was found to be maximum in September 1981 (10-60%) and again in January 1982 (5-35%). Thus, two peaks of maximum sexual activity were obtained in these hydras. In all these types, the percentage fell significantly in February and became zero in March 1982 (Fig. 3c).

The hydras from Santiniketan developed testes again in July 1982 and reached a phase of maximum testes production (10%) in August and September 1982. The number of hydras with testes decreased gradually till November (5%), remained constant till December 1982, and then dropped gradually to 1% level in March 1983 (Fig. 3c)

(ii) Position and arrangement of testes : In all types where testes production was observed, the testes were distributed on the column, between the subhypostome and the budding region (Fig. 4). In the hydras from Pune and Shillong the testes were arranged alternately on the column, presenting a spiral form (Fig. 4a, b).

In the hydras form Trivandrum, Lucknow, Madurai, Jammu and Santiniketan no such spiral arrangement was observed. The testes were either closely positioned in some region of the column and widely spaced in other region (Fig. 4c, d).

(iii) Detailed view of the testes : In the hydras from Santiniketan, the testes were broadly round in shape (Fig. 5a). In the Green hydra from Hyderabad the testes were very small and conical in shape. Each testis was provided with a conspicious papilla (Fig. 5b). In the hydra from Shillong the testes were large and broad with an extremely flat outer surface (Fig. 5c). In the hydras from Trivandrum the testes were of a low rounded form

(Fig. 5d), In the hydras from Madurai, the testes were conical in shape and each was provided with a tiny papilla (Fig. 5e) In the hydras from Pune, the testes were round in shape while in Jammu hydras the testes were of very low elongated form (Fig, 5f, g). In hydras from Lucknow the testes were small and broadly round (Fig. 5h).

II. *Ovaries*

(i) Frequency of ovary production : In the hydras from Bolpur, number of polyps with ovaries was 5% in Aug. 1981. It increased to 30% in November and dropped to zero in February 1982 (Fig. 6). The hydras from Tirupati exhibited a peak level (10%) of ovary production in October 1981 and stopped completely in December 1981. In the Chandan Nagar ecotype, very few hydras (1%) produced ovaries in November and December 1981, and stopped altogether in January, 1982. The hydras from Santiniketan showed very few ovaries (1%) in August and September 1981 and again 1—2% in August and October 1982. The hydras from Calcutta became sexual in July and reached a high peak level (78%) in November 1982. Ovary production dropped significantly to 4% in February and halted completely in March 1983 (Fig. 6).

In this case also the Green hydra from Hyderabad showed a very characteristic pattern. Ovary production decreased suddenly from 2% in July 1982 to 0.5% in August 1982. It continued at a low level (0.5%) till January 1983, after which it increased to 4.5% in February and remained at this level till March 1983.

(ii) Position of the Ovary : In the Green hydra from Hyderabad, and hydras from Tirupati, Calcutta, Santiniketan and Bolpur only one ovary was observed on the column at one time (Fig. 7). In the hydra from Chandan Nagar, 2—3 ovaries developed and ripened simultaneously. In the Green hydra from Hyderabad, ovary developed in the budding region. Quite often an ovary and a bud were seen in the same position, arranged opposite to each other (Fig. 7a). In the hydras from Tirupati, Calcutta, Santiniketan and Chandan Nagar ovary was normally found attached in the middle of the body region, slightly above the budding zone (Fig. 7).

In the hydras from Santiniketan, often the ovary exhibited some kind of abnormality. In some cases, it failed to detach from the column, while in some organism a very long peduncal was observed (Fig. 7d).

III. *Hermaphrodites*

(i) Frequency of occurrence of hermaphrodites : Only the Green hydra from Hyderabad and hydra from Santiniketan exhibited hermaphroditic condition in the laboratory. The former remained predominatly hermaphroditics from April 1982 to January 1983 and showed a large number of hermaphrodite individuals (80-85%) from August to October 1982. In January

1983, the number dropped to 5% which further reduced to 1% in February 1983 and then remained constant till March 1983. The hydras from Santiniketan showed a very low level of hermaphrodites (1%) from August to September 1981 (Fig. 8).

(ii) Location of the gonads in hermaphrodites : In the Green hydra from Hyderabad and hydra from Santiniketan which exhibited hermaphroditic condition, testes were always found to distal in location and ovary was in the middle of the column (Fig. 9a, b).

b. *Temperature Effects on Sexual and Asexual Hydras*

The Green hydra from Hyderabad, and hydras from Lucknow, Candanh Nagar and Calcutta which were bearing the gonads when shifted to 29°C ; or to 15°C ; continued to remain in the sexual condition. Hydras without gonads when transferred from 23°C to 29°C showed no tendency to become sexual. However, when they were transferred to 15°C only one type of hydra i. e., hydra from Bolpur became sexual and developed ovary within 2 weeks at the low temperature. All other ecotypes remained in the asexual state.

In all the 12 hydra types in which gonads were observed, the bud production continued quite normally during the period of sexual activity.

4. *Regeneration*

(a) Effect of temperature on hypostome and basal disc regeneration *in situ* : The regeneration timings were observed to vary inversely with the shift in temperature from the optimum in all cases. However, the degree of change was found to be different in the various ecotypes (Table 5 and 6).

Table 5 : Time taken for hypostome regeneration at different temperature (h)

S. No.	Hydra types	Time (h) ± S. E. M. at		
		23 ± 1°C	22 ± 1°C	15 ± 1°C
1.	Green Hyderabad	16.36 ± 0.50	13.5 ± 0.5	20.26 ± 0.37
2.	Imphal	19.12 ± 0.31	20.35 ± 1.35	25.5 ± 0
3.	Trivandrum	22.5 ± 0.76	18.8 ± 0.79	25.0 ± 1.0
4.	Bolpur	20.3 ± 0.35	17.0 ± 1.0	25.0 ± 0.5
5.	Srinagar	20.76 ± 0.44	20.05 ± 0.64	26.5 ± 0.5
6.	Pune	20.75 ± 0.62	18.75 ± 0.74	25.2 ± 1.19
7.	Chandan Nagar	20.86 ± 0.13	18.0 ± 0.58	27.25 ± 0.74
8.	Lucknow	20.5 ± 0.28	18 4 ± 0.39	27.0 ± 1.0
9.	Delhi	21.5 ± 0.28	20.83 ± 0.72	25.25 ± 0.74
10.	Calcutta	23.0 ± 0.28	20.83 ± 0.72	26.4 ± 0.39
11.	Shillong	23.5 ± 0.5	19.05 ± 0.50	26.25 ± 1.19
12.	Madurai	23.49 ± 0.28	18.76 ± 0.14	26.1 ± 0.59
13.	Santiniketan	22.5 ± 0.28	17.83 ± 0.44	25.5 ± 0.5
14.	Jammu	24.5 ± 0.94	18 33 ± 1.20	26 35 ± 0.34
15.	White Hyderabad	24.09 ± 0 5	19.85 ± 0.83	27.25 ± 0.19
16.	Tirupati	27.33 ± 0.33	24.0 ± 0	28.83 ± 0.6

TABLE 6. Time taken for basal disc regeneration at different temperatures (h).

S. No.	Hydra types	Time (h) ± S.E.M. at		
		23±1°C	29±1°C	15±1°C
1.	Green Hyderabad	7.31±0.31	6.5 ±0.5	15.3 ±0.40
2.	Imphal	13.91±9.46	10.25±0.24	19.75±0.87
3.	Trivandrum	16.1 ±0.49	11.8 ±0.19	22.56±0.43
4.	Bolpur	13.17±0.11	10.1 ±0.09	20.0 ±0.5
5.	Srinagar	12.81±0.42	9.9 ±0.09	19.0 ±0.24
6.	Pune	13.8 ±0.30	9.75±0.74	19.0 ±0
7.	Chandan Nagar	13.62±0.29	10.8 ±0.19	22.93±0 53
8.	Lucknow	15.43±0.21	11.25±0.24	21.0 ±0
9.	Delhi	12.75±0.32	9.6 ±1.09	19.5 ±0.5
10.	Calcutta	12.89±0.27	11.0 ±1.0	21.02±0.39
11.	Shillong	18.25±0.62	9.5 ±0.5	20.0 ±0.5
12.	Madurai	12.66±0.20	11.0 ±1.0	19.8 ±0.79
13.	Santiniketan	16.63±0.72	11.8 ±0.19	19.25±0.24
14.	Jammu	14.71±0.36	10.5 ±0.5	21.75±1.24
15.	White Hyderabad	15.25±0.27	11.7 ±0.74	20.75±0.24
16.	Tirupati	20.31±0.23	11.0 ±0	20.25±0.27

(b) Regeneration of the midgastric isolate at 23 ± 1°C : In all ecotypes except the Green hydra from Hyderabad, the hypostome regeneration occured earlier than basal disc (Fig. 10).

DISCUSSION

1. *Effect of Temperature on Growth*

On keeping the hydras at 34°C, shortening of the body column and reduction in tentacle length occurred. It has been proposed by Reisa (1973) that tentacle clubbing when exposed to unfavourable condition results in the reduction of the surface of the polyp in contact with the unfavourable environment without metabolic requirement which would be involved in muscular contraction. On keeping the hydras at 34°C for 96h, an abnormally large number of tentacles were observed to develop within the oral ring in the hydras from Pune, Delhi, Trivandrum, Chandan Nagar and Imphal. It appears that the different hydra types differ in their sensitivity to different temperature. In this context, it is worth noting that the exact upper and lower limit to temperature tolerance has been found to be characteristic for each species of *Xenopus laevis* embryos (Nelson *et al.*, 1982). A growth stimulating extract from the hypostome of hydra was found to induce supernumery heads whenever it was induced into the body column (Lesh and Burnett, 1964). It also induced heteropolarity in regenerating annuli of *H. pirardi* by the induction of large number of tentacles in the oral ring.

The decrease in the time taken for the bud to develop and detach from the parent, at 29°C as compared to 23°C in all hydra types, except the green hydra from Hyderabad, is rather striking. Schulze and Lesh (1970) have shown that in *H. viridis*, time taken for bud detachment was longer at 25°C as compared to 20°C. Keeping the bud maturation time at 23°C as 100% at 29°C it decreased to 79% in the hydras from Imphal and 73% in the hydras from Delhi. In the hydras from Calcutta and the Trivandrum it decreased to 47-49%. In all other types comparitively a larger decrease (upto 28%) was observed. At 15°C, bud maturation time increased to 297% in the hydras from Calcutta, while in the Srinagar and the Imphal types it increased only to 192% and 195% respectively.

Comparison of the population doubling time in the various ecotypes at 23°C shows 5 clusters (Table 1)

Ecological clusters	*Ecotype*
I	Calcutta, Shillong, Chandan Nagar, Tirupati, Srinagar.
II	White Hyderabad, Jammu.
III	Bolpur, Delhi, Lucknow, Madurai, Trivandrum, Imphal, Santiniketan.
IV	Pune
V	Green Hyderabad.

Comparison of the populative doubling time of the 16 ecotypes at 29°C, shows 7 clusters (Table 1)

Ecological clusters	*Ecotype*
I	Calcutta, Jammu, Pune, White Hyderabad.
II	Delhi.
III	Imphal
IV	Trivandrum
V	Chandan Nagar, Green Hyderabad, Shillong, Madurai.
VI	Tirupati, Lucknow, Santiniketan
VII	Bolpur, Srinagar.

On comparing the response of hydras at 34°C to the doubling time at 23°C and 29°C, some interesting correlating features emerge out. In hydra types more sensitive to higher temperature (34 ± 1°C), i. e., in the Pune, Delhi, Chandan Nagar, Imphal, Trivandrum, Calcutta and Green Hyderabad ecotypes, doubling time increased significantly at 29°C as compared to 23°C. In the Shillong, Jammu, Tirupati and Madurai ecotypes, it remained either same or showed a slight decrease at 29°C, but in the Bolpur, Srinagar, Santi-

niketan and white Hyderabad ecotypes, the doubling time at 29 ± 1°C. was significantly less than at 23 ± 1°C. David and Campbell (1972) showed that in *H. attenuata* the epithelial cells have a long and variable G_2 period suggesting it as a possible point of control of hydra growth. The long and variable G_2 of large interstitial cells (Campbell and David, 1974) suggests it as a possible control point in the proliferation and / or differentiation of these cells.

The response of the different hydra types at 34°C doubling time and time taken for bud development and detachment, bud maturation and for initiation of successive buds when considered together leads to some obvious conclusions regarding the temperature threshold for hydra growth and survival. While the Green hydra from Hyderabad, Imphal, Trivandrum, Pune, Chandan Nagar and Delhi ecotypes showed less growth at high temperature, in the Bolpur, Srinagar and Santiniketan ecotypes high temperature favoured growth. At low temperature (15 ± 1°C) the Green hydra from Hyderabad, White hydra from Hyderabad, and Lucknow and Jammu ecotypes showed a favourable response, while the Calcutta and Delhi hydra revealed their sensitivity to low temperature. Hydras with temperature tolerance over a wide range include the Lucknow, Shillong, Jammu, Tirupati, Madurai, Santiniketan and White Hyderabad, ecotypes, while the Delhi, Imphal, Pune, Trivandrum, Chandan Nagar and Calcutta ecotypes showed good growth only at 23°C. High growth rate was observed in the Green hydra from Hyderabad and hydras from Bolpur, Srinagar, Santiniketan, Lucknow, Madurai, Chandan-Nagar, Shillong, Calcutta, Pune, Madurai, and Tirupati. In the Trivandrum, Delhi, Jammu, White Hyderabad and Imphal, ecotypes, growth rate remained low at all temperatures. Thus, it appears that different ecotypes exhibit faster growth under different temperature conditions.

The mechanism controlling the decrease or cessation of growth must be a basic one and sensitive to a variety of stimuli. Lesh and Burnett (1964) extracted a fraction from the hypostome of hydra, which induces growth (induction of supernumery tentacles) whenever, it is introduced into the body column. This active fraction could not be extracted from *H. pirardi* after it had remained for 8 weeks in the cold. This was found to coincide precisely with the time the animal enters complete sexuality. Also if sexual *H. pirardi* (which did not produce buds) were brought from 8°C to 20°C growth processes were resumed and tentacles or supernumery heads arose at the level of gonads. On the basis of the data obtained in the present study, it appears that in different ecotypes, the mechanism controlling growth has different temperature tolerance threshold. This may therefore give a clue in the identification of different species.

2. *Sexual Reproduction*

Under stable laboratory conditions hydras became sexual at different periods of the year (Fig. 4, 6, 9). In some ecotypes, like the Santiniketan, Madurai, Shillong and Jammu, the two periods of maximum sexual activity were September 1981 and January 1982. In the Lucknow and Chandan Nagar ecotypes maximum number of hydras with gonads were observed in September 1981. Specificity in the periodic occurence of gonads under laboratory condition has been noted in *H. pirardi* and *H. pseudooligactis* (Burnett and Diehl, 1964) while the former has 1 phase, the latter has 2 phases of maxium sexual a ctivity. Under field conditions also, Hyman (1929), Rowan (1930) and Forrest (1963) and Caleb (1956) found maximum number of sexual forms in *H. americana*, *H. canadensis*, *H. brauri* and *H. gangetica* in August/ September. It therefore, appears that periodicity of gonad occurence may give an indication of the species nature of the hydra.

In the Santiniketan ecotypes, the hydras became spontaneously sexual at the same time of the year, both during 1981 and 1982. The occurrence of an endogenous cyclic phase has been noted in *H. pirardi* (Burnett and Diehl, 1964). The polyps maintained under constant conditions of temperature 20-21°C for three years entered sexually spontaneously every year only during September. Thus, it seems that the endogenous cyclic phase has a predominant role in some species. It may therefore be used as a clue in identification of species.

The present study has demonstrated that temperature has an important role in the induction of gonads in the Bolpur ecotype. Since these hydras developed ovaries when kept at 15°C, it means that the sexual state in this ecotype is induced at low temperature. The role of low temperature in gonad induction has been shown in *H. robusta*, *H. parva*, *H. psuedooligactis*, *H. pirardi*, *H. oligactis* and *H. fusca* (Hyman, 1928 ; Ito, 1954 ; 1955 ; Burnett and Diehl, 1964 ; Park *et al.*, 1965). Rise in temperature has been found to induce sexuality in *H. plaudicola* (Ito, 1952) and *H. magnipapillata* (Ito, 1952, 1954). Thus, the stimulus for gonad induction may be used for diagnostic purpose.

Hermaphroditic hydras were found both in the Santiniketan and in the Green Hyderabad ecotypes. However, very few hermaphrodites, were found in the hydras from Santiniketan while in the Green hydras from Hyderabad maximum number of polyps exhibited the hermaphroditic condition at 23°C. At 15°C, no sexual forms were observed. It has been observed earlier that *H. virdis*, enters sexuality spontaneously at 22°C and exhibits hermaphroditism (Burnett and Diehl, 1964). Thus, the spontaneous induction of the hermaphroditic state in maximum number of

polyps at 23°C appears to be a characteristic feature of Green hydras from Hyderabad as well as *H. viridis*. Spontaneous occurrence of gonads under laboratory conditions only during August / September, October, November and January, is intriguing and may be related to the seasonal variations under field conditions. Whitney (1907) and Hyman (1930) have suggested that changing ambient temperature during spring and autumn may be responsible for sexual development under the field conditions. Griffin and Peters (1939) Adshead *et al.* (1963) have suggested that drying up of bodies of water and concommitant increase in pH are important factors. A number of investigators (Burnett and Diehl, 1964 ; Mc Connell, 1938) found that the encysted embryos of hydra are extremely resistant. Thus, hydra resort to sexual reproduction under unfavourable stress conditions in nature. The existence of sexual cycles in some hydra types which enables them to drift repeatedly in and out of sexual condition has been thought of as a mechanistic safeguard against the possibility of insufficient forewarning of a prolonged warm crises (Reisa, 1973).

In the Srinagar, Delhi and White Hyderabad hydra types where no sexual forms were observed, under the laboratory conditions, it is suggested that apart from temperature some other factors singly or in combination may play an important role in inducing sexual state. In this context it must be noted that the stimuli for gonad formation in different species have included temperature, photoperiod, pH, oxygen tension, carbon dioxide tension, starvation, overfeeding, stagnation of medium and endogenous cyclic phase (Kanaev, 1952 ; Ito, 1952a, b, c ; Burnett, 1961a ; Burnett and Diehl, 1964).

The ecotypes were observed to form 4 clusters on the basis of striking differences in the shape and size of the testes (Fig. 8). Earlier the shape and size of the testes has been found to be typical for each hydra species by Schulze (1917), Hyman (1929), Ewer (1948), Forrest (1963). The consistency and specificity of shape and size of testes in different ecotypes observed in this investigation, confirm its reliability as a diagnostic character.

Observations of spontaneous induction of gonads under laboratory conditions, the influence of low temperature (15°C) on gonad induction in the hydra from Bolpur, the non-appearance of gonads in the polyps from Srinagar, Delhi and White hydra from Hyderabad have given significant clues for the identification of different ecotypes. The presence of hermaphroditic, stable or unstable dioecious nature and the shape of testes in the different types have emerged as reliable diagnostic characters.

3. *Regeneration*

(a) Hypostome and basal disc regeneration : At 23°C different ecotypes showed specific hypostome and basal disc regeneration time. The regeneration

study shows that the rate of regeneration increases at 29°C and decreases at 15°C (Table 5 and 6). Burnett *et al.* (1964) have demonstrated that at room temperature neuro-secretory droplets increase in the nerve cells within 4 h period in regenerating hydra. If hydras are excised and placed in the cold, this build up of neurosecretory droplets may be inhibited or may proceed slowly. The reverse may be thought to occur at high temperature.

(b) Regeneration of the midgastric isolate : In the present study, time taken for hypostome and basal disc determination in the midgastric annulus has been found to vary in the different ecotypes. While the basal disc regeneration occurred much earlier (6.98 h) than hypostome regeneration in the midgastric isolate of Green hydra from Hyderabad, the reverse was observed in the remaining hydra types. Although hypostome regeneration preceded basal disc regeneration in hydras from Pune, Chandan Nagar, Lucknow, Delhi, Calcutta, Shillong and Madurai ; all nonsymbiotic ecotypes in some cases the difference between the two events was very small (0.1-1 h) while in the hydras from Imphal, Trivandrum, Bolpur, Santiniketan, Jammu, White hydras from Hyderabad and hydras from Tirupati and Srinagar, it was appreciable (1.5-3.37 h). Thus, depending on whether basal disc regeneration occurs before or after hypostome regeneration, the ecotypes can be put in two well defined ecological clusters.

Ecological Cluster	*Ecotype*
I	Green hydra from Hyderabad
II	All other hydra types.

The decision to form first the head or the foot is linked to the circuit of cell communication across the regenerating middle piece, which leads to the formation of morphogenetic field, which in turn activates the future hypostome or basal disc. Schaller (1976b) has shown that the head activator has effects on interstitial cell behaviour. Not only does the head activator increase the rate of head regeneration, it also has a mitogenic effect by shortening the cell cycle of interstitial cells and other proliferating cell types in hydra. The morphogen causes an increase in the number of interstitial cells differentiating into nerves and a decrease in nematocyte formation (Schaller, 1976b). This easily correlates with regeneration phenomenon since shortly after amputation large amount of head activator released at and near the cut surface (Schaller 1973, 1976a). At the same time nerve cell differentiation increases and nematocyte formation decreases in the area. Berking (1979b) has characterized the effects of the inhibitor activity on interstitial cell behaviour as well as on regeneration. At concentration where it blocks head regeneration it also blocks both interstitial cell division and its differen-

tiation into nerves. Removal of the inhibitor results in a burst of mitotic activity in the interstitial cells and a sudden increase in the number of nerve cells formed as well as resumed head regeneration. The parallel influence of the morphogens on regeneration and interstitial cell behaviour suggest the temporal differences in the regeneration of the hypostome and basal disc in the midgastric section of different ecotypes may be either at the level of interstitial cells or neurosecretory cells or both. However, since regeneration has been shown to occur in nerve free hydra (Marcum and Campbell, 1978), the role of epithelial cells cannot be ruled out.

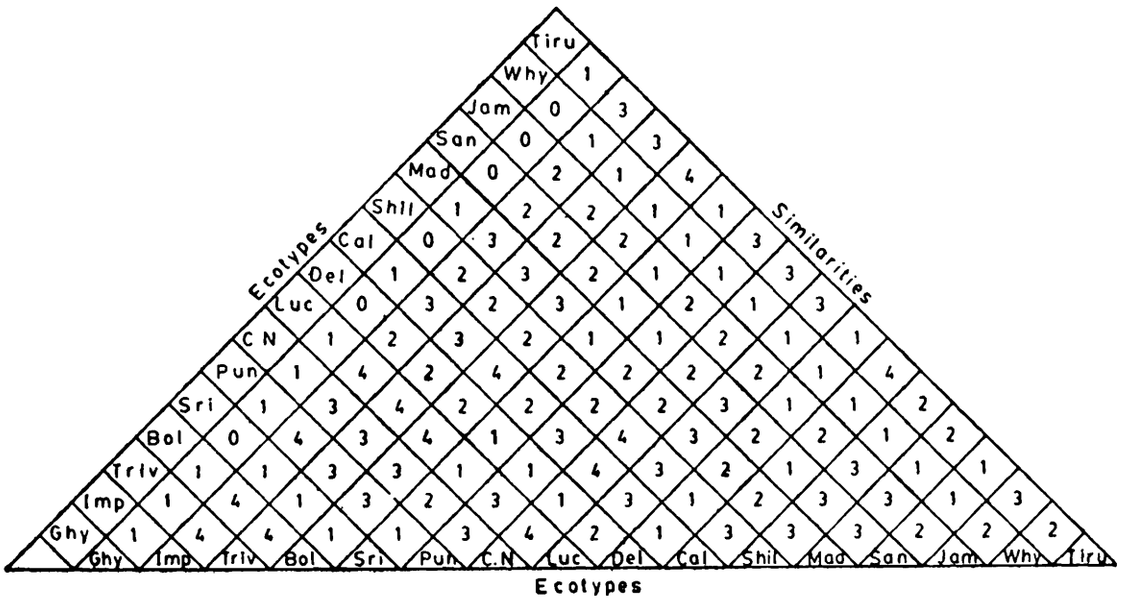
Taking into account 5 diagnostic physiological characters—sexual nature, number of sexual phases, presence and shape of testes or ovary, growth at 23°C and time taken for regeneration of the mid-gastric isolate (Table 7), the extent of resemblance among the 16 ecotypes have been analysed (Table 8). This directly provides the magnitude of interecotypic affinity among the various ecotypes on the basis of characters at the physiological level.

TABLE 7. A Projection of major physiological characters of the various ecotypes

S. No.	Hydra type	Sexual nature	Number of sexual phases	Testis ovary	Growth at 23°C	Midgastric isolate regeneration time
1.	Green Hyderabad	H	1	TCP	V	I
2.	Imphal	SD	1	TR	III	II
3.	Trivandrum	SD	1	TLR	III	II
4.	Bolpur	SD	1	O	III	II
5.	Srinagar	—	—	—	I	II
6.	Pune	SD	1	TR	IV	II
7.	Chandan Nagar	SD	1	TBR	I	II
8.	Lucknow	SD	1	TBR	III	II
9.	Delhi	—	—	—	III	II
10.	Calcutta	SD	1	O	I	II
11.	Shillong	SD	2	TBR	I	II
12.	Madurai	SD	2	TCP	III	II
13.	Santiniketan	UD	2	TBR	III	II
14.	Jammu	SD	2	TE	II	II
15.	White Hyderabad	—	—	—	II	II
16.	Tirupati	SD	1	O	II	II

Abbreviations : I to V—Ecological cluster number of each character (see p. 121 to 129)
H— Hermaphroditic, SD— stably dioecious, UD— Unstably dioecious, TCP— Testes conical in shape with papillae, TR—Testes round in shape, O— ovary, TBR— Testes broadly round in shape, TE— Testes elongated in form, TLR— Testes of low rounded form.

Table 8
Magnitude of inter-ecotypic affinities at physiological level



SUMMARY

Physiological characteristics of hydra at three levels, namely sexual reproduction, asexual reproduction and regeneration, revealed that a number of features can be used for the purpose of classification. Under the category of sexual reproduction are a number of characteristics such as presence of gonads, type of gonads, shape of testis, periodicity of gonad occurrence and the effect of temperature on induction of gonads. In the second category of asexual reproduction, reliable diagnostic characters which emerged are : sensitivity to high or low temperature, change in population doubling time on transfer from control to high or low temperature, bud detachment time, number of buds per hydra, and high or low population doubling time in general. Study of the time taken for hypostome and basal disc development was also found to be characteristic for each hydra type under constant temperature conditions.

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FIGURES

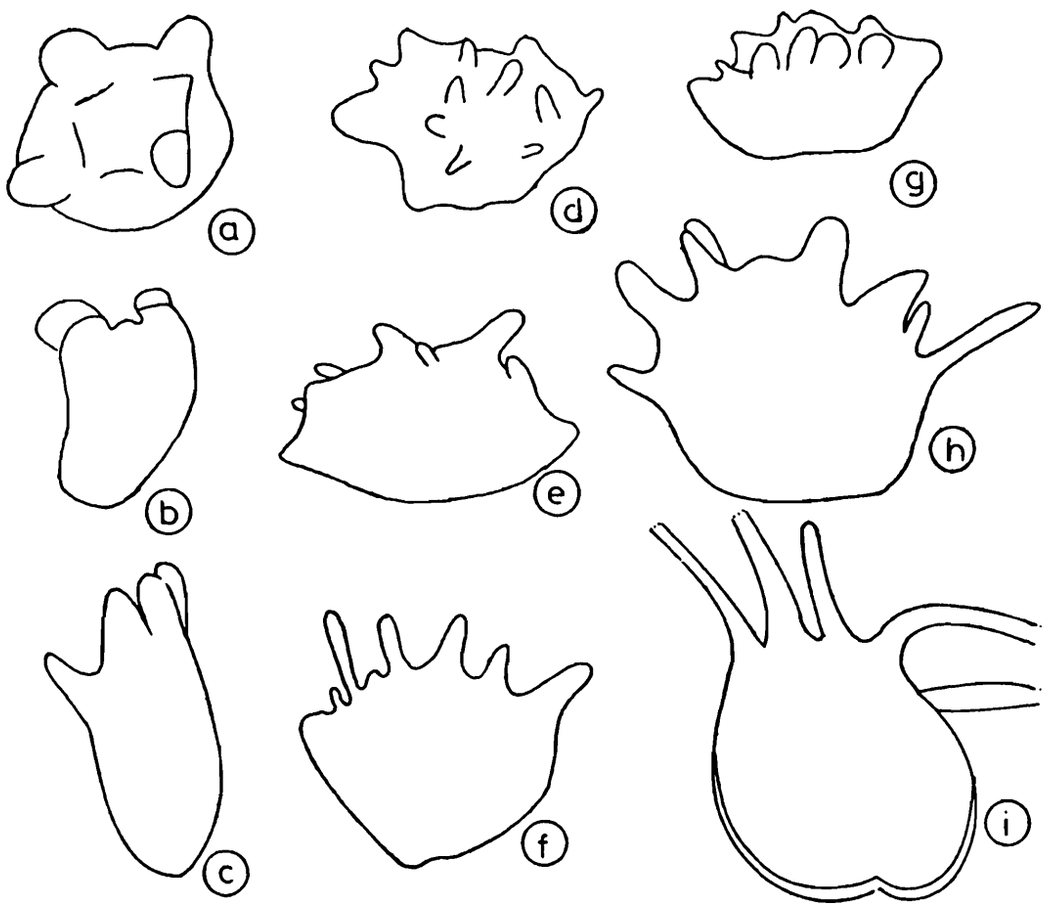


Fig. 1. Effects of high temperature (34°C) on the morphology of various hydra eco types (camera lucida drawings, X 22) :
After 24 h : Hydras from (a) Pune, (b) Imphal and (c) Delhi hydras show reduced body size and short stunted tentacles.
After 96 h : Hydras from (d) Pune, (e) Imphal, (f) Delhi, (g) Chandan Nagar, (h) Trivandrum and (i) Shillong hydras show extreme reduction in body size and occurrence of short slender tentacles in between the pre-existing tentacles. In Shillong hydra the body column is divided into 4 incomplete segments by vertical constrictions.

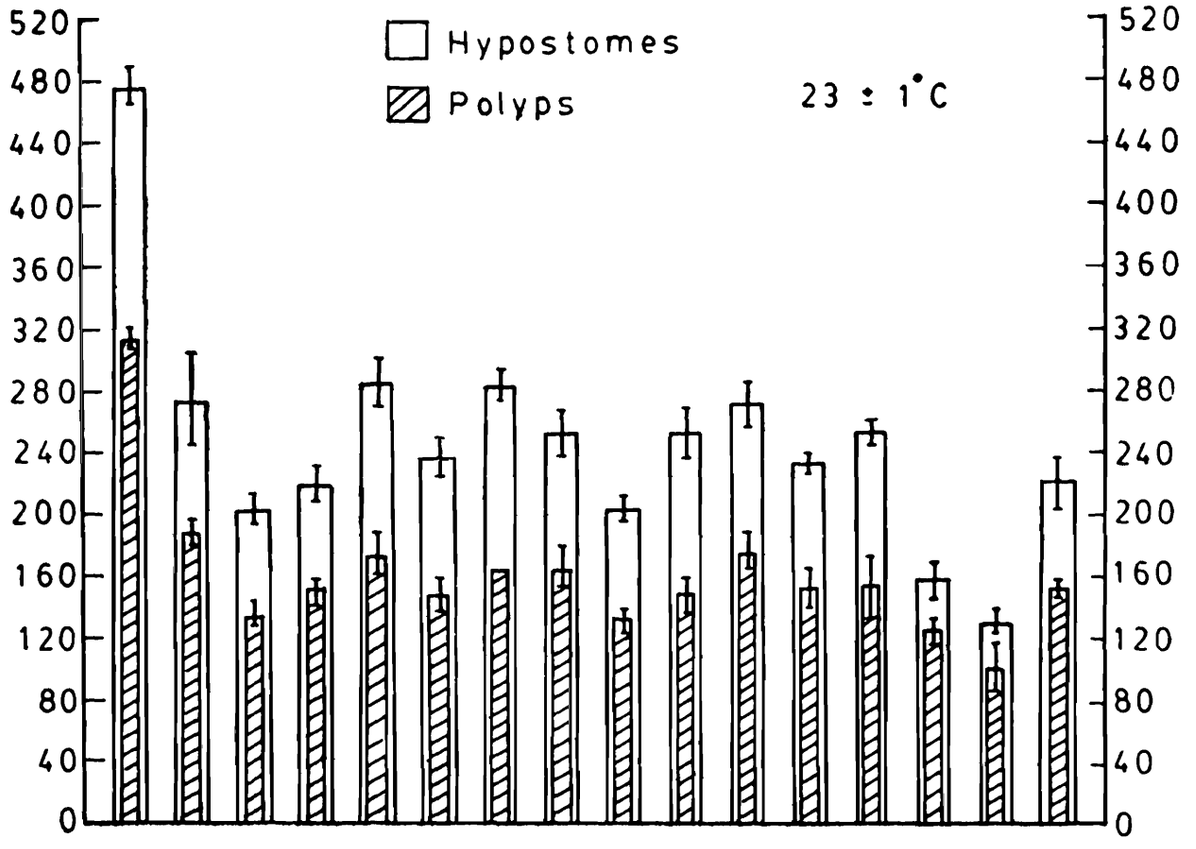

Hypostomes
Polyps
23 ± 1°C
520
480
440
400
360
320
280
240
200
160
120
80
40
0

Fig. 2a.

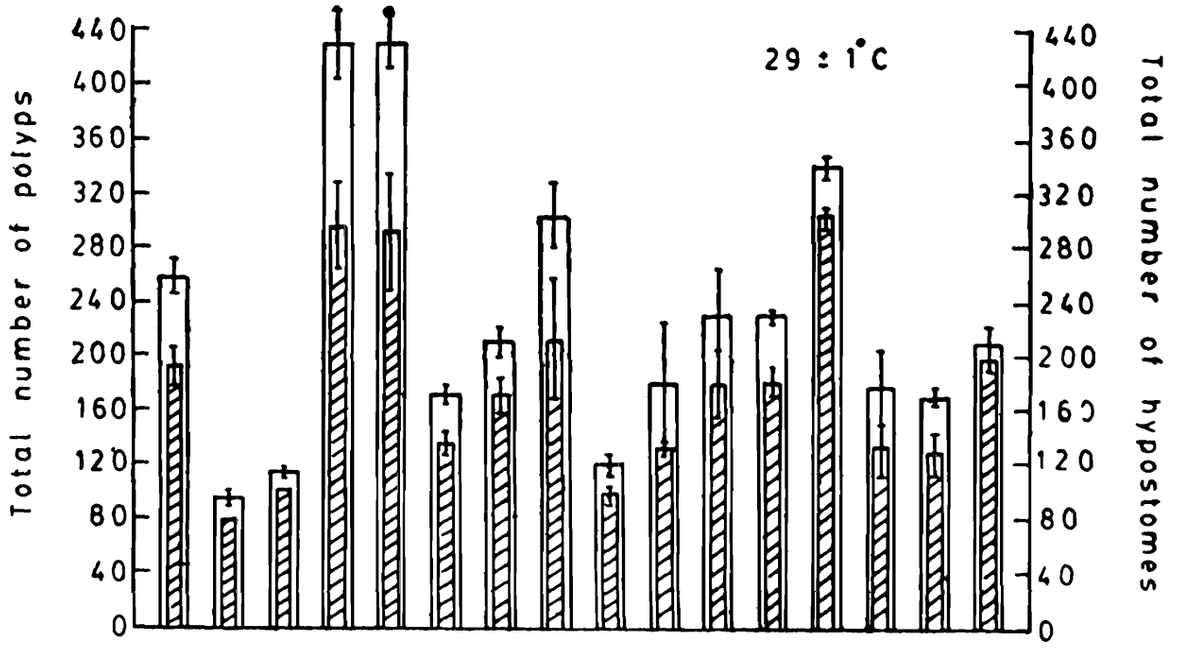

29 ± 1°C
Total number of polyps
Total number of hypostomes
440
400
360
320
280
240
200
160
120
80
40
0

Fig. 2b.

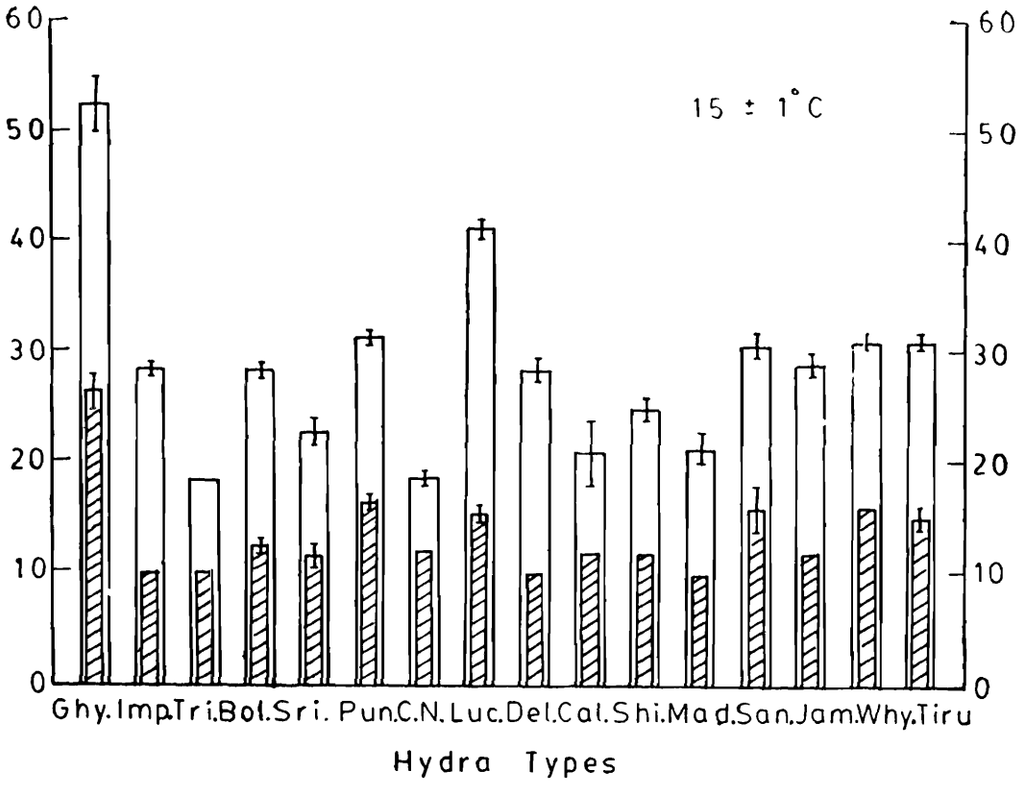


Fig. 2c.

Figs. 2a, 2b & 2c. Effects of temperature (23°C, 29°C and 15°C) on growth of 16 hydra ecotypes in terms of the total number of polyps and total number of hypostomes after 10 days. Bars denote standard error of the mean (± S. E. M.).

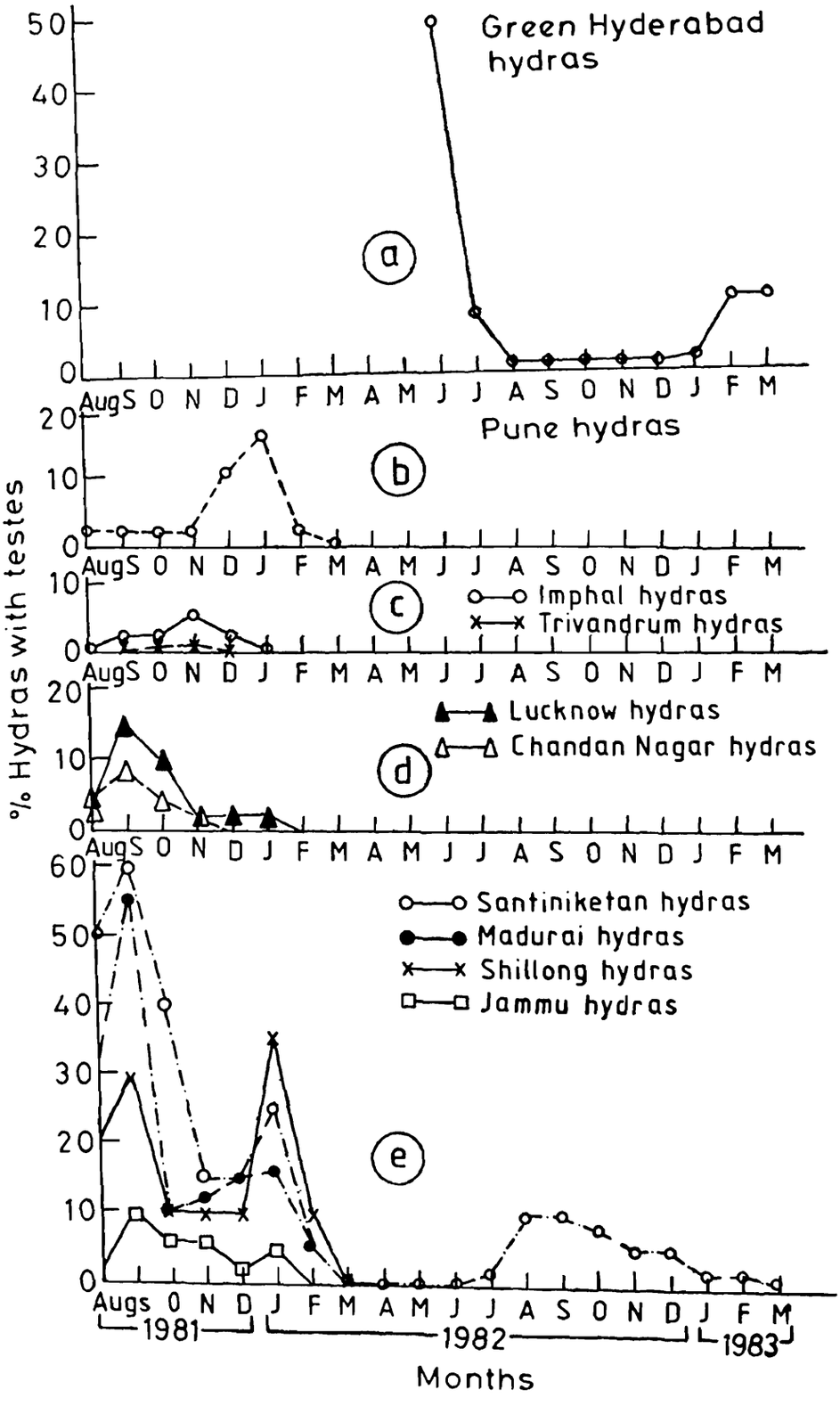


Fig. 3. Frequency of occurrence (%) of hydras with testis in (a) Green hydra from Hyderabad, and hydra from (b) Pune, (c) Imphal, Trivandrum, (d) Lucknow. Chandan Nagar ; (e) Santiniketan, Madurai, Shillong and Jammu ecotypes between August 1981 and March 1983.

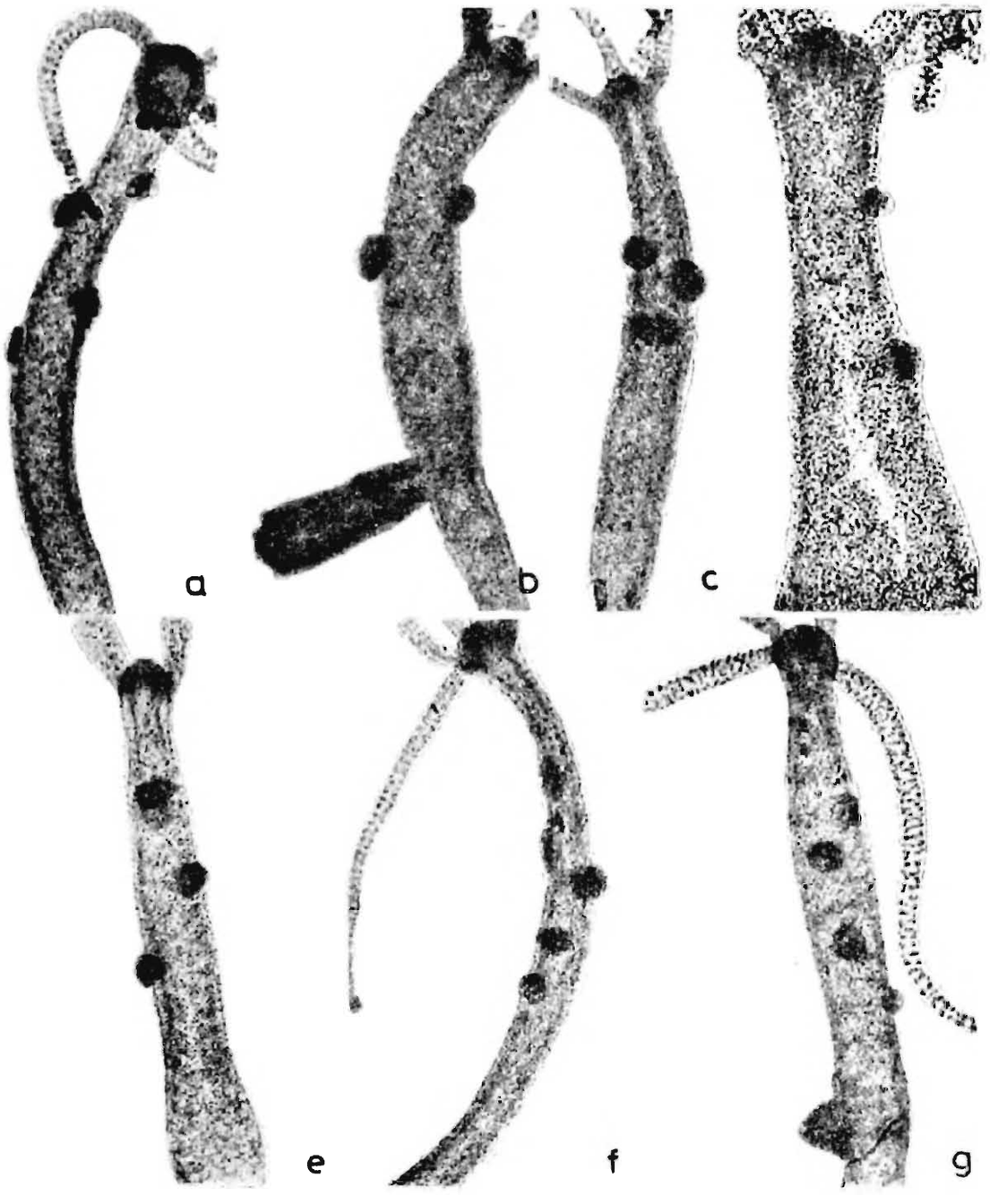


Fig. 4. Position and arrangement of testes in various hydra ecotypes (whole mounts X 39) :
(a) Pune ; (b) Shillong ; (c) Trivandrum ; (d) Lucknow ; (e) Madurai ; (f) Jammu and (g) Santiniketan.

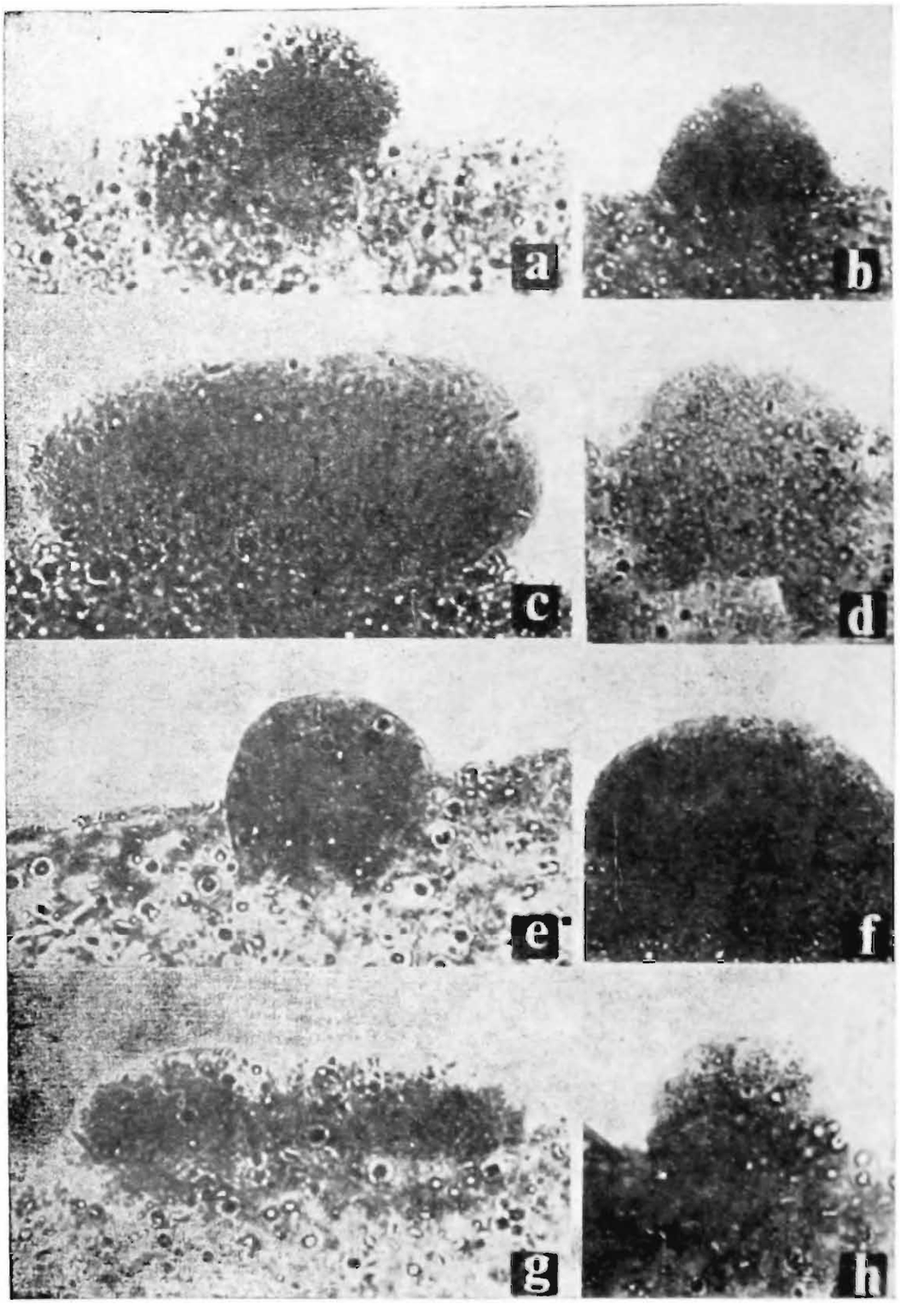


Fig. 5. Magnified view of the testis in the various hydra ecotypes (whole mounts X 176) :
(a) Santiniketan ; (b) Green hydra from Hyderabad ; (c) Shillong ; (d) Trivandrum ; (e) Madurai ; (f) Pune ; (g) Jammu and (h) Lucknow.

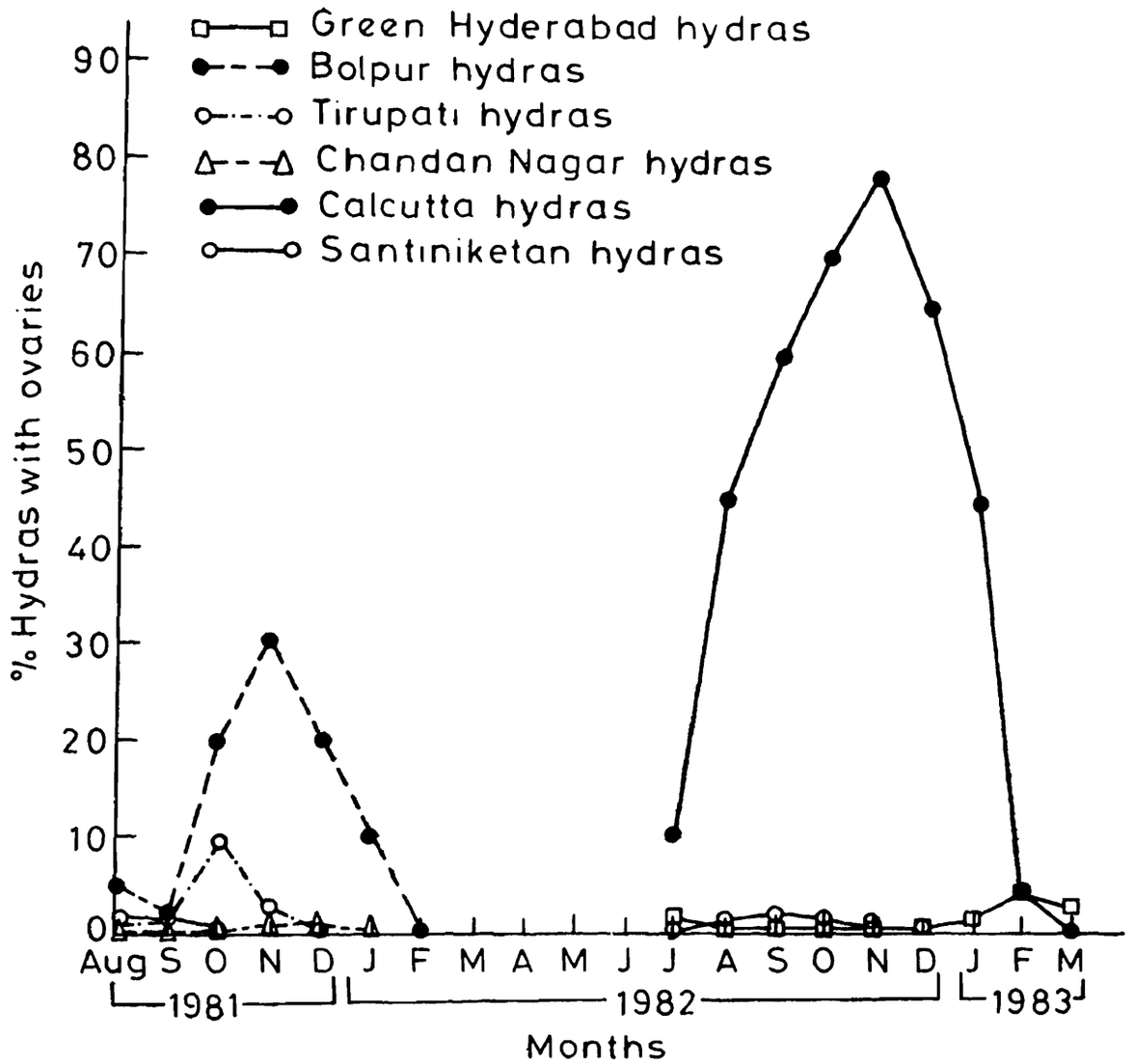


Fig. 6. Frequency of occurrence (%) of hydras with ovaries in Green hydra from Hyderabad. Bolpur, Tirupati, Chandan Nagar, Calcutta and Santiniketan hydra types between August 1981 and March 1983.

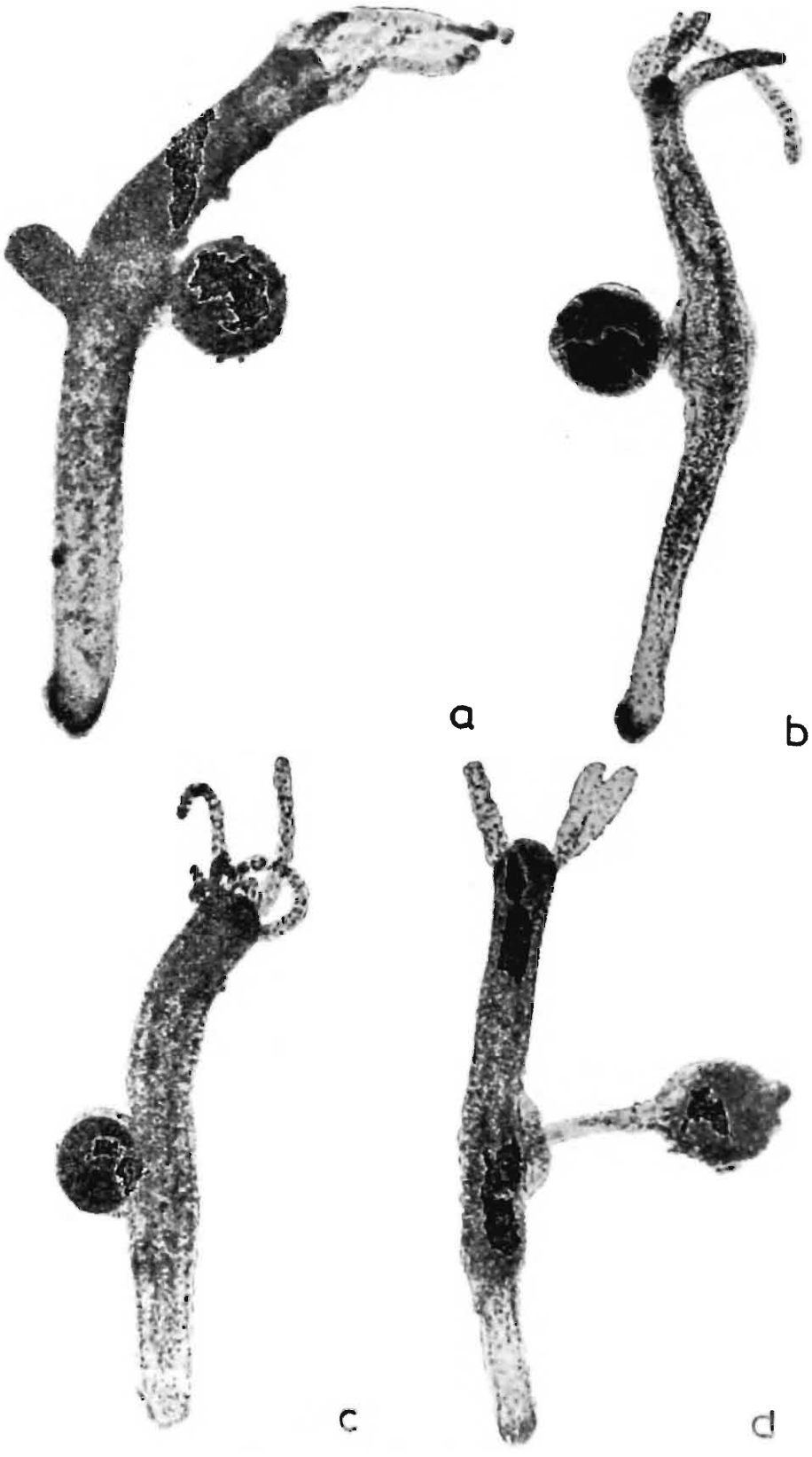


Fig. 7. Position of ovary in various hydra types (whole mounts:X 51) :
(a) Green hydra from Hyderabad ; (b) Tirupati ; (c) Calcutta and (d) Santiniketan.

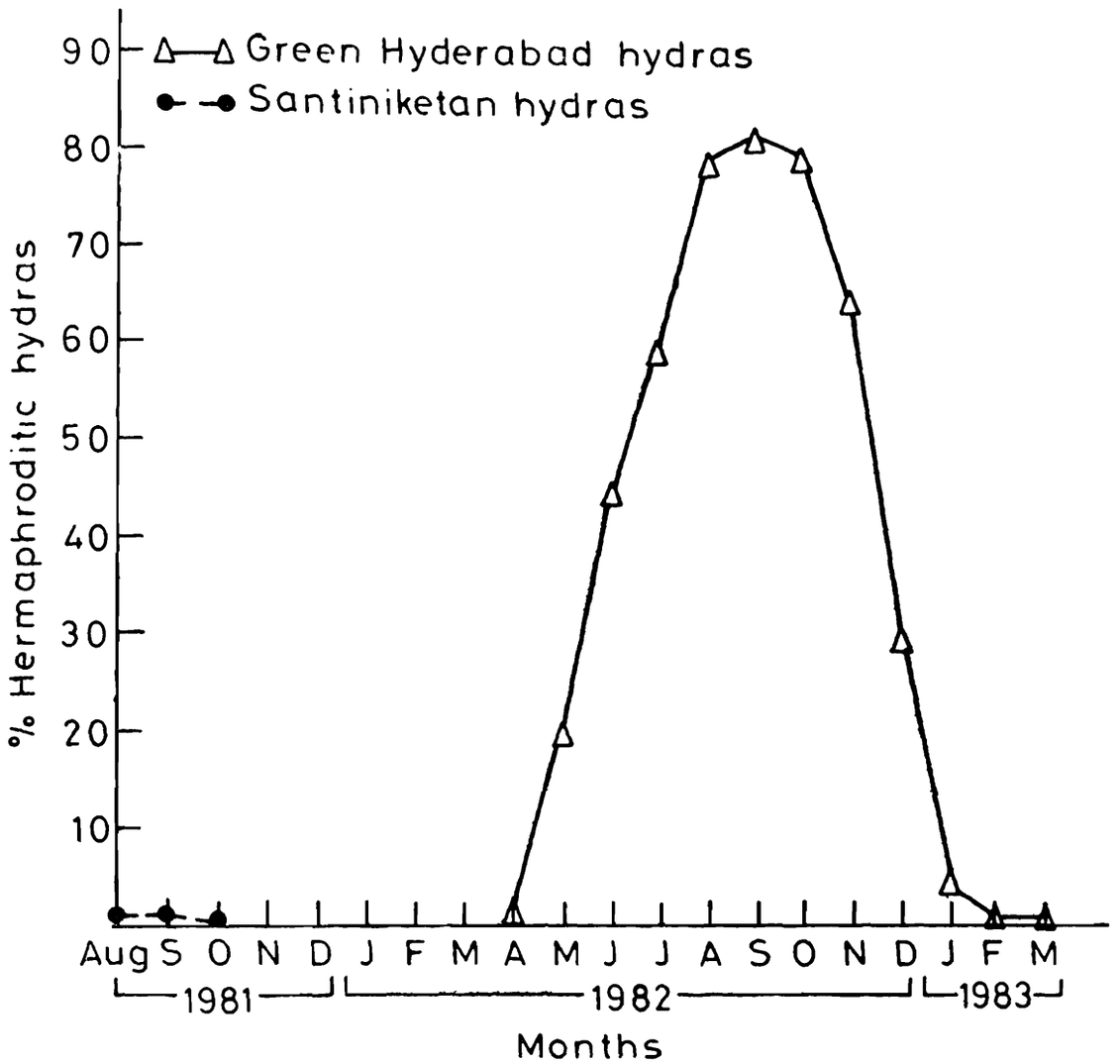


Fig. 8. Frequency of occurrence (%) of hermaphrodites in Green hydra from Hyderabad and Santiniketan hydra ecotypes between August 1981 and March 1983.

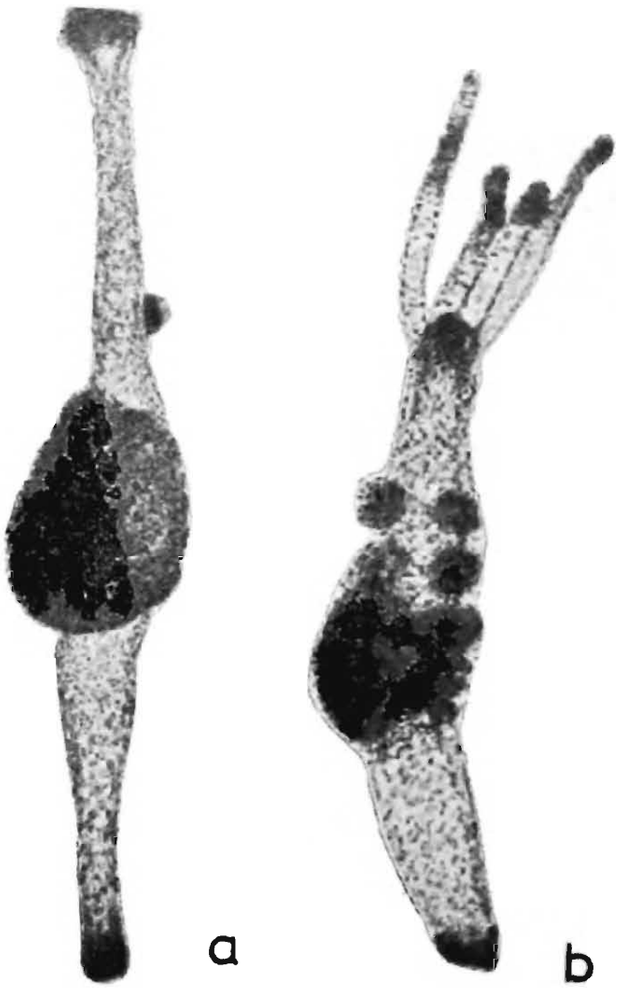


Fig. 9. Position of gonads in hermaphroditic hydras (whole mounts X 60) : (a) Green hydra from Hyderabad ; (b) Santiniketan hydra ecotype.

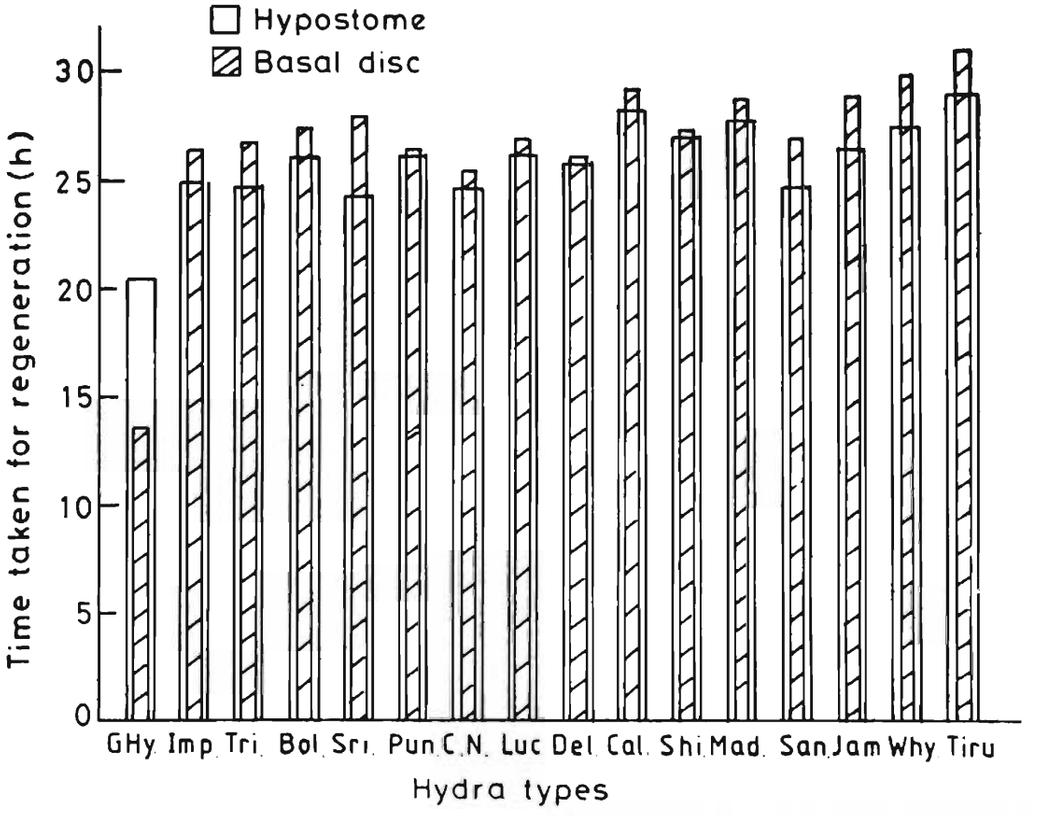


Fig. 10. Average time taken for hypostome and basal disc regeneration in the midgastric annuli of the 16 hydra ecotypes.

MORPHOGENETIC ANALYSIS OF ECOTYPES OF INDIAN HYDRA

Part VI : Some theoretical considerations about species problem in Hydra.

Introduction

The study conducted on 16 Indian hydra types under constant temperature, culture and feeding conditions has revealed that at morphological level, only a few characters which remain constant within a population include length of the tentacles, position of the bud zone and size of the basal disc (Prasad and Mookerjee, Part II). These features have also been found to differ significantly between one and more ecotypes. Other characteristics like the nature of the subhypostome and peduncle, have been found to be constant within some populations (Green hydras has been observed to have a distinct 'neck like' subhypostome, and Delhi hydras have a cylindrical column), while varying gradations of the 'neck like' subhypostome and 'stalk like' peduncle have been seen within most ecotypes. It is also worth noting that not only the ecotypes exhibited differences in the nature of stimuli inducing gonad production, but also showed clear cut differences in the shape of the gonads and in the frequency of gonad production (Prasad and Mookerjee, Part V). The range of temperature, optimum for growth, and the time taken for bud development and its detachment, bud maturation and time interval between successive bud initiation at the different temperature studied also differed in the different ecotypes. Time taken for regeneration of the midgastric annulus and for hypostome and basal disc regeneration in *in situ* hydra ; showed significant differences only between some ecotypes (Prasad and Mookerjee, Part V). The pattern of RNA synthesis (Prasad and Mookerjee, 1985) during growth and in regenerating hydras also showed a number of interesting similarities and dissimilarities. Our data on the RNA synthesis pattern was almost similar during growth in nonregenerating hydras of the five ecotypes, during regeneration, the green hydras showed an accelerated rate of RNA synthesis compared to the other four types (Prasad and Mookerjee, under preparation). Meaurement of DNA for cells of ecotypes of *Hydra* point out about its remarkable constancy (Prasad *et al.*, under preparation).

The crucial questions which one has to answer whether different ecotypes that we are dealing here, belong to the same species or to different species ?

If we follow the earlier line of thinking adopted before by Schulze (1914, 1917), Hyman (1929, 1930, 1931, 1938) and Campbell (1983), our problem will appear less troublesome and we can unhesitatingly pronounce them as different species since they show sufficient number of differences at morphological and physiological (reproductive) levels, to fulfil their requirement of distinct species. Campbell (1983) on the other hand, advances the view that species of hydra fall into four groups, two of which correspond with Schulze's two genera *Chlorohydra* and *Pelmatohydra* ; and the remaining two groups (both in Schulze's genus *Hydra*) are claasified on the basis of the width of the holotrichous isorhiza.

Even this approach will however bo too simplistic an approach for the classification of a complex metazoan like hydra due to two reasons : Firstly, it is now known that the presence of the stalk is not a reliable character and a number of investigators have questioned its validity (Prasad and Mookerjee, ibid, part I). Secondly, the selection of the width of the holotrichous isorhiza, as a sole diagnostic character, over-riding the importance of all other character, will also not have any obvious justification. One has to bear in mind that it has become increasingly certain that one or two structural features in hydra cannot meet the normal criteria for taxonomic validity satisfactorily (Reisa, 1973), more so in a somatic replicating system like Hydra.

Range of variations

From the standpoint of our findings on the concept of species in hydra, several features of importance emerge from the results obtained by the multi-faceted approach taken in the present investigation :

1. Under constant culture conditions, only a few structural features are found to remain consistent within a population. Characteristics like the number of tentacles, and shape of the organisms in general are unreliable for identification of species in hydras because they also show at times a degree fluctuations (Prasad and Mookerjee, ibid., Part II).

2. Some characters like the presence or absence of subhypostome, and cylindrical column (uniform diameter) may be consistent and well defined in some ecotypes and inconsistent in other ecotypes (Prasad and Mookerjee, ibid, part II).

3. Some ecotypes of hydra are hermaphroditic, some unstably dioecious and some stably dioecious (Prasad and Mookerjee, ibid, part V).

4. The temperature tolerance range of an ecotype is not necessarily related to the temperature conditions of the geographical locality from which it had been collected (Prasad and Mookerjee, ibid., part V)·

5. The shape of the holotrichous isorhiza varies in different ecotypes (Prasad and Mookerjee, ibid., part III). However, since the shape may be found to be different in ecotypes resembling in other characteristics such as bud zone, shape of the basal disc, nematocyts density (Prasad and Mookerjee, ibid., part II) growth rate, temperature, tolerance, regeneration of the mid-gastric annulus (e. g., Lucknow and Chandan Nagar ecotypes) (Prasad and Mookerjee, ibid., part V), it cannot be considered as a diagnostic characters of greater importance than other character.

6. Different ecotypes vary in the number of epithelial cells, density of stem cells, nerve cells and nematocysts. However, the density of gland cells remains same in the five ecotypes (except in hypostome of Calcutta hydras) studied (Prasad and Mookerjee, ibid., Part III).

7. The pattern of RNA synthesis during growth, did not differ significantly in non-symbiotic and symbiotic (dark grown) hydras (Prasad and Mookerjee, under preparation).

8. The pattern of RNA synthesis during regeneration, however, did not vary between non-symbiotic ecotypes but the overall synthetic rate was much faster in the symbiotic Green Hyderabad hydra. (Prasad and Mookerjee, 1985).

9. The mean absorbance of DNA per unit area was found to remain same in similar cell types of 6 nonsymbiotic ecotypes, indicating the conservation of the genomic entity of the cell types in Hydra (Prasad *et al.*, under preparation.

Necessity of multifaceted approach

It is clear that to arrive at any decision regarding the overall position of an ecotype in relation to other ecotypes, instead of one absolute criterion, reliance on morphological, behavioural, physiological, cellular and biochemical characters must be given due consideration. This is mainly because no single character has emerged to be of especial taxonomic significance and of sufficient reliability to stand alone as a systematic character above all others for the identification of species.

The Green Hyderabad hydra has been shown to differ significantly from all other ecotypes in all aspects, whereas the differences are not so striking between the other ecotypes. The presence of symbiotic algae in the epithelial

cells (Prasad and Mookerjee, Part III), extremely short tentacles (Prasad and Mookerjee, Part II), sensitivity to high temperature, hermaphroditic nature (Prasad and Mookerjee, Part V), and the presence of slipper shaped holotrichous isorhiza in Green Hydra ecotype (Prasad and Mookerjee, Part III) shows its resemblance to *Chlorohydra Viridissima* and thus puts it under the genus *Chlorohydra* (Schulze, 1917).

Thus, while the Green Hyderabad ecotype appears circumscribed and differentiated from other groups by a sharp boundary, whereas the other nonsymbiotic ecotypes have many over-lapping characters. Thus, the decision to assign a species, subspecies or strain designation to the different ecotypes containing nonsymbiotic hydra, must be approached with great caution. Moreover, the comparison of the nonsymbiotic hydra ecotypes studied here, with the known species of hydra has not yielded any satisfactory resemblance. Thus, it makes difficult to arrive at a definite conclusion, regarding their taxonomic status at this stage ; until more biochemical work particularly on immunological differences between the ecotypes are carried out. However, a comparison of the major characteristics among the 16 ecotypes as the basis of similarities in the 13 characters investigated, has been given in Table 1. This not only facilitates the identification of the different ecotypes found in India but also shows the affinity between the various groups.

Points of ecotypic similarities and dissimilarities

Hydra appears as a unique metazoan which seriously merits for a redefinition of the concept of species. Since this organism drifts in and out of the sexual state both in nature and under laboratory conditions, characters at asexual level assume equal importance. This is unlike the higher animals where the 'biological species' concept (Mayr, 1963) is readily applicable since sexual reproduction is the only means of reproduction.

A survey of the world distribution of described hydras show a large number of species many of which have been reported from restricted localities. Hyman (1929) reported that *H. americana* was found to occur in nature in company with the green hydra *H. viridis* and *H. oligactis* in a number of ponds, lagoons and streams within a radius of fifty miles from Chicago. Rowan (1930) found that *H. carnea* and *H. canadensis* occur together in some lakes in Canada. In the present investigation, green and white hydras were collected from the same pond in Hyderabad. These two hydra types, however, not only differ significantly in phenotypic characters but also give different asexual and sexual response under simila conditions.

TABLE 1 : A projection of major characteristics of various ecotypes

Sl. No.	Hydra type	Algal cells	Hydra size	Tentacle length	Nematocyst density	Holotrichous isorhiza	Bud position	Origin of tentacle rudiments	Basal disc size	Sexual nature	Sexual phases	Testes / ovary	Growth at 23°C	Midgastric isolate regeneration time
1	Green Hyderabad	+	IV	IV	II& III	S	II	SO	M	H	1	TCP	V	I
2.	Imphal	–	II	III	IV	C	IV	UO	L	SD	1	TR	III	II
3.	Trivandrum	–	II	II	I	C	IV	UO	s	SD	1	TLR	III	II
4.	Bolpur	–	III	III	V	C	IV	UO	M	SD	1	O	III	II
5.	Srinagar	–	III	II	II& III	C	V	UO	M	–	–	–	I	II
6.	Pune	–	III	II& III	IV	C	IV	UO	L	SD	1	TR	IV	II
7.	Chandan Nagar	–	III	II	III	C	IV	UO	s	SD	1	TBR	I	II
8.	Lucknow	–	III	III	II	S	I	UO	s	SD	1	TBR	III	II
9.	Delhi	–	II	III	IV	P	I	UO	s	–	–	–	III	II
10,	Calcutta	–	I	I	I	C	I	UO	M	SD	1	O	II	I
11.	Shillong	–	III	II	III	C	I & III	UO	M	SD	2	TBR	I	II
12.	Madurai	–	II	II	V	C	I	UO	s	SD	2	TCP	III	II
13.	Shantiniketan	–	III	III	II	S	III	UO	M	UD	2	TBR	III	II
14.	Jammu	–	II	III	I	S	I	UO	s	SD	2	TE	II	II
15.	White Hyderabad	–	II	II& III	II& III	S	I & III	UO	L	–	–	—	II	II
16.	Tirupati	–	III	II	I	B	IV	UO	s	SD	1	O	II	II

Abbreviation : S—Slipper shaped ; SO—Simultaneous origin ; UO—uneven origin ; C—Cylindrical ; P—Pyriform shape ; s—small ; M—Middle ; L—Large ; H—Hermaphroditic ; SD—Stably dioecious ; UD—Unstably dioecious ; TCP—Testes conical in shape with papillae ; TR—Testes round in shape ; O—ovary ; TBR—Testes broadly round in shape ; TE—Testes elongated in form ; TLR—Testes of low rounded form

Papenfuss (1934) attempted to obtain regenerates from a mixture of tissue fragment from different species. She reported that although tissue fragments of the green and brown hydra unite, all the aggregates eventually disintegrated. Noda (1970) obtained regenerates from the fragments of two different species of hydra—*Hydra magnipapillata* and *Pelmetohydra robusta*. He reported that the tissue from the two species in a mosaic hydra never separated ; although the *magnipapillata* tissue was sent into a state of depression in most cases. These results suggest that there is a certain degree of compatibility between the tissues of *magnipapillata* and *robusta* which is missing or is much less between the green and brown hydra used by Papenfuss (1934). Our own experiments involving mixing the tissues of two ecotypes between green hydra and nongreen Santiniketan hydra show an incompatiblity leading to separation of the two kinds of tissues (Nangia and Mookerjee, unpublished). Thus, it appears that the species-specificity between cells is maintained between some ecotypes and not in others.

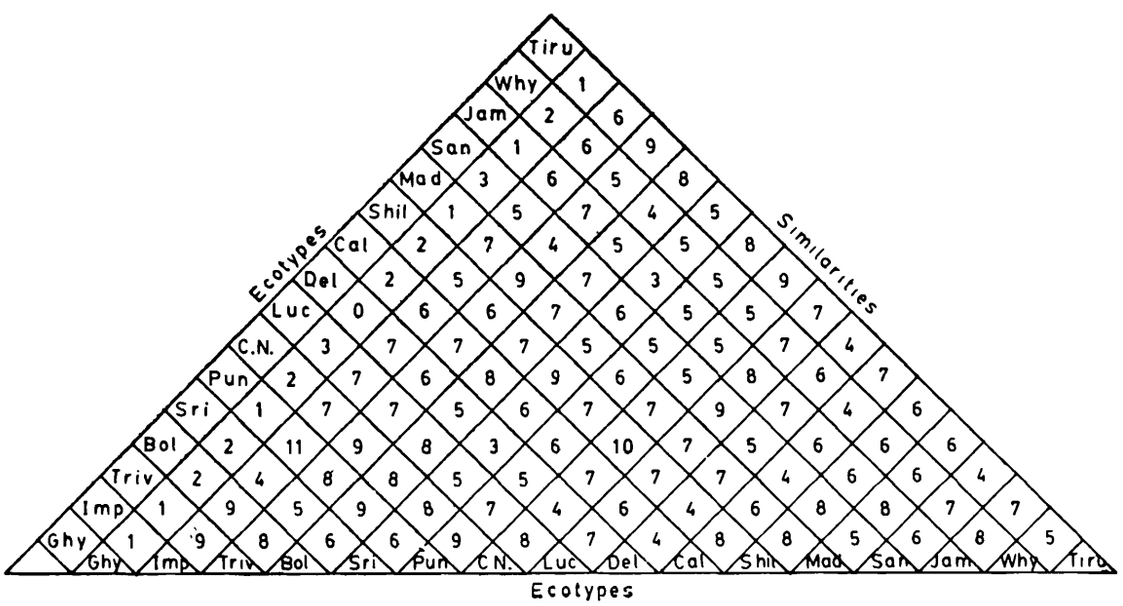
Regulative nature of the genome

From this study one has to take lesson that a better understanding of the role of environmental cues in development in Hydra has to be understood to appreciate the species problem particularly between allied ecotypes. Although analysis at each level has provided a valuable insight into the biology of *Hydra*, two of the most important areas of investigation of ours have focussed on cellular and biochemical characterisation of the different ecotypes. The trend of remarkable constancy of the transcription process (Prasad and Mookerjee, 1985) and the amount of DNA per cell (Prasad *et al.*, under preparation). Earlier studies in our laboratory showed that in each of the two species of Hydra—*Hydra vulgaris* and *Pelmatohydra oligactis* 32 chromosomes are present without identifying any sex chromosome (Datta, 1970). The stability of the number of chromosomes is indicative of the genetic togetherness of the ecotypes studied in this case. One can therefore, only point out for endogenous control system in this organism the threshold of which could be altered by physiological or environmental cues, leading to regulation in hydra. A complex system for determining hydra size, tentacle number, growth and reproductive behaviour would seem well suited for hydra's way of life which subjects it to a wide variations in environmental conditions.

Neurons and neurosecretions have been implicated in such processes and regeneration cell differentiation, budding and sexuality (Burnett and Diehl, 1964 ; Lentz, 1965 ; Davis *et al.*, 1968). If hydras are 'imperfect test tubes' (Schaller, 1976), it is because hydra has evolved mechanisms for moderating

the effects of environmental contingencies. It is not unlikely that some kind of thermolabile protein is there to regulate genetic expression within the ambit of species. Geographic variability of ecotypes may be result of such action. The present study might become a reliable frame of reference for the identification of ecotypes found in India since it is the first detailed study of Indian hydras. This idea will thus seriously challange the significance of morphology as the only guide for *Hydra* taxonomy. Modern approach to *Hydra* taxonomy is desirable and almost inevitable in order to resolve distant relationship and to classify hydra. It has shown that not all described characters can be tested for all hydras. Many characters may be present in some hydra types and not in others. Thus, sexual characters, such as the shape of the gonads, presence or absence of stable diocious nature cannot be always conveniently available to study since the asexual nature dominates over sexual nature in hydra, and the emergence of sexual forms is influenced by a number of in dependent stimuli, which in turn differ for different hydra types. Therefore, the significance of study of this organism at the cellular and molecular level have to be intensified.

Table 2
Magnitude of inter-ecotypic affinities



With our limited source of instrumentation at our disposal and knowing the magnitude of the species problem as it is in *Hydra*, we like to place for the time being the Green hydra from Hyderabad in a genus already ascribed to as *Chlorohydra*, and the rest of the ecotypes of nonsymbiotic hydra ecotypes to the genus *Hydra*, mostly structured ecotypic variations under the influence of genetic regulation.

Summary

A theoretical consideration to the concept of species problem in *Hydra* has been provided based on the data generated by the authors and also taken into account from others. It was found inevitable to suggest that geographical variabilities of the ecotypes might be the result of an endogenous control mechanism, perhaps mediated by heat shock protein type of regulator which renders differential morphogenetic expression as a result of the genome. Further, the semblance of the different ecotypes was determined by the different morphological criteria between the different ecotypes of Hydra and a selective reference for identification of ecotypes has been provided. The symbiotic green hydra was not only morphologically different from all the nonsymbiotic hydra but also the genomic expression was found to be singularly different from others. It was suggested that the green hydra be placed in genus *Chlorhydra* and all other nonsymbiotic hydra ecotypes in the genus *Hydra*.

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