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Cytological Studies on Indian Cyperaceae
II. Tribe Cypereae

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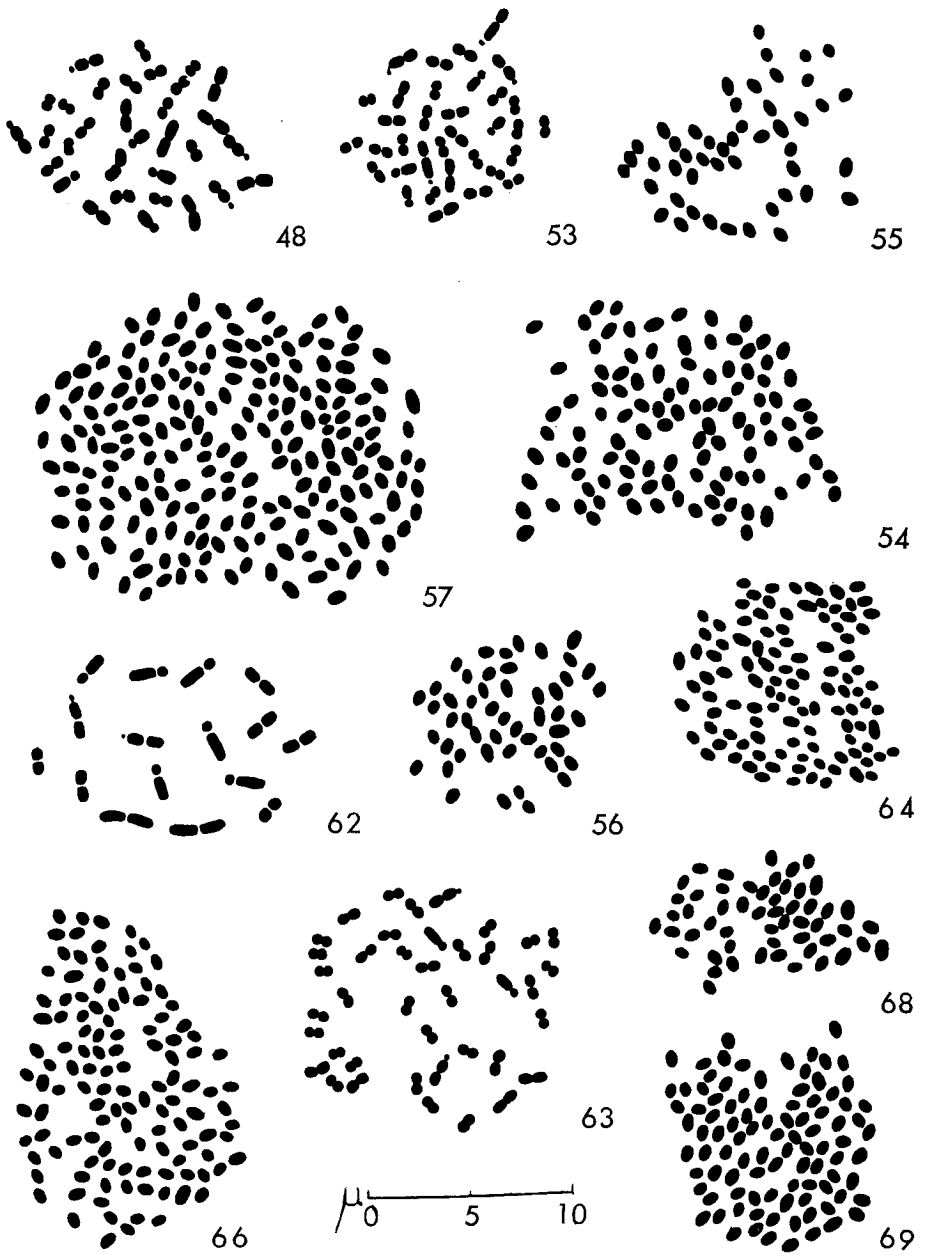
As mentioned in a previous paper (Sanyal and Sharma, in press), dealing with the tribe Scirpeae, cytological data on the Indian members of the family Cyperaceae are rather meagre, inspite of the taxonomical interest of the family. The origin of the family, whether through Juncales from the primitive Liliaceous stock, as postulated by Hutchinson (1959) or otherwise, is controversial. The delimitation of the different tribes and their relative primitive or advanced status are also the subjects for discrepant opinions. The reports of diffuse and localised centromeres within the same genus, as in *Eleocharis* and *Scirpus* of the tribe Scirpeae add to the importance of detailed cytological investigations on the family. The present investigation was undertaken on the Indian representatives of the tribe Cypereae available under different ecological habitats, as a part of a comprehensive programme to collect and study the members of the family Cyperaceae distributed in different localities of India, with a view to elucidate their interrelationships and the lines of evolution.

Materials and methods

Sixteen species under three genera of the tribe Cypereae have been analysed cytologically, collected mainly from marshy habitats in different areas of Eastern India. Chromosome studies were made principally from root-tip squashes, following the usual schedule of pretreatment, fixation and squashing. Of the different pretreatment chemicals used, excellent results were obtained in the materials belonging to the three genera, *Cyperus*, *Kyllinga* and *Pycreus* with a mixture of aqueous solutions of aesculine and (0.002 M) oxyquinoline or aesculine and isopsoralene (1:1) at 8-10°C for $3^1/_2$ to $4^1/_4$ hours. After pretreatment, the root tips were fixed in acetic acid-ethyl alcohol mixture (1:2) for one to three hours, and subsequently heated in a mixture of 2% acetic-orcein and N.HCl (9:1). In some cases, specially in *Cyperus*, where the cytoplasmic contents were heavy and the root-tips very hard, the tips after fixation were hydrolysed in (N)HCl for 10-12 minutes at 60°C and rinsed in 45% acetic acid prior to heating in the orcein-HCl mixture. Subsequently, the root-tips were squashed in 45% acetic acid, sealed and observed. In the study of meiotic chromosomes, inflorescences were fixed for varying periods

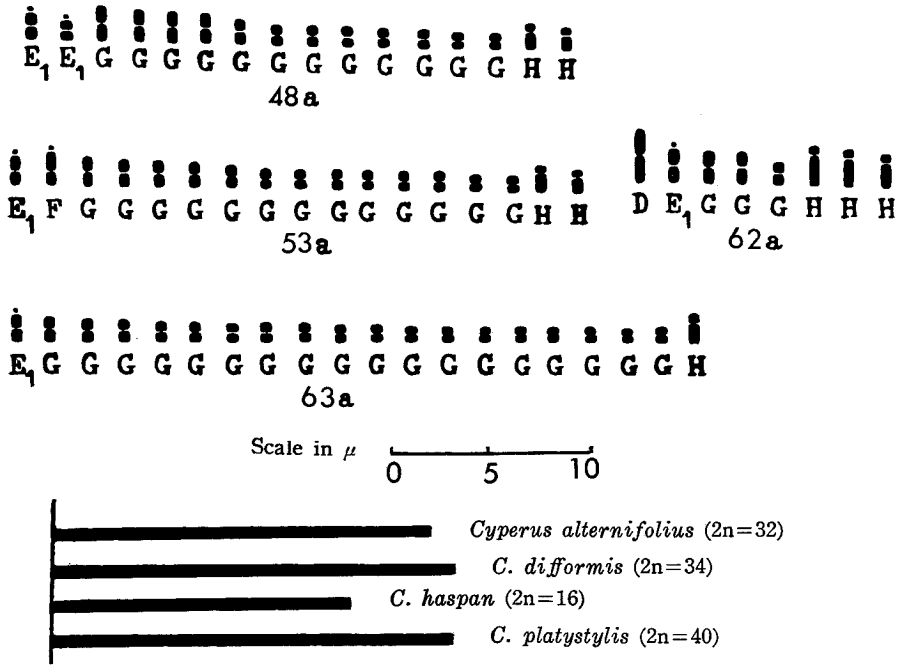
in acetic acid-ethyl alcohol (1 : 1) mixture before smearing in acetic-carmine solution. Pretreatment in 0.002 M oxyquinolene solution at 8–10°C for 30 minutes to 1 hour, prior to fixation, was employed in some cases. The figures were drawn at a table magnification of approximately 3,000×, with a compensating eyepiece of ×20, an apochromatic objective and an aplanatic condenser.



Indian Cyperaceae II: explanation of figures in the text.

Observations

The somatic chromosome numbers with Cypereae present a large range of variations. The minimum and maximum numbers of chromosomes counted in the different species of *Cyperus* range from 2n=16 to 2n=208. The single species of *Kyllinga* studied has 2n=120 while the two species of *Pycreus* have 2n = 54 and 2n = 96. The width of the chromosomes is smaller in these three genera than that of the members of Scirpeae observed previously. From a detailed karyotype analysis, a gross morphological resemblance is observed between the chromosomes of these genera and those of Scirpeae investigated previously. Certain types are common to both, indicating



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the homogeneity of the family Cyperaceae as a whole. For a comparative analysis, the descriptions of the different chromosome types of the two tribes are being maintained on a common basis, though only five of the fifteen chromosome types observed in Scirpeae, are present in the members of Cypereae analysed. These types, designated as D, E_1, F, G and H to match the corresponding types previously described under Scirpeae, are given below:

Type D—Comparatively long chromosome with median to nearly median primary constriction.

Type E_1—Medium-sized chromosome with median primary constriction and a satellite at the distal end of one arm.

Type F—Medium-sized chromosome with submedian to nearly submedian primary constriction and a satellite at the distal end of the long arm.

Type G—Medium-sized to short chromosome with median to nearly median primary constriction.

Type H—Short chromosome with nearly subterminal to nearly submedian primary constriction.

On an average, the chromosomes are small in size with little size difference. Different combinations of these types are present in the different species, indicating the role of structural alterations in their origin. The figure numbers have been maintained in continuation of the previous paper on the tribe Scirpeae of this series.

Genus—*Cyperus*

Thirteen species of this genus have been worked out.

C. alternifolius L. $2n = 32 = 4E_1 + 24G + 4H = 1.9-0.9\mu$ $n=16_{II}$ (Figs. 48, 48a, 49).

C. articulatus L. $n=56_{II}$ (Fig. 50).

C. compressus L. $n=64_{II}$ (Fig. 51).

C. corymbosus Rottb. $n=64_{II}$ (Fig. 52).

C. difformis L. $2n=34=2E_1+2F+26G+4H=2.1-1.0\mu$ (Figs. 53, 53a).

C. digitatus Roxb. $2n=108=1.2-0.7\mu$ (Fig. 54).

C. distans L. $2n=48=1.2-0.7\mu$ (Fig. 55).

C. elegans L. $2n=50=1.2-0.7\mu$ (Fig. 56).

C. esculentus L. $2n=208=1.3-0.6\mu$ (Figs. 57, 58), $n=104_{II}$.

C. flavidus Retz. $n=10_{II}$ (Figs. 59, 60, 61).

In addition to the normal first metaphase the presence of third metaphase division in PMCs has also been noticed. A regular third metaphase shows the presence of ten chromosomes and three organising nuclei within the more or less oval PMC. An abnormal third metaphase division has also been observed, showing thirteen chromosomes and three organising nuclei within the pollen mother cell.

C. haspan L. $2n=16=2D+2E_1+6G+6H=2.8-1.3\mu$ (Figs. 62, 62a).

C. platystylis R. Br. $2n=40=2E_1+36G+2H=1.7-0.9\mu$ (Figs. 63, 63a).

C. rotundus L. $2n=108=1.1-0.5\mu$ (Figs. 64, 65), $n=54_{II}$.

Genus—*Kyllinga*

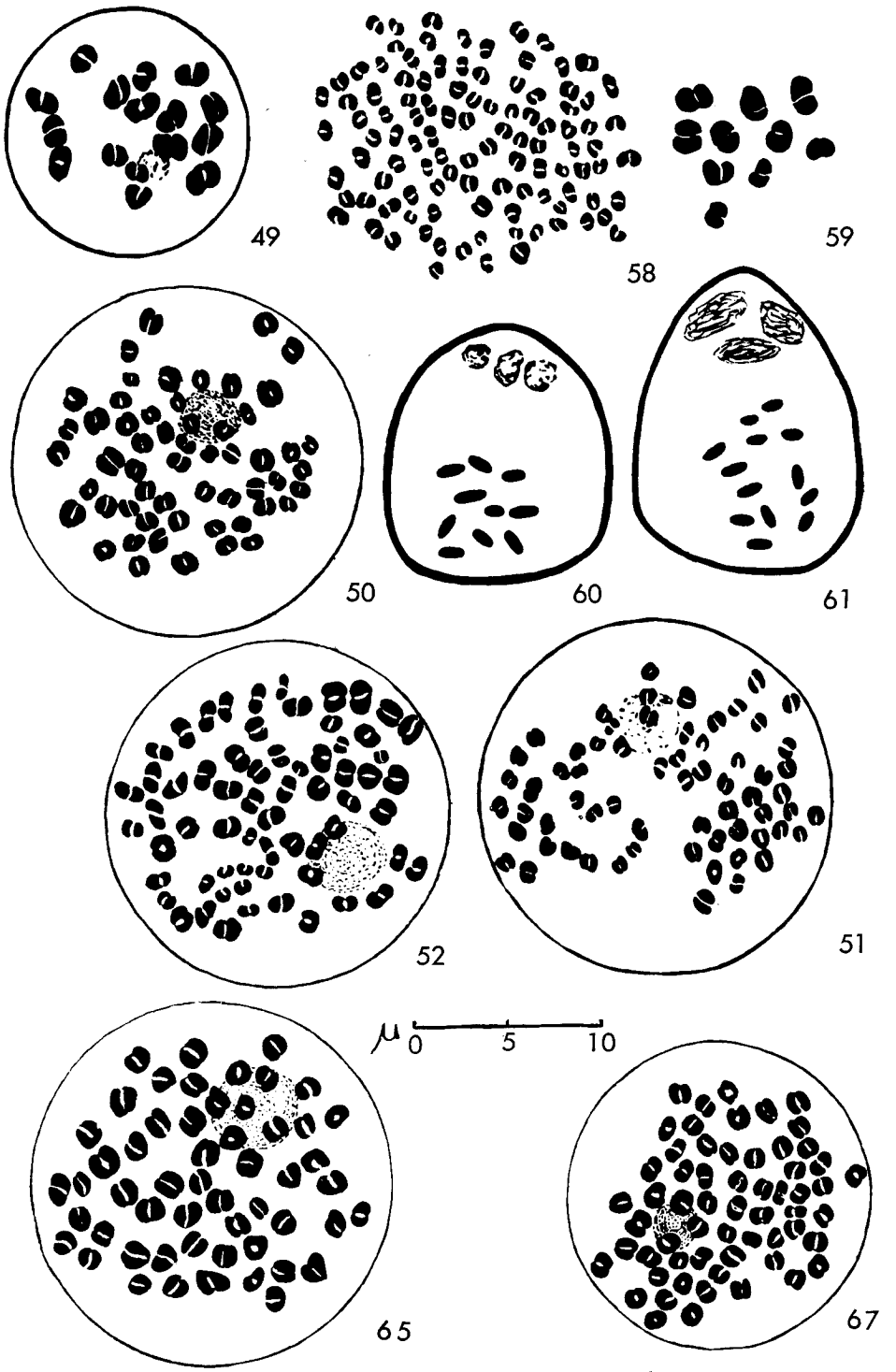
K. brevifolia Rottb. $2n=120=1.2-0.6\mu$ (Figs. 66, 67), $n=60_{II}$.

Genus—*Pycreus*

Only two species of the genus have been worked out.

P. nitens Nees. $2n=54=1.1-0.7\mu$ (Fig. 68).

P. polystachyus Beauv. $2n=96=1.2-0.7\mu$ (Fig. 69).



Indian Cyperaceae II: explanation of figures in the text.

Discussion

Evolution and interrelationships within Cypereae

Within the tribe Cypereae of Hutchinson (1959) three genera have been investigated during the present work namely, *Cyperus*, *Kyllinga* and *Pycreus*. In *Cyperus*, varying chromosome numbers, starting from n=8 as reported for the first time in *C. haspan* to a very high chromosome number as 208 in the somatic cells of *C. esculentus*, have been recorded (Gadella and Kliphuis 1964, Hicks 1929, Löve and Löve 1944, 1956, Reese 1957, Sharma and Bal 1956, Suzuka 1953, Tanaka 1937). Incidentally, this report too has been made during the present investigation. In *C. esculentus* previous workers have reported 2n=18 and 108 chromosomes respectively (Hicks 1929, Suzuka 1953, Tanaka 1937). In the present work both meiotic and mitotic cells were studied in which details of chromosome structure were clarified through special treatments. Meiosis showed 104 bivalents and correspondingly 208 chromosomes were noticed in the somatic cells. On the basis of previous findings, 216 should have been the somatic number arising through polyploid. But since even a careful study of a large number of plates did not show 216 chromosomes, the importance of aneuploidy in this genus cannot be ruled out. An analysis of the different chromosome numbers in *Cyperus* shows that inspite of wide occurrence of aneuploidy, multiples of 8 and 9 chromosomes are most frequent in this genus. It would, therefore, not be unreasonable to assume that the basic set has been represented by n=8 chromosomes noticed in *C. haspan* from where duplication of individual and whole sets of chromosome has resulted in further diversification. Intraspecific variations have been noticed in *C. distachya* (80-84) as recorded by Reese (1953, 1957), which no boubt have been facilitated by extensive vegetative reproduction of species of *Cyperus*. Karyotypes of the different species studied also show that, as in other genera, inspite of a gross similarity between the different species, there are differences in the minute details of the morphology of chromosomes. This observation indicates the importance of structural alterations in evolution. A feature worth noting is the size of the chromosome in *C. haspan* (2n=16). Even though the species is primitive among *Cyperus*, as evidenced in its chromosome number, size of the chromosome is rather short compared to the chromosome size of the primitive species of Scirpoideae, such as, *Fimbristylis*. This reduction in length is rather remarkable.

In *Pycreus* two species have been studied one showing 2n=54 and the other 2n=96 chromosomes respectively. In addition, previous records indicate widely varying chromosome numbers in different species such as 50, 80 and 94 chromosomes in somatic cells (Avdulov 1931, Levitsky 1931, Sharma, B. R. 1962). All these are clear evidences of the occurrence of extensive aneuploidy in the genus, which in Cyperaceae has played an important role

specially due to its vegetative propagation.

In *Kyllinga* also the chromosome number is widely variable being 2n=108, 112 and 120 respectively in three different species, the last one being observed in the present investigation as well (Sharma, B. R. 1962, Tanaka 1939, 1941). It appears that in both the genera *Kyllinga* and *Pycreus* polyploids and hybridization have resulted ultimately into the occurrence of such aneuploid numbers which have survived specially due to their vegetative means of reproduction.

Of the different genera of Cypereae of Hutchinson (1959) the chromosome number and karyotypes, so far studied, indicate clearly their advanced level as compared to the genera included under Scirpeae *sensu stricto.* However, as far as the advanced species (with high chromosome numbers) of both the tribes are concerned, there is a gross similarity in the chromosome morphology specially in the short size, median to submedian primary constriction and in the presence of a graded karyotype. This cytological similarity may be considered to show affinity between the two tribes of Hutchinson (1959).

Taxonomic status of the genera studied

In Hooker's (1897) system *Kyllinga* and *Cyperus* have been included under the first tribe Eucypereae followed by the second tribe Scirpeae including *Scirpus*, *Fimbristylis* and *Eleocharis*. The tribe Rhynchosporeae is placed last in the series.

Hutchinson's (1959) classification starts with the tribe Rhynchosporeae followed by Scirpeae, Cypereae, Hypolytreae, Sclerieae, Cryptangieae and Cariceae.

In the classification adopted by Pax (Engler and Prantl 1887–1931) Scirpoideae forms the first subfamily followed by Rhynchosporoideae and Caricoideae. In Scirpoideae there are two tribes Scirpeae and Hypolytreae, and within the former, subtribes Cyperinae and Scirpinae are placed including in them the genera constituting the tribes Cypereae and Scirpeae of Hutchinson (1959).

Holttum (1948), on the basis of spikelet and other morphological characteristics, mostly regarded the tribe Hypolytreae as the most primitive one leading to Scirpeae which includes *Cyperus* as one of the advanced genera. Further evolution led to Rhynchosporeae, Sclerieae, Cryptangieae and Cariceae.

Cytological data gathered during the present investigation undoubtedly suggests that the tribe Scirpeae of the subfamily Scirpoideae of Pax (as adopted by Rendle) is undoubtedly primitive. The most primitive characteristics are to be found in the subtribe Scirpinae including the genera *Fimbristylis*, *Scirpus* and *Eleocharis*. The cytological affinities between subtribes Scirpinae and Cpyerinae have been pointed out indicating the evolution of the latter from the former. In absence of any data on the genera included under Hypolytreae,—the other tribe under the subfamily Scirpoideae and the

most primitive one according to Holttum (1948)—it is not possible to assign its systematic status at present. In view of the affinities of the subtribes Scirpinae and Cyperinae, that is, tribes Scirpeae and Cypereae of Hutchinson (1959), it appears unnatural to keep the constituent genera under two distinct tribes. On the other hand their inclusion under the two subtribes within the tribe Scirpeae as followed by Rendle appears to be justified on cytological grounds. The present work is also in agreement with Holttum's (1948) contention that Scirpeae represents a very primitive group.

Hutchinson (1959), as mentioned above, considered Scirpeae as a second tribe preceded by Rhynchosporeae. Some amount of work has so far been done on the genus *Rhynchospora* and seven species have shown seven different numbers. Moreover, excepting in *R. tenuis*, where $2n=10$ chromosomes has recently been reported (Gadella and Kliphuis 1964), a number of species show a basic number of $n=13$ chromosomes.

In view of this fragmentary knowledge on the cytology of *Rhynchospora*, which also does not support its primitiveness and also in view of the contention of different taxonomists (Rendle 1953, Hooker 1897) including Holttum (1948), that critical analysis of spikelet morphology suggests *Rhynchospora* and its allies to be advanced representatives, there seems little reason at present to consider Rhynchosporeae as the most primitive tribe of Cyperaceae as contended by Hutchinson (1959). On the basis of the available data it would be reasonable to start the family with Scirpoideae representing as the most ancient stock with Scirpineae and Cyperineae as the subtribes.

Origin of the family Cyperaceae

With regard to the origin of the family, as pointed out in the introductory part of the work, there has been a considerable difference of opinion. Juncales has been regarded by Hutchinson (1959) to represent an intermediate step in the evolution of Cyperales from the Liliaceous stock. At present there are very little cytological evidences for or against the different views expressed so far. But the cytological data obtained indicate the possibility of a relationship existing between Juncales and Cyperales. The species of *Juncus* and *Luzula* are characterized by wide aneuploidy (in *Juncus*), high variability in chromosome number, intraspecific variations, short size in advanced types and lastly by diffuse centromeres (in *Luzula*). All these features find parallel in the primitive tribe Cypereae. In view of these cytological facts, taken in conjunction with the morphological data which led Hutchinson (1959) to derive relationships between the two, the possibility of the origin of this family through Juncales appears probable. However evidences from other aspects of study are needed to substantiate this contention.

Summary

The structure and behaviour of chromosomes of sixteen species belonging to three genera (*Cyperus*, *Kyllinga* and *Pycreus*) of the tribe Cypereae under the family Cyperaceae have been studied. Taxonomic status of the genera was dealt with in the light of cytological findings. Cytoecological correlation has been made. The advanced status of the genus *Cyperus* of the tribe Cypereae has been indicated. The advanced status of the tribe Cypereae of Hutchinson (1959) as compared to that of the tribe Scirpeae has been discussed. The question of giving them subtribe status in the tribe Scirpeae of Pax has been also suggested. The probability of the origin of the family through Juncales from Liliaceae as adopted by Hutchinson (1959) has been supported.

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