

CONTRIBUTIONS TO THE STUDY OF ALGAE WITH PARTICULAR REFERENCE TO THEIR KARYOLOGY¹

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Our contributions to the study of algae (to which I shall confine myself in to-day's address) extending over a quarter of a century which are published through 136 research papers to date, fall into the following categories :

1. Cytology and Cytotaxonomy of several groups of algae.
2. Cytogenetics of algae, Effects of radiations; Modifying action of certain radio-protective chemicals; Effects of chemicals.
3. Physiological studies in relation to germination of akinetes; conjugation in certain desmids and cultivation of certain dinoflagellates.
4. Systematics/Morphology and Reproduction.

Since it is not possible to survey the entire range of work at this juncture, I shall try to present only a bird's eye-view of the totality.

1. Cytology and Cytotaxonomy of several groups of algae : The investigations in this area cover 300 taxa of algae, largely belonging to green algae and some to Dinophyceae. The cytologically investigated genera included under various Classes/Orders are listed in Table I. The number of taxa investigated under each genus is indicated in brackets following name of the genus. The range of chromosome numbers has also been indicated for each Order/Class investigated. It

may be emphasised here that outstanding karyological characters of the Orders Volvocales, Ulotrichales, Chaetophorales, Chaetophorales, Oedogoniales, Conjugales, Siphonales, included under Chlorophyceae (Fritsch, 1935), Charophyceae, Bacillariophyceae, Euglenophyceae and Dinophyceae are summarised by the author in various reviews (Sarma, 1973, 1977, 1979, 1982 and 1983). The chromosome numbers of members of the above groups published in the world are listed by the author (Sarma, 1982). It is gratifying to record that chromosome numbers recorded from our School of about 200 algal taxa are first reports. The lowest and highest chromosome numbers in algae both recorded during the course of our studies are $n=2$ in certain taxa of *Spirogyra* to $n=c.600$ in a species of *Gymnodinium*.

On the basis of the above extensive studies, four chromosome types in algae, categorised as under, may be recognised (Sarma, 1983).

- (i) Chromosomes appreciably long with localised centromeres e.g., members of Cladophorales, Oedogoniales, Charales.
- (ii) Chromosomes extremely small without any indication of the position of the centromere e. g., many Volvocales, Chlorococcales, Chaetophorales, Siphonales, many spe-

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- cies of desmids and some species of filamentous Conjugales.
- (iii) Polycentric chromosomes e. g. several species of *Spirogyra* and some of desmids.
 - (iv) Chromosomes fairly large, but with no evidence of centromere and not associated with a spindle during mitotic cycle, e. g., members of Dinophyceae and Euglenophyceae.

On the basis of chromosome number, breadth and length of individual chromosomes, N. O. chromosomes, if detectable, it has been possible to prepare idiograms of individual taxa belonging to Charales (Khan and Sarma, 1967 a, b; Ramjee and Sarma, 1971) and Oedogoniales (Srivastava and Sarma, 1979), which proved to be of immense value in discussing the evolutionary trends within these groups. Although a large number of taxa of Cladophorales were investigated by other workers, no concerted attempt has as yet been made by any worker to depict idiograms of karyotypes, although the chromosomes are appreciably large and centromeric position of individual chromosomes identifiable.

Chromosome number within a particular group of algae give an insight to probable lines of evolutionary diversification. Coupled with other karyological characteristics they provide good evidence for decisions on taxa of disputed systematic position, either at specific, generic or even at supra generic levels. Thus, karyological information has proved to be an excellent tool, if judiciously used, for cytotaxonomic studies in algae (Sarma, 1978). To quote just a few examples from our own studies, cytological evidence lent support to the elevation of the Order Conjugales to Conjugatophyceae and the Order Oedogoniales to Oedogoniophyceae as proposed by Round (1963), but did not favour the

inclusion of Cladophorales along with Siphonales under Bryopsidophyceae by the latter author, of Fott's treatment of Oedogoniales (Fott, 1959) under Ulotrichales (cf. Sarma, 1964). Studies made by Vedajanani and Sarma (1978 a, b, c, d) and Abhayavardhani and Sarma (1981 a, b, 1983) on Conjugales, of Srivastava and Sarma (1979) on Oedogoniales, of Chowdary and Sivaji Singh on Siphonales are relevant to this context. The view of chaetophoralean origin of charophytes put forward by Desikachary and Sunderalingam (1962) has been refuted by Sarma, Khan and Ramjee (1970) on cytological grounds. The principle laid down by Wood and Imahori (1965) that monoecism and dioecism are not to be considered as valid taxonomic criteria in Charophyta on the basis of which they revised certain taxa of this group, was found to be totally unacceptable on cytological evidences by Sarma and Khan (1967) and later by Proctor (1971).

Karyological studies have served as a reliable means of determining the systematic position of certain genera of controversial systematic position. To cite a few examples, *Sphaeroplea* has been shown to be more closely related to Ulotrichales than to Cladophorales or Siphonales (Sarma, 1962), on the basis of its karyology. Similarly, *Microspora* was also shown to be having close affinities with Ulotrichales on cytological grounds (Sarma, 1963). The contention by Campbell and Sarafis (1972) that *Schizomeris* did not deserve a generic status, but could be considered as an ecophene of *Stigeoclonium tenuit* was opposed by cytological studies of *Schizomeris* by Sarma and Chaudhary (1975). The indentify of *Gaespitella pascheri* with *Stigeoclonium pascheri* was established by the studies of Shyam and Sarma (1979).

Broadly speaking, chromosome numbers within a group of algae have also revealed a clue to certain evolutionary trends within the group. Thus in general, euploidy played a distinctive role in the evolution of Cladophorales and Charales. In a majority of algal groups so far investigated, however, aneuploidy proved to be of greater significance in evolution of those groups. Exceptions do occur in both the cases. The occurrence of cytological races within a species was found to be a common phenomenon in many of the unicellular, colonial and filamentous algae. As examples could be cited the cases of *Euastrum cornubiens*, *Glosterium moniliferum*, *Gonium pectorale*, *Microspora amoena* and *Schizomeris leibleinii*. Comprehensive lists of algae exhibiting euploidy within a genus, aneuploidy within a genus and cytological races within a species have been documented by Sarma (1978).

Recently on the basis of cytogeographic and cytosystematic survey of world literature on Charophyta, Khan and Sarma (1981-82 and 1984) have come to the conclusion that most probably, India (particularly Uttar Pradesh and Bihar) may be considered as the primary centre of origin for world charophyte flora.

The karyology of dinoflagellates (Dinophyceae) assumes importance in the direction of bridging the gap between the two well established levels of genetic organisation viz., Prokaryota and Eukaryota where the gap is astounding. The dinokaryon (nucleus in Dinophyceae) has a nuclear organisation similar to the eukaryon with a nuclear membrane, nucleolus, chromosomes and undergoes mitosis. However, the DNA of the chromosomes of a dinokaryon is not complexed with the conventional histones of the eukaryotes, the chromosomes are

in a highly condensed state even in the interphase nucleus, and the mitosis is an aberrant one not conforming to the events well established in eukaryotes (cf Shyam and Sharma 1978, Sharma, 1978, Sharma, 1983), particularly in not being involved with a typical spindle apparatus, and the distribution of chromosomes in some mysterious way being a membrane mediated event as has been postulated in prokaryotes. A further probe into the finer aspects of the karyology of Dinophyceae is bound to prove rewarding particularly in relation to the evolution of the chromosome itself.

2. *Cytogenetics of Algae* : Cytogenetical studies relate to the use of radiations and chemicals on certain algae. Sarma (1981-82) reviewed the work of irradiation studies on green algae, including several contributions from his own laboratory. It was concluded that most of the green algae are highly resistant to ionising radiations (in comparison to higher plants) of which members of Conjugales, Chlorococcales and euglenoids are notable. These algae are characterised by either typical chromosomes or with minute chromosomes. Only such algae as are characterised by appreciably long chromosomes with localised centromeres such as *Oedogonium* spp. and the members of Charales seem to be comparatively more sensitive. Concerning studies on the effects of UV, they fall into several categories such as (a) determining the levels of tolerance to UV dose, (b) morphological and karyological effects, (c) inducing mutants (d) survival patterns, photoreactivation and repair of UV damage and (e) radioprotection. Various studies on the levels of tolerance of algae to UV radiation have established that sensitivity of different organisms to UV light differs at generic as well as specific levels. One of the most common

observations pertaining to karyological effects of any type of radiation (ionising or non-ionising) is mitotic delay, which progressively increases with increasing doses of radiation applied. No gross qualitative differences were observed between the karyological effects caused by X-rays and UV light in the types of chromosomal observations, although ionising radiations are more effective than non-ionising radiations, in view of the greater penetrating power of the former in comparison to the latter. Certain observations, such as chromatid and isochromatid exchanges, formation of bi-, tri-tetra-, and even penta-nucleate cells, infrequent formation of transverse septa unrelated to karyokinesis resulting in the formation of anucleate cells are of special interest in the UV irradiated *S. azygospora* (Vedajanani and Sharma 1979). Survival curves, in general, fall exponentially with increasing dose. It has been shown by Vedajanani and Sarma (1979 b) that UV induced damage even at high doses is repairable by exposure to visible light in *Spirogyra azygospora*. However, different periods of dark incubation seem to be having differential effect on the extent of photoreactivation. Many organisms respond to UV irradiation by a transient lag phase in growth during which period repair systems seem to operate. While considering the relative efficiency of photorepair and dark repair in reversing UV lethality on four species of desmids, Shashikala and Sarma (unpub.) came to the conclusion that photorepair was more efficient means of overcoming the damage caused by UV light in these desmids.

Several chemicals were employed in various concentrations on various experimental algae to study their modifying effects on UV survival in pre-treatments (before exposure to UV) and post-treatments (after exposure to UV). All the

chemicals tried (Acridine orange, acriflavine, thiourea, caffeine, cysteine, cystamine, 2-mercaptoethanol) were antagonistic to UV killing there by providing protection against UV irradiation. Acridine orange, acriflavine and caffeine proved to be distinctly synergistic to UV killing/damage. However, cysteine and cystamine proved to be protective even in post-treatments. Mercaptoethanol as posttreatment agent functioned as protective agent at lower concentrations and synergistic to UV at higher concentrations (Abhayavardhani and Sarma, 1983; Chowdhury and Sarma, 1983, in press; Sarma, 1983).

Sarma and Agarwal (1981) studied for the first time the effects of ultrasonic waves on the karyology of two green algae, *Rhizoclonium heiroglyphicum* and *Oedogonium gunnii*. The karyological effects were found to be akin to those obtained with radiations. The latter alga was found to be more sensitive than the former to ultrasonic waves. Sarma (1982) reviewed the work on the karyological effects of antibiotics (Chloramphenicol, oxytetracycline, gentamycin, penicillin, streptomycin, mitomycin-C, rifamycin and polymixin-B) on green algae, *Oedogonium gunnii*, *Spirogyra azygospora* and *S. paradoxa*. Of the various antibiotics used, gentamycin and polymixin-B were found to be more potent with respect to lethality as well as in the extent of production of comparable nuclear and chromosomal aberrations. Our School has also studied the karyological effects of several chemicals, besides antibiotics on some members of green alga. The chemicals include colchicine, caffeine, theobromine, maleic hydrazide, gibberellic acid, chlorol hydrate, coumarin, 2-4-D, acenaphthene NTG and EMS). Colchicine effects on algae seem to substantially differ as compared with

those or higher plants/animals, particularly in the absence of C-pairs characteristic of C-mitosis in the latter, and only very high concentrations of colchicine being capable of inducing polyploidy (Sarma, 1962). It is of interest to observe that mitosis and cell division in the dinophyceam alga *Gymnodinium inversum* var. *elongatum* have not been affected by colchicine in any concentration, including 3% conc. suggesting the absence of spindle mechanism which, however, is operative in eukaryotic organisms (Sarma and Shyam, 1957). From the various studies, it has been possible to conclude that, with very few exceptions, green algae are more resistant to chemicals as compared to higher plants. Several interesting morphological and cytological abnormalities were recorded in the studies on the effects of NTG on *Cosmarium obtusatum* (Sarma and Shashikala unpub). Of particular interest is the induction of giant cells with doubled chromosome number, besides formation of variously shaped longer cells than the control organisms, formation of chains of incompletely separated cells and occasional formation of triradiate cells. *Spirogyra paradoxa* was subjected to treatment of NTG (Vedajanani, Abhayavardhani and Sarma, 1980) and EMS (Abhayavardhani and Sarma, 1980). Anaphase bridges, laggards, micronuclei, chromosomal/chromatid breaks and chromatid exchanges are reported for the first time in the alga, as a result of treatment with NTG and EMS, which are considered as potent mutagens.

In view of complete paucity of information on the karyological effects of pesticides on algae, which form important constituents of the aquatic environment and constitute a link in the food chain, effects of four insecticides (Ekatin, Anthio, Dimecron and Nuvan), two

fungicides (Cuparmar, Phygon-XL) and two herbicides (Simazine and Atrazine) were studied. The experimental algae in these studies were *Spirogyra paradoxa* and *Oedogonium gunnii*. The lethal concentrations as well as those which bring about karyological aberrations have been determined in each case. The latter were assessed both qualitatively and quantitatively. Chromosome breaking capacity of all these pesticides was unequivocally established in all the cases.

3. *Physiological Studies* : The physiological studies include the effects of physical and chemical factors on spore germination, colony formation and sporulation in the green alga *Stigeoclonium pascheri*, since conditions affecting sporulation and spore germination have been little understood in green algae unlike in bacteria and blue green algae in which they have been sufficiently studied. The effects of UV light, gamma radiation and other factors such as quality of light, intensity of white light, temperature, pH and nutrients were studied on the various phases of the life cycle in the alga (cf. Sarma and Agarwal, 1980; Agarwal and Sarma, 1981, 1982, etc.). Such studies provide an insight into the factors which inhibit promote germination of spores such as akinetes, zygospores etc. Mode of germination, effects of UV light on germination and the extent of recovery of UV effects through photoreactivation were studied on the akinetes of *Pithophora* by Sarma *et al.* (1983), while effects of different factors on the sporulation of *P. oedogonia* were highlighted by Agarwal and Sarma (1983). Sporulation up to the extent of its natural population was achieved in this study with white light between 2 to 3.5 K lux, temperature between 20 to 30°C and pH between 4 to 9, the range being very wide for each of the factors

instead of being critical at a particular level.

Other studies include some observations on sulphur selenium antagonism on two desmids, *Cosmarium obtusatum* and *Glosterium lanceolatum*. Percentage survival of the algae decreased linearly with the increasing doses of selenate, the selenate toxicity to the algae being more pronounced in sulphur free medium as compared with sulphur containing medium (Sarma and Shashikala Jayaraman, 1983 in press). Effects of three metabolic inhibitors viz., sodium azide, sodium arsenate and cadmium chloride on the growth of two species each of *Cosmarium* (*C. leave*, *C. obtusatum*) and *Glosterium* (*Gl. moniliferum* and *Gl. lanceolatum*) have been studied by Shashikala Jayaraman and Sarma (unpub.). Of the three inhibitors used, cadmium chloride proved to be the most toxic to the growth of all the four species of desmids, both in terms of lethality and percentage inhibition.

Notable success was met with in the formulation of new synthetic nitrogen depleted medium in which conjugations was induced in two desmid taxa *Glosterium acerosum* and *Cl. turgidum* (Chowdhury and Sarma, unpub.).

Cultivation of freshwater dinoflagellates under controlled cultural conditions has proved to be a frustrating experience for many a worker. Successful culturing of these organisms gains significance particularly in view of the fact that both cytological and cytochemical studies on Dinophyceae will have to be carried out on pure cultures of these organisms. To determine critical conditions for their fast growth leading to bulk yield of these algae, a detailed study of the physical and chemical parameters governing the growth of eight freshwater dinoflagellates, drawn from three genera,

Gymnodinium, *Katodinium* and *Peridinium* were undertaken and finally a synthetic medium adjusted to pH 8 designated as 'modified Carefoot medium' was developed in which most of the freshwater dinoflagellates tested in the study could be cultivated at a much faster rate than any of the culture media known so far, including Carefoot medium which is considered to be the best so far for freshwater Dinophyceae. The other culture conditions are $22 \pm 2^\circ\text{C}$ and 2000 lux of light intensity for 16 h daily (Sarma and Khan, unpub.).

That the duration of light and dark regimes within 24 h LD cycle exercise a profound influence on the mitotic processes particularly, the mitotic index and mitotic peak have been shown with respect to *Eudorina elegans* (Sarma and Shyam 1973). *Gonium pectorale* (Shyam and Sarma, 1975) and on the desmid taxa *Cosmarium quadrum*, *Euastrum inermius* and *Staurastrum forficulatum*, such studies some times prove useful in determining the conditions under which mitotic figures are obtained in higher frequencies.

4. Systematics, Morphology and Reproduction : While our studies are mainly concentrated on the karyology of algae, a few notable contributions have emerged on the systematics, morphology and reproduction of some algae, of which reference is made to some of the more significant ones.

Of the systematic studies that have been made on the North Indian Volvocales, is the fascinating discovery of a new colonial genus *Lundiella indica* gen. et. sp. nov., in which the coenobia are obovoid in shape with slightly flattened apical region. The cells of the colony are polyhedral in shape arranged on the periphery without any sequence of tiers, and joined to one another by mucila-

genous pads between laterally produced portions of the cells but without a common mucilaginous envelope. This genus is at present known only in its vegetative state (Sarma and Shyam, 1974).

Eight unicellular taxa of Volvocales, belonging to the genera, *Papenfussiomonas*, *Chlamydomonas*, *Haematococcus*, *Sphaerellopsis* and *Phacotus* have been described by Shyam and Sarma (1976), of which two new varieties: *S. fluviatilis* var. *striata* var. nov. and *P. lenticularis* var. *undulata* var. nov. were established. Two new species of *Pyrobotrys* (*P. acuminata* sp. nov. and *P. desikacharyi* sp. nov.) have also been described by Sarma and Shyam (1974). A new species of *Tetraspora* viz., *T. apiocystioides* was established by Chowdhury et al. (1967) with characteristics of the genus *Tetraspora* but with colony shape of *Apiocystis*.

Some new facts concerning the sexual reproduction in *Eudorina californica* (Shaw) Goldstein (earlier described as *E. indica* Iyenger but was merged into the *E. californica* (cf. Goldstein 1964) were brought to light by Shyam and Sarma (1976), with reference to the place of actual union of gametes, details of actual fusion and time taken to complete the process. Certain observations on the formation, liberation and fusion of biciliate gametes have been made in the alga *Tetrasporidium javanicum* by Sarma and Suryanarayana (1969).

Four new species of freshwater dinoflagellates were described viz., *Gymnodinium dodgei* Sarma and Shyam, *Gymnodinium lalitae* Sarma and Shyam (Sarma and Shyam, 1974), *Gymnodinium indicum* and *Woloszynskia stochii* (Shyam and Sarma, 1975). Asexual reproduction through zoospore formation and sexual reproduction resulting in the formation of planozyotes (whose fate however, could not be followed) in *Woloszynskia*

stochii (Shyam and Sarma, 1975) and formation of cysts in *Gymnodinium dodgei* (Sarma and Shyam, 1974) are some of the notable observations made on reproduction of dinoflagellates.

Morphological observations were made on 22 taxa belonging to eight genera of Dinophyceae collected from freshwater habitats of North India. All the taxa described are first reports from Indian freshwater (Shyam and Sarma, 1980).

A detailed study on the process of conjugation in two species of *Closterium* viz., *C. acerosum* and *C. turgidum* was made and important differences in the process even between two species of the same genus were identified. Further, sexual activity and burst frequency were also calculated by appropriate formulae. Homothallism was not observed in any of the strains of both the algae. Heterothallism was established in 20 strain combinations of *Cl. acerosum* and 11 combinations of *Cl. turgidum* (Chowdhury and Sarma, unpub.).

Shyam and Sarma (1974) made a detailed investigation on the effects of the various concentration of nitrate and variations in the intensity and duration of light period in 24h LD cycles on cultures of the alga *Stigeoclonium pascheri* in relation to the type and extent of branching and occurrence or absence of hair formation. It was concluded that these characters are not of reliable taxonomic significance, since they are influenced largely by environmental conditions. The view of Cox and Bold (1966) in abandoning the generic name of *Caespitella* and its merger with *S. pascheri* has been upheld.

A new species of *Chara* (*C. bharadwajae* sp. nov.) and a new form of *Lychnothamnus* (*L. barbatus* f. *iyengari* f. nov.) were established by Sarma and Khan

(1966 a, b) while carrying out cytotoxic studies on Charophyta.

Cultural studies were made with respect to cultural characteristics, developmental morphology, reproduction and cytology of *Compsopogon*, a freshwater red

alga, which indicated a great caution to be exercised over the identification of the species of the genus. While the taxon resembled *C. coeruleus*, it also agreed with *C. corinaldii* in several respects (Shyam and Sarma, 1980).

TABLE I

KARYOLOGICAL STUDIES ON ALGAE

Name of the Class/Order (Fritsch 1935) with range of chromosome numbers in each Order)	Name of the genus (No. of taxa investigated in brackets)	Lowest chromosome number (Name of the taxon in brackets)	Highest chromosome number (Name of the taxon in brackets)
<i>Chlorophyceae</i>			
Volvocales (n=4—33±4)	<i>Volvolina</i> (1), <i>Gonium</i> (1), <i>Tetraspora</i> (1), <i>Tetrasporidium</i> (<i>olvolina pringsheimii</i>)	n=7	n=17 (16-18) (<i>Gonium pectorale</i>)
Ulotricales (n=3 to 44—48)	(1) <i>Ulothrix</i> (2), <i>Uronema</i> (4), <i>Microspora</i> (5), <i>Cylindrocapsa</i> (2), <i>Ulva</i> (2), <i>Enteromorpha</i> (3), <i>Hormidium</i> (4), <i>Klebsor-</i> <i>midium</i> (1), <i>Sphaeroplea</i> (1), <i>Prasiola</i> (1)	n=9 (<i>Enteromorpha compressa</i>)	n=46±2 (<i>Hormidium crenulatum</i>)
Chaetophorales (n=4—56)	<i>Stigeoclonium</i> (6), <i>Chaetophora</i> (2)	n=5 (<i>Stigeoclonium</i> <i>helveticum</i>)	n=14 (<i>Stigeoclonium subsecundum</i>)
Oedogoniales (n=9—46)	<i>Oedogonium</i> (19), <i>Bulbo-</i> <i>chaete</i> (1)	n=9 (<i>O. howardii</i> var. <i>minus</i>)	n=46 (<i>O. macrandrium</i> var. <i>hohenckerii</i>)
Conjugales (n=2—130±2)	<i>Spirogyra</i> (37), <i>Sirogonium</i> (2) <i>Zygnema</i> (8), <i>Mou-</i> <i>geotia</i> (3), <i>Desmidium</i> (4) <i>Spondylosium</i> (1), <i>Cosmarium</i> (2), <i>Closterium</i> (7) <i>Evastrum</i> (5), <i>Micrasterias</i> (3) <i>Stauras-</i> <i>trum</i> (9), <i>Xanthidium</i> (2) <i>Orychonema</i> (1)	n=2 (<i>Spirogyra</i> VRG 80) n=6 (<i>Staurastrum pilosum</i>)	n=92±2 (<i>S. nitida</i>) n=130±2 (<i>Closterium acerosum</i>)
Charales (n=6—56)	<i>Chara</i> (30), <i>Lychnothamnus</i> (2), <i>Lamprothamnium</i> (1), <i>Nitella</i> (33), <i>Tolypella</i> (1)	n=6 (<i>N. flexilis</i>)	n=56 (<i>C. zeylanica</i> f. <i>beutori</i>)
Euglenophyceae (n=4—182±5)	<i>Euglena</i> (3)	n=16 (<i>E. limnophila</i>)	n=182±5 (<i>E. spirogyra</i> var. <i>fusca</i>)
Dinophyceae (n=81—22 to c.600)	<i>Peridinium</i> (4), <i>Katodinium</i> (3) <i>Gyrodinium</i> (12), <i>Woloszy-</i> <i>nskia</i> (2), <i>Gyrodinium</i> (1), <i>Glenodinium</i> (1)	n=50.±2 (<i>P. penardiformae</i>)	n=c. 600 (<i>Gymnodinium</i> OBG 70)

Finally, an attempt was made to compile a check list of algal taxa published from India upto 1976 (by no means absolutely complete) along with a list of source references (Sarma and Khan, 1980). The sole object of providing such information about the scattered literature at one place is to extend a helping hand to beginners in algal systematics.

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