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INSTRUCTIONS TO AUTHORS

Manuscripts should be submitted in triplicate, typed double spaced on one side of the paper (A4 bond) with 3 cms. margin on all sides. The arrangement of the manuscript should be as follows : Title page, Abstract, Key words, Introduction, Methods, Results, Discussion, Acknowledgements, References, Tables, Figures legends and Figures. Full length of paper should not exceed 10 printed pages.

Title Page :

It should contain the following information :

(i) The title of the paper which should be concise but informative, (ii) a short running head of not more than 40 characters placed of the foot of the title page, (iii) first name, middle initial and last name of each authors, (iv) name of department(s) and institution(s) to which the work should be attributed, (v) name and address of author for correspondence.

Abstract : The second page should carry an abstract of not more than 200 words. The abstract should state the purpose of the study, basic procedure, main findings and principal conclusions. Abstract should be followed by relevant Key Words.

Introduction : This should contain a concise statement of the purpose of the article. Only pertinent references should be given.

Methods : The methodology, apparatus and procedure in sufficient detail should be identified to allow other workers to repeat the experiments. Standard methods can, however, be identified by proper references. The new or substantially modified methods should be described giving reasons for using them.

Results : Results should be quoted in SI units. The results should be presented in logical sequence in the text, tables and illustrations. Unnccessary repetition should be avoided. Only important observations may be emphasized in the text.

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Photographs must be in black and white. A clear print in glossy paper, large enough to be ligible after 25% reduction is necessary for reproduction. These should be submitted unmounted.

EDITORIAL NOTE

Diversity and unity are the two underlying principles that seem to pervade life in general. Diversity in all phases of life activity is found in this vast array of life-ranging from simple viruses through unicellular organisms, to man, that most complex of all biological entities. Equally typical of all living organisms is unity manifesting a common similarity in all life forms. A full realisation of diversity and unity and the variety of ways they operate could only be possible through the combined efforts of all biological sciences. The ***Vidyasagar Universiity Journal of Biological Sciences*** *is a small attempt in that direction. The Journal has provided the much-needed platform for such biological sciences as Anthropology, Physiology, Botany, Zoology and allied disciplines to pursue a common objective. It would always be our endeavour to identify common areas of interest and interaction which could function as the meeting points for sciences engaged in gaining knowledge about life and its myriad pattern.*

The papers included in the inaugural issue of the journal are as diverse as the whole living world is. The studies cover such diverse fields of enquiry as demography, pathophysiology, taxonomic botany, cytology and biochemistry. One may question the wisdom of making the range so wide. But it has to be admitted that in their own distinctive ways all the papers share a genuine concern for the intricacies of life's diverse pattern.

In this particular issue emphasis has been given more on the involvement of scholars and researchers from outside Vidyasagar University. It is our firm realisation that only with the involvement of scholars working in reputed research institutes and departments of good standing and scholars, who have made and have been making significant contributions in their respective fields, the journal could shed its infancy sooner than expected. We would like to acknowledge our debt to the contributors for participating in our effort. The journal has taken shape with the encouragement and support provided by Prof. S. N. Ghosh, Vice-chancellor of Vidyasagar University. He has all along been enthusiastic and sympathetic to our cause. We are also thankful to our external members of the editorial board, the reviewers and to everyone who has helped us in some way or the other.

Rajat Kanti Das
Editor-in-Chief
VUJBS.

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Approaches to Survival

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Abstract

This presentation deals with the concept of "survival" of human groups, where survival is visualized at biological / demographic and sociocultural levels. The states of survival of groups in different physical environmental and socioeconomic settings have been described. The strategies for survival of these and other groups, including the broader, human rights approach to survival, has been discussed.

Key words : Survival, economic niche, group identity, human rights.

States of Survival

A necessary condition for life of any kind is survival. In this article, I shall be mainly concerned with survival of human groups, i.e., human aggregates of whatever kinds. "Survival" may be defined objectively (although not necessarily completely subjectively) in terms of the group's own perception of "being in existence". A perceived threat to "being in existence" is treated as a threat to survival. "Survival" may thus be considered at two levels : (I) biological and / or demographic; and (II) sociocultural.

I. BIOLOGICAL/DEMOGRAPHIC SURVIVAL

Biological/demographic survival may be defined in terms of a group comprising of a resonable size, such that it is not likely to become extinct in the short run, and the individual members of the group not having any gross health impairments. Several examples of threat to biological/demographic survival may be cited.

(i) *Population Size delcine :* As long ago as 1954, Cappieri (1) had shown, using estimated and census data, that some populations of the Andaman and Nicobar archipelago have gone extinct between 1854 and 1931. The cause of such extinction is likely to be biological, i.e. introduction of new infectious diseases by the mainlanders following establishment of the penal settlement, or even earlier. Obviously, the native Andaman and Nicobar islanders had no experience of the diseases and therefore had not developed any immunity to them either. The same phenomenon had occurred in case of the indigenous Americans who were subjected to a drastic population size reduction following the European intrusion. Fast (2), in a perceptive account, has described the struggle of the native Americans for survival and their ultimate fate, a fate that indigenous populations in other continents must have shared as well.

(ii) *Populations of inviable group size:* Some of the Andaman and Nicobar groups which had not gone extinct by the time Cappieri (1) conducted his study, are presently of such sizes which are unlikely to be viable. As of 1991, the Great Andamanese have a size of 29, the Onges,

96 the Sentinelese 80-100 (estimated) individuals (3). The problem of inviable group size is not restricted to island populations alone, as the data set out in Table 1 show. There are populations on the mainland which are on the verge of extinction.

Table 1. *The sizes of 10 small indian tribes (from 1971 census)*

Population	1971	1961
1. Arandan	5	44
2. Kochu Velan	8	47
3. Rona, Rena	12	23
4. Andamanese	24	19
5. Sentinalese *	82	50
6. Shompen *	92	71
7. Onge	112	129
8. Malakkuravan	275	248
9. Jarawa *	275	500
10. Toda	945	716

*Estimated population

(iii) *Differential growth rates of groups/subgroups* : It is common knowledge that different population groups grow numerically at different rates depending on many factors, biological and sociocultural. Some of these groups/ subgroups grow slower than the Indian population as a whole, and some may have even negative growth rates. I may readily cite the example of the Pahiras, a small group divided into three smaller subgroups, inhabiting the adjoining areas of the districts of Purulia, West Bengal and Singhbhum and Ranchi, Bihar. The growth rates of the Pahira subgroups were smaller than that of India, and the growth rate of the smallest subgruop was negative, when a study was conducted in 1963-64 (4). The data are set out in Table 2.

Table 2. *Intrinsic rate of natural increase (r) for the three Pahira subgroups*

Subrgoup	1961
Northern Pahira	-0.0028
Southern Pahira I	+0.0119
Southern Pahira II	+0.0131

Source : Basu, 1971 (4)

While the Pahiras provide an example at the micro-level, an example at the macro-level is given in Table 3. It is apparent that the populations of the different States of India grow at widely divergent rates. The observation seems to be similar at both the micro - and macro-level situations : some populations grow fast and become increasingly larger proportions of a wider aggregate, the tribe, state or country. By contrast, some others become increasingly smaller proportions of the wider aggregate and thereby tend to "fade out" into obscurity in the total scenario. Whether this phenomenon of "fading out" constitutes a threat to survival, i.e. "being in existence", at the level of perception, and leads to defensive responses of withdrawing into itself as in the case of the Sentinel islanders until recently (3) of the Onges (5) who seem to

be living on doles, or asserting group identity as in case of many indigenous peoples in india and abroad (I shall return to this phenomenon later) is a question of considerable import.

Table 3. *State/union territory-wise annual population growth differentials of scheduled tribes, 1971-81*

State/Union Territory	Total tribal population		Annual growth rate
	1971	1981	
India	79092841	104754623	0.02381
Andhra Pradesh	5774548	7961730	0.0321
Bihar	7950652	10142368	0.0243
Gujrat	1825432	2438297	0.0289
Hariyana	1895933	2464012	0.0262
Himachal Pradesh	769572	1053958	0.0314
Jammu and Kashmir	381277	497363	0.0266
Karnataka	3850034	5595353	0.0374
Kerala	1772168	2549382	0.0364
Madhya Pradesh	5453690	7358533	0.0300
Maharashtra	3025761	4479763	0.0392
Manipur	16376	17753	0.0081
Meghalaya	3887	5492	0.0346
Orissa	3310854	3865543	0.0155
Punjab	3348217	4511703	0.0298
Rajasthan	4075580	5838879	0.0360
Sikkim	9502	18281	0.0654
Timil Nadu	7315595	8881295	0.0194
Tripura	192860	310384	0.0476
Uttar Pradesh	18548916	23453339	0.0235
West Bengal	8816028	12000768	0.0308
Arunachal Pradesh *	339	2919	0.2153
Chandigarh	29073	63621	0.0783
Dadra and Nagar Haveli	1332	2041	0.0427
Delhi	635698	1121643	0.0568
Goa, Daman and Diu	16514	23432	0.0350
Mizoram	82	135	0.0499
Pondicherry	72921	96636	0.0282

* The poplulation figures (and the growth rate derived thereof) seem suspicious, but the population figures are taken from the census reports.

II. SOCIOCULTURAL SURVIVIAL

I have so far been considering the demographic/biological survival of "groups" without clarifying what is meant by "group". A "group" may be defined as a set of individuals who perceive of themselves as being related to each other by some common bond(s) [the bond(s) may be real or putativel]. A "group", therefore, is essentially a theoretical construct although many of the bonds, interrelations and interactions among the constituent individuals may be real and objectively ascertainable. A major dimension of the survival of a group involves the desire of the individuals concerned to retain,re-formulate or lose the group's identity.

It is intutively understandable that people generally tend to retain the group identity, e.g. religious, national, regional, "tribal", caste, etc., handed over to them from generation to generation. However, this tendency is not universal, and groups do sometimes consciously lose their identities or construct new identities — deliberately or under forces of circumstance. Examples of several kinds are cited below.

(i) *Conscious retention of atypical identity :* The village of Mirpur in southern Midnapur district, West Bengal was founded by 12 Portuguese gunmen and their local consorts. These men were hired by the then feudal landlady of Mahisadal to protect her estate from Maratha raiders. The man eventually "married" local women and settled down in the land given to them by the landlady in the mid/late - 1700's. The population of Mirpur, numberiong 320 in 1976 (6), retain their Portuguese ancestry through some Portuguese surnames, although sharing the same general socioecomomic environment involving occupation, education, income/expenditure, etc. with the dominant Hindu and Muslim neighbours. The point to note is the proud perception of the Mirpurians of their Western ancestry and their keenness to retain this identity.

(ii) *Conscious disowning of atypical identity :* In sharp contrast to the keenness of the Mirpurians to retain their Western identity, about 35 km. down south along the coast in a village called Khejuri, a group of comparable Portuguese - Bengali admixed ancestry chose to disown their admixed identity. Khejuri was a prosperous port and business centre in the mid - 1600's. The earliest European visitors to India, the Portuguese, frequented Khejuri and eventually founded an admixed group. However, with the disappearance of the Western (British) rulers, the admixed group with a Western ancestry which must have dominated the social scenario for long, felt insecure and deliberately chose to disown their atypical ancestry and claimed to be Muslim, even though the local Muslims were not keen to offer them the affiliation.

The contrast between Mirpur and Khejuri shows that the same phenomenon of submergence of an atypical identity into a wider one may be viewed as a risk to, or means of,survival by groups of similar ancestry under different sociocultural compunctions.

(iii) *Passive acceptence of identity withering away :* The Sherpas of Upper Khumbu, northeastern Nepal generally lived a secluded tradition-bound life up until the mid-1950's in their mountain abode. However, the suddden rise of the tourist trade resulted in a large scale shift from the traditional farming-herding to the new service-based economy involving activities like tourist guide,porter, inn-keeper, etc. Utilizing the new economic opportunities, children attending schools on a full-time basis, and enjoying the dual facilities of supplies of food and availability of medical care, appear to be positive signs enhancing the chances of survival. However, all these positive traits accompanying the opening up of a traditional society hidden in their mountain abode until recently, have their negative impacts on the traditional life-style and value system, in short, the unique features of the Sherpa identity. Weitz et al. (7), following Furer-Haimendorf (8,9), described the situation thus : "A large number of Sherpas have abandoned their traditional farming herding economy, mortality rate of yound Sherpa males in mountaineering accidents has increased ; many women live in Khumbu deprived of the security of their husbands who go on trekking tours for long periods or live in Kathmandu; some Sherpa men have married Western women or have a second wife in Kathmandu; presence of foreign tourists in most Sherpa homes prevent them from visiting neighbours for a casual chat and drink of beer; the Sherpa religious places (Buddhist) are often not treated with appropriate reverence by foreign tourists for lack of knowledge ; factionalism has cropped up in the generally harmonious Sherpa society, and so forth... In short, the tourist trade has disrupted the traditional life style, including social and familial ties".

Confronted with the new lifestyle accompanying the new economic pursuits, apprehends a Sherpa woman, the Sherpa language and eventually the unique Sherpa identity, may wither away.

Strategies for Survival

(i) *Conscious loss, or jealous maintenance, of group identity :* The strategies for survival may take different forms. In the example cited above, the Khumbu Sherpas chose to quickly occupy the new economic niche made available by the tourist trade. The shift to the new niche resulted into desertion of the traditional farming-hearding economy and endangered the traditional value system, social cohesion, language and, eventually, the unique Sherpa group identity. Perhaps, the Khumbu Sherpas consciously risked the perpetual pleasure of having a unique group identity to achieve the more materialistic individual economic prosperity. Going even a step further from there, the portuguese-Bengali admixed group in Khejuri disowned its historically atypical identity to ensure individual biological survival. Both the cases referred to above are examples of ensuring individual survival at the cost of survival of historically recognized groups. At the other extreme lies the case of the Sentinel islanders, who jealously guarded external contact until recently, presumably to preserve their group identity even at the cost of materialistic individual economic development.

(ii) *Re-formulation of group identity :* I shall now discuss a few cases of conscious efforts over a considerable period of time to ensure sociocultural survival, i.e. maintenance of identity, of extant ethnic groups. The efforts to form confederations of groups constitute composite indentities, and actively assert the rights of the confederated groups, as in the case of the Gorkhaland movement, may at least partly be aimed at ensuring the sociocultural survival of the constituent groups even if at a subordinate level of cognizance vis-a-vis the composite identity of the confederation. Several characteristics of the Gorkhaland movement can be readily observed (10) :

"First, the movement represents a general awekening among, and efforts for assertion of group identity by, various ethnic/linguistic/regional/etc. groups in West Bengal in particular and East and North-East India, the Indian region as well as the world as a whole in general. Second, the general cause behind these efforts, i. e. subordination of the ethos of these groups by more dominant groups of the neighbourhood, appear to be true for the Gorkhaland and movement as well. Third, similar urges for assertion of group identity must have existed in the past, as exemplified by several tribal movements in the recent historical periods. For instance, a social-historical account of the movement led by Birsa Munda in the last century to assert the rights of the Chotanagpur tribes to land and forest products against usurpation by local landlords and the British Raj has been given by Singh (11) ... Fourth, the very fact that the movemens are led by relatively well educated, well to do and well connected elites, which by difinition are somewhat isolated from the general masses within each group, may also imply a built in limitation. This limitation assumes a special importance in the Gorkhaland case because within the composite identity "Gorkha", which is intended to designate the hill people in general and juxtapose them with the plains people, there exists several traditionally discrete, disparate and often antagonistic ethnic entities".

The point I am trying to make at this point is that a composite identity may have been forged in the Gorkhaland case to protect the hill people's habitat, economy, traditional life and culture against the disruptive intrusion by the plains people. This may be interpreted as a conscious effort to ensure survival of the larger group by subordinating the smaller group identities, even though the smaller identities are discrete and may occasionally have antagonistic relations. The successively lower orders of group identities, however, remain in a state of animated suspension, as it were, in a hierarchical system of identity perception. The hierarchy may be described as follows :

"At one level all hill people felt a sense of identity, and felt dominated upon by the plains-people. However, at a lower level, the Sherpas, Bhutias, and Lepchas, the tribals felt dominated

upon by the Nepalis who were brought into this area by the British Raj a couple of hundred years ago; at a still lower level the Lepchas felt dominated by the Sherpas and Bhutias who were also migrants from Tibet via Nepal or Sikkim; at the even lower, intra-tribal leval occurs this feeling of inequality between the more-privilaged Christian and less-privilaged Buddhist Lepcha".

The hierarchical system of identity perception may have emerged by a conscious design as a compromise aimed at bringing all the discrete groups under one fold, yet allowing retention of their individual identities.

(iii) *Assertion of group identity and right to survive:* The Chittagong Hill Tracts (CHT) in southeastern Bangladesh have traditionally been inhabited by several Tibeto-Burman speaking ethnic groups, of whom the Chakmas are numerically the largest. Except for introduction of cash crops like opium, indigo. tea,tobacco and jute, followed by shrinkage of land available for subsistence agriculture and marginal infiltration by the neighbouring Bengali-speaking population, the British Raj did not infringe upon the local autonomy between 1860 and 1947. After 1947, this non-Islamic, non-Bengali-speaking, non-wet-ricegrowing region, which went to Pakistan was "opened up", trade and commerce passed on the Bengali hands, the Kaptai hydro-electric project submerged 20,000 hectares of good, cultivable valley land and displaced 100,000 of more people. With the emergence of Bangaladesh in 1971, large scale, subsidised Bengali colonization occurred, with the tribal : Bengali ratio changing from 90 : 10 in a total of 268,000 in 1950 to 50 : 50 in 746,000 in 1981. The 12 simple societies became captives to the Bengalis in their own native soil. Genocide took a crude form. The area was converted into a military camp. One-third of the Bangladesh army was deployed in CHT. "The army, the police force and the Bengali settlers cooperated in burning down villages and crops, raping women, killing people, dishonouring Buddhist temples and torturing prisoners" (12).

The indigenous CHT people in their desperation have organized a resistance movement, the "Shanti Bahini"; have sought refuge in, and protection from, the neighbouring States of India and Myanmar without much success; have appealed to international fora with varying degrees of success/failure. A case nearer home is represented by efforts to assert the confederated identity of the Chotanagpur tribes beginning with te movement led by Birsa Munda late last country. Without going into the intricacies of the Jharkhand movement and its zig-zig course, one may appreciate that the movement has recently achieved a limited success in the form of constitution of the Jharkhand Area Autonomous Council (JAAC).

Away from home, "Latin America has been the focal point for organizing indigenous rights...; documenting abuses by states against native peoples ...; and pressing for rights to land, culture, and self-determination in development" (13). The rabid jokes hurled at Rigoberta Menchu (14), the Guatemalan indigenous leader and 1992 Nobel Laureate (Peace), merely reflect the frantic efforts by the State power/colonial rulers to belittle the indigenous efforts to assert rights to subsistence, health and education, and human dignity... "Latin American anthropologists have perhaps been the most personally involved in pressing for human rights" (13). Such humanrights movements may be viewed as strategies for survival of disadvantaged groups.

Anthropology and Human Rights

"A conventional wisdom persists both inside and outside of anthropology that anthropologists have been largely uninvolved in human rights formulations" for reasons that (i) human rights perceptions differ among cultures ; (ii) they advocate collective rather than individual rights; (iii) they emphasize political economic, rather than human rights, approaches; (iv) they are overly

conscious about the political sensitivities of doing their field work; and (v) their involvement in small scale studies cause lack of familiarily with larger scale, universal human rights issues (13). Messer (13) has stoutly defended the anthropologists' role thus:

"The midcentury anthropologist struggled with questions of cultural relativism mostly as a debate over cultural values..., but changing world conditions, the clear violations of human decency and dignity on the part of non-Western political leadership under the banner of cultural relativism, as well as the expansion of the human rights concept... have all changed the human rights problematique and correspondingly, anthropologists' responses to it".

Messer (13) goes on the elaborate the point thus : anthropologists are contributing data relevant to human rights ; actively working to enhance institutional support to human rights; examining the contexts of human rights abuses in specific social settings; working with interpreters of local traditions in order that "people might be empowered to improve human rights in their own lives", and above all, contributing to enhanced cross-cultural understandings of local concepts of human rights, which alone may eventually lead to realistic formulations of human rights problems and their possible solutions.

It is not the purpose of this presentation to project the anthropologist as the new messiah in the arena of human rights. I nevertheless submit, in all humility, (i) that the necessary condition for all living beings, i.e., "survival", constitutes a total concept incorporating and interknitting three dimensions, viz. biological, cultural and their interactions, i.e. biocultural; (ii) that understanding and manipulation of these dimensions require information collection at the grass root level, and (iii) that while scholars from other disciplines may be better equipped to understand and manipulate the three dimensions in isolation from each other, the anthropologist is uniquely and professionally trained to handle the vast area of overlap of the three dimensions, collect information at the grass root level and promote cross-cultural understanding and tolerance, so essential for survival in the contemporary scenario charged with inter-ethnic conflicts at micro-, macro- and mega-levels. The human rights approach, incorporating a cross-cultural understanding of human rights perceptions, through information collected at the grass root level, may hopefully constitute a generalized global strategy for survival of disadvantaged groups, to which the anthropologists and other human scientists may dedicate themselves for proper discharge of their social responsibilities.

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A Contribution to the study of the *Gesneriaceae* Dum. in the Eastern Himalaya

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Abstract

The present work records 10 genera and 25 species of the Gesneriaceae from Darjeeling and Sikkim regions of the Eastern Himalaya, the principal abode of the taxon in India. *Didymocarpus* and *Aeschynanthus* are the most dominant genera with eight and six species respectively.

Key words : Gesneriaceae, Eastern Himalaya, India, *Didymocarpus* , *Aeschynanthus* .

Introduction

The Gesneriaceae, a family of Asteridae under Magnoliopsida (1,2) is represented in India by 19 genera (3), most of which are Himalayan. The morphological novelty of this taxon has been brought into light by Burtt and Jong (4). Information from such disciplines as cytology (5), chemistry (6), ontogeny (7) have added much to taxonmy of this family (8). In view of the absence of an exhaustive taxonomic review on the Gesneriaceae in India, this investigation was initiated in Darjeeling - Sikkim region. The work is aimed to contribute to the study of the Gesneriaceae in the Eastern Himalaya which is its main abode in India.

Methods

This work is based on careful study and scrutiny of pertinent literature, specimens preserved in the Lloyd Botanic Garden (LBG), the Central National Herbarium, Calcutta (CAL) and those collected from field trips in conformity with the earlier work (9). Comprehensive keys to the identification of genera and species and diagnostic features of the species are provided to facilitate their identification. The taxa are arranged alphabetically with correct nomenclature, citations and information regarding flowering and fruiting periods, distribution, voucher specimens etc.

Results

The present inventory of the family Gesneriaceae encompasses 10 genera, a key to the identification of which is presented below :

+ Corresponding Author

1. Seeds with hair :
   2. Stamens 4 perfect :
      3. Leaves fleshy, equal, almost entire ... 1. *Aeschynanthus*
      3. Leaves membranous, unequal, serrate ... 7. *Loxostigma*
   2. Stamens 2 perfect ... 8. *Lysionotus*
1. Seeds without Hair :
   4. Stamens 4 perfect :
      5. Fruit a berry ... 10. *Rhynchotechum*
      5. Fruit a capsule :
   6. Peduncle filiform and pedicel capillary ... 6. *Leptobaea*
   6. Peduncle not filiform and pedicel rigid ... 3. *Corallodiscus*
4. Stamens 2 perfect :
   7. Capsule globose, circumsciss; inflorescence subcapitate ... 5. *Epithema*
   7. Capsule ellipsoid or linear, longitudinally dehiscent; inflorescence otherwise :
      8. Leaves bilaterally unequal :
         9. Flowers bracteate, corolla cylindric ... 9. *Rhynchoglossum*
         9. Flowers bracteolate, corolla funnel-shapped ... 2. *Chirita*
      8. Leaves bilaterally equal ... 4. *Didymocarpus*

1. **Aeschynanthus** Jack. (nom. cons.)

Key to the Species

1. Leaves glabrous, corolla shortly two lipped :
   2. Corolla pubescent, seeds with two hairs near the hilum :
      3. Calyx tube longer than its lobes ... 4. *A. hookeri*
      3. Calyx tube not longer than its lobes :
         4. Calyx tube shorter than lobes ... 5. *A. parviflorus*
         4. Calyx tube as long as lobes ... 6. *A. sikkimensis*

2. Corolla glabrous, seed with one hair near the hilum :

5. Flowers scarlet ... 2. *A. bracteatus*

5. Flowers yellow-green ... 1. *A. acuminate*

1. Leaves hairy, corolla deeply 2-lipped ... 3. *A. gracilis*

1. ***Aeschynanthus acuminate*** Wall. [Cat. 218, n 6397 (1832), nom. nud.] ex DC., Prodr. 9 : 263 (1845); B.B. Clarke in Fl. Brit. India 4 : 341 (1884); Sen in Bull. Bot. Surv. Ind. 5 : 114 (1963); Hara in Enum. Fl. Pl. Nepal 3 : 133 (1982).

An epiphytic herb, glabrous; leaves 8-10 x 3.5-4 cm, elliptic, acuminate; peduncles subfasciculate, 1-or few-flowered; calyx deeply lobed, corolla 12-13 mm, yellow-green, glabrous; fruit a linear capsule, up to 15 cm in length; seeds with one hair near hilum and one at the apex.

Flowering and Fruiting : August-November.

Distribution : Eastern Himalaya : Sikkim, Bhutan; Upper Assam, Khasi and Jainti Hills.

Specimens examined : Mungpoo, 600m, R.M. Dutta and N.C. Majumdar 369 (CAL); Darjeeling, 2250m, Ribu and Rhomoo 16 (LBG).

2. ***Aeschynanthus bracteatus*** Wall. [Cat. 794 '1828), nom. nud.] ex DC., Prodr. 9 : 261 (1845); C.B. Clarke in Fl. Brit. India 4: 342 (1884); Burtt et Davidson in Not. Bot. Gard. Edinb. 21:230 (1955); Hara in Fl. E. Himal. 121 (1971); Matthew, Fl. Pl. Kurseong 74 (1981); Mukherjee, Fl. Pl. Darjiling 165 (1988).

An epiphytic herb, glabrous; leaves 9-10 x 3.5-4 cm, lax, elliptic, acminate, fleshy; peduncle 1-7 flowered, longer than potiole; calyx lobes 1.5-1.8 mm, red; corolla 3.3-3.5 mm, scarlet, almost glabrous, lower lip with reflexed lobes; capsule 10-15 cm long; seeds with 1 hair near the hilum and 1 at the apex.

Flowering and Fruiting ; July-October.

Distribution : Temperate Himalaya : Sikkim and Bhutan; upper Assam, khasi Hills, Burma, South Tibet and Yunnan.

Specimens examined : Darjeeling, 1800m, G. King 127 (CAL); Tarai, 800m, YHb 3837 (LBG); birch Hill (Darjilling), 2000m, Mukherjee 1308.

3. ***Aeschynanthus gracilis*** Parish ex C.B. Clarke, Comm. et Cyrt. Beng. 75, t. 48A (1874), in Fl. Brit. India 4 : 340 (184); Hara in Fl. E. Himal. 1 : 297 (1966).

An epiphyte with fulvous or reddish hairy branches; leaves small, 1-1.2 x 0.8-0.9 cm, broad-lanceolate, cuneate or rounded at base, hairy; flowers scattered, subsolitary; sepals 3-4 mm, linear, villous; corolla 25 mm, very oblique, villous outside, scarlet with orange-black marking at mouth, deeply 2-lipped, lower lip with reflexed margins; capsule 5-7.5 cm long; seeds with 1 hair at hilum and 1 at apex.

Flowering and Fruiting : February - May.

Distribution : Eastern Himalaya : Sikkim, Bhutan; Assam and Khasi Hills.

Specimens examined: Snighik, 1700m, R. Seshagiri Rao 6842 (Cal); Birik Dham, 2000m, YHb (LBG).

4. ***Aeschynanthus hookeri*** C. B. Clarke in DC., Monogr. Phaner. 5 : 21 (1883), in Fl. Brit. India 4 : 338 (1889); Hara in Fl. E. Himal. 297 (1966), 121 (1971) and in Enum. Fl. Pl. Nepal 3 : 133 (1982); Mukherjee, Fl Pl. Darjiling 166 (1988).

A. parasitica C.B. Clarke, Comm. and Cyst. Beng. t. 49, not of Wall. (1874).

Epiphytic harb, glabrous except corolla; leaves 8.7 x 2.5 cm, acuminate, fleshy, nerves obscure; flowers terminally umbelled; calyx tubular, scarlet, very shortly lobed; corolla more than 2.5 cm long, curved, orange red; seeds with 2 hairs near the hilum and 1 at the apex.

Flowering and Fruiting : June-September.

Distribution : Himalaya (Nepal to NEFA), Assam, North Burma and West China.

Specimens examined : Sittong; 1400m, W.G. Craib 159; Birch Hill, Darjeeling, 2000m, Mukherjee 1309; Lepcha Jagat 2300m (LBG).

5. ***Aeschynanthus parviflrus*** (D. Don) G. Don, Syst. Veg. 4 : 238 (1827); Hara in Fl. E. Himal. 297 (1966), in Enum. Fl. Pl. Nepal 3 : 133 (1982).

Trichosporum parviflorum D. Don in Edinb. Philos. J. 7 : 85 (1822), Prodr. Fl. Nep. 125 (1825).

Aeschynanthus ramosissima Wall. [Cat. 24, n799 (1829), nom. nud.], Pl. As. Rar. 1 : 55, t. 71 (1829), C.B. Clarke in Fl. Brit. India 4: 339 (1884); Sen in Bull. Bot. Surv. Ind. 5 : 114 (1963); Banerjee in Rec. Bot. Surv. Ind. 19(2) : 72 (1966).

A glabrous epiphyte; leaves 10x4 cm, broadly lanceolate; pedicels clustered; calyx ca. 0.8 cm, lobes lanceolate and shorter than tube; corolla ca. 2.5 cm, bright red, pubescent; capsule fleshy, long; seeds with 2 hairs near the hilum and 1 at the apex.

Flowering and Fruiting : September-December.

Distribution : Subtropical Himalaya : Kumaon, Nepal and Sikkim.

Specimens examined : Rongbee, 1700m, YHb 553 (LBG); Lepchakhowa 1500 m, J. K. Sikdar 948 (CAL).

6. ***Aeschynanthus sikkimensis*** (C. B. Clarke) Stapf in Bot. Mag. t. 8938 (1922); Spring, Fl. Sikkim Himal. f. 76 (1963); Hara in Enum. Fl. Pl. Nepal 3 : 133 (1982), Mukherjee in Fl. Pl. Darjiling 166 (1988).

A. maculatus var. *sikkimensis* C. B. Clarke in DC., Monogr. Phaner. 5 : 24 (1883).

A. maclulatus Lindley : C. B. Clarke in Fl. Brit. India 4 : 399.

A glabrous epiphyte; leaves 9.5-10 x 3.8-4 cm, lanceolate; flowers clustered; calyx 0.6 - 0.7 cm, lobes lanceolate, as long as tube; corolla pubescent, narrow, 1.8-2.0 cm long,

bright red; capsule linear; seeds with 2 hairs near the hilum and 1 hair at the apex.

Flowering and Fruiting : June-September.

Distribution : Himalaya; Nepal to Sikkim and Bhutan; Khasi Hills.

Specimens examined : Lachen, 2000m; Rammam, 1800m; Darjeeling, 2300 m (LBG); Birch Hill, 2000m, AM 1307.

2. ***Chirita*** Buch. - Ham. ex D. Don

Key to the Species

1. Peduncles many flowered ... 1. *C. macrophylla*

1. Peduncles 1-5 flowered :

   2. Calyx 5-cleft two-third way down ... 2. *C. pumila*

   2. Calyx 5-cleft half way down ... 3. *C. urticaefolia*

1. ***Chirita macrophylla*** Wall. Pl. As. Rar. 1 : 56, t. 72 (1830); DC., Prodr. 9: 269 (1845); C. B. Clarke, Comm. and Cyrt. Beng. t. 71 (1874), in Fl. Brit. India 4 : 358 (1884); Hara in Fl. E. Himal. 297 (1966), 121 (1971); Matthew, Fl. Pl. Kurseong 75 (1981); Hara in Enum. Fl. Pl. Nepal 3 : 133 (1982); Mukherjee, Fl. Pl. Darjiling 166-167 (1988).

An erect herb, about 10-45 cm; leaves 15-16 x 8-8.5 cm, ovate or elliptic, crenate-serrate, acute, sparsely hairy above and more densely on nerves beneath; peduncles many flowered, bracts almost glabrous; calyx connate at base or up to middle, nearly glabrous; corolla tubular, 5 cm, yellow.

Flowering and Fruiting ; June-October.

Distribution : Temperate Himalaya : nepal to NEFA.

Specimens examined : Sonada, 2200m, YHb 503 (LBG); Jalapahar, 2200m, Mukherjee 1292.

2. ***Chirita pumila*** D. Don, Prodr. Fl. Nepal 90 (1825); DC. Prodr. 9: 269 (1945); C.B. Clarke, Comm. and Cyrt. Beng. t. 74 (1874), in Fl. Brit. india 4 : 357 (1884); Matthew, Fl. Pl. Kurseong 75 (1981); Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982); Mukherjee, Fl. Pl. Darjiling 167 (1988).

A low, erect undershrub, about 50 cm; leaves 14x5 cm, distinctly unequal, serrate, elliptic, acuminate, mature pilose on both surfaces; peduncles 1-5 flowered, bracts ciliate; calyx deeply 5-fid, hirsute with white hairs; corolla tubular funnel-shaped, 3-3.5 cm, purple blue, yellowish below.

Flowering and Fruiting ; August-November.

Distribution : Subtropical Himalaya (Simla to NEFA), Southwest Tibet, North Burma, Thailand, Indo-China, West China.

Specimens examined : Sittong, 1000m, YHb 3835; Brich Hill, 2100m; Mungpoo, 1500m (LBG); Lebong Cart Road, 1900m, Mukherjee 1985.

3. ***Chirita urticaefolia*** Buch.-Ham. ex D. Don, Prodr. 90 (1825); DC., Prodr. 9 : 268 (1945); Clarke, Comm. and Cyrt. Beng. t. 69 (1874), in Fl. Brit. India 4 : 358-359 (1884); Matthew,

Fl Pl. Kurseong 75 (1981) ; Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982); Mukherjee, Fl. Pl. Darjiling 167 (1988).

An errect herb, up to 45 cm; leaves 12.5x5.0 cm, elliptic, serrate, elliptic, acuminate, sparsely hispid above and villous on nerves beneath; peduncles 1-5 flowered, bracts nearly glabrous; calyx 5-fid, half way down, hispid; corolla funnel shaped, 5 cm, purple with yellow lines inside.

Flowering and Fruiting : Aughst-December.

Distribution : E. Himalaya (Nepal to Bhutan); Assam North Burma and West Chana.

Specimens examined : Darjeeling town, 2000m, J. Parks 730 (LBG); Senchal, 1900m (LBG); Jalapahar, 2200m, Mukherjee 464.

3. ***Corallodiscuss*** Batalin.

1. ***Corallodiscus lanuginosa*** (Wall. ex DC.) Burtt in G. Chron. Ser. 3, 122 : 212 (1947); Hara in Fl. E. Himal. 298 (1966), in Enum. Fl. Pl. Nepal 3 : 134 (1982); Mukherjee, Fl. Pl. Darjiling 167 (1988).

Didymocarpus lanuginosa Wall. [Cat. 23, n. 791 (1829) nom. nud.] ex DC. Prodr. 9 : 268 (1845); C.B. Clarke, Comm. & Cyrt. Beng. 97, t. 67 (1874).

Didissandra lanuginosa (DC.) C.B. Clarke, in DC., Monogr. phan. 5 : 66 (1883), in Rec. Bot. Surv. Ind. 19(2). 73 (1966).

A scapigerous, acaulescent herb; leaves 7.5-8.0 x 3.5 - 4.0 cm, crowded, radical, elliptic to ovate, hirsute above and fulvous wooly beneath when young; cymes ultimately glabrous; corolla pale blue or purple, 1.8-2.0 cm, hairy; stamens 4 perfect; fruit a capsule, often curved, seeds glabrous.

Flowering and Fruiting : June-november.

Distribution : Temperate Himalaya (Kumaon to Bhutan), Khasi Hills.

Specimens examined ; Chenugdua, 1600m, W. Smith 3345 (LBG); Darjeeling 2200m, YHb 3416 (LBG), Observatory Hill (Darjeeling), 2150 m, Mukherjee 1356.

4. Didymocarpus Wall.

Key to the Species

1. Stem inconspicuous, never exceeding 4 cm, leaves mostly radical :
   - 2. Leaves ashy pubescent above, capsule stalked ... 4. *D. cinereus*
   - 2. Leaves not ashy pubescent above, capsule sessile :
     - 3. Leaves all radical, corolla 1.2-1.8 cm long, deep purple ... 5. *D. macrophylla*

3. Leaves cauline, corolla exceeding 2.5 cm, rose purple ... 2. *D. andersoni*

1. Stem conspicuous, exceeding 4 cm, leaves cauline :

4. Leaves alternate - opposite, scattered on stem ... 8. *D. subalternans*

4. Leaves opposite, 2-4 gathered at apex :

5. Stem ashy pubescent ... 7. *D. podocarpus*

5. Stem not ashy pubescent :

6. Corolla orange red, capsules exceeding 4 cm in length ... 3. *D. aurantiacus*

6. Corolla dark purple, capsules less than 1.5 cm in length :

7. Calyx lobes white, corolla violet-purple ... 1. *D. albicalyx*

7. Calyx lobes not white, corolla dark purple ... 6. *D. oblonga*

1. **Didymocarpus albicalyx** C.B. Clarke in DC., Monogr. Phaner. 5 : 78 (1883); Hara in Fl. E. Himal. 298 (1966); Matthew, Fl. Pl. Kurseong 75 (1981); Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982); Mukherjee, Fl. Pl. Darjeeling 168 (1988).

D. villosa auct. non D. Don : C.B. Clarke, Comm. & Cyrt. Beng. 89, t. 59 (1874).

D. leucocalyx C.B. Clarke in Fl. Brit. India 4 : 348 (1884).

Stem 2-leaved at the apex, hairs patent or deflexed; leaves 15x10 cm, ovate, serrate, villous above; inflorescence subumbellate cyme; bracts caducous; calyx deeply divided, white; corolla 0.9-1.2 cm, violet purple; capsule 1.0-1.3 cm, subsessile.

Flowering and Fruiting : June-December.

Distribution : Himalaya : East Nepal to Bhutan.

Specimens examined : Darjeeling town, 2200m, N.C. Majumdar 327 (CAL); Kalimpong, 1600m, YHb 993 (LBG); Pulmajoa 2200m, K. Biswas 5679 (CAL); Batasia, 2300m, S. Kurz 12723 (CAL).

2. ***Didymocarpus andersoni*** C.B. Clarke, Comm. & Cyrt. Beng. 92, t. 62 (1874); in Fl. Brit. India 4 : 346 (1884), Hara in Fl. E. Himal. 298 (1966), 122 (1971), in Enum. Fl. Nepal 3 : 134 (1982).

Stem very short, 2-4 leaved; leaves 15 x 10 cm, hairy above and on the nerves beneath; inflorescence capitellate; bracts connate, concealing pedicel, purple; calyx teeth short, obtuse; corolla exceeding 2.5 cm, tube narrow, rose-purple; capsule 1.25 cm, sessile.

Flowering and Fruiting : June-november.

Distribution : Eastern Himalaya.

Specimens examined : Dikeha valley, 2200m, Smith & Cave 833 (LBG); Kurseong, 1500m, YHB (LBG); Batasia, 2400m (LBG); Kalimpong, 1600m, Thornton 36 (CAL).

3. ***Didymocarpus aurantiaca*** : C.B. Clarke, Comm. & Cyrt. Beng. 90, t. 60 (1874); in Fl. Brit. India 4 : 346 (1884); Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982).

Stem short, villous, 2-4 leaved; leaves 7.5-15.0 cm, ovate, villous above; flowers pedicelled, bracts scarcely connate; calyx lobed half way down, purplish; corolla funnel-shaped, orange red; capsule linear, pedicelled, ca. 2 cm long.

Flowering and Fruiting : June-October.

Distribution : Eastern Himalaya : Nepal, Sikkim.

Specimens examined : Rongbee Jhora, on rock, 1200m, Choo 181 (CAL); Badamtam Road, 1300m (LBG); Mongpoo, 1400m (LBG); Reang, 1200m, Ribu & Rhomoo 5 (LBG).

4. ***Didymocarpus cinereus*** : D. Don, Prodr. Fl. Nep. 122 (1825), "cinerea"; C.B. Clarke in Fl. Brit. Ind. 4 ; 346 (1884); Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982).

Didymocarpus obtusa Wall. [Cat. 23, n 786 (1829), nom nud.] ex R. Br. in Bennett, Pl. Jav. Rar. 118 (1840); DC., Prodr. 9: 267 (1845); C.B. Clarke, Comm. & Cyrt. Beng. 91, t. 61 (1874).

Stem short or O, ashy pubescent; leves radical or opposite when cauline, ovate cordate, obtuse, crenate, glabrate beneath, peduncles hardly exceeding leaves; inflorescence cymose; calyx subcampanulate, sparsely pubescent, lobes ovate, obtuse; corolla purple, paler below, tubular; capsule pedicelled, 3.5-3.8 cm long.

Flowering and fruiting : July-December.

Distribution : Eastern Himalaya : Sikkim, Nepal.

Specimens examined : Great Rangeet, 1200m, YHB 974 (CAL).

5. ***Didymocarpus macrophyllus***: Wall.ex D. Don, Prodr. Fl. Nep. 122 (1825), "macrophylla" Wall., Cat. 23 n 784 (1829); C. B. Clarke in Fl. Brit. India 4 : 346 (1884) ; Hara in Enum. Fl. Pl. Nepal 3 : 134 (1982).

D. plicate D. Don, Prodr. Fl. Nep. 122 (1825).

D. aromatica Wall., Pl. As. Rar. 2 : t. 141. qucad f. 4-7 tantum (1831).

Stemless; leves all radical, ovate, crenate-serrate; inflorescence subcorymbose cyme, bracts ovate; calyx funnel shaped, upper one-third lobed, lobes obtuse; corolla 1-2 cm, deep purlpe; capsule sessile, 2.5 cm long.

Flowering and Fruiting : May-August.

Distribution : Eastern Himalaya : Nepal and Bhutan.

Specimens examined : Sonada, 2200m (LBG) ; Sikkim, 1800 m, G. King 2114 (CAL).

6. ***Didymocarpus oblonga*** : Wall. ex D. Don, Prodr. Fl.Nep. 123 (1825), "Oblonga" Wall., Pl. As. Rar. 2 : 34, t. 140 (1831); C. B. Clarke, Comm. & Cyrt. Beng. 86, t. 56 (1874), in Fl. Brit. India 4 : 346 (1884); Banerji in Rec. Bot. Surv. Ind. 19(2) : 73 (1966); Hara in Fl. E. Himal. 3 : 1055 (1975), in Enum. Fl. Pl. 3 :134 (1982).

D. verticillata Wall. [Cat. 23, n. 783 (1829), nom. nud.].

Stem up to 12 cm, villous pubescent, with 4leaves at apex; leaves oblong or elliptic, corsely crenate. pilose; flowers in subcorymbose, glabrous cyme; bracts coloured, connate; calyx lobes one third of tube rounded; corolla 0.8-1.0 cm, dark purple; capsule subsessile, less than 1.5 cm long.

Flowering & Fruiting : June- September.

Distribution : Eastern Himalaya (Nepal and Sikkim).

Specimens examined : Gangtok, 2300 m, G. King 119 (CAL); Chimangton, 1700m (LBG).

7. ***Didymocarpus podocarpus*** : C. B. Clarke in DC., Manogr. Phaner. 5: 76 (1883), ut podocazpa in Fl. Brit. India 4 : 347 (1884) p.p.; Banerji in Rec. Bot. Surv. Ind. 19 (2) : 73 (1966); Hara in Enum. Fl. Pl. Nepal 3 : 134-135 (1982) ; Mukherji Fl. Pl Darjiling 168 (1988).

Stem up to 20 cm, ashy pubescent, 4-leaves at the apex; leaves ovate-elliptic, crenate, minutely pilose above; cymes 2-4 flowered; bracts glabrous; calyx campanulate, upper one-third lobed, lobes obtuse; corolla 2.5 cm long, purple; capsule on long stalks, 2.5 3.8 cm long.

Flowering and Fruiting : August-November.

Distribution : Himalaya (E. Nepal to Bhutan).

Secimens examined : Zemu valley, 2500m, Smith & Cave 2791 (CAL);

Kalimpong, 2200 m,; Senchal, 2300m (LBG); Jalapahar, 2200m, Mukherjee 1355.

8. **Didymocarpus subalternans** C. B. Clarke, Comm. & Cyrt. Beng. 85, t. 55 (1874); in Fl. Brit. India 4 : 347 (1884); Hara in Enum. Fl. Pl. Nepal 3 : 135 (1982).

Stem up to 30 cm, almost glabrous; leaves cauline, scattered, opposite, alternate, ternate; peduncle subumbelled; bracts purplish, ovate; clyx funnel-shaped, semi 5-fid, purplish, lobes somewhat triangular; corolla tubular, ca. 1.3 cm long, purple; capule stalked, 2.5 cm long.

Flowering and Fruiting : June-November.

Distribution : Himalaya (Nepal to Bhutan).

Specimens examined : Kalimpong to Gangtok, 2500m, D. Chatterjee 206 (CAL); Lechen, 2300m, G. A. Gammie 690 (LBG).

6. *Epithema* (Blume) Benth.

1. *Epithema carnosa* Benth., Scroph. Ind. 57 (1835); "Carnosum" C. B. Clarke, Comm. & Cyrt. Beng. 129. t. 90 (1874), in Fl. Brit. India 4 : 339 excl. var.; Hara in Enum. Fl. Pl. Nepal

3 : 135 (1982).

A small, succulent, pubescent herb; lower leaf petioled and upper leaves subsessile, sinuate-crenate, thinly pubescent; flowers in scorpioid raceme, bracts truncate, bracteoles small, linear; calyx campanulate, pubescent, corolla tubular, 2-lipped, bluish; stamens 2-perfect; capsule globose, 2-3 mm in diam, circusciss.

Flowering and Fruiting : July-November.

Distribution : Eastern Himalaya : Nepal to Bhutan; Khasi Hills.

Specimens examined : Darjeeling, 1200m, G. King 12300 (CAL); Rishap, 1000m (LBG).

Leptobaea Gamble

1. *Leptobaea multiflora* (C. B. Clarke) Bentham ex Gamble, List Tr. Darjeeling 58 (1878); C. B. Clarke in DC., Manogr. Phaner. 5 : 165 (1883) in Fl. Brit. India 4 : 368 (1884); Hara in Fl. E. Himal. 298-299 (1966).

Championia multiflora C. B. Clarke, Comm & Cyrt. Beng. t. 68. (1874)

A shrub up to 1.5m; leaves opposite, crowded, elliptic acuminate, minutely crenulate, pubescent; peduncles few flowered, often fascicled on short lateral branches; calyx persistent, teeth linear, pubescent; corolla glabrous, white; stamens 4 perfect; capsule beaked by the style, 2-valved.

Flowering and Fruiting : February-June

Distribution : Eastern Himalaya : Sikkin, Bhutan; Assam, Khasi Hills.

Specimens examined : Labha, 1000m YHb 974 (LBG), Mungpoo, 1200m YHb 1003 (LBG); Kurseong, 1300m, YHb 1104 (LBG).

7. ***Lexostigma*** C. B. Clarke

1. *Loxostigma griffithii* (Wight) C.B. Clarke in DC. Monogr. Phaner. 5 : 60 (1883), in Fl. Brit. India 4 : 344 (1884); Sen in Bull. Bot. Surv. India 5 : 114 (1963); Hara in Fl. E. Himal. 299 (1966) ; Matthew, Fl. Pl. Kurseong 76 (1981); Hara in Enum. Fl. Pl. Nepal 3 :135 (1982) Mukherjee, Fl. Pl. Darjiling 169 (1988).

Didymocarpus griffithii Wight, Ill. Ind. Bot. 2 : 182, t. 159 (1850).

Dichrotrichum griffithii C. B. Clarke, Comm. & Cyrt. Beng. 79, t. 51 (1874).

A thinly pubescent herb; leaves opposite, unequal sided, subfalcate, base acute or unequally rhomboid, serrate, acuminate; cyme peduncled, bracts and bracteoles small; sepals oblong, acute, green; corolla tubular, inflated above base, yellow ; stemens 4, anthers connivent in pairs; capsule linear, loculicidal ; seeds with one hair at apex.

Flowering and Fruiting : August-December.

Distribution : Subtropical Himalaya ; Khasi Hills.

Specimens examined : Ramvijhora, 2200 m, W. Smith 339 (LBG); Kurseong, 1500m (LBG); Rimbick, 2300m (LBG); Birch Hill, 2000m, Mukherjee 1311.

8. *Lysionotus* D. Don

1. Lysionotus serratus D. Don in Edinb. Phil. Journ. 7 : 85 (1822), Prodr. Fl. Nepal 124 (1825); C. B. Clarke in Fl. Brit. Ind. 4 : 344 (1884); Hara in Fl. E. Himal. 299 (1966), p. p. ; 106 (1975); Matthew, Fl. Pl. Kurseong 76 (1981) ; Hara in Enum. Fl. Pl. Nepal 3 : 135 (1982); Mukherjee, Fl Pl. Darjiling 169 (1988).

L. ternifolia Wall., Pl. As. Rar. 2 : 20, t 118 (1931); C. B. Clarke, Comm. & Cyrt. Beng. t. 52 (1874).

As epiphytic herb, glabrous; leaves 15x5 cm, narrow lanceolate-elliptic, closely serrate or sinuate, nerves conspicuous, oblique ; inflorescence many flowered peduncled cyme, inconspicuous and bracteoles small; sepals narrowly lanceolate; corolla purple or white with purple veine ; stamens 2 perfect, connective with an oblong process.

Flowering and Fruiting : August-December.

Distribution : Himalaya (Kumaon to NEFA); Assam, North Burma.

Specimens examined : Kurseong, 1500m, Barin Ghosh (LBG); Gangtok, 1200m, G. King 199 (LBG) ; Jalapahar, 2250, Mukherjee 1354.

9. *Rhynchoglossum* Blume

Rhynchoglossum obliquum Blume, Bijdra 741 (1826) ; C. B. Clarke, Comm. & Cyrt. Beng. 124, t. 88 (1874), in Fl Brit. India 4 : 367 (1884); Banerji in J. Bombay Nat. Hist. Soc. 55 : 264 (1958); in Rec. Bot Surv. India 19 (2); 73 (1966); Hara in Fl. E. Himal. 299 (1966), in Enum. Fl. Pl. Nepal 3 : 135-136 (1982).

R. obliquum (Wall. ex D. Don) A DC. in DC ., Prodr. 9 : 274 (1845).

R. obliquum var. parviflora C. B. Clarke in DC., Monogr. Phan. 5 : 162 (1883) in Fl. Brit. India 4 : 367 (1884).

A succulent membranous herb, up to 50 cm; leaves alternate, elliptic, acuminate, unequal sided, cordate on one side of unequal base; raceme long; bract O, bracteoles minute ; calyx campanulate; corolla tubular, 2-lipped, lower lip much longer than upper and ovate; stamens 2 perfect; capsule ellipsoid.

Flowering and Fruiting : July-September.

Distribution : Eastern Himalaya, Burma.

Specimens examined : Loddah, 1000 m, YHb 1000 (LBG); Darjeeling, 1300m, G. King 13722 (CAL).

10. Rhynchotechum Blume

Key to the Species

1. Stem tomentose or wooly at apex; leaves whitened beneath, above tawny, silkily wooly; corolla not exceeding 5 mm .. 1. *R. ellipticum*

1. Stem patently hispid upwards, leaves hirsute on both surfaces; corolla 7-9 mm .. 2. *R. Vestitum*

1. Rhynchotechum ellipticum (Wall.) A. DC. in DC., Prodr. 9 : 285 in adnate (1845), "Rhyncothecum" Hook. f. in B. Mag. 96 : t. 5832 (1870) ; C. B. Clarke, Comm. & Cyrt. Beng. 131, t. 91 (1874), in Fl. Brit. India 4 : 373 (1884); Hara in Fl. E. Himal. 299 (1966), in Enum. Fl. Pl. Nepal 3 : 136 (1982).

Corysanthera elliqtica Wall. [Cat. 218, n 6411 (1832), nom. nud.] ex D. N. F. Dieter.

An erect undershrub ;stem tomentose upwards; leaves opposite, elliptic, acute, base cuneate, minutely dentate, whitened ventrally;cymes in lower axils, many flowered, somewhat umbellate; sepals ca. 4mm, lanceolate; corolla subcampanulate, 4-5mm, rose-purple; fruit a sebglobose berry.

Flowering and Fruiting : February-May.

Distribution : Subtropical E. Himalaya (Nepal to Bhutan).

Specimens examined : Mongpoo, 1000m, C. Biswas 1411 (CAL) ; Rishap, 1200m, YHb 739 (LBG).

2. *Rhynchotechum vestitum* Hook. f. et Thoms. ex C. B. Clarke, Comm. & Cyrt. Beng. t. 92 (1874), in Fl. Brit. India 4 : 373-374 (1884) ; Ghosh in Jour. Bengal nat. Hist. Soc. 8 (1) : 46-55 (1989).

An undershrub; stem patently hispid upward; leaves opposite, elliptic, acuminate, base caudate, slightly dentate, hirsute on both surfaces; cymes very hirsute; sepals ca. 6 mm, lanceolate; corolla subcampanulate, 7-9 mm, purple; fruit somewhat spherical berry.

Flowering and Fruiting : August-December.

Distribution: Eastern Himalaya : Sikkim, Bhutan, NEFA.

Specimens examined : Rishap, 1200m, YHb 769 (LBG); Rimbik, 2100m, YHb 434 (LBG); Sikkim, I. Hosunkill 37296 (CAL).

Discussion

The present work records 10 genera and 25 species which are mostly endemic to the subtropical to temperate conditions prevailing in the Eastern Himalaya. Among these plants, only two species each of *Aeschynanthus (A. bracteatus* and *A. hookeri)* and *Chirita (C. pumila* and *C. urticaefolia)* extend up to West China. *Didymocarpus* and *Aeschynanthus* are the most dominant genera each with eight and six species respectively. Species of *Aeschynanthus* and *Lysionotus* are epiphytes, while others are mostly litho-or chasmo-phytes growing in damp habitats with subdued illumination. Species of *Rhynchotechum, Rhynchoglossum, Epithema*

and *Leptobaea* and be seen in damp forest covered areas. The economic importance of the Gesneriaceae is little known. Leaves of *Rhynchotechum ellipticum* are used locally as vegetable. Decoction of leaves of some species of *Didymocarpus* are used in treatment of urine retention. Most of the representatives of the Gesneriaceae have become rare due to progressive paucity of their optimum habitat. Active steps to their conservation under proper surveillance are deemed essential since a thorough scientific research is certain to reveal their economic importance as well as ecological functions.

Acknowledgements

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Expression of Outer Membrane Proteins of *Vibrio parahaemolyticus* Under Different Cultural Conditions

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Abstract

Different strains of *Vibrio parahaemolyticus* were taken to find out the expression of their outer membrane proteins under different cultural conditions. Four kanagawa phenomenon positive and four kanagawa phenomenon negative strains of *Vibrio parahaemolyticus* were grown under *in vitro* and *in vivo* cultural conditions and their outer membrance proteins were prepared. Sodium dodecyl sulphate polyacrylamide gel electrophoresis revealed that there was no difference in migration of outer membrane proteins of both *in vitro* and *in vivo* grown bactera. *In vivo* cultural condition also could not exhibit a difference in protein profile between kanagawa phenomenon positive and kanagawa phenomenon negative strains.

Key words : *Vibrio parahaemolyticus*,outer membrane protein, rabbit ileal loop, *in vitro* & *in vivo* Kanagawa phenomenon.

Introduction

Vibrio parahaemolyticus (V. parahaemolyticus) is a gram negative, halophilic, estuarine bacteria, causing sporadic cases and common source of gastroenteritis due to infected sea foods (1,2). The pathogenicity of this organism is closely associated with its ability to cause hemolysis on a special high salt blood agar, Wagatsuma agar, i.e., Kanagawa phenomenon (3). The strains from clinical cases are generally Kanagawa phenomenon positive (KP^+) and those from environment are usually Kanagawa phenomenon negative (KP^-) (4).

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Adherence of bacteria to host cell is a prerequisite for virulence. The adherence of *V. parahaemolyticus* was studied and it was reported (5) that KP$^+$ strains showed adherence, whereas most of KP$^-$ strains were nonadhering to rabbit intestinal epithelial cell (RIEC) *in vitro*. Thermostable direct hemolysin (TDH) and cell surface hydrophobicity had no role on adherence of these strains. Antisera to the outer membrane proteins of KP$^+$ strains of *V. parahaemolyticus* could inhibit the adherence of these strains to RIEC suggesting that OMP might have some role on adhesion of these strains. Migration of OMPs of KP$^+$ and KP$^-$ strains of *V. parahaemolyticus* grown *in vitro* remained the same in sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) (6).

It is known that bacteria have a remarkable ability to alter their metabolism rapidly as they adapt to multiply in the environment of the host (7,8). It was reported (9) that pathogenic *Escherichia coli (E. coli)* expressed new outer membrane proteins (OMPs) when grown *in vivo*. Sciortino *et al* (1983) reported that *Vibrio cholerae* O1 *(V. cholerae O1)* when grown *in vivo* in the intestine of infant rabbit expressed novel outer membrane associated proteins similar to those observed on cholera vibrios grown under *in vitro* cultural conditions of iron-deprivation (10). The present study was, therefore, undertaken to identify the *in vivo* specific membrane proteins of *V. parahaemolyticus* and their role in adherence.

Materials and Methods

Bacterial strains : Four Kanagawa phenomenon positive (KP$^+$) strains of *V. parahaemolyticus* isolated from clinical cases admitted to the Infectious Diseases Hospital, Calcutta and four Kanagawa phenomenon negative (KP$^-$) strains isolated from environment water sources were taken for this study (Table 1).

TABLE 1. Characteristics of *V. parahaemolyticus* strains

Strain No.	Serotype	Source	Kanagawa phenomenon
NICED 17	04 : K55	Clinical	+
NICED 22	02 : K3	Clinical	+
NICED 37	05 : K17	Clinical	+
NICED 38	04 : K55	Clinical	+
NICED 40	40 : K42	Environment	-
NICED 42	07 : K19	Environment	-
NICED 43	03 : K30	Environment	-
NICED 44	04 : K42	Environment	-

Bacterial culture in vitro : Bacteria were grown in Brain Heart Infusion broth (Difco, Detroit, USA) supplemented with 1.5% NaCl under constant shaking at 37°C overnight with proper aeration.

Bacterial culture in vivo : Bacteria were grown in the rabbit ilium, a model popularly known as rabbit ileal loop (11). Briefly, rabbits weighing 1.5 kg were kept under fasting for 36

hours and supplied with 10% glucose adlibidum. The rabbits were then anaesthetized and abdomens were opened. A tie was made in the ilium at about 10 cms away from caecum. The iliums were thoroughly washed with normal saline. A second tie was made at the distal part of the ilium to get a large loop. 5 ml. of bacterial cultures were then injected into the loop. Abdomen was then stitched. The animals were sacrificed after 18 hours. The bacteria were collected from the loops by rinsing the loop with cold phosphate-buffer-saline (PBS; pH 7.4).

Preparation of outer membrane proteins (OMPs) : Cells of *V. parahaemolyticus* grown *in vitro* and *in vivo* conditions were pelleted, washed in PBS (pH 7.4) and subsequently disrupted by ultrasonic disintegrator (MSE). The unlysed cells were removed by centrifugation at 2,000xg for 10 minutes. The supernatant obtained was centrifuges at 1,00,000 xg for 40 minutes in cold (4°C). The pellet comprising the bacterial envelope was suspended in 1% sodium salt of N-lauryl sarcosine (Sigma Chemical Co., St. Louis, MO., USA) and incubated at 25°C for 30 minutes and again centrifuged at 1,00,000xg for 1 hour. The pellet (sarcosyl insoluble fraction) obtained comprised of the outer membrane protein (12,13,14).

Protein estimation : Protein estimation was made according to the method of Bradford (15). Bovine serum albumin was taken as the standard.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS—PAGE) : Vertical electrophoresis with polyacrylamide gel was done according to the method of Laemmli (16). Briefly, a 10% separating gel followed by a 4.5% upper stacking gel was cast and electrophoresis of OMPs was done at constant current. Low molecular weight protein standards (Pharmacia Fine Chemicals, Uppsala, Sweden) were run parallely for determination of molecular weight. After completion of electophoresis the gels were fixed by 100% methanol and 7.5% glacial acetic acid, stained by Coomassie brilliant blue and destained by 5% methanol and 7.5% glacial acetic acid until the bands become clear.

Results

Outer membrane protein profile of both KP⁺ and KP⁻ strains of V. parahaemolyhticus grown in vitro : SDS-PAGE was done with four KP⁺ and four KP⁻ strains grown *in vitro* (Fig 1). Migration of proteins in SDS-PAGE of KP⁺ and KP⁻ strains were found to be similar. The apparent molecular weight of proteins of both KP⁺ and KP- strains ranged between 102 kDa and 14 kDa. The major protein band was found at 38 kDa region.

Outer membrane protein profile of both KP⁺ and KP⁻ strains of V. parahaemolyticus grown in vivo : Four KP⁺ and four KP⁻ strains were grown in ileal loop of rabbit intestine. Outer membrane proteins were prepared from these *in vivo* grown cells and their SDS-PAGE was done (Fig 2). It was noted that there were no differences between OMP profiles of KP⁺ and KP⁻ strains between *in vitro* and *in vivo* conditions also no difference in protein profile between KP⁺ and KP⁻ strains was visible.

Discussion

Despite several studies, very little is known about the adhesins of *V. parahaemolyticus*. As a few proteins of outer membrane were thought to be adhesins thereby constituting one of the major virulence factors of *V. parahaemolyticus,* it was presumed that expression of novel outer membrane protein might take place if *V. parahaemolyticus* were grown under *in vivo* condition. However, in contrast to *V. cholerae* and *E. coli* (9) *in vivo* culture of KP⁺ and KP⁻ strains of *V. parahaemolyticus* could not enhance that expression of novel

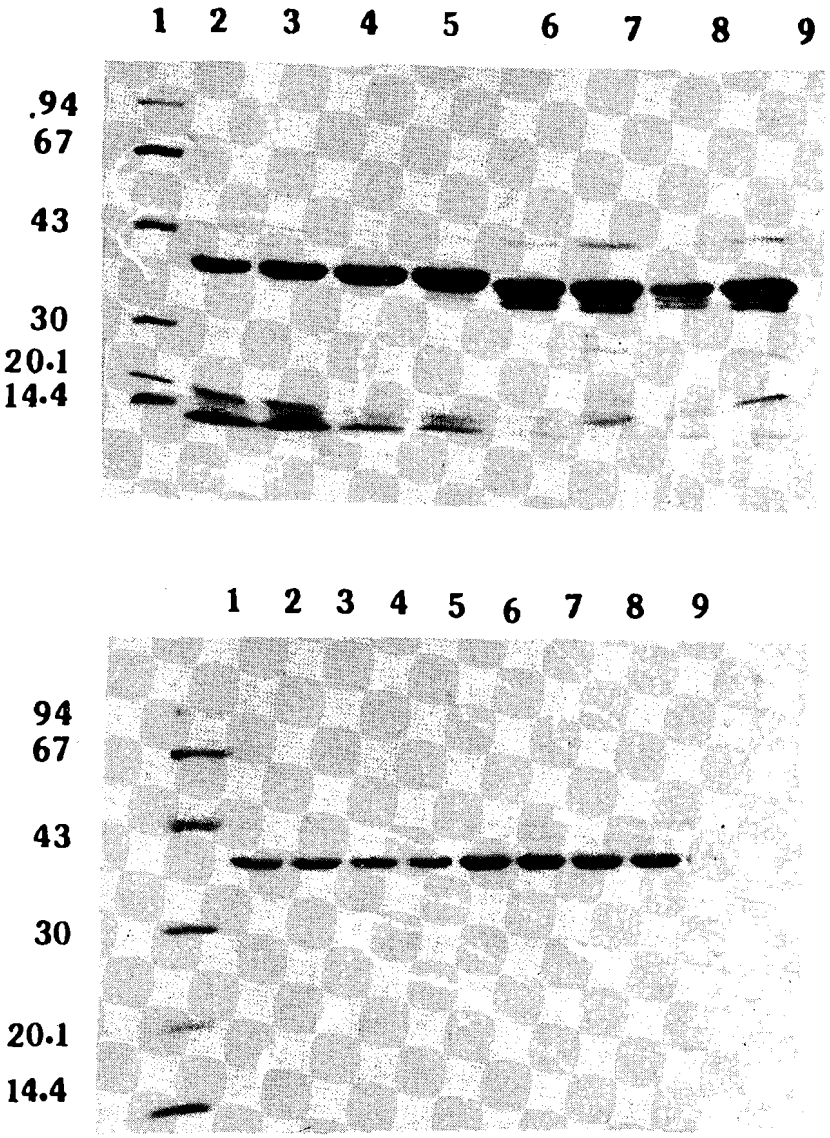

Fig. 1 : *SDS-PAGE analysis of outer membrane proteins of different strains of V. parahaemolyticus grown in vitro, Lane 2 to 5 represent OMP profiles of KP⁺ strains and Lane 6 to 9 represent OMP of KP strains. Lane 1 represents the low molecular weight markers (Pharmacia, Uppsala, Sweden).*

Fig. 2 : *SDS-PAGE analysis of outer membrane proteins of different strains of V. parahaemolyticus grown in vivo. Lane 2 to 5 represents KP⁺ strains and lane 6 to 9 represents KP⁻ strains. Lane 1 represents the low molecular weight markers (Pharmacia, Uppsala, Sweden)*

in vivo specific proteins. Migration of proteins in SDS-PAGE remained the same in both *in vitro* and *in vivo* cultural conditions. As migration of outer membrane protein remained the same under *in vitro* and *in vivo* vonditions, further studies are required to compare the antigenicity of these proteins under similar conditions.

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Regulation of Phosphorylation of Macromolecules in *Leishmania donovani* Promastigotes by cAMP and Calcium Ion

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Abstract

The growth of *L. Donovvani* promastigotes was inhibited by 73%, 95% and 65% by 100 μM dibutyryl cAMP, 100 μM verapamil and 4 μM Ca^{2+} - ionophore A21837, respectively. Multiplication of the parasite was stimulated by lower concentration (1μM) of Ca^{2+} -ionophore. The parasite growth was more susceptible to dibutyryl cAMP along with either Ca^{2+}-ionophore or verapamil. Drastic inhibition in the posphorylation of proteins and lipophosphopolysaccharide (LPPS) of the parasite was found in dibutyryl cAMP along with either Ca^{2+} - ionophore or verapamil as compared to effects of these agents individually. The synthesis of LPPS was gradually suppressed by the increased Ca^{2+} -ionophore, and the molecular mass of LPPS was also found gradually lowered as compared to control.

Key words : *Leishmmania donovani*, cAMP, LPPS, Ca^{2+} , Verapamil, Ca^{2+}-ionophore.

Introduction

Leishmania donovani is digenetic hemoflagellate, alternating between a flagellated promastigote form in the mid gut of the vector phlebotomine insect and a rudimentary flagellated obligatory intracellular amastigote form in macrophages causing visceral leishmaniasis (Kala-azar), a fatal disease that is endemic in eastern regions of india an d some other tropical and subtropical countries around the world.

The parasite expresses a major phosphorylated glycoconjugate, i.e., lipophosphopolysaccharide (LPPS) (1) and/or lipophosphoglycan (LPG) (2) which acts as ligand for macrophage receptor for attachment of the parasite, that is essentially the first step for the establishment of infection (2). These phosphorylated glycoconjugates play specific roles in the inactivation of defense mechanism of macrophages by chellating Ca^{2+} . However, the regulation in its synthesis is yet to be determined.

Cyclic AMP inhibits the growth of *Leishmania* promastigotes (3), but the nature of its action remains to be elucidated. Calcium ion is not recognized as a major intracellular messenger system which essentially regulates many cellular processes (4,5,6). Although, high-affinity Ca^{2+} ATPase has been demonstrated in *L. donovani* promastigotes suggesting its role as an extrusion pump for Ca^{2+} (7), the role of Ca^{2+} in the physiological processes

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is yet to be established in clear terms.

In this communication we have reported the regulation of phosphorylation of proteins and LPPS in *L. donovani* promastigotes by cAMP and Ca^{2+} .

Materials and Methods

(i) *Materials :* Adenine, hemin, D-biotin, L-glutamine, verapamil, Ca^{2+} -ionophore A23187 and dibutyryl cyclic AMP purchased from Sigma Co., USA, Medium 199 and HEPES obtained from Gibco Co., USA, bbrain heart infusion broth (BHI) from Acumedia, USA and agar from Difco Co. were used. Radioactive [^{32}P] was obtained from BARC, India. Other reagent grade chemicals were used in this study. Calcium ionophore A23187 was dissolved in a solution of ethanol and dimethyl sulfoxide (1:3, v/v) and the concentration was adjusted at 1 mM.

Promastigotes of *L. donovani* (MHOM/IN/78/UR6) (1), an Indian strain, highly subpassaged in solid blood agar medium consisting of BHI (3.7%, W/V), D-glucose (1.8%, W/V), agar (1.5%, W/V) and rabbit blood (2.0%, V/V) grown at 24 ± 1°C were used in this study. The parasite was cultured in S1-199 liquid medium (8) with or without experimental agents.

(ii) *Biosynthetically labelling of the parasite components with [^{32}P] :* Promastigotes of log phase culture in S1-199 medium were washed twice with sterile saline (0.85%, W/V) by centrifugation at 1000 x g for 10 min at 4°C. These washed parasites were allowed to grow in the S1-199 to which [^{32}P] (50 µCi/ml of culture) was added in presence or absence of experimental agents. Initial parasite concentration was adjusted to $1.0x10^7$/ml. After 6 h of incubation at 24 ± 1°C the parasites were harvested by centrifugation in eppendrof centrifuge. The [^{32}P] -labelled parasites were subjected to SDS-PAGE analysis and were autoradiographed using Kodak X-Omat AR film at -70°C.

Results and Discussion

(i) *The effects of dibutyryl cAMP, Ca^{2+}-ionophore and verapamil on the growth of* ***L. donovani*** *promastigotes :* The effects of these compounds on cell division are shown in Table 1. Multiplication of *L. donovani* promastigotes was inhibited by 37%, 50% and 73% at 96 h culture by 10, 50 and 100 µM dibutyryl cAMP, respectively. Intracellular levels of cAMP have been demonstrated to modulate metabolism and replication of prokaryotic (9) and eukaryotic cells (10).. The growth of *L. tropica* and *L. donovani* were inhibited by 70% by dibutyryl cAMP at 1 mM. We have found the same degree at inhibition at ten time lower concentration of the agent. This difference obtained may be due to the strain variation and / or culture condition. Then we tested the action of high intracellular Ca^{2+} level due to the effect of Ca^{2+}-ionophore A23187 (11) on the multiplication of promastigotes of UR6-strain. At lower concentration of this agent (1 µM), stimulation of the growth of the parasite was observed. But inhibition in the replication was exerted by higher concentration (4 µM) of Ca^{2+}-ionophore indicating that Ca^{2+} regulates the replication of the parasite. The true locomotion of the parasite and flagellar movement were enhanced by the increase of intracellular Ca^{2+} , as observed under a microscope. The indispensability of Ca^{2+} was also proved by the action of verapamil, an inhibitor of Ca^{2+} transport on the growth of promastigotes. The growth inhibitory effect of cAMP was enhanced by either verapamil or Ca^{2+} -ionophore indicating that the action of cAMP was largely regulated by intracellular Ca^{2+}.

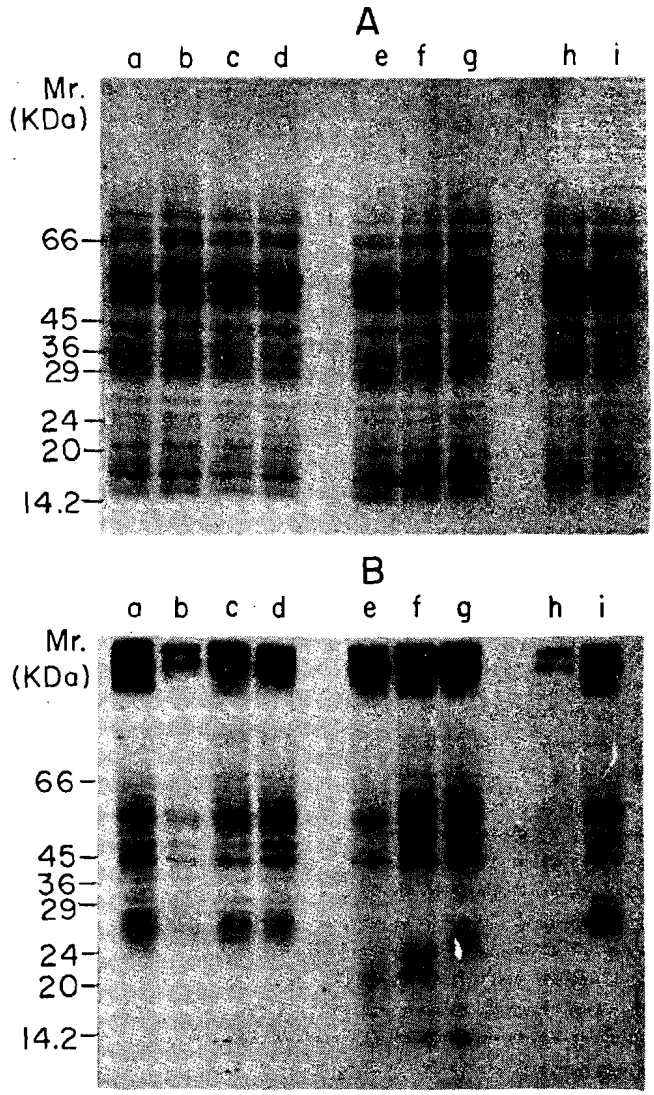

Fig. 1 : *Effect of dibutyryl cAMP, Ca^{2+} -ionophore, verapamil, dibutyryl cAMP with either Ca^{2+} -ionophore or verapamil on the protein profiles (A) and phosphorylated macromolecules (B) of UR6 promastigotes after SDS-PAGE (11% acrylamide) analysis and stained with Comassie blue and autoradiographed of the same gel after drying, control (lane a): dibutyryl cAMP (100μM) + Ca^{2+} - ionophore (1μM) (Lane b); dibutyryl cAMP (100 μM) (lane C) ; dibutyryl cAMP (50μM) (Lane d); Ca^{2+} - ionophore (2 μM) (Lane f) ; Ca^{2+} - ionophore (1 μM) (Lane g); verapamil (50 μM) + dibutyryl cAMP (100 μM) (Lane h) and verapamil (50 μM) (Lane h) and verapamil (50 μM) (Lane i).*

Table 1: Effect of dibutyryl cyclic AMP, Ca^{2+}-ionophore and verapomil in the growth of *L. donovani* promastigotes.

Experiments	Number of cells x 10^5/mL Duration of incubation (hours)						
	0	24	48	72	96	120	144
Control (Vehicle)	5	12	40	150	480	650	600
Dibutyryl cAMP (10μM)	5	13	35	125(-17)	300(-37)	550	500
Dibutyryl cAMP (50 μM)	5	12	35	95(-37)	240(-50)	350	350
Dibutyryl cAMP (100 μM)	5	12	30	70(-53)	130(-73)	220	200
Ca^{2+}-ionophore (1 μM)	5	15	55	240(+60)	600(+25)	750	750
Ca^{2+}-ionophore (2 μM)	5	13	45	200(+33)	520 (+8)	680	550
Ca^{2+}-ionophore (4 μM)	5	12	35	100(-33)	170(-65)	250	200
Verapamil (10μM)	5	12	30	85(-43)	200(-58)	500	550
Verapamil (50 μM)	5	12	25	55(-63)	85(-82)	70	50
Verapamil (100 μM)	5	10	20	30(-80)	20(-95)	0	-
Dibutyryl cAMP (50 μM) + Ca^{2+}-ionophore (1 μM)	5	12	35	75(-50)	90(-81)	55	20
Dibutyryl cAMP (50 μM) + Verapamil (50 μM)	5	10	20	20(-86)	5(-99)	0	-

*The above Table Shows the mean value of four experiments. The figures within the parenthesis are the relative percent of positive or negative growth as compared to control containing vehicle (ethanol and DMSO upto 4 μl/ml of culture).

(ii) *The effects of dibutyryl cAMP, Ca^{2+} -ionophore and verapanil in the phosphorylation of proteins and polysaccharides of* ***L. donovani*** *promastigotes :* Regulation of phosphorylation of proteins and phosphopolysaccharides in *L. donovani* promastigotes by Ca^{2+} and cAMP were visualized by autoradiography of SDS-PAGE analysed [^{32}P] -labelled parasites in presence or absence of dibutyryl cAMP, Ca^{2+} -ionophore or verapamil. Intracellular Ca^{2+} pool was manipulated by using Ca^{2+} -ionophore or verapamil. During six hour exposure of these agents to the parasite culture, the protein profiles remained identical with control (no agent except vehicle) (Fig. 1A). But, there were differences observed in the phosphorylated molecules (Fig. 1B). Prominent inhibition in the phosphorylation was noted with dibutyryl cAMP (100 μM) along with Ca^{2+} -ionophore (1 μM) (Fig. 1B, Lane b), whereas a slight inhibition was found with dibutyryl cAMP alone (Fig. 1B, lane c and d). In control, major phosphorylated macromoles were appeared in the top region of the gel and other phosphorylated proteins of approx. Mr 55 kDa doublet, 47 and 45 kDa were prominent (Fig. 1B, Lane 1). And, another macromolecule spanned in approx. 25-29 kDa region of the gel which was LPPS (1) confirmed by phenol extraction of the whole cell and analysed by SDS-PAGE and autoradiography (Figure not shown). LPPS gradually appeared as low molecular weight component and the intensity of the band was gradually reduced with the

increase of concentration of Ca^{2+} -ionophore (Fig. 1B, Lane g-e) indicating that synthesis of LPPS was down regulated by elevated Ca^{2+} pool in the cell. Phosphorylation of major proteins of Mr 55 (doublet), 47 and 45 kDa was found to be markedly reduced with higher concentration of Ca^{2+} -ionophore (4 μM). Phosphorylation of proteins and LPPS were affected to some extent by verapamil (50 μM) (Fig. 1B, Lane i). But the inhibition was most prominent in verapamil (50 μM) along with dibutyryl cAMP (100 μM) (Fig. 1B, Lane h).

This study has clearly shown that the phosphorylation of many proteins and LPPS was controlled by cAMP and the action of cAMP was largely regulated by intracellular Ca^{2+} . The relationship between calcium and cyclic AMP in *Leishmania* was thus clearly demonstrated.

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Nonlinear Inbreeding Effects on Secondary Sex Ratio in Relation to Birth Order and Parental Age

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Abstract

As inbreeding effects on secondary sex ratio are postulated but not yet demonstrated, the problem has been re-examined in pedigree data allowing for nonlinear effects. A consistent rise in male birth proportion in low inbreeding followed by its decline in high F-values has been observed in the Telaga sample and pooled data from two other Telugu-speaking populations of Kharagpur, W. Bengal, India and also in subsamples for separate birth orders and parental age-groups. A similar pattern is also traceable in five of the eight published results and the deviation in three samples could be ascribed to lack of control for other influences on live- birth sex ratio in all inbreeding levels. An apparent decline in male proportion in still births in inbred series and especially, at low F-values might be related to the wavy relationship between inbreeding and sex ratio. An increasing trend in sex ratio with paternal age continues upto an older age of fathers in unrelated than in related married couples, before reduction of male proportions, presumably due to differences in marriage age.

Key words : *Inbreeding effect, sex ratio, parental age, birth order.*

Introduction

Live -birth sex ratio has assumed some importance in anthropology and especially human genetics as an indicator of mutation, selection, migration and other processes of microevolution which has several bio-cultural implications. There are suggestions that predominance of males in prenatal mortality can be largely explained by lethality of X-linked genes. It is intended in this paper to re-examine the hypothesis of inbreeding effects on the proportion of male births, since study of inbreeding effects has been claimed to be a useful approach to quantitative genetics in human populations (1). A few causal explanations, such as, excess of females homozygous for 'X-linked' or 'partially sex-linked' lethal genes (2), and decline of immunity against Y-chromosome in mothers (3) in inbreeding have been offered for apparent excess of male births to consanguineous parents. But alteration in secondary sex ratio in the inbred individuals has not been so far found to be significant in any of the studies reported. Even then, Bittles et al. (4) have considered 'the estimated higher male birth proportion among the people of Karnataka region of India than in major human populations' to be suggestive of enhanced selection

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against X-linked mutations, in view of frequent marriages of men with daughters of their sisters or maternal uncles. The results of a few studies on inbreeding effects also appear to be contradictory. For example, there is a slight increase of secondary sex ratio in some Indian data (5) and its reduction in others (6,7) among the offspring of related couples.

Perhaps, the failure to arrive at unequivocal results concerning inbreeding effects on sex ratio at birth may at least partly be attributed to the nature of the studies themselves. The studies, except in the case of samples from two large endogamous populations (7,8), have been conducted in composite geographical populations which might have confounded genetical effects of inbreeding, if any. The analyses have generally been made on data which are not based on pedigrees and this must have introduced further errors in the relevant information. Studies have looked only for linear effects of inbreeding on sex ratio, and thus ignored the possible influences of natural selection and epistasis. Besides, the effects of birth order and parental age on secondary sex ratio, widely observed in both people of wide regions (9,10, 11, 12, 13, 14) and endogamous populations of India (5,15,16,17), remain to be considered in the analysis of inbreeding effects on sex ratio at birth. The present study tries to fill up some of these lacunae, and in addition, verifies its findings by comparing relevant results from other published reports.

Materials and Methods

The analysis is mainly based on the data regarding consanguineous marriages, and outcome of successive pregnancies of each marriage derived from large pedigrees of four generations collected by BKD during 1993-94 from three endogamous Telugu-speaking populations : the Telaga, the Kapu and the Sristi-Karnam, settled in the Kharagpur town of Midnapur district, West Bengal, India. There is a narrow range of variation in respect of socio-economic and educational status among the people. Over 90 per cent of the male members and widows are employed as low-grade skilled workers in the workshop or open lines of the Railway, the rest being engaged in shop-keeping. Most of the adult males have received technical training after their middle level schooling and adult females have generally attended upper primary schools. Age of the living individuals have heen verified from their school certificates, railway passes or horoscopes, and that of deceased relatives reconstructed with the help of pedigrees. The data for the Telaga population, which form the bulk of the sample, are analysed separately, that from the other two groups being pooled for examining the consistency of results.

Inbreeding coefficients (F) are calculated for autosomal genes by applying Wright's path-coefficient method and considering F-values of common ancestors of both parents. However, for utilising samples of reasonable sizes, the offspring of (i) unrelated parents ($F = 0$), (ii) relatives more remote than first cousins ($0 < F < 0.0625$), (iii) first cousins ($f \geq 0.0625$), and (iv) maternal uncle : niece pairs ($F \geq 0.125$) are grouped into successive classes, ignoring variation within each level. Sex ratio is calculated as' number of males/ number of females'. For comparison of results, the proportion of male births have been estimated for the same four inbreeding classes in the present as well as relevant published data for verification of the reliability of the findings.

Although the Chi-square test for homogeneity has been applied to adjudge the significance of changes in sex-ratio with inbreeding in the overall samples of Telaga and other Telegu-speaking groups of Kharagpur, consistency of results in sub-samples of different birth orders or parental age groups have also been duly recognised as a criterion for reliability of the findings.

Results and Discussion

(i) In all birth orders : Sex ratio at all births (live- and still-) of all parities appears to increase markedly in the low degree of inbreeding ($0<F<0.0625$) in both the samples from the Telaga and the other two populations (Pooled) under study Table 1.

TABLE 1. : Male (m)/Female (f) ratio at birth with different inbreeding levels (F) in Telugu speaking population of Kharagpur, West Bengal.

Populations	TELAGA						SRISTI—KARNAM & KAPU COMBINED		
	all births			live births			all birhts		
F	m	f	m.f	m	f	m/f	m	f	m/f
0.000	404	377	1.05	393	375	1.05	123	115	1.07
0.000< & <0.0625	33	18	1.83	33	18	1.83	10	4	2.50
0.0625≤ & <0.125	57	60	0.95	56	57	0.98	27	23	1.17
0.125≤	70	72	0.97	68	71	0.96	36	34	1.06

A similar amount of increase in male/female ratio is also observed in low values of non-zero F for live-births in the Telaga sample. A substantial decline of sex ratio at birth is, again, observed with progress of inbreeding upto the level $0.0625<F<0.125$ in each of the three samples. But with further intensity of inbreeding, the change in sex ratio at birth does not appear to be either consistent or appreciable in amount. Even though the Chi-square values indicate low significance at only ten per cent probability in the Telaga samples and at even higher probability levels in the other sample for the initial rise and first subsequent fall of sex ratio at birth with inbreeding, it is hardly possible to ignore the consistency of these results. The Chi-square values for change in sex ratio at birth with further inbreeding are, of course, negligible, being 0.01 in the Telaga and 0.08 in the other sample.

TABLE 2 : Sex ratio (m/f) in first seven live births at different levels with inbreeding (F) among Telaga and the total sample of Telugu speaking populations of Kharagpur, West Bengal.

	Telaga				All			
F	m	f	X^2_{1DF}	m/f	m	f	X^2_{1DF}	m/f
0.000	383	361		1.06	500	468		1.07
			3.92				5.82	
0.000< & <0.0625	30	15		2.00	40	19		2.10
			4.55				6.33	
0.0625≤	123	126		0.98	179	178		1.01
0.0625≤ & <0.125	56	55		1.02	78	75		1.05
			0.09				0.08	
0.125≤	67	71		0.94	101	103		0.98

(ii) In comparable birth orders : The elevation and subsequent depression of live-birth sex

ratio with low and high levels of non-zero inbreeding respectively indeed become significant at $p<0.05$ in the Telaga sample and at $p<0.02$ in the total sample, when births of the first seven offspring are considered (Table 2), excluding subsequent births which did not occur in some of the inbreeding classes. As shown in the table , different levels of high inbreeding are pooled for this comparison on account of negligible values of Chi-square for hemogeneity between the highest two inbreeding classes in respect of numbers of male and female births.

Table 3 : Proportion of live born males (P_m) at different birth orders with different inbreeding levels (F) among Telaga populations of Kharagpur, West Bengal.

		BIRTH ORDER				
F		1	2	3	4	5-10
0.000	n	203	184	138	94	149
	P_m	0.51	0.52	0.52	0.46	0.53
0.000< & <0.0625	n	12	12	7	6	14
	P_m	0.75	0.58	0.86	0.50	0.[illegible]
≥0.0625 & <0.125	n	30	29	24	14	[illegible]
	P_m	0.47	0.52	0.50	0.50	0.53
≥0.125	n	42	38	25	16	18
	P_m	0.48	0.42	0.52	0.56	0.56
≥0.0625	n	72	67	49	30	33
	P_m	0.46	0.46	0.51	0.53	0.54
<0.0625	n	215	196	145	100	163
	P_m	0.52	0.52	0.54	0.46	0.53
TOTAL	n	287	263	194	130	196
	P_m	0.51	0.51	0.53	0.48	0.54

(iii) In different birth orders : The hypothesis of a rise and subsequent fall of male birth proportion in low and high levels of inbreeding in order, in the Telaga population, is further strengthened by the remarkably consistent results obtained in each birth order at least upto the third, while the increase of male propotion with low inbreeding continues even in later live-births (Table 3). Similar results have been obtained from the combined Kapu-Karnam data.

(iv) Comparison with published data : Some published data on live-birth male proportion in different levels of inbreeding are re-calculated for the four inbreeding classes, considered in the present analysis, in order to verify how far the observed pattern of nonlinear inbreeding effects on secondary sex ratio in the Telugu speaking populations of Kharagpur holds good in other human populations (Table 4).

Table 4 : Proportions of male live-births (Pm) at different inbreeding levels (F) among Telugu populations of Kharagpur, West Bengal and other populations derived from published data :

Populations/ Area	F	0.00	0.0625> & 0.00<	≥0.0625 & <0.125	≥0.125	Source
INDIA.						
1. Telaga, Kharagpur						
(All births)		0.517	0.647	0.487	0.493	Present
(Live births)		0.512	0.647	0.496	0.489	study '95
2. Sristi-Karnam & Kapu, Kharagpur		0.517	0.714	0.540	0.514	"
3. Vadde*						
Kolleru lake (1)		0.516	0.535	0.484	0.534	Reddy,
Andhra Pradesh (2)		0.503	0.519	0.511	0.434	1992 (8)
4. Karnataka		0.522	0.519	0.526	0.519	Bittles et al 1988 (4)
5. Tamil Nadu						Rao and
Urban		0.515	0.525	0.504	0.479	Inbaraj
Rural		0.512	0.486	0.508	0.525	1977 (19)
JAPAN						
6. Nagasaki		0.519	0.532	0.526	—	Schull 1958 (2)
7. Kure		0.513	0.545	0.475	—	"
8. Hiroshima		0.521	0.501	0.511	—	"

* (1) represents mothers aged <40 years
(2) represents mothers aged 40+ years.

It is noteworthy that at least seven of the ten samples compared (considering only one sample of the Telaga population) agree well with the hypothesis of the rise and fall of male birth proportion in low and high levels of inbreeding respectively. In this background, the deviation from the expected pattern in the three samples from large regional populations should not pose any serious obstacle to the hypothesis of an initial rise and a subsequent fall in the proportion of male births with the progress of inbreeding. Apart from the problem of errors in the data collected without pedigrees, those samples represent large and composite regional populations and would therefore, sometimes fail to provide a reasonable amount of uniformity in gene pool, socio-economic status, birth order, parental age and other factors that could influence sex ratio, in different classes of inbreeding. It has been shown in Tables 1 and 2, that inclusion of data for certain birth orders only in a few inbreeding classes can reduce the significance of the findings.

(v) *Two-level comparison :* The phenomenon of a wavy pattern of inbreeding effect on secondary sex ratio can also explain the apparent contradictions in the direction of inbreeding effect on a few Indian populations. The live-birth sex ratio would be elevated in the inbred offspring if the frequency of low inbreeding is high, and it would be lowered if the frequency of high inbreeding is large. It would also depend on the manner of classification of different inbreeding levels. For example, in the Telaga sample, one could observe a reduction of male births in high inbreeding for the first three birth orders by grouping F-values from zero to below 0.0625 (Table 3).

(vi) *Birth order effect :* The data presented in table 3 do not reveal any influence of inbreeding on the relationship between birth order and sex ratio. They rather display a poor agreement with the expectation of declining male births with ascending birth order. Recent socio-cultural changes altering age at parenthood, especially in relation to brith order, adoption of birth control measures and induction of abortions having implications for

Table 5 : Male proportions (Pm) in first two parities in different inbreeding levels (F) considering pregnancy outcome among Telegu speaking population of Kharagpur, West Bengal.

F	Parity	Telaga		All	
		n	Pm	n	Pm
0.00	1	201	0.52	260	0.54
	2	189	0.51	243	0.51
0.00<	1	80	0.53	118	0.53
	2	83	0.47	118	0.47
0.00<F<0.0625	1	11	0.82	17	0.76
	2	12	0.58	18	0.61
0.0625≤ F < 0.125	1	29	0.45	44	0.48
	2	30	0.53	44	0.45
0.125≤	1	40	0.50	57	0.51
	2	41	0.39	56	0.45

change on coital frequencies or hormonal conditions during child-birth might have led to the perturbation of the negative correlation between birth order and sex ratio (14). One technical problem is the assignment of birth orders to live-births in a sibship. When the first two birth orders are assigned to live-births considering all parturitions including abortions and still births (Table 5), live-birth sex ratio appears to decline in the second birth order especially in the total sample of Telugu speaking populations of Kharagpur, irrespective of the inbreeding level.

(vii) *Sex differential foetal loss :* In pedigress collected from the Telaga and from all the three populations under study, the excess of males in the still births is limited to the non-inbred foetuses (F=0). The total inbred series (F>0) especially in the Telaga data reflect a relativly greater proportion of loss in female foetuses. There is a marked excess of abortions (which must, of course, include some induced ones) in the inbred series, especially in that with low F-values below 0.0625 (Table 6).

Table 6 : Prenatal mortality and male proportion (Pm) in still births at different inbreeding levels (F) in a samples of Telugu speaking population of Kharagpur, West Bengal.

F	Populations	Parturitions	Abortions		Still births		
		N	f	%	n	%	Pm
0.00	Telaga	790	9	1.14	13	1.65	0.92
	All	1029	10	0.97	16	1.55	0.81
0.00<	Telaga	321	11	3.43	7	2.18	0.43
	All	461	17	3.69	19	4.12	0.53
0.00<& <0.0625	Telaga	57	6	10.53	0	<1.75	—
	All	65	6	9.23	0	<1.54	—
0.0625≤ &<0.125	Telaga	118	1	0.85	4	3.38	0.25
	All	173	6	3.57	12	6.94	0.50
0.125≤	Telaga	146	4	2.74	3	2.05	0.67
	All	217	5	2.30	7	3.23	0.57

In view of the fact that spontaneous abortions do not generally exceed with inbreeding (18) and various cultural factors, it could be assumed that the foetal deaths recorded with unknown sex would also include a greater proportion of female foetuses. The apparent absence of still births with low inbreeding level (F<0.0625) along with high rate of abortion might be related to the observed increase in male proportion in live-births in that inbreeding level.

(viii) *Parental age effects on live-birth sex ratio :* While verifying the influence of inbreeding on the ralationship of paternal and maternal ages with secondary sex ratio (Table 7), it is noticed that the suggested pattern of nonlinear effect of inbreeding on the proportion

Table 7 : Parental age effect on proportion of male live-births (Pm) in different degrees of parental consanguinity and inbreeding levels (F) in a sample of Telaga population of Kharagpur, West Bengal.

		Parental Consanguinity							
		Non-consanguineous		Beyond 1st cousin		1st Cousin		Uncle Niece	
Parental age in years	F	0.000		<0.0625		0.0625<		0.125<	
		n	Pm	n	Pm	n	Pm	n	Pm
	Father								
20-24		103	0.48	8	0.62	18	0.39	8	0.38
25-29		287	0.51	18	0.61	42	0.57	41	0.44
30-34		214	0.53	15	0.73	34	0.56	50	0.58
35-39		96	0.56	2	0.50	15	0.40	25	0.40
40-44		46	0.52	0	-	2	0.00	11	0.54
45-49		21	0.38	8	0.62	1	0.00	2	0.00
50-54		01	0.00	-	-	-	-	0	-
55-59		-	-	-	-	-	-	2	1.00
Low*		700	0.52	41	0.66	94	0.53	99	0.50
High*		68	0.47	10	0.60	18	0.33	40	0.45
	Mother								
<15		11	0.45	1	1.00	2	1.00	5	0.20
15-19		271	0.48	16	0.62	43	0.42	47	0.51
20-24		271	0.54	19	0.74	41	0.54	59	0.49
25-29		138	0.57	6	0.50	23	0.61	21	0.43
30-34		60	0.47	5	0.40	2	0.00	6	0.67
35-39		17	0.41	4	0.75	1	0.00	1	1.00

* low father's age represents below 40 years for F=0, and below 35 yrs for F>0.

of male births is consistently maintained in individual paternal and maternal age-groups of five years in the pedigree data from the Telaga population. The distribution of the male proportion in live births for five-yearly age-groups of paternity tends to conform to the suggestion that male births are proportionately less in higher ages of fathers. But this effect of paternal age on secondary sex ratio occurs earlier from 35 years onwards in all levels of inbreeding with non-zero F-values than in the offspring of unrelated parents (F=0). This suggestion of an enhancement of the paternal age effect in reducing male births would require further verification and causal explanation.

Interestingly, there is a positive rather than negative correlation between paternal age and proportion of malebirths in younger ages of fatherhood until decline of sex ratio with increase of paternal age begins in early fifth decade of life in nonconsanguineous males and in late fourth decade in consanguineous ones. Teitelbaum (13) suggests that the apparent effects of paternal age on live-birth sex ratio is a derivative of real effect of birth order. That assumption would explain the increasing sex ratio with father's age in

younger fathers below 35 years among the Telaga, because there is an apparent trend of increasing age at marriage in the males of the population. Novitski and Kimball (12) have, on the other hand, reported a positive paternal age effect on sex ratio in the highest birth order group. Together with that report, the present finding would suggest that the relation between father's age and birth order is sensitive to socio-cultural changes in the particular population under consideration. The suggestion of some difference between the related and unrelated couples in respect of paternal age effects on sex ratio might also be reflective of such socio-cultural differences between the two groups of married couples. A similar increase of live-birth ratio is observed with increase of maternal age upto about 30 years. which is followed by the decline in sex ratio with further increase of maternal age in the non-inbred series (F=0). Since maternal age in this subsample is fairly correlated with paternal age, similar underlying processes are likely to be operating to determine the variation in male/female ratio at birth. The maternal age and sex ratio interrelationship in different inbreeding levels are not so clearly defined perhaps due to the absence of strong correlation between paternal and maternal ages in related couples and particularly maternal uncle and niece marriages. This suggestion is based on the understanding that maternal age effects on live-birth sex ratio are derived from the paternal age effects (12).

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Byssus and Byssal Gland of *Mytilopsis sallei* (Récluz) (Bivalvia : Dreissenidae) : An Ultrastructural study

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Abstract

The foot of many adult bivalves is glandular and secretes the byssus used for anchoring to any solid substratum. *Mytilopsis sallei*, a dreissenid bivalve, also produces numerous firm byssal threads. The species colonizes heavily and fouls the Visakhapatnam harbour area of Andhra Pradesh.

The scanning electron microscope study shows that the foot has a midventral groove with a semicircular distal depression. The byssus threads of this species branch off from a very short stem. The thread is flat and it splays into a round attachment disc. The transmission electron microscope study reveals that the secretory granules of the Type 1 cell are ellipsoidal in nature having parallel arrays of microfilamentous components as the collagen granules of mytilid biivalves. The Type 2 cell has ovoid to cylindroid granules of nearly uniform electrondense in nature resembing the phenol gland of other byssated forms. Such cells are chiefly distributed near the heel region of foot. The Type 3 cell is found near the periphery of foot and contains electron lucent secretory granules of irregular shape like that of mucous cells of other bivalves. It is assumed that the main component of byssus in this species is a collagen-type protein with certain amount of polypheolic protein acting as tanning agent. This maiden investgation on the fine structure of byssal apparatus of *M. sallei* unfolds some species-specific features, while comparing it with other byssated forms studied so far.

Key words : Byssus, Dreissenidbivalve, Collagen granules.

Introduction

Basides the importance of the bivalve foot in settlement, its structure or the glandular nature and use of its secretions have been considered as important arrangements for adhesive strategies to resist the impact of waves and buoyant effect of water. The proteinaceous byssus, a complex pedal secretory product, is employed as a semipermanent or permanent anchorage system in many adult bivalves. One end of the byssus thread is attached to the stem or directly into the foot, its other end being fixed to the substratum by a funnel or disc-shaped byssal plaque. Many of these have become the most potent organisms to cause aquatic fouling. They remain attached to any underwater stationary objects like rocks, floats, buoys, concrete jetties,conduits, raw water supply systems and ships moored in harbours. Some have so strong attachment that they maintain their community on the hull of an active, cruising ship. *Mytilopsis sallei* is such a dressenid

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bivalve whcich is believed to be accidentally introduced from Central America around late sixties (1,2) and now the species fouls the Visakhapatnam harbour area, Andhra Pradesh, India, in enormous number. This species is also posing a threat to other harbours throughout the Indo-Pacific areas (3). Apart from many physiological adaptations (4,5,6) its secret of success in fouling lies probably with the unique firmness of the byssus and their remarkable power of ecesis. This species has virtually dominated all other groups (7) and it maintains harmonious balance with many macro and microfaunal associates (8). Yonge (9) noted widespread but sporadic occurrence of byssus in many adult bivalve groups including Dreissenacea, who had highly developed byssus. Although, plenty of reports on the anatomy of byssal apparatus of the mytilids are available, that of the dreissenids has hardly been studied in detail. Mesenheimer (10) and Seydel (11) gave an account of the byssal gland of *Dreissena polymorpha* indicating the presence of pedal mucous gland and other groove glands. Kerney and Morton (12) noted the merit of the byssus as a primitive feature in Dreissena enabling the bivalve to colonise stony places inaccessible to other bivalves. Morton (2) observed the byssal notch and multi-threaded byssus complex of *Mytilopsis sallei* while describing the biology and functional morphology of the species. Banu *et al.* (13) in their histological and histochemical study of the foot of *M. sallei* characterised two types of glands viz., "white" gland and "enzyme" gland. Banu *et al.* (14) previously described the structure of byssus of the said species as a fine, cylindrical thread having an attachment disc.

The present study describes the fine structure of byssiferous foot and glandular components of *M. sallei* so as to examine the byssal complex in greater detail. Further, taxonomic confusion of *Mytilopsis* (15) and its allied species (16) have prompted this study with a view to identify the specific feature of byssal complex and also to compare it with other byssated groups studied so far.

Material and Methods

Specimens of *M. sallei* were collected from the concrete jetty of Visakhapatnam harbour, Andhra Pradesh (India). The animals were brought into the laboratory and kept in sea water. The severed feet with byssal apparatus were cleaned with filtered sea water.

The entire feet with a portion of byssus stem were immersed in 4.5% glutaraldehyde buffered at pH 7.4 with 0.1 M phosphate. The samples were then washed for 1 hour in the same buffer. This was followed by dehydration in graded ethonols. The specimens were dried in Polarson CPD with liquid Co_2 (purified) and then mounted on aluminium stubs by colloidal silver. For larger samples pressure sensitive double-sided adhesive tapes were used to hold the samples on the stubs. The specimens were coated with gold-palladium by a Polaron sputter coater at a 10^{-1} torr. The samples were studied with a JSM T200 and SX-25 scanning electron microscope.

The severed feet were cut into small slices (1 mm^3) and placed in the fixative consisting of cold 5% glutaraldehyde buffered at pH 7.2 with 0.1M phosphate for one hour (17). These were postfixed for 1 hour in cold 1% OsO_4 buffered at pH 7.2 followed by usual dehydration in graded ethanol. Then the tissue was treated with propylene oxide with two changes each of 15 minute duration. Initial infiltration was carried out in small volume of propylene oxide and araldite mixture of equal parts for overnight. Subsequently, the proportion of araldite mixture was increased at regular intervals over a period of 2 to 3 hours. Following infiltration, the tissues were embedded in araldite mixture (18). The mixture was allowed to polymerise at 60°C for 48 hours. Ultrathin sections were stained with uranyl acetate and lead citrate (19). The sections were viewed in the transmission

electron microscope JEOL 100 CX.

Observations

The byssiferous foot *of M. sallei* is cylindrical in shape with a narrower distal end. The scanning electron microscopic observation shows that the ventral pedal groove at the distal end forms a small (dia. 250μm) semicircular sucker-like depression. The depression is oval in shape. The centre of the depression is deep. The distal depression is situated well behind the apical part of foot so that a narrow conical, grooveless terminal portion is left forward (Fig. 1). At the heel region on the foot the pedal groove becomes wide and forms a crater-like stem cavity. The posterior margin of the cavity is slightly elevated. The byssus stem of the species is not a well defind component. The byssus threads are seen to branch off from a short, cylindrical stem. The stem hardly grows beyond the cavity (Fig.2). The proximal part of the byssus thread near its origin from the stem is much wider but devoid of any surface corrugation. The threads are nearly flat in form. The distal part of thread shows smooth texture. Each thread at its terminal free end has an attachment disc (av. dia. 300 μm). The attachment discs are approximately round in outline. The thread gradually widens and meets the attachment disc near the centre (Fig. 3).

Apart from the ciliated and microvillous epithelium, muscles, the entire foot of *M. sallei* consists of subepidermal gland cells. The gland cells which appear to be ultrastructurally distinct are denoted by 1, 2, 3 in the following description.

Type 1 Cell : This group of cells are positioned deeply in the distal part of the foot and contain a large (av. dia 5.2 μm), ovoid to round nucleus with dispersed heterochromatin materials (Fig. 4). The nucleus often shows a distinct nucleolus. Each cell body is characterised by greatly developed rough endoplasmic reticulum (RER), whose cisternae are often dilated and contain a microgranular component. The cell has further sparsely dispersed ribosomes and a good number of mitrochondria distributed throughout the cytoplasm. No structures which could be designated as the typical Golgi complex are traced in such a cell. The secretory granules of this cell are ellipsoidal (length ranges from 1-2 μm) in shape and apparently contain a microfilamentous and granular component. Some secretory granules have a structural substance alike to microgranular component as found in the cisternae of RER. The secretory granules also show parallel electron dense areas at linear intervals (av.100 μm) through the long axis. The orientation pattern of electrondense and electronlucent areas appears to differ in various granules of the same cell as seen in sections having different obliquity. This also suggests that the bundles of microfilamentous components are oriented in wavy pattern. These gland cells often have long cell processes which end near the pedal groove to discharge their products. The apices of such cell processes are crowned with microvilli. The cell processes lying between the epithelial cells have terminal bars. It is also to be noted that the ellipsoidal nature of the secretory granules is apparently lost when these are discharged into the byssal groove(Fig.5).

Type 2 Cell : Such cells are mostly found near the multipartitioned ducted complex or byssal stem forming zone situated at the heel region of the foot. The cell has a round nucleus (av. dia 3.7 μm) with a prominent nucleolus. Such a cell is packed with ovoid (dia. ranges from 0.45 - 0.65 μm) to cylindroid (length upto 1.6 μm) secretory granules (Fig. 6). The granules are nearly homogeneous, electrondense in nature including an even denser thin rim of peripheral ones. Two different phases of granules are apparent in such a cell. The granules having smooth peripheral outline may be designated as mature type

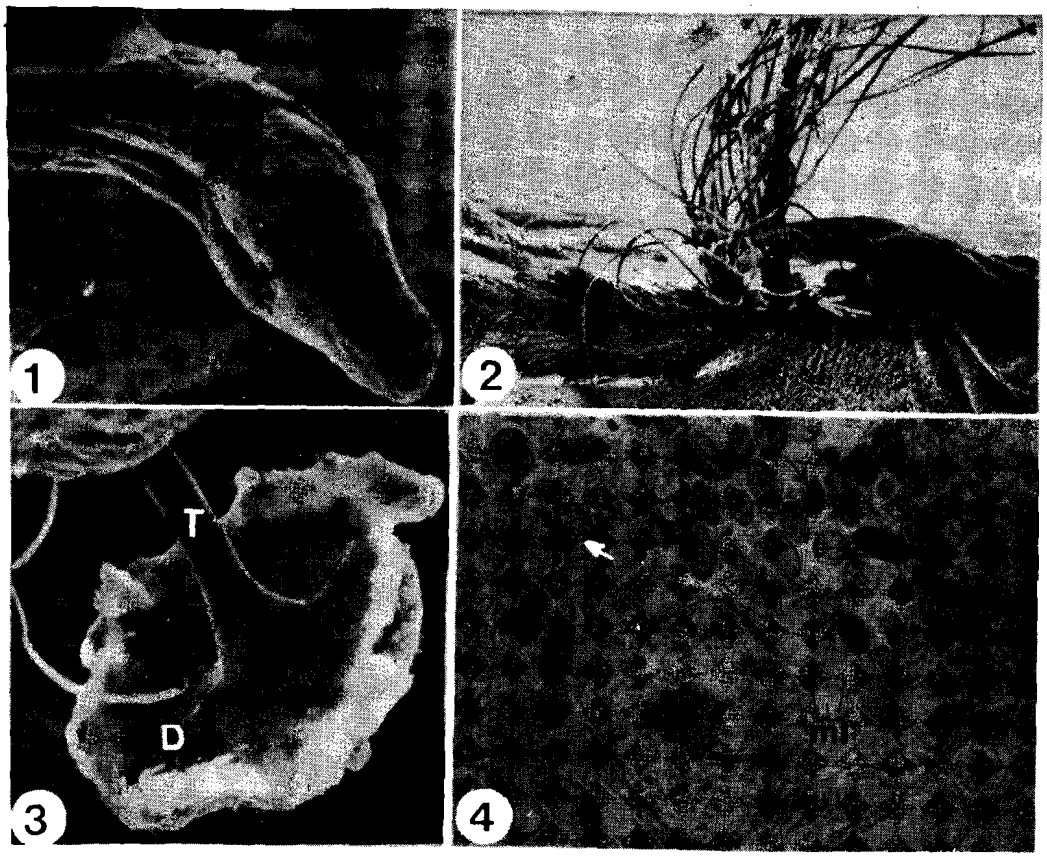

Fig. 1 : *Lateroventral view of foot Mytilopsis sallei. Note the distal depression (small arrow) and vetral pedal groove (big arrow). X 28.*

Fig. 2 : *Lateroventral view of foot of M sallei at heel region to show elevated margin of stem cavity (arrow) and a short stem from which byssus threads branch off. X 30.*

Fig. 3 : *Dorsal profile of attachment disc of M. sallei showing a byssus thread meets to its disc at the centre. Tethread, D-disc. X 220.*

Fig. 4 : *Portion of a cell body of the type 1 cell showing greatly developed RER cisternae (white arrow), mitochondria (mi) and ellipsoidal secretory granule (black arrow). X 5100.*

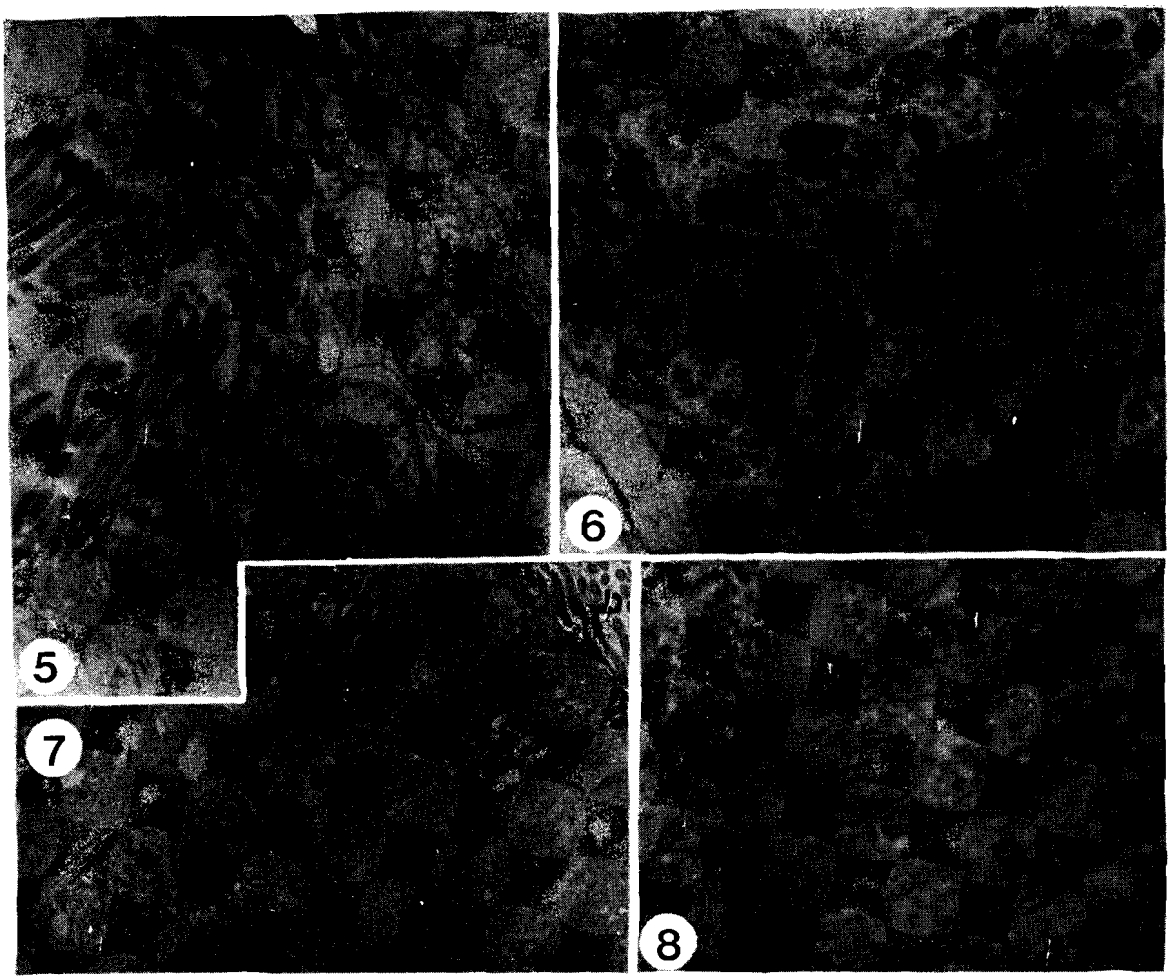

Fig. 5. : *Microvillous apex of a type 1 cell. Note the secretory granule (c) and terminal bar (small arrow). The ellipsoidal nature of granules is apparently lost when these are secreted in the byssal groove (big arrow). X 9500.*

Fig. 6 : *Portion of a cell body of the type 2 cell packed with ovoid to cylindroid secretory granules of nearly homogeneous electrondense in nature. X 7700.*

Fig. 7 : *Portion of cell group of the type 3 cell found at periphery of foot and byssal grove (b). Note varying shapes of secretory granules (m) . X 5250.*

Fig. 8 : *Portion of stem forming zone of the heel region of foot showing a single duct (d) filled with stem forming meterial secreted by bordering cells. Cylindroid secretion granules are shown by double arrow and cell nucleus by N. X 5400.*

and those showing fuzzy outline are the immature secretory granules. The cell has variably developed RER and few scattered mitochondria. Some of the cisternae are greatly swollen and appear to contain a much denser component. Transitional elements deprived of ribosomes are often seen. The cytoplasm of this cell also contains the Golgi complex in which the cisternae are prominent.

Type 3 Cell : This cell group is distributed at the periphery of entire foot along with the ciliated epithelial cells. These cells tend to be columner type. The nucleus is ovoid (av. length 7.0 µm) and cytoplasm contains electronlucent secretory granules of varying shape (Fig. 7). Other cytoplasmic organelles include a nonextensive RER and some mitochondria located especially at the lateral and basal protions of the cell. Golgi complex is discernible among the populations of secretory granules. The apical border of the cell has microvilli which are simple, unbranched and shorter (av. length 0.5 µm).

Ultrastructure of the stem forming zone : The stem forming zone of *M. sallei* is situated at the heel region of foot. The pedal groove meets here in a spaceous depression called the stem cavity. Inside this cavity the byssus stem broadens and forms multibranched root portion. The root is formed within a multipartitioned ducted complex separated by tissue septa. The upper peripheral part of this zone is occupied by thick blocks of muscle. The primary source of stem forming materials is the apical cell processes of gland cells bordering the ducts. The processes of Type 2 secretory cell are evident in this region since ovoid to cylindroid granules (av.dia 0.6 µm) are found here (Fig.8). The ducts are lined by ciliated epithelial cells. The proximal part of each duct is branched and even broader when compared to its distal extremities. The byssus stem forming material is found in the form of dense homogeneous substance with no sign of any granular component.

Discussion

Microanatomical studies with the help of scanning electron microscope on the byssiferous foot are meagre. There is always a possibility of obtaining more information from such studies over the light microscopic observation. Thus it becomes easier to interprete morphological specialities in more believable functional terms. Further, an adequate appraisal of the homologies of byssyal structure of differnt bivalves becomes possible.

The micromorphological observation made in this study is comparable to those given by earlier authors (20,21) on various mytilid bivalves. Although, the general shape of the distal depression of *M. sallei* shows certain apparent similarities with that of mytilids, especially with *Modiolus striatulus* (22). The shape of such a depression is quite different in isognomonid and pteriid bivalves (23). Moreover, the depression of *M. sallei* is not at all comparable to arcid bivalves studied so far (24).

As the foot of *M. sallei* like most byssated bivalves is not used for locomotion, its use is restricted to the formation of byssus threads and attachment. The byssus production and plantation are mediated by an efficient pedal neuro-musculoglandular coordination.Bairati and Vitellaro Zuccarello (20) have pointed out that the length of thread and pedal groove may vary and this is due to difference in active length of foot when a thread is being formed. Bruzone (25) has found that *Mytilus edulis chilensis* (Hupe) requirs 3-8 minutes to spin a byssus thread. This clearly suggests that byssus is not instantly produced and probably the secretion is released (appropriately triggered by nerve), moulded (by pedal fluidmuscle system), and fastened to an object in sequential manner.

One of the important components of the byssus appartus is a stem formed at the

heel region of foot, although this region is not prominent in *M. sallei* when observed under scanning electron microscope. That's why, Banu *et al.* (13,14) and Morton (2) did not mention about a byssal stem in their description of diagrams of the species. The anatomic relationship of foot with stem is also well documented in *M. sallei* under TEM observation The multipartitioned musculo-glandular complex at the heel region of foot generates the stem in the form of numerous leaf like septa. These septa are later inseparably moulded into a stem on which byssus threads are attached. The two terms stemgenerator (21) and stem gland (26) are interchangealby used to designate this zone. As the Type 2 secretory cells are found predominantly in this zone, so, apparently, such secretion contributes to formation of a stem component. The precise explanation of stem growth in the species is beyond the scope of present study and which probably remains a conjecture by Tamarin (21).

The byssus thread of *M. sallei* like other mytilid bivalves can be distinguished into proximal, middle and terminal disc-tipped portions. The surface feature of proximal part of thread does not show any distinct corrugations as found in mytilid bivalves. Such a smooth nature of thread is also found in *Isognomon legumon* (23) and *Barbatia* spp. (24). There appears a distinct difference in the stem-thread relationship between *M. sallei* and other mytilid bivalves studied so far (20,21,27). The attachment disc of *M. sallei* is also notable as the thread meets the disc nearly at its centre.

The byssiferous foot of *M. sallei* is adequately composed of different types of gland cells of exocrine in nature. The fine structure of such gland cells clearly shows the active synthesis of at least three types of secretory products. The secretory product of Type 1 cell is essentially identical to that of collagen gland cell of *Mytilus californianus (28,29) M. edulis (30) M. galloprovinicialis (31)*and *Modiolus striatulus* (22). The secretion granules of such a cell have a consistent ellipsoidal shape and internal structures demonstrate evenly spaced periodic microfilamentous components parallel to the long axis. It is yet to be ascertained whether the granules of collagen gland contain collagen or procollagen (29). As far as the structure of secretion granules of such gland cells of *M. sallei* are concerned, these probably bear similarities with the type B of *Mytituls galloprovincialis* (32). With the evidence of greatly developed RER and scant development of the Golgi complex, it appears that most of the formed materials of collagen granules originate from the RER cisternae. This situation also finds support from the observation in mytilid bivalves (23,30). The process of byssus collagen fibrogenesis from procollagen in the extracellular phase still remains unanswered. However, the present findings confirm that the secretion granules of collagen gland undergo structural changes as soon as these are liberated into the groove. The ovoid secretion granules of Type 2 cell are unique as these have often intense electrondense rim at the periphary. These are neither similar ultrastructurally to phenol gland nor enzyme gland secretion granules of other mytilid bivalves (23,28). It is likely that such cells produce both phenolic compounds and phenolic oxidase. As a matter of fact, the denser of peripheral zone and the core of secretion granules may contain those two compounds in variable amount. A detailed cytochemical study on this particular gland cell will make this issue clear.However, the histochemical study on byssal gland of *M. sallei* made by Banu *et al* (13) have shown the presence of extensive white (=collagen) gland and a few enzyme glands. Vitellaro Zuccarello (31) in a detailed cytochemical study on the enzyme gland of *Mytilus* has shown that the secretory granule core contains protein rich in acidic and aromatic amino acids, whereas the outer layer contains mainly aromatic amino acids possibly indicating a polyphenolic protein. Therefore, Banu et al (13) detected

numerous tyrosine in Millon's reaction and fuchsinophilic substances probably due to the presence of aromatic protein in the enzyme gland of *M. sallei.* This situation further supports the statement of Vitellaro Zuccarello (31) that the enzyme gland may produce both phenolic compounds and a phenol oxidase. So this gland is truly equivalent to other main glands like collagen and phenol gland secreting structural substances of the byssus but not merely catalising the tanning process. The fine structure of Type 3 cell bears resemblance to the mucous cell in general. This group of cells is found at the periphery of the foot often sandwiched between ciliated epithelial cells. The granules are electron-lucent and moreover, these donot have any organized structure resembling mucous cells of other bivalves. The physiologists have long assumed the presence of a protective covering of mucus over the epithelial cells in a variety of animals. Besides, many functions are performed by mucosubstances (33). According to Tamarin *et al.*, (34) mucus secretion may help in the initial application of byssal disc to the substratum. The specialised morphology of the microvillous apices of mucous cell in relation to granule conduction needs further attention. The junctional complexes in between the epithelial and mucous cells are always evident.

The present study has revealed that at least two glands are involved in the secretion of byssus thread and disc of *M. sallei.* It is assumed that the main bulk of the byssus in this species is a collagen-type protein and this is probably a reason why the thin (25-30 µm) threads are capable of withstanding strong wave action. The outer component of byssus is a phenolic protein secreted by a gland, which also produces a polyphenol oxidase enzyme initiating quinone crosslinking (35). The synthesis and elaboration of these secretory products probably require the carefully controlled environment of the cell interior and several other factors of the cell exterior.

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Iron-deficiency of Female Athletes in Response to Exercise During Phases of Menstruation

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Abstract

Iron-deficiency is a common problem in athletes who do not increase their iron-intake above that of general population. This study was made to evaluate the changes of serum iron level of female athletes and non-athletes in response to exercise during their successive two phases of menstruation. The study was conducted on 12 athletic and 12 non-athletic female subjects their age ranging from 17 to 24 years. Exercise test was done on them by magnetic brake bicycle ergometer under the work intensity of 900 kgm/min upto exhaustion in successive two phases of menstruction. Blood was collected before and after exercise in two phases of menstrual cycle (follicular and luteal phase) for estimation of serum iron (µg/dl) and haemoglobin level of blood (gm%).

Results indicate that, except in luteal phase of menstruation in pre-exercising condition of both athletes and non-athletes, serum iron level decreases significantly in response to exercise condition.

Key words : Iron-deficiency, menstrual cycle, exercise.

Introduction

Iron status is associated with exercise training. Anemia can impair exercise performance because reduced haemoglobin concentration is associated with a reduced blood oxygen content.

Iron deficiency is usually attributed to an inadequate iron intake, high demands during the adolescent growth spurt and during menstruating blood loss coupled with inadequate absorption. There are evidences from both animal and human studies that iron deficiency reduces physical work capacity (1). Both physical work and exercise would require additional iron for oxygen transport and cytochrome mediated ATP production.

Weaver (1) reviewed the stages of iron deficiency in endurence - trained athletes, both males and females, the most severe of which is the point where haemoglobin production falls, resulting in anemia. Kilbom (2) observed small decreases in haemoglobin and haematocrit values and 25% decrease in serum iron in adult women engaged in bicycle ergometer training.

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Serum iron concentration is influenced by many pathologic and physiologic states. It decreases at about the time of menstrual bleeding.

The aim of the present study was to compare the iron-status of competitive female athletes, who were trained for different games, with that of less active women in their two successive phases of menstrual cycle in response to exercise.

Methodology

Twelve women athletes (6 of them long distance runners and 6 were sprinters), age ranging between 18-24 years, volunteered for this study. The athletes included in the study had been engaged in athletic activities for at least 6 consecutive years. All of them were physically fit at the time of the study.

The non-athletes numbering twelve were also physically fit. All the non-athletes had normal and regular menstrual cycle with no history of discomfort during menstruation, but out of twelve atheletes eight reported greater occurrence of oligomenorrhea, a missed menstrual period at least theree times in previous year. Their behavioral characteristics during successive menstrual cycles were determined with the help of a questionnaire.

All the female subjects were invited to the laboratory for a practice trial of exercise on magnetic brake bicycle ergometer. Next day, after reporting in the laboratory their height, weight and pre-exercise heart rate were recorded. They were allowed to do exercise at 900 kgm/min on bicycle ergometer till exhaustion in successive two phases of menstrual cycle on two different days. Blood samples were collected before exercise and just after exercise for the (i) determination of haemoglobin content by the method of Wong (3) and (ii) estimation of serum iron by the method of Colorimetric Bathophenanthroline with deproteinization (4) in two successive phases of menstruation, follicular phase (6-9 days after the onset of menstruation) and luteal phase (6-9 days after ovulation).

Results

Table 1 : Physical characteristics of female athletes and non-athletes (Mean ± S.D.)

	Age (year)	Height (cm)	Weight (kg)
Athletes (N=12)	19.6 ± 1.9	159.6 ± 4.1	49.1 ± 4.3
Non-athletes (N=12)	18.9 ± 0.9	156.8 ± 4.2	56.2 ± 7.0

It may be noted from Table 1 that the mean body weight of athletes is significantly lesser ($p<0.01$) than that of their non-athlete counterpart. Post-exercise serum Hb levels, both in the cases of athlete and non-athlete groups, are significantly decreased ($p<o.oo1$) from that of pre-exercise level (Table 2). Moreover, in both luteal and follicular phases of menstruation serum iron levels show significantly lower values ($p<0.001$) after excrecise than that in pre-exercise condition.

Table 2 : Changes of Hb (gm%) and serum iron level (µg/100 ml) in response to exercise during two successive phases of menstruation of both athletes and non-athletes. (Mean ± S.D)

	Before exercise				After Exercise			
	Serum Iron		Haemoglobin		Serum Iron		Haemoglobin	
	Luteal	Follicular	Luteal	Follicular	Luteal	Follicular	Luteal	Follicular
Athletes (N=12)	75.9 ±21.2	63.3 ±17.9	13.7 ±0.8	13.1 ±0.8	70.4 ±14.1	54.0 ±17.8	13.1 ±0.8	12.6 ±0.7
Non-athletes (N=12)	87.9 ±17.8	80.6 ±17.1	12.6 ±0.2	12.6 ±0.6	82.5 ±18.5	73.8 ±16.0	12.1 ±0.4	11.3 ±0.5

Discussion

On analysing the data it was found that a comparison of the means revealed the pre-exercise value of serum iron of athletes to be lower than that of non-athletes. This low value might be due to poor iron status which is called 'Sprots anemia'. Yoshimura *et al* (5) indicated the occurrence of athletic anemia during hard physical training and its causal mechanism may be sought with special reference to protein nutrition. A number of factors have been described as possible causes of iron deficiency in athletes including intravascular haemolysis (6,7,8). Haemoglobin released from the haemolysed cell is picked up by the haptoglobin and removed by the liver. Subsequent loss of iron from haemoglobin through the urine and sweat could deplete iron-stores and lower serun ferritin (9). It was also found that transport of oxygen by the blood was a key link in the delivery of O_2 to the tissues. When Hb concentration is reduced, maximal O_2 delivery to the tissues becomes limited (10). Again, when the amount of haemoglobin released into the plasma following a homolytic episode exceeds the binding capacity of haptoglobin, the excess haemoglobin may be excreted by the urine resulting in haemoglobinuria (11). Thus the proportion of plasma haemoglobin that is lost in the urine depends upon the concentration in the plasma and the concentration of unbound haptoglobin at the time of haemolysis (12).

Some workers (13) noted low serum iron in athletes with injuries of the achilles tendon. In our study it was also obersved that athletes, who shortly after the examination suffered a muscular tedinous injury, had hyposideremia. It may be possible that a slight iron deficiency may primarily affect the function of some iron containing enzymes involved in the collagen synthesis. Recent reports indicate that iron deficiency without anemia may reduce mitochondrial iron-dependent enzymes causing higher lactate level and increased heart rate response during submaximal work (13). Ohira *et al* (13), observed that work capacity of female athletes could be improved after iron treatment.

By comparing and analysing the data it was observed that the average value of serum iron fell significantly after exercising condition both in the case of athletes and non-athletes during luteal and follicular phases of menstrual cycle. This iron deficiency might have been influenced by the intensity of training. Hard training induced a reduction of iron level.

The mean value of serum iron, both in the case of athletes as well as non-athletes, decreases more in follicular phase than in luteal phase. This reduction may be due to blood loss in secretory period. The initial stage of iron deficiency is caused by depletion of iron stores in the liver, spleen and bone-marrow. Serum ferritin levels also get reduced both in athletes and non-athletes between the post-menstrual and mid cycle tests (14).

From the above findings and discussion, conclusion might be drawn that in training period, female athletes would require additional iron for their better physical performance. So, a regualr checking of their serum iron level in successive phases of menstrual cycle should be planned during their training programme and adequate iron supplementation should be prescribed to prevent iron deficiency affecting their performance.

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Changes in Juice and Sucrose Contents (Pol%) in *Saccharum officinarum* L. Grown in Soil Amended with Vesicular Arbuscular Mycorrhizal Fungi and Phosphates

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Abstract

Juice and sucrose contents in plants were enhanced due to VAM infection. An irregular increase in these contents was observed during different phases of plant growth. The increase was more significant upto 9 months of plant growth but it slowed down afterwards. Out of the three phosphates employed, maximum increase in juice (162.70 to 341.90 ml/plant) and sucrose (8.75 to17.90 pol %) contents were recorded when VAM infected plants were grown in soil amended with rock phosphate, whereas minimum rise in juice (156.60 to 328.70) ml/plant) and sucrose (8.40 to 17.55 pol. %) contents were recorded in plants treated with single super phosphate.

Key words : VAM, phosphates, juice, sucrose, *Saccharum officinarum.*

Introduction

Sugarcane is an important cash corp which fulfills the sugar requirement of our country. The common sugar which is sold in the market is sucrose. It is disaccharide having two monosaccharide molecules, i. e., glucose and fructose. A number of enzymes like sucrose synthetase and sucrose phosphatase synthetase are involved in the synthesis of sucrose.Together with these, uridine disphosphate synthetase glucose compound (UDPG) are also associated with sucrose synthesis. Since VAM fungi enhance the plant biomass, the juice contents may also rise in plants due to increase in cane heght and biomass. Thus VAM fungi indirectly affect the sugar level in plants. The present paper deals with the individual and combined effects of VAM fungi and phosphates on juice and sucrose contents of VAM fungi infected and VAM fungi free plants during different phases of plant growth.

Methods

The sugarcane sets of B. 0.109 variety (showing maximium VAM infection during preliminary survey) were allowed to grow in soil having inoculum of *Glomus fasciculatum* (200 VAM spores/seedlings). They were allowed to grow for three months to develop infection of VAM in their roots. The control cane sets were grown in sterlized soil without VAM fungus. The VAM infected and VAM free plants were grown in soil amended with different phosphates.

Pots (60 x 50 x 50 cm) were filled with sterilized normal soil (50 Kg/pot) and

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uniform dose of various sources of phosphate (di-ammonium phosphate, rock phosphate, single super phosphate) @ 50 Kg/ha. The different nutrients were amended to the soil after regular intervals of three months. Five plants were grown on each treatment. They were provided with normal conditions for growth in green house.

The juice content of fresh sugarcane was extracted. The treated and untreated plant samples were uprooted after 3,6,9 and 12 months of growth. Both VAM fungus infected and VAM fungus free plants were washed in water and then crushed through hand operated crusher machine.The total juice obtained from a plant was collected and filtered through sieve and finally through musline cloth. The total juice was transferred to measuring cylinder and its volume was measured. After measuring the volume, the juice was used for sucrose analysis.

The juice was filtered through glass wool and then centrifuged at 3000 rpm for 30 minutes. The sucrose content was analysed by using Brix hydrometer and polariscope. The necessary correction for temperature was done and purity was determined as coefficent of purity.

$$\text{Purity Co-efficient} = \frac{\text{Pol (\%)}}{\text{Corrected brix reading}} \times 100$$

The analysis was completed according to the procedure adopted by Spencer and Meade (1).

Results and Discussion

The data presented in Table 1 show that there was increase in volume of juice and in the percentage of sucrose content in cane shoots of both VAM fungus infected and VAM fungus free plants. However, the increase was more significant in VAM fungus infected plants as compared to VAM free plants. Among the various sources of phosphates employed, the amount of juice and the percentage of sucrose was the highest in rock phosphate followed by di-ammonium phosphate, single super phosphate and control (without nutrient) during different phases of plant growth, i. e., upto 3 months, 6 months, 9 months and 12 months. The rate of increase was higher upto 9 months of plant growth but it slowed down afterwards (Figs.1 and 2). Maximum VAM infection in roots and infestation in soil was also recorded in rock phosphate treatment. So, juice (162.70 to 341.90 ml/plant) and sucrose (8.75% to 17.90%) synthesis in plant might have been stimulated due to higher uptake of nutrients by VAM fungus. Increase in juice of sucrose percentage in cane by phosphates have also been reported by Kanwar and Kochar (2), Misra *et al.* (3), Rao (4), Nandu *et al.*(5), Pannu *et al.*(6), Shivalingam *et al* (7), Mengel and Kirkby (8).

The results of the present investigation reveal that the metabolic activities associated with juice and sucrose synthesis remained the highest between 6 to 9 months of plant growth. Increase in juice and sucrose contents in cane by VAM treatment is an interesting phenomenon which may help in better recovery of sugar form sugarcane. The extra nutrients may trigger the synthesis of juice and sucrose but the effect of VAM fungus was always found to be superior to the effect of nutrient used. It was also observed that the joint effect of VAM fungus and phosphate was always superior to the effects of VAM and

phosphate taken separately. Indeed, the dose of fertilizers should always be within the prescribed limits which varies from soil to soil and site to site.

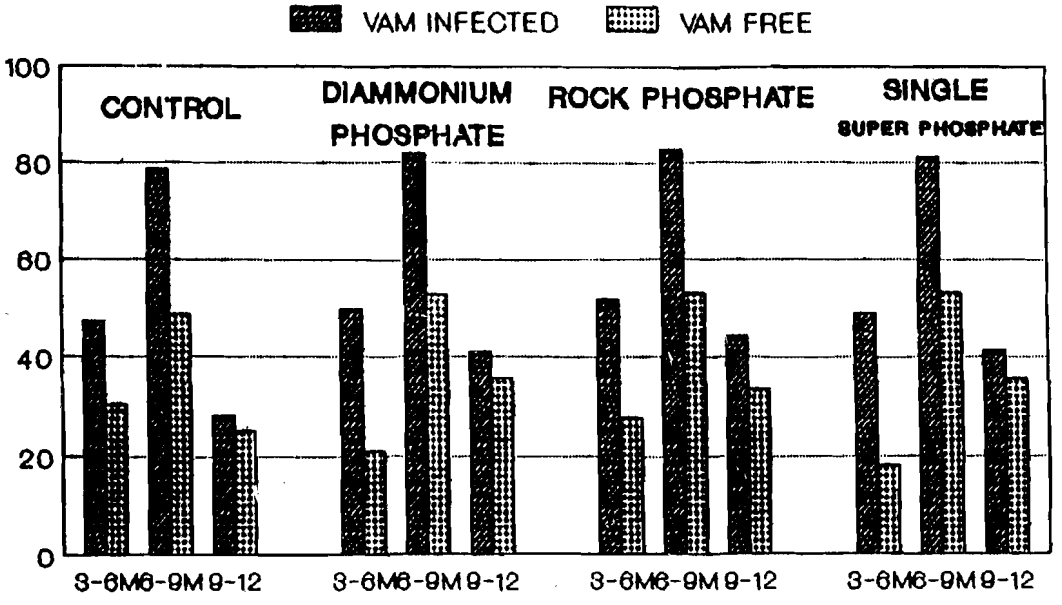

Fig. 1 : *Rate of increase of juice content (ml/plant) in VAM infected and Vam free cane plants grown in phosphate amended soil and different phases of growth.*

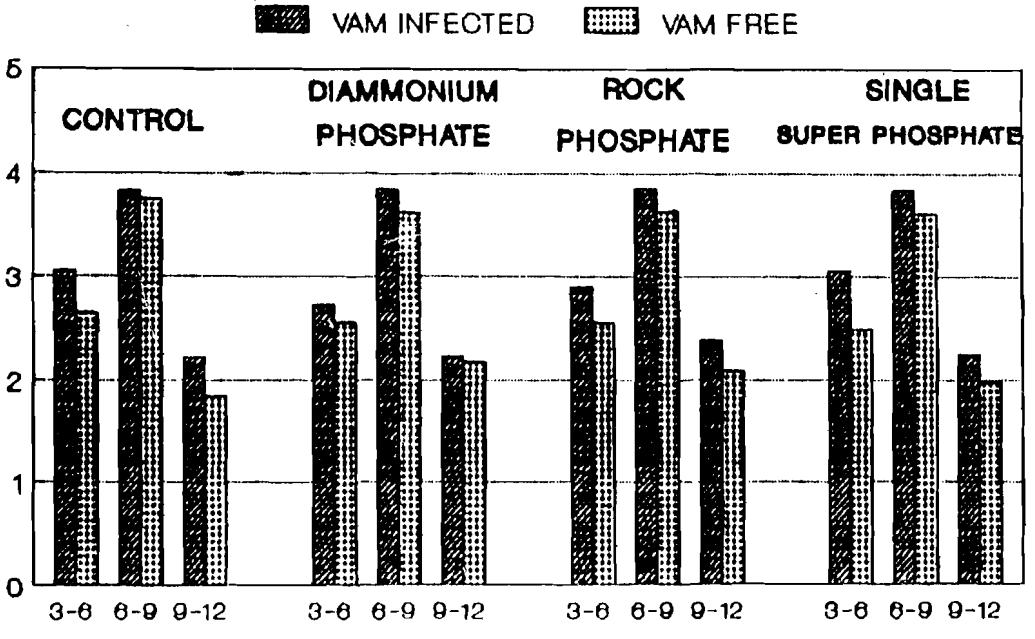

Fig. 2 : *Rate of increase of sucrose content (pol%) in VAM infected and VAM free cane plants grown in phosphate amended soil at different phases of growth.*

Table 1. Showing changes in juice (ml/plant) and sucrose (pol%) contents during different phases of growth in VAM infected and VAM free plants *(Saccharum officinarum)* grown in soil supplemented with different sources of phosphates.

Treatment/ content	Juice content (ml/plant) and sucrose content (pol%) in cane after 3 months VAM infected	3 months VAM free	6 months VAM infected	6 months VAM free	9 months VAM infected	9 months VAM free	12 months VAM infected	12 months VAM free
Control (without nutrient)								
Juice	145.50	66.30	192.90	96.70	271.50	145.40	299.90	170.70
Sucrose	8.40	6.15	11.45	8.80	15.28	12.55	17.50	14.40
Diammonium phosphate								
Juice	158.70	85.60	208.60	106.70	290.60	159.70	331.70	195.60
Sucrose	8.72	6.24	11.55	8.79	15.40	12.41	17.63	14.59
Rock phosphate								
Juice	162.70	81.60	214.60	109.70	297.50	162.90	341.90	196.70
Sucrose	8.75	6.33	11.65	8.88	15.51	12.51	17.90	14.60
Single super phosphate								
Juice	156.60	86.50	205.70	104.60	286.90	157.70	328.70	193.80
Sucrose	8.40	6.32	11.46	8.81	15.30	12.42	17.55	14.40

Value of 't' of between VAM infected and VAM free treatment (*Significant p 0.05)

Control 2.691* Diammonium phosphate 2.643*

Rock phosphate 2.648* Single super phosphate 2.629*

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