DISCUSSION

a) Systematic considerations:

The tribe Orchideae of Dressler and Dodson (1960) comprises of four subtribes and 16 genera. However, the systematic position of these subtribes and their components have been a subject of controversy among the orchidologists. The genus Habenaria included in the present study belongs to the subtribe orchidinae of the above tribe. Engler and Prantl (1889) included this genus Habenaria under the tribe ophrydineae. Hooker (1890) places this genus under the subtribe Habenarieae in the tribe ophrydeae. The genus Habenaria as expressed by him is exceedingly polymorphic. Further, he has included the species of Platanthera, Peristylus, Gymnadenia, etc., under Habenaria only. But, Smith (1905) splits the genus Habenaria of Hooker (1890) into 3 taxa, namely, Habenaria, Peristylus and Platanthera. Holtum (1953) following Hooker, retains the genera Platanthera and Peristylus of Smith (1905) under the genus Habenaria itself.

Schlechter (1926) has maintained the genus Habenaria in the subtribe Habenarinae of the tribe ophrydoideae. Blatter and MacCann (1931-32) while revising the classification of Orchidaceae members of the erstwhile
'Bombay Presidency' have given a status of separate genus to Habenaria. Santapau and Kapadia (1959) following Schlechter (1926) and Blatter and MacCann (1931-32) also suggest a separate position for this genus.

The tribe Epidendreae (Dressler and Dodson, 1960) chosen for the present study, has also undergone various changes regarding its taxonomic position.

Of the seven tribes described by Lindley (1830), we are mainly concerned with the tribe Malaxideae. Members belonging to this tribe are characterised by having waxy pollen mass which communicates directly with the stigma. According to him, the tribe Malaxideae consists of two sections.

1) Pleurothalleae.
2) Dendrobieae.

The genera Dendrobium, Eria and Porpax included in the present investigation belong to the section Dendrobieae of Lindley (1830).

Hooker (1838) treats Dendrobieae and Erieeae as two separate subtribes under the tribe Epidendreae of Lindley (1830). The subtribe Dendrobieae of Hooker includes Dendrobium as a major genus along with ten other genera.
Another subtribe Erieae includes the genus *Eria* and three other genera. However, he has not described *Porpax* as a separate genus. He has given the following Key to identify the genus *Eria*:

Peduncles, 1-many folowered, axillary or subterminal on a leafy stem or on a pseudobulb, column short, produced into a foot. . . . . . . . *Eria*.

But, in contrast to the system of Hooker (1890), Santapau and Kapadia (1966) have suggested the following artificial Key to identify the genera *Eria* and *Porpax* separately:

Pseudobulbs flattened, discoid, rounded:

pseudobulbs with distinct reticulate, lace-like sheaths; flowers orange or deep brown-red; sepals united to form a tube at least at the base. . . . *Porpax*.

Pseudobulbs without lace-like sheaths; flowers greenish yellow or white; sepals completely free. . . . . . . . . . . . *Eria*.

In the systems of classification followed by the earlier workers, for example, Lindley (1830) and Hooker (1888) the characteristic feature of the caudicle or stipe-like structure (gland) was taken as the criterion in separating the tribes. While as, the structure of the anther and rostellum were considered as basic characters in delimiting the subtribed. Up to the level of subtribes,
the classification given by Hooker (1888) appears to be more natural. But, the characters taken into consideration to identify the taxa at the level of genera and species seem to be artificial.

The main characters used by Lindley (1830) and Hooker (1888) to separate the species in the genus Eria were shape and extent of articulation, especially of lateral lobes of the lip. It can also be seen that the same criteria were also used in separating the species belonging to the genus Dendrobium. Thus, the above classifications not only reveal the artificial nature of their system, but also are quite far from the phylogenetic approach in their treatment.

Although, Schlechter's (1926) scheme of classification is considered to be most practical one, still it lacks in details of classification at the generic level. He brings in all the orchidaceous members having waxy pollinia under one tribe, Karosphaereae and splits this tribe into many series, subseries and subtribes. There are also found some nomenclatural changes done, at the subtribal and generic level. For instance, the earlier subtribe Erieae of Hooker (1888) has been changed to Dendrobieae and the genera Eria and Dendrobium are placed under the subtribe Dendrobieae.
Dressler and Dodson (1960) recognise 5 tribes in contrast to 4 of Schlechter (1926) and 7 of Lindley (1830) in the family Orchidaceae. According to them, the tribe Vandeae of Lindley (1830) cannot be precisely separated from their tribe Epidendreae on the basis of a feeble character like the presence of a stipe-like structure. Similarly, the tribe Malaxideae of Lindley (1830) also cannot be separated for lack of precisely defined characters. Although, Dressler and Dodson's (1960) scheme of classification basically agrees with Schlechter's (1926), they have sought more of similarities in forms than differences. Thus, they have proposed only 40 subtribes in contrast to 80 of Schlechter (1926). The nomenclature of tribes and subtribes have been critically reviewed and have been brought in line with the international rules of Botanical nomenclature (1959). Accordingly, the subtribes Dendrobieae and Erieeae of Hooker (1888) which were modified by Schlechter (1926) as only subtribe Dendrobieae have been revised and renamed as Dendrobinae and Epidendriniae respectively. The subtribe Epidendriniae includes the genera Eria and Porphax and the subtribe Dendrobinae includes the genus Dendrobium. However, in doing so, they have pointed out the need for a further critical study in the subtribes, since the systematic position of these genera is not clear.

Ghose (1965), tracing the history of development of
orchid classification, agrees with Oakes Ames (1915) who opines that the system devised by Lindley (1830) and modified by Bentham (1885) with the arrangement suggested by Pfitzer (1907) and with slight modification, will be as acceptable as the system proposed by Schlechter (1926).

A retrospect of the different systems of classification and the various views by eminent botanists (loc.cit) show that, there still persists a dearth of clear knowledge regarding the orchid classification. Obviously, it becomes imminent need to take up a thorough investigation of the orchid members chiefly at the lower levels.

As mentioned earlier, in the subtribe Epidendrinae, the genus Eria is a natural group with the following characters: 1) Pseudobulbs flattened, discoid, rounded and without lace-like sheaths, 2) terminal inflorescences and 3) greenish yellow flowers. Though the generic characters are uniform, the separation of the species is done based on lip character with its shape and articulation. Secondary importance is usually given to the vegetative and other floral parts. Careful observation of the lip characters, such as, side lobes, margin, midlobe and gland-dots on the surface of the lip, shows a progressive specialization from the primitive to an advanced species. The lip characters are utilised not only to differentiate
the species, but also to understand the probable phylogeny. Adams (1959) and Dressler and Dodson (1960) are of the opinion that, the primitive lip is more like a petal, while the advanced is variously modified and is unlike the petal. Thus, in the present study, considering the lip as a taxonomic character in speciation, a comparatively simple lip of *E. reticosa* is further found modified with lobation and serration on the central segment of the lip as in *E. mysoresensis*. In *E. exilis* such a tendency of lobation is found reduced. But, there is comparatively more serration of margins which take it to a higher level than *E. mysoresensis*. On the other hand, when the lip of *E. exilis* is compared with that of *E. microchilos*, there is more of similarity in their morphology by the absence of lobation. But, *E. microchilos* can be said to be more advanced than *E. exilis* by looking to the crenulated margins and a few gland dots on the lip. From the above observations, though *E. exilis* seems to be closely related to *E. mysoresensis*, it might have given rise to an advanced form like *E. microchilos*, long back in the history of its evolution. *E. microchilos* however, shows close relationship with the next species, Viz., *E. dalzellii* with its crenulated margin and presence of gland dots. But still, *E. dalzellii* is found to show more advanced characters over *E. microchilos* by having dense gland dots, incurved side lobes, decurved midlobe and crenulated margins. Looking at the most complex lip of *E. dalzellii* it can be
assumed that *E. dalzellii* is a highly advanced form amongst the 5 species of *Eria* studied.

Although, the species of *Eria* simulate each other in many of the morphological appearances, variations in lip character reveal the individual identity of species. In other words, the species of the genus *Eria* in consideration are well defined.

Thus, based on the morphological observations made (Fig. 153 and table 30), the evolutionary trend amongst the species of the genus *Eria* may be graphically presented in chronology of primitive to advanced status as:

? → *E. reticosa* → *E. mysorensis* → *E. exilis*  
----→ *E. microchilos* → *E. dalzellii*

The species in the genus *Habenaria* are readily distinguished with the help of their superior lip which is continuous with column, often shortly adnate to it, producing a short or elongate spur. Morphological characters found in the species of the genus *Habenaria* are able to decide their individuality (table 29).

The species of the genus *Porpax* can be distinguished by their minute, clawed, deeply lobed and yellowish orange coloured lip. From the observations made in table No. 29
for the Morphological characters for the two species of the genus *Porpax*, it can be easily made out that the species maintain their individuality.

The genus *Dendrobium* is characterised by having long lip with denticulated margins. Morphologically, each species is well defined in its characters which are already described in table. No.30.

b) **Cytological Considerations:**

Role of cytology in modern approaches to taxonomy forms an outstanding feature of cytotaxonomy. It is a discipline which seeks to study variations, explaining variational discontinuities and relationships in terms of cytology. A review of the foundation of the subject is given by Babcock in *Crepis* (1947), Blackslee in *Dathura* (1959) and Heimberger (1959) in *Anemone*. We find today a vast literature pertaining to the subject. Cytological treatment could be considered more comprehensive and to a greater extent accurate. A special role is claimed for cytological data in taxonomy, since the chromosomes are the seat of hereditary material. Warburg (1938) comments, "this is true to some extent, since the chromosome number and morphology often give evidence regarding the origin of forms as in polyploids."
a) **Significance of chromosome number:**

It has been long since realised that the chromosomes in a plant are important to taxonomy in connection with their numbers, size and shapes. Such chromosome characters have been utilised as classificatory criteria in addition to the other morphological features. In certain cases like *crepis* (Babcock, 1947) and *Anemone* (Heimberger, 1959), they have provided direct evidence relating to the nature and origin of variations. There have been innumerable number of cases which speak of the significance of chromosome study in evolution of the species. The works of various cytotaxonomists and evolutionists like Love and Love (1942, 1944 and 1948) in boreal plants, Babcock (1947) in *crepis*, Goodspeed (1954) in *Nicotiana* and Stebbins, Jenkins and Walters (1953) in compositae members are some of the monumental works.

In the family Orchidaceae, there has been considerable cytological work. Miduno (1940,b) was of the opinion that aneuploidy is a characteristic feature of this family. According to Duncun (1953), the extent of chromosome number variation among genera in a group may be as extensive as it is among the species within the genus. Further, he adds that speciation among Orchid genera may be due to, chromosome number variation accompanied by aneuploidy or euploidy as in case of *Phaphiopedilum*, *Goodyera*, etc., or may be caused by the number of supernumeraries as in *Cyprepedilum acaule*. 
Karyotypic relationships of Dendrobieae and Vandeae have been extensively studied by Kosaki and Kamemoto (1961), and Shindo and Kamemoto (1963). Tanaka (1965) reports the result of chromosome counts in 111 species of orchids and he speculates that reduction of chromosome number might have played an important role in speciation. Later, in 1967, Jones reporting on the chromosome number for 123 species of Vandeae, emphasises the importance of chromosome number in orchid taxonomy.

Other workers who have emphasised the need for further orchid cytology are chennaveraiah and Jorapur (1966), Sharma and Chatterji (1966), Chatterji (1968, 1977), Mehra and Vij (1970), Roy and Sharma (1972), Hegde and Boraiah (1972, 1974), Jorapur and Hegde (1974), Vij and Gupta (1975), Mukerjee (1975) and Biswas (1977) etc.

In spite of the considerable cytological work on Orchidaceae, information on the subtribes like Orchidinae, Epidendrinae and Dendrobinae is still felt wanting.

So far, as the chromosome number reports on the genus Habenaria are concerned, it is surprising to note that only about 40 species have been worked out, of about 500 species available all over the world. The first cytological work on Habenaria species is found done by Humphrey (1933) in
H. blephariglottis as 2n=42. Later workers namely, Miduno (1939 and 1940), Sampathkumaran and Rangaswamy (1931), Mehra and Bawa (1962), Jorapur (1968), Arora (1971), Vij and Gupta (1975), Banerji (1975) and Mukerjee (1975) have also reported both on the gametic and somatic numbers. The somatic chromosome numbers like 28, 32, 42 and 62 and gametic numbers like 16 and 21 have been reported by the above said workers. In the present study, however, all the 4 species of the genus Habenaria studied show the diploid chromosome number as 2n=42. It is further indicative of the uniform diploid number for this genus. The meiotic studies made, have also revealed the haploid complement of chromosomes to be n=21.

The earlier records of chromosome numbers have shown in majority of the species to be 2n=42 and in some as 2n=28, revealing 7 as base number. The chromosome number reports made in the present study have also revealed the diploid number to be 2n=42, thus, confirming the base number as 7. But the other reports like 2n=62 in H. geniculata by Miduno (1939) and 2n=16 and 2n=32 by Sampathkumaran and Rangaswamy (1931) in 2 species of Habenaria have suggested a different base number like 8. However, investigations in many more species is felt necessary to decide the various base numbers that may have been responsible for speciation in the genus.
No records on the chromosome numbers are found made on the species of the genus Porpax. However, in the present investigation 2 of the 6 species available are worked out and found to have the diploid number as $2n=24$. Interestingly, a tetraploid in the species *P. reticulata* is worked out with the somatic number as $2n=48$. As there are no earlier reports on the chromosome numbers of this genus, the base number may be suggested as $n=6$ or $12$. Occurrence of an accessory chromosome in the tetraploid species is quite interesting and thus confirms the affinity with the other genus *Eria* a member of the same subtribe Epidendrinae.

So far as the chromosome number reports on the genus *Eria* are concerned, there are different haploid and diploid numbers, recorded in few species. Chardard (1963) reported $n=19$ in *E. convallarioides* and *E. paniculata* and $n=20$ in *E. giungli*. Certain diploid numbers like $2n=34$, $36$, $38$, $40$, $42$, $44$ and $66$ are also reported (Tanaka, 1965; Pancho, 1965; Sharma and Chatterji, 1966; Mehra and Vij, 1970; Mehra and Sehgal, 1974, 1975; Mukerjee, 1975 and Biswas, 1977). In the present study, however, two species, *E. mycroensis* and *E. exilis* show $2n=38$, while *E. reticosa* reveals $2n=42$. The other two species Viz., *E. microchilos* and *E. dalzellii* show the diploid number as $2n=24$.

The diploid number $2n=42$ for *E. reticosa* is in accordance
with the report made on the species *E. biflora* by Biswas (1977). Further, the number 2n=38 for *E. mysorensis* and *E. exilis* is also in accordance with the diploid number 2n=38 reported by Biswas (1977) in a species of *Eria* and the gametic count of n=19 by Chardard (1963). But the somatic number 2n=24 as found in *E. microchilos* and *E. dalzellii* seems to be a deviation from the earlier reports of 2n=34, 36, 38, 40, 42, 44, 66 and n=19, 20 by different investigators (loc. cit.). Thus, it can be inferred that, the genus *Eria* is having different base numbers for its species.

For the genus *Dendrobium*, different chromosome numbers are reported by Hoffmann (1929), Miduno (1940), Effinein-Heim (1941), Ito and Mutsuara (1957), Kosaki (1958), Vajrabhaya and Randolph (1960), Kosaki and Kamemoto (1961), Dorn and Kamemoto (1962), Jones (1963), Chardard (1963), Shindo and Kamemoto (1963), Pancho (1965), Kamemoto and Sagarik (1967), Chatterji (1968). Mehra and Vij (1970), Maxwell (1970), Wilfret and Kamemoto (1971), Banerjee and Chaudhuri (1972), Hegde and Boraiah (1974) and Mukerjee (1975). Out of 133 species for which chromosome numbers have been reported to date, 111 are with 2n=38, 20 with 2n=40 and 2 with 2n=76. In the present study, the somatic count made for *D. mabelae* and *D. microbulbon* agree with the earlier reports for
diploid number as 2n=38. Considering the majority of the species with diploid number 2n=38 the base number can be suggested as n=19 for this genus.

b) **Significance of structural changes:**

Structural specificity of chromosomes is of prime importance in deciding the individuality of a taxa. Any alteration in the structure of a chromosome or chromosomes would alter the phenotypic expression of it. This has been well illustrated from the studies on *Crepis* (Babcock, 1947), *Dathura* (Blackslee, 1959) and others. A comparative study of the Karyotypes of different species has not only provided the information on the trend of evolution of orchid species, but has also revealed the interrelationships between them. The significance of structural alterations of chromosomes in the evolution of orchid species have been pointed out by Sharma and Chatterji (1966), Wilfret and Kamemoto (1969), Jorapur (1968), Tara and Kamemoto (1970) and Roy and Sharma (1972).

In the genus *Eria*, the significance of structural changes of chromosomes can very well be demonstrated from the present study. In other genera like *Habenaria*, *Porpax* and *Dendrobium* studied, it is rather difficult to ascertain the trend of evolution among the species at this stage,
where, only a few species are studied. However, a gross idea regarding the differences in the Karyotypes at the generic level are perceived.

All species of the genus Habenaria studied show that their somatic chromosome number to be $2n=42$. Among these 4 species studied, H. crinifera shows most primitive characters by possessing more number of long chromosomes (5), maximum chromatin length (54.20 μ) and less number of satellited chromosomes (1). Absence of short chromosomes is also one of the primitive characters observed. In H. crassifolia also, there are no short chromosomes in the compliment, revealing its primitivity. But compared to the earlier species, i.e., H. crinifera, it does show advanced characters in the Karyotype, like, the presence of 3 pairs of satellited chromosomes and lesser chromatin material. Thus, H. crassifolia can be considered as an advanced member than H. crinifera. Regarding the other two species, Viz., H. panchaganensis and H. grandifloriformis, there is not much difference in the total chromatin length, from 47.26 μ in H. panchaganensis to 47.29 μ in H. grandifloriformis. Total number of long chromosomes present in both the above mentioned species is also the same, having only one pair in each of them. But, it would be interesting to note that, there are 3 pairs of short chromosomes and 5 pairs of satellited chromosomes in H. grandifloriformis as against
only one pair of short chromosomes and four pairs of satellited chromosomes in *H. panchaganensis*. These Karyotype variations are sufficient to delimit the above two species as separate entities. Further, it can well be conceived that structural changes have effected speciation in the genus.

In the genus *Porpax* studied, both the species Viz., *P. jerdoniana* and *P. reticulata* have diploid chromosome number as 2n=24. The structural changes found in the Karyotypes will clearly prove their independant status as separate species in the genus. *P. jerdoniana* exhibits maximum chromatin length of 33.11 \(\mu\), more number of long chromosomes as 5, less number of short chromosomes (1) and the presence of only 2 pairs of satellited chromosomes. All these characters depict this species as primitive one, against *P. reticulata* which has less chromatin length of 23.30 \(\mu\), more number of short chromosomes (2) and presence of 3 pairs of satellited chromosomes and complete absence of long chromosomes. Thus, *P. reticulata* is more advanced amongst the two species studied in the genus *Porpax*. Occurrence of tetraploid plant in the species *P. reticulata* with an 'accessory chromosome' in the compliment, also supports the view that, *P. reticulata* is a more active and evolved form, among the species studied.
Thus the structural changes in chromosomes have helped in understanding the mode of speciation in the genus *Porpax*.

Considering the chromosome patterns in the genus *Eria*, a tendency towards a gradual reduction in the size of the medium chromosomes is observed, followed by an increase in the number of short chromosomes. This is further accompanied by a gradual diminution in the total chromatin length (table 31). Thus, as can be seen from the Karyotype of *E. reticosa*, of the 21 pairs, 16 are of medium sized chromosomes. Further, the compliment shows comparatively less number of short chromosomes (5) than other species analysed. It can also be noted that this species has maximum total chromatin length of 35.66 μ.

In case of *Crepis*, it has been shown that, the occurrence of more number of long-sized chromosomes in the Karyotype indicate the primitiveness of a species (Babcock, 1947). Thus, the Karyotype observations made above on *E. reticosa*, with its primitive lip characters, evidently reveals its lower status among the species of the genus.

The second species studied, Viz., *E. mysorensis* shows in its Karyotype a sudden increase in number of short chromosomes and a consequent decrease in the number of medium chromosomes. There are only 3 pairs of medium sized and 16 of
short chromosomes. Increase in number of chromosomes with subterminal constrictions and decrease in the length of chromatin (22.86 μ) keeps this species next to *E. reticosa* in the line of evolution. Comparing this cytological data to the morphological observations, the idea that *E. mysorensis* might have originated from the comparatively primitive species *E. reticosa*, gets enough support.

The third species studied in the genus *Eris* is *E. exilis*. When the lip specialization and the reduction in size of the plant are correlated with Karyotype features, *E. exilis* seems to have attained a higher status over *E. mysorensis*. This statement can aptly be justified looking to the highest number of short chromosome pairs (18) and the minimum chromatin length (21.56 μ). Thus, *E. exilis* shows more closer relationship with *E. mysorensis* in possessing the characters like, sudden increase in number of short chromosomes, decrease in number of medium sized chromosomes and nearly equal length of chromatin (21.56 μ in *E. exilis* and 22.86 μ in *E. mysorensis*).

In the remaining two species, Viz., *E. microrchilos* and *E. dalzellii*, a progressive reduction in total chromatin length is noticeable (Fig. 152). Although, these two species show a close relationship with each other, the fact that *E. dalzellii* possesses a pair of short chromosomes with
terminal constriction separates the two species. Other than this, the species *E. dalzellii* proves its highest evolutionary status among other species, by having highest number of short chromosomes and minimum chromatin length (13.48 μ). This is further supported by the morphological character of the lip, which is the most complex one.

Another interesting feature of the two above mentioned species is the occurrence of 'accessory chromosomes' in their somatic compliments. *E. microchilos* is found to have five 'B' chromosomes while *E. dalzellii* has ten. These are the only two species that are found to have accessory chromosomes, which confirms their closer relationship. Presence of more number of 'B' chromosomes also suggests the maximum evolution of *E. dalzellii* among the 5 species studied.

Thus, it is evident that the structural changes in chromosomes have a significant role in evolution of the species in this genus. The Karyotypic evolution can be correlated with the lip specialisation and can be presented from an unknown origin as:

? —→ *E. reticosa* —→ *E. mysorensis* —→ *E. exilis* ———

—→ *E. microchilos* ———→ *E. dalzellii*. (Fig.153).
Duncan (1953) noted that 'B' chromosomes have not been identified with certainty in Orchids. However, there are a few reports like, in *Listera ovata* by Mac Mahon (1936), *Paphiopedilum insignesylhetense* by Duncan and Maclead (1953), *Cyrepedium acaule* by Belling (1924), *Epipactis latifolia* by Hagerup (1947) and *Tainia laxifolia* by Tanaka and Matsuada (1972) of their occurrence in the compliment. However, report on the occurrence of 'B' chromosomes in the genera *Eria* and *Porpax* made in the present study is the first time record. Grouping of these two genera, Viz., *Eria* and *Porpax* under the same subtribe *Epidendrinae* by Dressler and Dodson (1960) gets support by the above made observations. Though, the observations made on the morphology of leaves and flowers showed no particular variations with the plants having accessory chromosomes, they seem to have contributed to the furtherance of evolution and maintenance of these species.

Both the species studied in the genus *Dendrobium*, Viz., *D.mabelae* and *D.microbulbon* show diploid number as 2n=38. Both of them show more or less same chromatin length. For *D.mabelae* it is 23.00 μ and for *D.microbulbon* 22.51 μ. The number of short chromosomes in *D.mabelae* is found to be 17 while it is 16 in *D.microbulbon*. Thus, there are certain similarities observed in both the species regarding their
However, presence of 10 pairs of short chromosomes with subterminal constrictions in *D. mabelae* separates it from *D. microbulbon*. Seven pairs of short median and nine short submedian chromosomes are observed in *D. microbulbon* in contrast to only 3 short median and 4 short submedian pairs in *D. mabelae*. Thus it is the structural change in chromosomes that decide their individual status.

**Meiosis:**

In the present investigation meiosis could be studied in only 8 species. In the genus Habenaria the species *H. crassifolia, H. panchaganensis, H. grandifloriformis* and *H. crinifera*, while in Eria; *E. microchilos* and *E. dalzellii* and in Dendrobium, *D. mabelae* and *D. microbulbon* are investigated. The meiotic behavior in all the above species is mostly regular. However, a few abnormalities like secondary associations, formations of laggards and precocious movements of chromosomes are noted.

Secondary associations were observed only in one species, Viz: *H. crinifera*. Formation of 4 quadrivalents and 13 bivalents are observed at metaphase-I. Occurrence of secondary associations is a common feature in polyploids (Lawrence, 1931). In case of orchids, chromosomes involved
in secondary associations are in accordance with the degree of polyploidy (cf. Duncan, 1959).

The study of secondary associations has been utilised to determine the extent of affinity/proximity between the taxa (Riley, 1960) and to deduce the base numbers (Lawrence, 1931; Sharma and Chatterji, 1966). In case of orchids, the probable causes for the formation of secondary association has been attributed to 1) Change in habitat conditions, 2) Chemical affinity not related to homology of genetic loci, 3) extrinsic factors such as heat or drying and 4) Consequence of presence of mycorhizal fungus (Duncan, 1959; Mehra & Vij, 1972). Looking to the secondary associations found in the present study it appears that, such a phenomenon may be due either to, change in the physical or chemical factors of chromosomes or due to change in the environmental conditions, as many of the orchid species are found to be aneuploids with 24, 38 & 42 as diploid numbers.

In the genus Habenaria, 21 bivalents could be made out in all the 4 species studied. The fact may be taken into consideration in supporting our view for the base number for the genus as $X=7$ and for Dendrobium as $X=19$.

Precocious movements of chromosomes are observed in H. crassifolia, H. crinifera, E. dalzellii and D. mabelae.
Occurrence of laggards is observed in all the species of Habenaria, Eria and Dendrobium studied. Unequal distribution of chromosomes at anaphase-I is also observed in E. dalzellii. Presence of micronuclei along with tetrads is noted in H. crassifolia, which results in the unequal distribution of chromosomes, leading to aneuploidy. Such findings of abnormal behavior of chromosomes in meiosis may also be due to environmental causes (Swanson, 1963) and might induce genetic imbalance affecting the phenotypes.

Study of microsporogenesis, which could be studied in some details in species of Habenaria, Eria and Dendrobium show that the microspores do not separate but remain in a tetrad condition which in many instances resembles an octant. Microspore division in all these cases is observed to be simultaneous. The microspore wall is laid down even after the first division of each of the tetrad nuclei is completed. This type of division at pollen mitosis has been considered to be an indication of the advance stage in evolution, confirming the highly evolved status of orchid genera.

c) Chemical considerations:

Invaluable importance of chemical knowledge in understanding and evaluating the taxonomic problems has been realised since the pioneering work of Bate-Smith (1954,
1956, 1959). Working on the species of \textit{Lathyrus}, Packet (1959) has convincingly demonstrated, how valuable this information on the constituents of leaf extracts would be, in elucidating the interrelationships among the species.

The present cytotaxonomical investigations have provided certain supporting clues for the phylogenetic relationships between the species. There are numerous compounds present in a particular organ of a plant. The knowledge of their presence or absence in plants belonging to a group of species would help to consolidate the concept of their phylogeny. However, the present study has been confined to chiefly two groups of such chemical compounds, \textit{Viz}: 1) Free amino acids and 2) Flavonoids (secondary phenolic substances).

1) \textbf{Free amino acids:}

In the genus \textit{Habenaria}, the presence of l-\textit{glycine} and l-\textit{valine} in all the species speaks of the compactness of this genus. The amino acid patterns between the species are distinct. While the species \textit{H.\textit{crassifolia}} is characterised by the presence of an "Uncommon" amino acid with \textit{Rf} value 0.11 and reddish brown colour (RBr), the other species \textit{H.\textit{pancheganensis}} is specific in having an "Uncommon" amino acid with \textit{Rf} value 0.13 and yellow (Y) colour. The other
two species, Viz: *H. crinifera* and *H. grandifloriformis* are also characterised by having specific "Uncommon" amino acids, with reddish brown colour (RBr). But these species maintain their individuality by having two different Rf values as 0.53 for *H. grandifloriformis* and 0.24 for *H. crinifera*.

The species of *Porpax* also show variations in their amino acid contents. In case of *P. jerdoniana*, glycine, valine and leucine have been identified. On the other hand, in *P. reticulata*, proline and glycine are observed which show their affinity. However, *P. reticulata* is characterised by having an "Uncommon" amino acid with Rf value 0.48 and pink (P) colouration in contrast to *P. jerdoniana* where no specific "uncommon" amino acid is observed.

In the genus *Eria*, presence of glycine and alanine in all the species studied shows the compactness of the genus. In case of *E. reticosa*, it is characterised by the presence of an "Uncommon" amino acid with 0.25 as Rf value. This species also reveals its individuality by possessing l-leucine with Rf value 0.54, which is not found in any other species of this genus.

Presence of a yellow coloured spot (Y) with Rf value 0.63 in the patterns of both *E. exilis* and *E. mysorensis* reveals their closer relationship. But, these species
distinguish themselves by having "Uncommon" amino acids with different Rf values as 0.26 for \textit{E. mysorensis} and \textit{E. exilis} with 0.19. In \textit{E. exilis} the presence of 1-valine is noted which is also found to occur in \textit{E. microchilos} and \textit{E. dalzellii}. Thus, the position of \textit{E. exilis} in the line of evolution is well defined with the other two species, Viz: \textit{E. microchilos} and \textit{E. dalzellii}.

Cytotaxonomical studies have already revealed that \textit{E. microchilos} and \textit{E. dalzellii} are closely related. This fact is further substantiated by the chemical findings, like presence of glycine, alanine, valine and isoleucine in both the species. Not much difference is observed in the Rf values of "Uncommon" amino acids for \textit{E. microchilos} (=0.21) and \textit{E. dalzellii} (=0.23). However, these two species maintain their distinct entities, by having two different amino acids, Viz: 1-lysine monohydrochloride in \textit{E. microchilos} and 1-arginine monohydrochloride in \textit{E. dalzellii}.

Thus, these finding for free amino acids also justify the evolutionary trend for the genus \textit{Eria}, which is proposed earlier. According to Bell (1971), the presence of an "Uncommon" amino acid in a species distinguishes it from the others. Thus occurrence of an "Uncommon" amino acid in different species helps to evaluate their phylogenetic relationships. This is convincingly evidenced by Packet (1959) in \textit{Lathyrus}. 


The two species of *Dendrobium*, viz: *D. mabeae* and *D. microbulbon* show similarities in having a pink spot with same Rf value as 0.39. But *D. mabelae* is characterized by having an "Uncommon" amino acid with Rf value 0.45 and bluish pink (Blp) colouration in contrast to *D. microbulbon* which has Rf value 0.39 with pink (P) colour.

2) Flavonoids (Secondary phenolic substances):

The flavonoid spot analysis made in the 4 genera Viz: *Habenaria*, *Porpax*, *Eria* and *Dendrobium* show individual characteristic spot patterns. The genus *Habenaria* is characterised by two "marker spots" with brown (Br) and yellowish green (YG) colours. Of the 27 spots detected for the 4 species, brown (Br) and yellowish green (YG) fluorescent spots are found occurring in all of them, revealing their interrelationships (Table.No.33). Presence of brown (Br), greenish brown (GBr), reddish brown (RBr) and yellowish green (YG) spots in both the species namely *H. crinifera* and *H. crassifolia* confirms their close relation.

Totally, 9 spots are detected for two species of *Porpax*. Presence of three "marker spots" with red (R), reddish green (RG) and green (G) fluorescence in both the species show their compactness. However, *P. jerdoniana* differs from *P. reticulata* in having reddish brown (RBr) spot.
P. reticulata also maintains its distinct entity by possessing greenish yellow (GY) and brownish green (BrG) fluorescent spots.

For the species in the genus Eria, reddish brown (RBr) and greenish yellow (GY) spots are characteristic and are noted as "marker spots". However, the individual species in the genus show specific pattern of "marker spots" which vary in their Rf values (table, No.33). E. reticosa maintains its individual status by having brownish green (BrG) and brownish red (BrR) spots along with the "marker spots." It shows relationship with other species namely, E. mysorensis by having greenish yellow (GY) spots and with very close Rf values as 0.10 in E. reticosa and 0.11 in E. mysorensis.

On the basis of the presence of greenish yellow (GY) and reddish brown (RBr) spots in the two species, viz., E. mysorensis and E. exilis show their close relationship. Further, E. micrhochilos proves its distinctness with yellowish blue (YBl) fluorescent spot with a Rf. value of 0.20. E. dalzellii is also quite distinct in having brownish yellow (=0.21) and green (=0.59) spots. But, these two species again show their close relationship by possessing reddish brown (RBr) spot with Rf value 0.54 and greenish yellow (GY) spot with Rf value 0.51 respectively.
Hence, the flavonoid spot patterns substantiate the morphological and cytological findings. Similar works have also been done on *Baptisia* (Harborne, 1971), where the flavonoid analysis has been utilised to establish the phylogenetic relationship. In barley, Fröst and Hdlm (1971) also have made the same type of study with long wave UV on TLC which gives a clue even to understand the probable center of origin. Therefore, the comparative flavonoid patterns in species of the genus *Eria* lends a good support to the proposed view of the line of evolution amongst the species.

Thus, from the above correlative study of morphological, cytological and biochemical characters, it is evident that, in the subtribe Epidendrinae, the two genera *Eria* and *Porpax* are distinct morphological entities.

In the other genus *Dendrobium*, both the species, Viz, *D. mabelae* and *D. microbulbon* show different fluorescent spots showing that they are separate entities. But, the reddish brown (RBr) and green (G) coloured "marker spots" found in both of them prove their affinity.

It is known from the studies on Lotus (Harney and Grant, 1964) and *Tragopogon* (Belzer and Ownbey, 1971), that the
flavonoid synthesis is genetically controlled. In the present study, however, structural changes of chromosomes have shown to play an important role in speciation. Such a phenomenon may in turn have its influence on the variations of flavonoid patterns of the species. Therefore, the view of Turner and Alston (1959) that, the relative value of biochemical characters, supplement other characters is evidenced by the present study.

The subtribes Epidendrinae and Dendrobinae when compared, establish themselves as different entities on the cytological and biochemical grounds. We find both these subtribes included in the tribe Epidendreae by Dressler and Dodson (1960) on the basis of their having waxy pollinia. This treatment of them gets justification with the findings in the present study also.