KARYO-SYSTEMATIC STUDIES IN HELOBIALES

IV. THE GENUS OTTELIA PERS.
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I. INTRODUCTION.

The genus *Ottelia* consists of about 40 species, which have a wide range of geographic distribution (Dandy, 1934). Hooker (1885) reported only a single highly polymorphic species of this genus, namely *O. alismoides* from India. A cytological basis for the great variation in this species was first established by the present writer (Sunder Rao, 1950 and 1951), who revealed the existence of two intraspecific chromosome races with \(2n = 22\) and 66 chromosomes. Janaki Ammal (1945) reported about 40 somatic chromosomes for the same species. On the assumption that \(11\) is the basic number, there are thus diploid, tetraploid and hexaploid chromosome races within the species. During the present investigation, not only an attempt was made to gather details concerning the cytology of the three chromosome races and their mode of geographic distribution in India and elsewhere but also a correlation was sought between the morphological features and the degree of ploidy of each race. Such studies may ultimately lead to a taxonomic evaluation of the intraspecific chromosome races in the species.

During the course of the present study several collections were also made of certain unisexual forms, which are morphologically more or less similar to *Ottelia* Pers., and which were formerly treated under a separate genus *Boottia* Wall. Since there is no cytological knowledge about the latter and a better understanding of these unisexual forms has some bearing on the problems of evolution and generic boundaries in *Ottelia*, available material has been examined with the hope of obtaining cytological evidence as to their relation to *Ottelia*. For the
sake of convenience, however, the original distinction between the two genera is still maintained in the present paper and all the monoclinous and diclinous plants studied cytologically are referred under \textit{O. alimoides} and \textit{Boottia} species respectively. In order to avoid the difficulties in connotation, it may be mentioned at the outset that the words 'form' and 'race' are used as synonymous and are only meant to denote sub-specific categories.

II. DESCRIPTION OF THE FORMS OF OTTELIA STUDIED CYTOLOGICALLY.

The genus \textit{Ottelia} presents great difficulties to the herbarium worker, as certain characters easily recognisable only the fresh material are difficult to detect in the dry state. Furthermore, the inherent variability underlies the taxonomic complexity of at least certain of its species. However, an admirable revision of the genus has been presented by Dandy (1934) but his treatment needs supplementing in the light of observations made on material collected from different parts of India. As the determination of the taxonomic status of the chromosome races is one of the objects of the present investigation and since the degree of the morphological divergence is decisive of their status, the individual description of such races is likely to be an useful appendage to this paper and is given below. In order to understand the range of morphological variation of the species, herbarium sheets collected from different parts of S.E. Asia and Australia were also examined and the ploidy of such specimens inferred, though such inferences are extremely unreliable.

The quantitative characters of the diploids and polyploids
and their comparative statement have been presented in a tabular form (Table 1) in the subsequent pages. It requires, however, to mention that the height of certain forms given below and therefore the size of the plant parts like leaves is not probably true to the maximum heights which the plants naturally attain. Collectors of them might have pressed shorter specimens, which are always convenient to handle. This is also possibly true of the measurements given in the various regional floras of India. Despite the fact, such measurements are mentioned below and are thought useful as the proportion of any two dimensions of any plant part or the proportion of one part to the related one, always follow a characteristic trend, which is specific and which varies from one species to the other (Anderson, 1951).

All the plants described below are submerged, flacid, rooted, fresh-water herbs, occurring in different parts of India and Malaya in semipermanent and permanent ponds and lakes and sometimes in the slow running waters.

(A) The Indian forms.

(i) The Diploid forms. These were collected mostly from the various parts of Andhra Desa. They were, however, not found in Vizagapatam Dt., where only polyploids occur. Diploid forms are also found in Poona and Junagarh.

Type I: The Andhra forms. Plants of variable heights depending on the depth of water; Leaves: radical, crowded, highly variable
in length, invariably with a distinct differentiation of the lower completely submerged and the upper at least partially floating leaves; sometimes tiny depauperate plants wholly consisting of the submerged type; rarely leaves showing transition between both the types; submerged leaves, sessile with linear-lanceolate lamina; floating leaves petiolate; petiole highly variable in length, usually 4"-6" long, flattened, a little trigonous; margins of leaf-bases with membranous wings; entire; Lamina: concave and spoon-like, on account of which it breaks at two or three points along the margin while pressing; variable in size, usually about 3" long and 3" broad or about 3.6" long and 3.2" broad or a little smaller or larger with proportional dimensions; broadly ovate with almost straight and less prominent lobes tending towards triangular shape or slightly cordate with broad and rounded basal lobes; rarely elongate-ovate 2.7" long and 1.4" broad or extremely broad 2.8" long and 4.2" broad; if smaller or larger with proportional dimensions; entire; undulate; usually 9-veined, rarely 7 or 11 - veined depending on the breadth; obtuse or almost blunt; Flowers: solitary, raised above the the surface of the water by long axillary peduncles of variable lengths, the length depending on the depth of the water; hermaphrodite; sessile; about 1.5" to 2" long; enclosed in a tubular bifid spathe; usually 1" long with crisp, wavy and unequal wings designated as (i) very broad: with broad rounded basal lobes, measuring about 0.3" to 0.4" across; (ii) narrow: with no conspicuous basal lobes; (iii) small: narrow and short, sometimes not extending the whole length of the spathe; (iv) very short: rudimentary ones; usually fine in number (2 broad, situated opposite to each other, one probably developing along the midrib of each bract, sometimes
asymmetrical, one outgrowing the other in size; 2 narrow and one small; the former on one side and the latter on the other side of the spathe with reference to the two broad ones; rarely 6, with one extra very short wind co-existing either with the narrow ones or with the short one; rarely also 4 or 3 with one small or one small and one narrow undeveloped respectively; sometimes the size variations not well-marked; Sepals: 3, linear, oblong, about 0.5" long, bright green, reticulate veination, margins fringed with narrow membranes; Petals: 3, always larger than the sepals, 1" long or a little smaller, white with yellowish base; obovate, or orbicular with fleshy basal appendages; Stamens: 6 - 9 in number; slightly unequal in length; filaments flat; broad above with short blunt closely set hairs; narrow and smooth below; filament distinctly articulated at the point of attachment with anther lobes; anther lobes: 2, narrow and elongated, parallel, more or less equal in length with the filament, basal rounded parts of the anther lobes slightly projecting below the point of attachment with the filament; Ovary: inferior, oblong, 3 to 5-sided, 6 - 9 carpels, numerous ovules on the projecting placentas; Styles: as many as carpels; flat and smooth at the base; each deeply bifid at the top; lobes pretty long, sometimes unequal, flat tip subacute, with a midrib-like structure running all along the length; the two margins of each lobe studded with closely set short hairs; Fruit: large with numerous seeds; 1"- 1.5" long; oblong; spathe and calyx lobes persisting; wings becoming enlarged but maintaining the same size relations as in the flower; Seeds: many, oblong, testa pulpy. (Photograph, 1).

Type 2: Andhra forms. resemble the Type 1 except for the absence of wings (Photograph, 2).

Type 3: Poona forms. resemble the Andhra forms in all respects;
the spathe wings two and not broad, rarely rudimentary or nil.

Type 4: Junagarh forms: these are short statured plants when compared with the diploids described above; Leaves: deeply cordate, about 2.4" long and 2.4" broad; almost round but cordate at the base; sometimes elliptic - ovate about 2.2" long and 1.6" broad; mostly 7 - veined; entire; Flowers: whitish or yellowish, enclosed in a spathe about 1.8" long; bifid; 2 or 3 - winged; wings not prominent; sometimes rudimentary (photograph, c).

(ii) Hexaploid forms.

Benares forms. Extensive collection of dried and pressed specimens from Benares and nearby villages was observed with a view to compare them with the diploid forms. All are medium-sized plants with bright green and crisp leaves. They are exactly like the diploid forms but for the gigantism found in certain parts of the plants and certain other minor points of distinction mentioned below. Like diploids, they are also divisible into two following types:

Type 5: Winged forms. Leaves: of variable lengths upto 12" long; petiolate; petiole trigonous; serrulate, serrulation confined to petioles only; lamina, usually about 3.5" long and 3.5" broad; or rarely a little bigger or smaller; usually ovate or orbicular with a highly cordate base; basal lobes usually almost rounded; entire, wavy; Flowers: solitary in spathes of 1" to 1.5" long; raised above by trigonous peduncles of various lengths; Wings: usually five; but variable between 3 - 5; their breadth equal; the two larger wings so prominent in the diploids could be detected in some specimens but they are not
so conspicuous; wavy and crisp; Sepals and Petals: similar to those of diploids but a little larger in size; petals pink or white in colour; Hooker (1885) did not describe the pink coloured flowers; Stamens: 6 - 10; filaments unequal in length; not so flat as in diploids; a little broad at the top and narrow at the base; covered by closely set long unicellular hairs (hairs longer than those of diploids), gradually becoming smaller and sparingly distributed at the base; no marked constriction at the point of attachment of the anthers; anther lobes as long or a little shorter than the filaments; Ovary: 6 - 10 carpels; styles as many as carpels; markedly unequal in length and smooth at the base and forked at the top; lobes of the style equal or unequal; the two margins of each lobe fringed by closely set long hairs (hairs longer than those of the diploids)(Photograph, 4).

Type 6: Wingless forms. Similar to the winged forms but for the absence of the wings on the spathes; Leaves: similar to the winged forms but for a little difference in the shape of the lamina; usually elliptic - ovate or ovate with a cordate base; 5" long and 3.5" broad or 3.7" long and 2.7" broad or rarely linear - lanceolate, 5" long and 2.3" broad; sometimes these forms differ from the winged forms in the external appearance of the sepals also; there are black spots or streaks arranged in longitudinal rows on the sepals; such streaks are absent in diploids also(Photograph, 5).

Type 7: Sagar forms. They resemble the Benares forms in all essential respects, falling roughly under the same two categories, except for the fact that the flowers are only white in colour. The shape of the leaves is similar to the winged
forms of Benares and leaves with and without serulation are found in one the same plant; the stamens are fewer, either 5 or 4, rarely even 3 in number; the ovary consists of usually 4 or 5 carpels.

Type 8: Nagpur forms. Exactly like Sagar forms; leaves sometimes reniform with blunt or almost rounded apex.

Type 9: Patna forms. Leaves; with petioles which gradually merge in the laminas; Lamina: lanceolate 3" long and 1" broad; or ovate, 2" long and 1.3" broad; Flowers: in spathes > 0.8" long; wings rudimentary.

Type 10: Bengal forms. Gigantic specimens with huge leaves; Leaves: petiolate; petiole 5" to 11" long finely serulate all through the length of the petiole, teeth being prominent near the leaf base or sometimes entire; Lamina: usually deeply cordate or ovate - lanceolate, 5.5" long and 5.5" broad or 6.5" long and 7.5" broad or 4.5" long and 3.3" broad respectively or a little smaller or bigger with proportionate dimensions; finely serulate or denticulate, the teeth confining themselves only to the basal part of the lamina; sometimes entire; wavy; mostly 11 - veined; Flowers: large with spathes, 1.5" long; spathe wings large; 5 - 6 in number; roughly of equal size.

Type 11: Cuttuck forms. Look like Bengal forms in all respects, as is the case with the Sagar and Nagpore forms.
Type 12: **Andhra forms.** Leaves: with lanceolate, ovate or cordate laminas; entire; spathes about 1.2" long; winged or unwinged; if winged, 5 in number roughly of equal lengths (?)..

(iii) **Bisexual hexaploid forms resembling Bootitia sp.**

These were collected from Narayanapur, Benares, U.P., India; gigantic with large leaves, which are petiolate; petiole about 9" long; serulate or entire at the base; Lamina: ovate or ovate - lanceolate; a little cordate at the base or lamina gradually merging in the petiole; usually 9" long and 2" broad or 7" long and 4.5" broad; a little larger or smaller; usually 9 - veined; entire; wavy; acute or a little acuminate; Flowers: bisexual; large; enclosed in spathes measuring about 1.5" - 1.7" long; mouth with many teeth; wings usually 5 or 6 in number; narrow and of equal breadth; rarely 2 or 3 ill-developed; **Sepals; Petals, and Stamens** : similar to the other polyploids; **Carpels**: usually many, up to 11 in number; styles as many as carpels and have the same structure as those of the other polyploids (Photo. 10).

These forms resemble **Bootitia** Wall., in almost all external morphological features but for their bisexual flowers Dandy (1934) emphasising the similarity of certain diclinous and monoclinous species of **Ottelia** cited B. Rodimieri (Lev. & Van.) Lev. & Van., which has several spathes wings veering also a close relationship with **O. alismoides**. According to him, the same is the case with **E. brachyphylla** Gurke and **O. Multifolia** (Planch) Walp.,
by two-winged spathes.

(iv) Bootitia Wall.

Some male specimens of a dioecious species of Bootitia (now called Ottelia, Dandy, 1934) were collected from Narayangapur, Benares, U.P., India and they were found coexisting with the other hexaploid forms of Ottelia alismoides. The leaves are bright dark green and exactly resemble the leaves of the previous forms. Flowers: unisexual and male; several occurring in a single spathe; the spathes raised above the surface of the water by trigonous peduncles; spathes up to 2.5" long; a little flat; five-winged; 2 broad ones with rounded basal lobes (not so conspicuous as in the diploids) the rest narrow and small, sometimes not extending the whole length of the spathe; rarely two-winged, in which case wings rudimentary; or three-winged, out of which one rudimentary; Sepals: fairly long; 0.8" long; Petals: larger than sepals; white or pink in colour (the latter colour not mentioned by Hooker, 1885); Stamens: always 12; dimorphous; six outer, shorter stamens with narrow filaments which are hairy; six inner, longer stamens, filaments flattened and broad at the base or in the middle, studded all over with long hairs (hairs longer than those of the other hexaploids); pistillode present; Styles: $\phi$, narrow and flat; stigmas bifid, flat and rounded; petoloid; hairy all over (Fig. 35, 11, 1-10).

(B) The Malayan forms.

A large number of herbarium specimens collected from different parts of Malaya were observed and all of them resemble
the hexaploid forms described above, particularly those collected from Benares and Bengal. Ridley (1924) recorded the occurrence of pink and white coloured flowers, which is the feature of only the Benares forms. Such a similarity between the Malayan forms and the plants of the upper Gangetic plain, India is interesting (Photographs).

(C) The forms collected from other regions of S.E. Asia.

Herbarium sheets of specimens collected from Indi-China (Pho.to 1) and certain parts of India look like polyploids. *Quercus japonica* is known to occur in China and it also looks like a hexaploid (Photo. 1).

From the foregoing account it is clear that *Q. elismaoides* consists of a large number of types, which occur in different parts of India and elsewhere. The descriptions given above fairly correspond with those given in the various regional floras of India and Malaya. The characters, like the height of the plants,

the shape of the leaves, the number and the size of the spathe wings by which they are apparently found to differ overlap in the various chromosome races. It is this fact that makes the taxonomic recognition of the chromosome races difficult. Nevertheless, it is interesting to note that the forms having the same chromosome number and occurring in a particular locality are fairly constant within a narrow range of morphological features. Forms occurring in two contiguous areas look alike (compare the hexaploid Bengal and the Orissa forms) and types that geographically isolated maintain their identity and distinctness (compare the diploids of Junagarh and Andhra). It is difficult to say at present whether this is an indication of their genetic constancy or not, as there are no cytogenetical data. They have been therefore called by the name of the locality from which they were collected.

III. CYTOLOGICAL TECHNIQUE.

In the case of the hexaploid _Ottelia alismoides_ and _Boottia_ sp., satisfactory somatic metaphase plates were obtained when the root - tips were fixed in Belling's Navashin or "Craf". However, for the study of the morphology of the somatic chromosomes of the diploids, root - tips were fixed in Benda, 2 BE, the different types of chrome - acetic - formalin fluids and Lewitsky's chromic - formalin (1:1; 1:2). The last mentioned fixative with a greater proportion of formalin was proved useful for accentuating the sharpness of the constrictions.

For meiotic stages, in both diploids and polyploids, flower buds known to contain dividing pollen mother - cells
were pretreated in Carnoy's fluid for 20 - 30 seconds, washed in water and then fixed in Belling’s Navashin and "Craf". Certain observations recorded in this paper were also made on smeared material. Pollen mother - cells were gently squeezed out of the anthers between two clean slides, which were immediately inverted in a petri dish containing Belling's Navashin. After three hours' fixation, the slides were stained in crystal violet, using 1 % chromic acid as a premordant. A few smears were also prepared and fixed in the fluid recommended by Catcheside (1934). Both the fixatives gave satisfactory results. In the case of the latter, the slides were bleached before staining with crystal violet.

Rapid counts of the chromosome numbers of hundreds of plants collected from different parts of India and Malaya were made from temporary acetocarmine smears, prepared out of the root - tip material fixed in acetic - alcohol (1:3). The same method was also found valuable for the study of side views of metaphases I in order to understand the relative frequency of the multivalents and the bivalents in the polyploid forms.

IV. DETAILS OF MITOSIS AND MEIOSIS

A. *Ottelia alismoides* Pers.

1. The diploid race. Figs. 1 and 2 show the somatic metaphase and the idiogram respectively. The twenty two chromosomes are characterised by distinct morphology. On the basis of length alone, they can be classified into three groups namely, (a) one long (b) seven medium and (c) three short pairs of chromosomes. Probably, they can be further classified into the following seven types based on the length, the position of the
the attachment constrictions and the satellites:

(a) **Long chromosomes**:
   (i) one pair of chromosomes (A), longest in the complement with a distinctly median constriction;

(b) **Medium-sized chromosomes**, which vary in length at the same time forming graded series:
   (ii) one pair of chromosomes (B) with subterminal constrictions but satellited. The satellite knobs are small and are attached to the short proximal arms with short threads;
   (iii) two pairs of chromosomes (C and D) with subterminal constrictions; the proximal arms of these chromosomes form distinct segments about 1/3 the length of the long distal arms; their full lengths become visible only in flat acetocarmine smears;
   (iv) three pairs of chromosomes (E, F and G) with subterminal constrictions; the proximal arms are rounded and knob-like;
   (v) one pair of chromosomes (H) with either median or slightly submedian constrictions;

(c) **Short chromosomes**, forming graded series among themselves;
   (vi) two pairs of chromosome (I and J) with sub-terminal constrictions almost resembling E, F and G but for their short length;
   (vii) the last pair having a submedian constriction.

Fig. 8 represents an exceptional metaphase, where most of the chromosomes show a tendency towards peripheral arrange-
ment and only eight of them are found in the centre of the plate as in *Tenagocharis latifolia* (Sundar Rao, unpublished observations). They lie flat on the metaphase plate (cf. Upcott, 1936, Fig. 23). and in addition to this, the centromeres of the chromosomes of the peripheral circle are disposed at almost equidistant spaces with the proximal arms of the chromosomes projecting into the interior of the spindle, appearing as though they are in a state of equilibrium and mutual repulsion. This indicates that there is a tendency to eliminate as far as possible the long distal arms from the spindle, presumably for want of space. A study of the disposition of the long chromosomes with median constrictions leads to the same conclusion. They are not only peripherally arranged but assume a V-shape with both the arms outside the spindle and the centromere falling in line with the rest (Figs. 1 & 3).

Some of the somatic chromosomes of the diploid *Q. alismoides* are characterised by a specialised property of differential staining reaction, which becomes manifestly clear in old preparations and invariably escapes observation in freshly made preparations. The differential behaviour consists in certain segments of such chromosomes losing stain more rapidly than the rest of the chromosome and hence giving a false appearance of secondary constrictions in old preparations (cf. discussion on the secondary constrictions in *Polygonatum*, Therman - Suomalainen, 1949). An intercalary region of the long medianly constricted chromosomes (type A in fig. 2) and a subterminal portion of the medium-sized chromosomes with median or slightly submedian constrictions (type H in fig. 2) are of this nature. It is not improbable
that other types of chromosomes also possess such segments. This differential reactivity, however, did not manifest itself consistently in materials treated with different fixatives and on different occasions. Perhaps, their appearance is partly determined by the environmental factors, like temperature (cf. also Darlington and La Cour, 1938, pp. 618) or various individuals differ in this respect (Trillium erectum, Bailey, 1949).

Chromosomes with differential staining reaction have been reported from time to time in both plants and animals. Kaufmann (1948) referred some of the cases known to date in the literature. Lam (1945) in Solanum acule (2n = 48) and Hakansson (1950) in Godetia found unstained regions in chromosomes. It is now more less definitely established that whole chromosomes or chromosome segments, which are heterochromatic, behave in such a manner (Darlington and La Cour, 1940 and 1941) and that the differential reactivity is sometimes associated with the genetical inertness (Heitz, 1935). The special property of the chromosomes of the diploid Ottelia alismoides responsible for the appearance of the unstained regions may, therefore, be attributed to the heterochromatic nature of such regions. Darlington and La Cour (1940) suggested that their appearance is due to the inhibition of the nucleic acid formation.

Yet another but a specific type of differential reactivity was shown in Paris polyphylla (2n + 10 f). It consists
in the coiling of constant parts of chromosomes into a finer thread of a smaller diameter than the rest of the chromosome thread and hence appearing less deeply stained. Such regions are localised at the ends of the distal arms of the chromosomes and this property was correlated with the genetic function of such regions (Darlington and La Cour, 1938).

In having the lowest chromosome number 2n = 22 so far reported in the genus, two SAT - chromosomes and a pair of nucleoli in the late telophases (Fig.4), there is no doubt that this is a diploid race of *Ottelia alismoides*. The exact determination of the number of nucleoli in the early telophases was found to be sometimes difficult due to the nucleolar globules, along with two big "true" nucleoli (Fig.5). However, there are only two nucleoli in the resting nucleus (Fig.6).

The meiosis of the diploid race was studied somewhat in detail; but stages prior to late diplotene were not fixed well and hence critical examination could not be made. The nucleolus at this stage is big and has no fixed position in the nucleus. It may be found very close to the nuclear membrane or somewhere near about the centre of the nucleus (Fig.7). Whenever it takes the former position, it is flattened towards the side of the nuclear membrane (Fig.6). There is a vacuole in the centre of the nucleolus. Out of the 11 bivalents that are formed regularly, the one with satellites is constantly associated with the nucleolus. Depending on the distance at which the two nucleolar chromosomes are attached to the nucleolus, the chromosomes are paired (Fig.7) or remain
unpaired (Fig.8) at the region of the attachment. Such a phenomenon was observed by Iyengar (1939) in *Cicer*.

During diakinesis, 11 bivalents were constantly observed (Fig.9). At this stage also, one bivalent is attached to the nucleolus.

As a rule 11 bivalents were found at metaphase I but in one P.M.C., however, 12 bivalents were found (Fig.15) — an abnormality similar to that of *Tenagocharis latifolia* and *Sesbania aculeata* (unpublished observations). The bivalents are not only large but are of various sizes, which is in keeping with the size variations of the somatic chromosomes. Table I shows the various types of configurations assumed by the 11 bivalents at the equatorial region:

<table>
<thead>
<tr>
<th>Type of arrangement</th>
<th>No. of cases</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) No definite arrangement.</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>(2) 1 in the centre and 10 all round.</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>(3) 2 in the centre and 9 all round.</td>
<td>8</td>
<td>11 &amp; 13.</td>
</tr>
<tr>
<td>(4) 3 in the centre and 8 all round.</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

It is clear that the arrangement of common occurrence is that with 2 in the centre and 9 all round. As suggested by Cannon (1923), the 11 bivalents in *Ottelia* no doubt assume a configuration with 3 in the centre and 9 all round, but it is not found in a majority of cases. Apparently, the size of the bivalents and their relative position at the previous prometa-
phase or even at diakinesis might be the determining factors.

Analysis of a few favourable side-views showed that the bivalents are characterised by variable number of chiasmata, which is proportional to the length of the chromosomes. The single long bivalent with five or six chiasmata, the seven medium-sized bivalents with four to one chiasmata and the three short bivalents with two or one are clear.

In two cells, however, the long bivalent showed an abnormal behaviour as regards chiasma frequency and the degree of spiralisation, while the rest of the bivalents of the same nucleus showed almost a normal behaviour (Fig.17). The chromosomes of this bivalent are attenuated and are as long or even longer than the second metaphase chromosomes, still retaining the major spirals. Pollen mother cells with abnormally long chromosomes at metaphase I were reported by Darlington (1936) in Fritillaria. Upcott (1937) sketched an abnormal anaphase with long chromosomes in a triploid garden variety of Tulip. Similar cells were also observed by Levan (1939) in Allium, which was treated with colchicine.

Unlike Fritillaria and like Allium, the remarkable feature of these abnormal bivalents as revealed by a comparison of the figs. 16 and 17 is the considerable reduction in the chiasma frequency. The number of chiasmata is only two, either terminal or interstitial, as against five or six chiasmata in the normal bivalents of this type. Inspite of this fact, the total number of chiasmata in the abnormal cell is roughly equal to that of a normal cell. That means reduction in one bivalent
is sometimes compensated by a slight increase in the other bivalents of the same nucleus and for this there is at present no statistical evidence. This probably provides an example of unitary control of chiasma frequency (Mather, 1936; Mather & Lamm, 1935). This phenomenon is similar to that of *Drosophila melanogaster*, where prevention of crossing-over in two of the long chromosomes resulted in the increase of the crossing-over in the third chromosome (Morgan, Bridges and Schultz, 1933). Mather (1936) gave an extensive survey of the problem. The unique behaviour of this long bivalent in *Ottelia* having a reduced number of chiasmata may be also due to its reaction towards the various external factors. Environmental factors like temperature (Stow, 1926 & 1927; Katayama, 1931; Sax, 1931) or genic factors (Goven, 1926; Beadle, 1930) may inhibit crossing-over. Sometimes varietal difference with regard to chiasma frequency may occur (Sax, 1935). Mather (1934) showed that X-ray treatment brings about an alteration in the chiasma frequency.

The disjunction of the bivalents is normal at anaphase I. During telophase I, the two nuclei contain rather conspicuously large nucleoli, one in each nucleus (Fig. 20). After a short interphase, the nuclei pass into the II division and at metaphase II (Fig. 18) the haploid set of chromosomes show exactly similar morphology as those of the somatic cells and such a set consists of:

(i) 1 long chromosome with a median constriction;
(ii) 6 medium-sized chromosomes with subterminal constrictions;
(iii) 1 medium-sized chromosome with either a medium or a submedian constriction;
(iv) 3 short chromosomes with subterminal constrictions.

At anaphase II, lagging (Fig. 22) and bridge formation (Fig. 21) were observed. There are rare phenomena. Pollen grains are formed in a normal manner and they are highly fertile (see Table II).

2. The hexaploid forms.

Bisexual hexaploid forms of a variable morphological pattern were collected during the present investigation. All of them do not, however, differ from each other as also from the unisexual Boothia sp., in the chromosome number, morphology and meiotic behaviour. Hence, some of the cytological details have been presented under the unisexual forms. The following are some of the facts which are not mentioned there.

There are 66 chromosomes in the root tip cells (Figs. 23 & 31). On account of the fact that a large number of chromosomes were crowded in a relatively small cell, the chromosome morphology could not be worked out. It is nevertheless apparent that the winged and wingless forms are both characterised by the same type of chromosome complements. Figs. 27-30 show the nucleolar number in the resting nuclei. Six is the common number (Figs. 27 & 22), although numbers lower and higher than six were also met with (Fig. 29 & 30).

While making an extensive study of the acetocarmine
smears of pollen mother cells, a few cells showing no indication of division were met with. They coexist with normal actively dividing cells and one such cell is sketched in the fig. 24. There is no clear differentiation of the chromation threads or chromosomes in the nucleus and signs of degeneration are also visible. Probably, a mutation is responsible for a derangement of the metabolic activity, generic balance and hence for a pathological condition of the nucleus in such cells. Yasui (1935) working on the triploid Hosta observed various stages of degeneration of the pollen mother cells and it is remarkable to note that in this case normal and healthy pollen mother cells were in close proximity with the tapetum and the degenerating ones in the centre of the anther loculus. Such a location of the abnormal pollen mother cells in the anther loculus could not be verified in the case of the hexaploid O. alismoides.

Meiotic stages earlier than diakinesis were not studied because of the large number of chromosomes. In almost all cells 33 bivalents could be counted; in some cases, however, the bivalents were found sticking together and were connected by much drawn out thread-like structures, rendering the exact determination of the number of bivalents difficult (Fig. 25 a). Diakinesis is a stage of maximum repulsion on account of which the bivalents tend to move away from each other resulting in the attenuation of the connecting threads, which seem to emanate from the matrix of the chromosomes.

Fig. 32 shows the polar view of metaphase I. The
bivalents are very much condensed and are of variable sizes. Conspicuously enough, two of them are specially large. In one metaphase, a bivalent with satellites was noted (Fig. 25 b) as is the case in Calceolaria (Srinath, 1940) and Sesamia bispinosa (Sundar Rao, unpublished observations).

Figs. 35 - 37 represent the side views of metaphase I with different types of bivalents depending on the number and position of chiasmata. It is clear that the number of chiasmata per bivalent varies from five to one, either terminal or interstitial. Notwithstanding the fairly high chiasma frequency, formation of bivalents almost appears to be the rule in the hexaploid Ottelia alismoides. Working with the American species of Polygonatum, Therman (1950) found that in P. commutatum only bivalents were formed during meiosis, despite the fact that the chiasma frequency in the species was also high. In O. alismoides, however, a single instance of a trivalent was observed at metaphase I (Fig. 36). In this metaphase, a total number of 32 bodies including the trivalent mentioned above could be counted. Obviously, one of the other bodies is also a trivalent, though it could not be ascertained with certainty. The sporadic formation of trivalents probably implies autosynthesis.

In fig. 37, six bivalents are seen connected with each other by fine thread-like structures, which are certainly reminiscent of the previous diakinesis stage. That means stickiness developed in the previous prophase persists up to the metaphases and probably even to the subsequent stages.
Bivalents, which are not connected with others also show processes of the same type. This phenomenon of stickiness has been variously termed by different cytologists and Resende (1941) substituted all of them with chromatic "a glutination". It is postulated by Resende and Luisote Rijo (1948) that genetic unbalance and physiological changes may initiate chromatic agglutination. Pinto - Lopes and Resende (1949) summarised the then existing knowledge on the subject and recognised spontaneous, functional and provoked chromatic agglutinations. The credit of ascribing for the first time genic and chromosomal mutations to agglutinations goes to Resende (1941) and since *O. alismoides* is a high polyploid, it is just possible that genic unbalance might have brought about the stickiness. The stickiness was shown by Muntzing (1948) as a symptom of the probable heterochromatic nature of B- chromosomes in *Poa*.

Perhaps such an explanation is also tenable in *O. alismoides*, since segments of chromosomes, possibly heterochromatic nature showing differential staining behaviour have been found in the diploid races, their presence in the hexaploid race is not precluded.

Anaphase I is normal (Fig.23) and interphase nuclei contain three nucleoli, which are in a state of fusion (Fig.26) at metaphase II, the chromosome morphology is clearly recognisable and an examination of a large number of metaphases II revealed that there are only two long chromosomes with median constrictions (Fig.34).
3. **Hexaploid forms, which resemble Boottia.**

The somatic chromosome number is 66 (Fig. 39), as is the case with the hexaploid forms previously described. In this respect, these forms also resemble unisexual *Boottia* sp. Four long chromosomes with median constrictions could be picked out easily from the diploid complement of these forms (Fig. 41).

4. **Malayan forms.**

A study of the root-tip material of *O. alismoides* from Sungi Rusa Dt., Penang, Malaya collected by Mr. H. Ritchings, Horticultural officer, Penang has revealed that all the forms are hexaploid. Morphologically they resemble some of the Indian forms and attention has been drawn towards this fact in the beginning of this paper.

5. **Unisexual male Boottia sp.**

The resting nucleus is characterised by six nucleoli of different sizes (Fig. 42). Fig. 43 shows a telophase nucleus with numerous globules of nucleolar material. Irregular fusion of these globules led to the formation of heteromorphic nucleoli, whose size relations and number are not a constant feature in different resting nuclei of these species. On the other hand, Bhaduri (1944) brought forward conclusive evidence in the case of *Scilla* to show that size variation is a specific character and that in a species the segregation of the nucleoli of differ-
-ent sizes will always result in four microspores with different size combinations of nucleoli.

The somatic chromosomes are exactly similar to those of bisexual forms both in number (2n = 66) and morphology (Fig. 40). *Bootia* exhibits great regularity in its meiotic behaviour and thirty-three bivalents are always formed as shown in fig. 44. In twenty-two well-spaced metaphase plates observed during the present investigation, no multivalents of any type or univalents were encountered and as such no variation in the number of bodies could be detected in the polar views. Despite the fact that the species is a high polyploid, the bivalents exhibit but little secondary association and this fact must be correlated with the total absence of multivalents. Whenever the phenomenon of secondary association is manifestly clear, groups of twos and threes were found and in any case not more than three bivalents were found associated (Figs. 46, 47 & 48).

Segregation of the metaphase bivalents into two groups simulating anaphase I is a phenomenon of sporadic occurrence observed in certain pollen mother-cells. In all such cases, there is no numerical correspondence between the two groups of bivalent. For example, fig. 48 represents such a metaphase I with 19 14 arrangement. At other times still, the two groups are found bridged by a third group as is the case in the fig. 44, which roughly shows 10 + 9 + 5. It could not, however, be verified whether this is associated with spindle abnormalities or not. Vaarama (1949) gave an extensive survey of the problem connected
with spindle abnormalities and chromosome reduction in *Ribes nigrum*.

Working with *Primula kevosea*, Upcott (1939) found pollen grains with "double plates", each plate having exactly half the number of chromosomes. Probably, the same phenomenon of segregation of chromosomes explains the formation of triploid cells in a hexaploid *Triticum vulgare* (Love, 1936) and for the formation of haploid tissues with recessive genes in *Drosophila* (Brad as, 1926; Hornight, 1937), where it was presumed to provide a mechanism through which the elimination of recessive genes was effected. Huskins and his school have recently reported reduction groupings in somatic tissues of diverse plant genera (also called somatic-meiosis), when such tissues are subjected to sodium nucleate treatment (Huskins, 1946; Wilson and Cheng, 1949 and the literature cited therein). It is also known that the formation of the same type of reductional groupings can be induced by low temperature treatment (Huskins and Cheng, 1950).

The causes leading to the segregation of bivalents into groups at metaphase I and the consequences of such a phenomenon in *Boottia* sp., are little understood at present. This is perhaps an expression of its hybrid character, the chromosomes tending to segregate into two groups, each corresponding roughly to the genetic number of the parents and this is probably one of the mechanisms by means of which unbalanced polyploids revert back to the original diploid
number of the putative parents (cf. Cotton, Brown, 1947). This spontaneous segregation of the bivalents into two groups is in all probability inherent in the chromosomes of all the cells of Boottia but in some it is brought to the threshold of expression on account of some disturbances in the cell, coming either from within or from without, genetic or otherwise, or as pointed out by Wilson and Chang (1949) due to the unusual accumulation of the substances similar to Sodium nucleate.

Side views of metaphase I (Fig. 49) show different types of bivalents in variable proportions. In the case of Boottia sp., interesting is the fact that the proportion of the rod-bivalents is increased when compared with the diploid O. alismoides. While there are none or rarely one in the diploid, there are about 2 - 14 rod-bivalents in Boottia. Associated with this fact is the marked reduction in the chiasma frequency of the bivalents when compared with the related bisexual diploid and hexaploid O. alismoides. During the present investigation, it was found that the maximum number of chiasmata per bivalent is 3 in Boottia as against the corresponding number 5 or 6 in the diploid O. alismoides. As can be inferred from a comparative study of the figures 36, 37 & 38 with 49, hexaploid O. alismoides differs from Boottia sp., in having a lower proportion of rod-bivalents and a higher maximum number of chiasmata for these bivalents, which come very close to that of diploids.

There appears to be no doubt that Boottia is one of those polyploids, which show a marked reduction in the chiasma
frequency. Upcott (1939) made a comprehensive study of this reduction factor in *Primula kewensis*, *Solanum lycopersicium*, *Primula sinensis*, *Kniphofia Nelsonii*, *Campaena perscifolia* and *Allium Schoenoprasum*. Chin (1946) in *Sorghum vulgare*, *Secale cereale* and *Hordium vulgare*, Anderson and Sax (1936) in *Tradescantia virginiana* and Walters and Gerstel (1948) in *Rhoeo discolor* found reduction in chiasma frequency in the tetraploids when compared with the diploids. Among the several reasons that were adduced to explain this observed phenomenon, mention may be made of Upcott's view (loc. cit.), namely that the reduction in the chiasma frequency is due to delay and therefore partial failure of pairing following an increase in the volume of the nucleus in the polyploid, the number of chiasmata being proportional to the amount of pairing. According to her, auto- and allopolyploids do not show detectable difference in effect on the reduction factors. Anderson and Sax (1936) were of the opinion that completion delays pairing in polyploids and hence inhibits chiasma formation.

The afore-mentioned consideration while providing an explanation for the differences in the chiasma frequency between the diploid *O. alismoides* and hexaploid *Boottia*, does not, however, adequately explain the difference in chiasma frequency between the hexaploid *O. alismoides* and *Boottia* sp., both of which have the same chromosome number and morphology. Since they differ in sex, the observed differences in chiasma frequency may be related to genic causes. Or it may be correlated with 'cryptic' structural changes, which might have progressed unobtrusively in *Boottia* sp. During the course of poly-
logy and which are probably responsible for a reduction in chiasma frequency. Such an explanation is based on the assumption that the degree of pairing is proportional to the amount of homology of the paired threads and the homology is liable to change along with the structural changes in the chromosomes. Cryptic structural changes bring about a change in the homology without at the same time altering the chromosome morphology. This assumption brings to *Boottia* a higher evolutionary status on account of the greater differentiation of chromosomes, when compared with hexaploid *Q. alismoides*. This is in consistency with the unisexuality of *Boottia*, which is regarded as an advanced character as against the bisexuality of *Q. alismoides*.

Disjunction of the bivalents at anaphase I is normal, but for a few lagging bivalents (Fig.55), which vary from one to four. During interphase, a maximum number of three nucleoli was observed in each nucleus. (Fig.61).

At metaphase II, 33 chromosomes could be counted (Fig.50) and out of 20 metaphases observed only two plates, one with 34 (Fig.51) and the other with 35 chromosomes (Fig.52), showed a deviation from the usual number. This may not be ascribed to the irregular division of the multivalents, as no such multivalents were observed during the present investigation, but probably to the non-disjunction of bivalents themselves during the first division. Sometimes variation in the chromosomes number may also arise on account of cytomixis (Fig.53). In fig.52, segregation of chromosomes into groups is clear even at metaphase II.
The second metaphase chromosomes show distinct morphology. In fig. 50 two distinctly long chromosomes with median constrictions, two medium-sized chromosomes also with median constrictions and the rest of variable lengths with subterminal constrictions are clear. Since there is only one long chromosome with a median constriction in the idiogram of the diploid Q. alismoides (Fig. 2), 3 such chromosomes must be expected at the second metaphase of Boottia, on the assumption that Boottia is a hexaploid based on 11 series. But significantly enough, there are only two such chromosomes. This fact lends support to the view that structural changes in the chromosomes mark the evolutionary trend of the hexaploid races.

In an anther locule, where all the cells were at metaphase II or anaphase II, a single cell was found with 66 chromosomes (Fig. 54). This is a case of the formation of restitution nucleus as a result of either the failure of the second division or fusion of second anaphase chromosomes after division; and if such cells divide, they eventually form diploid gametes. Non-division was observed by Belling (1925) and the fusion of mitotic figures was reported by Gates (1915) in Oenothera, by Rosenberg (1917) in Hieracium, Blackburn and Harison (1921) in Rosa, Ljundahl (1922) in Papaver, McClintock (1929) in triploid Zea and Frywer (1931) in Beta.

The second division proceeds normally to form four microspores. Very rarely lagging chromosomes were observed (Fig. 56) and sometimes bridges which were not found at anaphase I
were noted at this stage (Fig. 57). However, fragments usually associated with such bridges were not seen. They persist up to telophase II as fine strands connecting the two nuclei (Figs. 59 & 60). Chromatic agglutination, described elsewhere in this paper in connection with \textit{O. alismoides}, is perhaps responsible for their formation. Chromosomes at anaphase II go sometimes astray and lie outside the spindle (Fig. 58).

At the end of second division linear, T-shaped and isobilateral type of tetrads are formed (Fig. 62-64). The nuclei show a variable number of nucleoli associated with the nucleolar globules - a fact which is in accordance with the observations made in the telophase in the somatic cells.

V. DISCUSSION.

(a) Cytotaxonomic considerations in \textit{Ottelia}.

A brief survey of the taxonomic literature shows that there has been considerable diversity of opinion concerning the generic delimitation of \textit{Ottelia}. When Persoon (1805) first founded the genus, it was thought to be wholly characterised by hermaphrodite flowers occurring singly in the winged spathes. The generic diagnosis was then based on \textit{Ottelia alismoides} (L.) Pers. Later on Wallich (1830) described the genus \textit{Boottia} and based it on \textit{Boottia cordata} Wall., which was the only species known at that time. The chief characters of this genus are: "flowers monoclinous, spathes unwinged, male many - flowered, the female one - flowered,
stamens 12, styles 9-15, the leaves with clearly defined floating laminas". In view of the obvious similarity between the two genera, Ascherson and Gurke (1889) included *Ottelia* and *Boottia* in the tribe Otteliaceae and based the generic distinction on monocliny and declivity of the two genera respectively.

Ever since that time, several new species have been described under both the genera and our knowledge about them has been enlarged. After a careful evaluation of all the characters of the species known to date, Dandy (1934), however, questioned the validity of the generic distinction of Ascherson and Gurke (1889). According to him, such a method of classification of the genera, though convenient, is highly artificial as the tendency of dioecium must have acted independently not only in the tribe Otteliaceae but also in other tribe of Hydrocharitaceae and hence may not be of generic importance. Emphasizing the fact that it would only lead to the separation of two closely allied plants of the same genus, Dandy (1934) merged the unisexual *Boottia* in the older bisexual *Ottelia*. If the concept laid down by Dandy (*loc. cit.*) is acceptable, then the genus *Ottelia* has a much wider generic boundary than that envisaged by Persoon (1805) and the tribe Otteliaceae is monotypic (*cf.* key to the Subfamilies, Tribes and Genera of Hydrocharitaceae by J.E. Dandy, in *The Families of the flowering plants*, J. Hutchinson, 1934, Vol. II, pps. 30 & 31).

From the foregoing account it is clear that the group
of unisexual forms in question has thus had a varied taxonomic history, being regarded either as distinct species of a large genus *Ottelia*, or as constituting a distinct but closely related genus *Boottia* Wall, of the tribe *Otteliaceae*. That there exists a certain degree of affinity between the two types of forms is certain and cannot be questioned and in fact it was recognised by Ascherson and Gürke (1889), when they founded the tribe *Otteliaceae* embracing both the genera. But the entire previous confusion in carving out the generic boundary of *Ottelia* was due to the difference in the emphasis laid by the different systematists on the unisexuality and the bisexuality of the different forms.

\[ \text{Monocotyledons are replete with instances, where non-} \]
\[ \text{adaptive specific and generic differences have arisen as a} \]
\[ \text{result of spontaneous mutations due to inherent factors (Arber,} \]
\[ \text{1925); in some cases, the specific differences were found to be} \]
\[ \text{of the same character as differences which were used to distinguish the largest groups (Eg. *Eriocaulon*, Fyson, 1921; *Trillium* and *Paris*, Gates, 1917; *Yucca* and other sub-families of *Liliaceae*, Baker, 1875). In all such cases, over-emphasis on a single differentiating feature may elevate a species to generic status as is the case with *Boottia*. In such circumstances, cytology is a valuable guide to taxonomy.} \]

It has been pointed out earlier that the basic number of the genus *Ottelia* is 11, and that there are diploid, tetraploid and hexaploid chromosome races within the species *O. alismoides*. Available cytological data on *Boottia* sp., show that
it exists only as a hexaploid and that the chromosome numbers \( n = 33 \) and \( 2n = 66 \) are also based on 11-series. In this respect, *Boottia* sp., comes very close to *Ottelia alismoides*. A careful analysis of the chromosome morphology at metaphase II of these unisexual forms in comparison with the hexaploid *Ottelia* confirms the same conclusion. In hexaploid *Ottelia alismoides* there are only two long chromosomes with median constrictions (Fig. 34) at metaphase II and the same number of long chromosomes was also found in *Boottia* sp., (Figs. 50 & 51). This evidence, though not conclusive in itself, is cogent enough tending to provide cytological confirmation for the amalgamation of the genus *Boottia* in *Ottelia* and for the consideration of the unisexual forms as species of the much older genus *Ottelia*. Conversely, it may be argued that two different genera of the same tribe as *Ottelieae* may have the same basic number and hence need not necessarily imply amalgamation. But the close resemblance in the external features of the two types of forms along with the similarity in the chromosome types lend special credence to such a view. In fact, when one is confronted with *Boottia*, there appears to be something almost persistently reminiscent of *Ottelia*, both from morphological and cytological points of view. To add to this totality of evidence, *Boottia* has approximately the same range of geographic distribution as *Ottelia*.

That the genus *Boottia* shows a natural tendency to merge with *Ottelia* may also be shown by certain intermediate forms collected during the present investigation.
These look exactly like *Boottia* sp., in all essential features like the height and the shape of the leaves but for their bisexual *Ottelia* - like flowers. This morphological similarity is also borne out by their chromosome complements. All of them are characterised by 2 n = 66 chromosomes and two pairs of long median chromosomes in the karyotypes (Fig. 41) like the hexaploid *Ottelia* and *Boottia* sp. In view of this morphological and cytological coincidence between the bisexual forms and the forms of *Boottia* sp., even a casual observer is led to think that *Boottia* sp., is probably polygamo-dioecious. Whether this is a fact or not, it must be admitted that such bisexual forms act as connecting links between *Boottia* on the one hand and *Ottelia* on the other (Sunder Rao, 1951).

All the above mentioned evidence goes to show that if the several unisexual and bisexual forms be not grouped under a single composite genus *Ottelia*, it would result in 3 or 4 unnatural genera, like the former *Boottia* Wall; *Ottelia* Pers., *Xystrolobus* Gangep and *Oligolobus* Gagnep, which would certainly prove cumbersome to any student of phylogeny. It must, however, be mentioned that the ultimate test of this problem lies in the hybridisation experiments involving species of *Ottelia* and *Boottia*. If they form at least partially fertile hybrids, then the amalgamation of both the genera will be based on the genetic foundation. As remarked by Stebbins (1950), "if two species supposed to belong to different genera do form partially fertile hybrids, as with maize X teosinte (*Zea X Tripsacum*) the two 'genera' are probably artificial groups, which should be merged".
A parallel instance is provided by the two genera *Hebe* and *Veronica*, which were formerly separated according to the mode of dehiscence of their capsules. However, Frankel (1941) working on the cytology of the two genera found that certain New Zealand species of *Hebe*, which were formerly assigned to *Veronica*, are characterised by the same basic number as *hebe* (two polyploid series, based on \( x = 20 \) and \( x = 21 \)) and are different from the typical *Veronica* in this respect. A re-examination of the type of capsular dehiscence showed a close similarity to that of *Hebe* and hence all the species were transferred to that genus.

The use of cytological data to decide and to illuminate the taxonomic relationships at the generic level has been emphasised by Smith (1932) in *Anchusa* and by Menzel (1950) in certain Solanaceae like *Margaranthus*, *Saracha* and *Quincula*. Senn (1938) evaluated the generic status of *Chamaecrista* Moench on the basis of the chromosome numbers and observed that within the limits of this genus fall all those species of *Cassia*, which show a polyploid series based on 2 and in contrast to the numbers \( n = 6, 13 \) & 14 which also exist in the genus *Cassia*. Babcock (1947) brought forward and integrated the morphological, cytological and genetical evidence to bear upon the two former monotypic genera *Phaeacium* and *Cymbosperm* and reduced them to two closely related species of *Crepus*, namely *C. pulchera* and *C. palaestina*. He considered these two species under the same subgroup of the same section, which bears the name of the older of the monotypic genera, *Phaeacium*. 
(b) **External morphology and taxonomic status of the intra-specific chromosome races.**

It was formally assumed and taken for granted, both by cytologists and taxonomists, that the so-called Linnaean species are stable units of classification with regard to the external morphological features and the chromosome numbers. In fact, the older concept of the species is based on this fundamental assumption of stability (cf. Darlington, 1951). But as a result of closer and intensive studies of species populations with the advent of newer and easier cytological techniques, it is now becoming increasingly evident that some of the highly polymorphic species with a wide range of ecological preferences and geographic distribution may be characterised by more than one chromosome number.

This fact has been amply demonstrated with reference to a large number of species. For instance, Münzting (1936) estimating such species at one hundred at the lowest, listed about 58 instances and gave an admirable 'resume' of the whole subject. Later on Löve and Löve (1942) have showed that 7% of the Scandinavian species contain karyological races. Recently Tischler (1950) has remarked that 7% of the total flora of the northern Europe has species with oscillating chromosome numbers. More recently, Löve (1950 and 1961) has discussed about 30 such instances in order to bring out their taxonomical importance. References to species having chromosome races were also given by Clausen (1931), Brunn (1932), Darlington (1937), Fagerlind (1937), Arwidsson (1935 and 1943), Manton
(1950) and Hulten (1950), who dealt with some of the problems connected with intraspecific polyploidy. Gustafsson (1947) has considered the intraspecific polyploidy in apomitic species.

The discovery of this fact has brought in its wake a serious problem of the taxonomic status of such plants with variable number of chromosomes. Varied are the opinions expressed in this connection and diverse is the terminology that has been multiplied with a view to designate such plants. They have been referred to in literature as sub-species, cryptospecies, polyplootypes, varieties, forms and types but a majority of cytologists have described them as 'intraspecific chromosome races' (Müntzing, 1936 and Darlington 1937). No attempt has been made by the previous authors, except Müntzing (1936, p.326) and to some extent Löve (1951) to clarify the connotation of the different words mentioned above with a special reference to the already existing biological unit, namely the species, in which they are supposed to exist and with which they are intimately connected. Maybe, the exact taxonomic status of the 'chromosome races' is difficult to comprehend and not easy to define, especially when the concept of the species itself is in a fluid state. For the taxonomic evaluation of the chromosome races, the formulation of any single concept, which would find universal applicability may not be possible, just as Gates (1951) has recently pointed out at "an inclusive definition of species which would apply to all crops of organisms is———of no value, even if attainable" (cf. also Turrill, 1936 and 1938).
To some extent personal judgment also comes in while deciding such cases as some taxonomists are "ultralumpers" and others "ultrasplitters".

There are primarily two schools of thought with regard to the taxonomic status of the "chromosome races". Müntzing (1936) adducing strong evidence in favour of auto-polyploidy as a factor in speciation emphasised that the morphological variation exhibited by the races is always continuous and as such it is difficult for the taxonomists to recognise them from the distinctly separated units called species. In all such cases, he therefore preferred the usage of a perfectly neutral term "race". It must, however, be pointed out that he very carefully weighed the evidence and cautiously expressed the opinion that "if a new polyploid chromosome race is formed and observed by taxonomists it will get a taxonomical value, which is proportional to the degree of morphological divergence and to the sharp sightedness of the observer". He further added that if also the new ecological properties and the new distribution are observed, the race will get a fairly high taxonomical value and even be considered as new species, unless morphological differences are too slight". On the other hand, Nannfeldt (1935) was strongly of the opinion that "as soon as the chromosome races are morphologically distinct and thus recognisable to the taxonomist, they had better to be recognised as species, even if the morphological characters are small". Following this idea, Löve (1950 and 1951) showed that the chromosome races of certain
collective species, in the flora of the northern Europe were once treated as separate species or at least as varieties by the classical taxonomists and that in all such cases the re-establishment of the chromosome races as distinct species is essential. In order to illustrate the point in question, he brought forward evidence from nearly thirty instances of such collective species, admittedly saying that this concept is applicable only to floras that are well investigated. Gustafsson (1947) expressed the opinion that when two populations are no longer able to recombine freely, they function as the two distinct species even though they are not morphologically differentiated. According to him such conditions are often obtained in plant kingdom, when a simple increase in the chromosome number takes place. Among others, who discussed the taxonomic importance of the chromosome races, mention must be made of Hall (1937), Turrill (1938) and Ruxley (1944). Darlington (1941 and 1951) also discussed the importance of intraspecific polyploidy in taxonomy.

If chromosome races are to be regarded as species, the concept of species must be applied to them. Geneticists and taxonomists appear to be agreed now that isolation is an important aspect of speciation. The change in the number of genomes, which is an essential property of all intraspecific chromosome races leads to one type of isolation of plants, namely the reproductive isolation. Diploids and hexaploids always fail to cross and even if they cross the offspring would be sterile. This is the bone of the contention of all
those, who affirm that polyploid chromosome races must be regarded as distinct species, despite the fact that the morphological dissimilarity is little. However, the taxonomist is in main concerned with the phenotypic characters which must be distinct so as to enable him readily to recognise the races in the field. As Gates (1951) has recently pointed out, "the science of taxonomy is founded on the existence of visible differences by which forms can be classified". To designate two morphologically more or less identical forms as two species because they are characterised by two different chromosome numbers" involves contradiction in terms" (to borrow the wording of Gates). It is true that two intraspecific chromosome races do not cross. Apparently, this sterility barrier is of no value to the taxonomist, when it is not accompanied by distinctly visible morphological differences. Reproductive isolation as the criterion of specification has been the subject of scathing criticism by Gates (1951), who has brought forward a volume of objections against it. If this is so, the other types of isolation, namely geographic isolation (as in Biscutella laevigata, Manton, 1937; other ref. in Høntzing, 1936) and ecological isolation (as in Eysotis, Geitler, 1936) are also of no avail to the taxonomist and do not constitute valid criteria for erecting new species out of intraspecific chromosome races. This concept laid down in this paper, which is at variance with that of Nannfeldt (1938) may not be applicable to races of all collective species but appears to be true in the case of O. alismoides, as borne out by the following discussion. Before splitting a "collective species" into different biological units called
species, judgement must therefore be exercised in each individual case. In general morphological features, diploid forms of *O. alismoides* appear almost similar to the hexaploids as there are no sharp or marked differences distinguishing them. This lack of differentiation is interesting and may be due to the origin of the hexaploids from two putative parents, which closely resemble the hexaploids themselves. It is, therefore, not surprising that intraspecific chromosome races of *O. alismoides* escaped the attention of the systematists. Notwithstanding this fact, diploids present certain differences when compared with the polyploids, no matter whether the differences are striking or not to merit taxonomic recognition. While drawing the following comparison between the different polyploid chromosome races, considerable emphasis has been laid on the various characters that were brought into play by Dandy (1934) in the speciation of the genus *Ottelia*.

**Gigantism:** The statements of Münzinger (1936) that "on an average there is a marked positive correlation between the chromosome number and gigas characters and that the gigas characters of polyploid chromosome races are caused by the higher chromosome numbers" are applicable to *O. alismoides*. Many of the diploids are a little shorter in stature and generally have slightly smaller leaves and flowers than those of the polyploids, though the size relations are not always absolute. The gigantism is particularly manifest when diploids and polyploids occur together in the same pond, as is the case in certain areas of Andhra Deza. Whenever it is so, diploids are invariably marginal and polyploids are deep water forms.
TABLE II.

Showing a comparative quantitative estimate of the various characters of the diploid and hexaploid forms to evaluate their taxonomic importance.

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<td>No. of units studied</td>
<td>$\bar{X}$</td>
<td>S.D.</td>
</tr>
<tr>
<td>1.</td>
<td>Pollen diameter</td>
<td>313</td>
<td>52.96 $\mu$</td>
<td>1.15</td>
</tr>
<tr>
<td>2.</td>
<td>Pollen sterility</td>
<td>600</td>
<td>1.18 $%$</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Length of the leaves (including petiole)</td>
<td>73</td>
<td>8.31</td>
<td>1.90</td>
</tr>
<tr>
<td>4.</td>
<td>Breadth of the Lamina.</td>
<td>68</td>
<td>3.51 $%$</td>
<td>0.63</td>
</tr>
<tr>
<td>5.</td>
<td>Length of flowers.</td>
<td>24</td>
<td>1.77 $%$</td>
<td>0.13</td>
</tr>
<tr>
<td>6.</td>
<td>No. of wings.</td>
<td>23</td>
<td>4.50 $%$</td>
<td>0.31</td>
</tr>
<tr>
<td>7.</td>
<td>Length of sepals.</td>
<td>23</td>
<td>0.48 $%$</td>
<td>0.046</td>
</tr>
<tr>
<td>8.</td>
<td>Length of petals.</td>
<td>18</td>
<td>0.81 $%$</td>
<td>0.083</td>
</tr>
<tr>
<td>9.</td>
<td>Number of stamens.</td>
<td>24</td>
<td>6.67 $%$</td>
<td>0.87</td>
</tr>
<tr>
<td>10.</td>
<td>Number of styles.</td>
<td>24</td>
<td>7.25 $%$</td>
<td>0.94</td>
</tr>
<tr>
<td>11.</td>
<td>Number of carpels.</td>
<td>24</td>
<td>7.25 $%$</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Hexaploid significant over diploid at 0.1 %

Difference not significant.
This also is in agreement with the general rule that the height of the plant is dependent on the depth of the pond at which it grows. Though diploid forms of \textit{Boottia} have not been discovered so far for comparison, the hexaploids forms of the same approach the hexaploid \textit{Ottelia} in the gigas characters.

The gigantism is particularly clear in the case of certain unicellular structures like pollen grains. Comparatively the pollen grains of the diploids are invariably smaller than those of the polyploids. The same is the case with the unicellular hairs that are found on the filaments of the stamens. They are always short and stumpy in diploids while those of hexaploids are long and sometimes forked at the apex. Apparently, ever since the origin of the polyploids, sufficient time has not elapsed for segregation and mutation of the polyploids back to the original diploid size. One of those other reasons adduced by Darlington (1937) for the loss of gigantism is the structural changes of chromosomes. There is a slender thread of evidence to show that relatively the chromosomes of the polyploids have undergone some structural changes. Inspect of them, they have presumably still retained the gigas characters.

If at all a distinction is to be sought between the diploids and hexaploids, it must be based on this cellular gigantism. It is for the taxonomists to decide how far this is a valid systematic character.
Shape of the leaves. Broadly - ovate or elongate - ovate leaves with less cordate bases are the chief types of the leaves encountered in all the diploid forms. Such leaves appear to differ superficially from those of polyploids, which are found to possess large, broadly - ovate but with deeply cordate bases. A closer examination, however, shows that all the types of leaves encountered in polyploids are only exact but enlarged replicas of the diploid forms, as deeply cordate leaves are also a feature of certain diploids, collected from Junagarh. Moreover leaves of two different shapes may be found in one and the same form, depending on their position on the plant body. All these facts obviously point to the conclusion that the shape of leaves is not by itself a taxonomic character in O. alismoides, as the distinction, if any, is of a negligible nature. It must be remarked here that speciation in certain polymorphic genera of Hydrocharitaceae like Ottelia, based on the shape of the leaf alone will be highly artificial and will lead to chaos in taxonomy, unless it is uniformly consistent and associated with other morphological differences.

Serrulation of the leaves. Polyploid forms collected from Bengal, Orissa, Sagar and Malaya are characterised by very fine serrulation all along the two margins of petioles, sometimes confining to the lower part of the petioles or only to the leaf bases. At other times still, it extends to the basal part of the laminae also. On the other hand, there is no serrulation at all in the diploids. This point of distinction, however, breaks down, if it is remembered that leaves with and
without serrulation are found in one and the same polyploid specimen. The entire absence of serrulation in certain other polyploid specimens reveals at once the unreliability of this character in the taxonomy of the species.

The spathè wings. Dandy (1934) laid considerable emphasis on the presence or the absence of the spathè wings and their number in the speciation of the genus. Based on these facts, he recognised three groups of species, which are of subgeneric rather than of generic status, namely (i) those without wings (ii) those with two wings and (iii) those with 5 - 10 wings. Sometimes, males and females of dioecious species are respectively characterised by the absence and the presence of wings, as is the case in P. Thorellii Gagnep., (Dandy, 1934). Comparing the diploids and the polyploids from this point of view, a correlation between the nature of the spathè wings and the chromosome number is possible. It has already been pointed out earlier in this paper that in all the diploid forms, the usual number of wings is five, of which two are conspicuously large and are situated opposite to each other on the spathè. Probably one is developed along the midrib of each bract. The rest, which are always a little smaller in size, are disposed in a characteristic way with reference to the bigger wings. It has also been pointed out that significantly enough if the number increases or decreases, it is due to the addition or the non-development of the smaller wings but not of the larger ones. In the case of the hexaploids, however, the inequality of the spathè wings, so
pronounced in the diploids, is not marked at all. Conversely, the hexaploids are usually characterised by five or rarely six wings, all of which are more or less equal in size. At any rate, the two large wings found in the diploids are not so conspicuous in the polyploids. It is interesting to note that the increase in the chromosome number can be correlated with the equality in the size of the spathe wings. Thus, while a line of demarcation can be drawn between the diploids and the hexaploids on the basis of the wings, such a distinction, however, breaks down when wingless diploids and polyploids are considered. Furthermore, the range of variation in the number of wings in both the groups of chromosome races is 0 - 6, a fact, which obliterates the supposed distinction between the two groups of forms. Winging, therefore, appears to afford little clue to the separation of the various chromosome races studied during the present investigation and as such it must be discarded.

The time of flowering: The hexaploid forms of *Q. alismoides* bloom a little later than their diploid relatives. It is difficult to assess at present how far this little change in the flowering time is geographically advantageous to the polyploids over the diploids. It is in all probability due to the slowness of the growth rate and hence of the development of the polyploids. From time to time, such observations have been made in some comparative studies of the diploid and polyploid races and species. For example, Blackeslee (1941) in tetraploid *Petunias*, Manton (1935) in the polyploid *Nasturtium officinale* and Anderson (1941) in
the tetraploid American *Tradescantias* found the same. However, the evidence presented by Muntzing (1936) in the case of *Nymphaea* and Clausen, Keck and Kiesey (1940) in *Artimisia* and *Potentilla* does not consistently lead to the above conclusion but tends to show that sometimes opposite is the case in certain polyploids.

**Colour of the flowers:** All the diploids are white flowered, while the hexaploids are either white or pink. Since white colour is found in all diploids and also hexaploids, pink colour is a derived character owing probably to gene mutation. Pink coloured flowers were not mentioned by Hooker (1899). This variation in the colour of flowers, which is characteristic of hexaploid *Ottelia* is shared by the hexaploid *Boottia* species. That a similar type of mutation is also responsible for the change in the colour of the hexaploid *Boottia* sp., resulting in the same type of colouration is most interesting.

**Stamens:** In diploids, the number of stamens vary from 6 to 9. Approximately, the same variation (5 to 10) is also shown by the hexaploids. The stamens are of unequal lengths in the diploids but this variation was found to a greater extent in the polyploids. It has reached an extreme expression in the case of *Boottia*, which is also a hexaploid, where there are constantly six long and six short stamens.

There is, however, a point of distinction in the shape of the filaments and the mode of the attachment of anther lobes.
to the filaments. Invariably, there is a marked constriction at the region of attachment of all diploids and it is absent in the polyploids. The reliability of this character as a point of taxonomic distinction between the chromosome races must remain a matter for further scrutiny and verification. The hairs on the filaments of the diploids are short and stumpy, while those of the polyploids are long and sometimes bifurcated at the tip.

**Pollen grains**: Pollen grains are invariably bigger in polyploids (cf. Table II) than those of diploids.

**Styles**: Styles are usually as many as carpels and no distinction is possible between the diploids and the polyploids from the point of view. They are unequal in length in both the races and perhaps the inequality is more distinct in polyploids than the diploids and as such it cannot serve as a distinguishing character. As is the case with the staminal hairs, the hairs fringing the margins of the styles and stigmas of the polyploids are always bigger than those of the diploids.

**Carpels**: The number of carpels vary from 6 to 9 in the diploids whereas in polyploids the range of variation is 6 - 11. This difference between the two is not significant, so as to form a taxonomically important character.

**Fruits**: They are longish-obleng in the polyploids and in the diploids they are a bit broad and short. The wings to
the spathe persist and they confirm to the same pattern described elsewhere in this paper.

From the foregoing consideration of the morphological characters, some of which are alleged to have played a part in speciation of the genus *Ottelia*, it is reasonably evident that the diploid and the polyploid forms of *O. alismoides* cannot be separated from each other into distinct units called species, merely on account of the difference in the chromosome number. There appears to be no character or a group of characters, which can be regarded as criteria in recognising the two groups of forms as constituting distinct species. With regard to certain characters, the diploids are no doubt characterised by certain range of variability but the same type of variation is also shown by the polyploids but for the difference, namely, that the range of variation is wider and complex in the polyploids. A distinction based on these characters fails at one stage. In other words, the range of variability from the diploids to polyploids instead of being sharply discontinuous is of a continuous nature with an intergradation of characters. This is not unexpected as all polyploids tend to be continuous owing to the presence and the cumulative action of many genes, which govern the expression of the same character.

Hence, it is best to regard *O. alismoides* as a "collective species" containing diploid, tetraploid and hexaploid chromosome races, instead of separating the races into distinct species. While considering the species from a comparative rather than evolutionary point of view, Darlington (1933) recognised eight
types of species, among which mixed species containing polyploids chromosome races is one. *Ottelia alismoides* approaches this type according to Darlington's terminology. In the present case, the application of the terminology of Clausen, Keck and Hiesey (1945) and so of Gustafsson (1947) is unwarranted for want of cytogenetical data, although intraspecific chromosome races seem to approach ecospecies and coenospecies. Perhaps, they may appropriately be described as "Cryptic species" (Stebbins, 1950, p.193; Darlington, 1944, p.148), which are still a long way off from the species.

Intraspecific chromosome races, which are morphologically indistinguishable and taxonomically belonging to one unit are known in literature. Baldwin (1936) discovered diploid and tetraploid chromosome races in *Sedum ternatum* and emphasised that taxonomically all the seven lines of plants reported in his paper belonged to the same species. The two forms of *Citrus microcarpa* reported by Nakamura (1939) are similar. After a careful study of a number of morphological and physiological characters of diploid, triploid and tetraploid strains of *Canä aurea - vittata* and *C. humils*, Oomen (1948) came to the conclusion that the respective races belong to the same species. Hall (1937) working with *Tulipa* pertinently remarked: "It still remains a matter of dispute, whether polyploids should be assigned specific names of their own. For example, *T. Clausina* is a pentaploid and the corresponding tetraploid from the N.W. Frontier of India, though it has been named as *T. chitralsensis*, cannot be distinguished macroscopically from the pentaploid;
are diploid and triploid forms to have specific names? The distinction between diploid and triploid is absolute, something different in kind from that between two closely related species, 'both diploid'. Anderson and Sax (1936) in the case of the diploid and autotetraploid races of *Tradescantia hirsutiflora*, *T. occidentalis* and *T. canaliculata* and Winge (1938) in the case of the different chromosome races of *Narcissus Bulbocodium* also expressed the view that intraspecific chromosome races should not be regarded as distinct species.

(c) *Polyploidy and the geographic distribution of Ottelia alismoides.*

The part played by polyploidy in the geographic distribution of species has been the subject of considerable discussion for the past two decades and several are the conflicting views that have been expressed in this connection. Except certain aspects of this problem, which are thought pertinent from the point of view of *Ottelia alismoides*, no attempt has been made here to review the vast literature that is now available on this subject. Instead reference is given to such exhaustive reviews and important publications as those of Cain (1944), Fagerlind (1937, 1944), Flovik (1938, 1940), Gustafsson (1947, 1948), Hågerup (1932, 1939, 1940, 1941), Löve and Löve (1943, 1949), Münzing (1936), Rohweder (1937, 1938), Stebbins (1940, 1942, 1950) and Tischler (1935, 1937, 1942, 1946).
While drawing attention to the differential behaviour of the auto- and allopolyploids in their reactions towards geographic distribution, Stebbins (1940) pointedly remarked that "the strictly autopolyploid forms are almost as restricted in distribution as the diploids, while the farther edges of the range of the group are occupied by allopolyploid forms of complex origin". In order to indicate that this tendency varies in different genera, he further added; "In *Crangis*, allopolyploidy is the important factor, while in *Tradescantia* the increase in the distribution is the result primarily of chromosome doubling or autopolyploidy". Therefore, it seems imperative to take into account the mode of origin and the nature of species before evaluating the role of polyploidy as a factor in determining the range of geographic distribution.

In the words of Charles Darwin, *Ottelia alismoides* is a wide ranging, much diffused and common species. Its variability is in main reflected in the presence of diploid, tetraploid and hexaploid chromosome races. There is no doubt that this intraspecific polyploidy is responsible for its wide spread geographic distribution in S.E.Asia, eastern tropical Australia and N.E.Africa. The related unisexual species of *Bouttia*, which is hexaploid, is not only found within the above mentioned range of distribution but has also extended to New Caledonia and S.E. South America. Apparently, chromosome reduplication has produced remarkable changes in the reaction norms of the plants,
which enabled them to extend their geographic boundaries.

Hagerup (1931, 1932, 1939, 1940) has come to the same conclusion and emphasised that "with polyploidy, there may follow a change in the ecological and plant geographical value of the plant". (c.f. also Navashin, 1929).

Detailed study of the geographic distribution of the chromosome races of *O. alismoides* in India, which will be described in the later part of this paper, has revealed that not only the species as a whole has a wide distribution but that each race has a geographic range of its own, which is directly proportional to the chromosome number. Relatively, the diploid forms are extremely localised to particular regions while hexaploids practically cover almost all parts of India. Out of curiosity, this investigation was extended to other regions of S.E. Asia and the examination of the root-tip material obtained from Malaya showed that only hexaploids occur in that region. Later on, this fact was confirmed by the examination of a large number of herbarium specimens collected from different localities of Malaya.

Furthermore, a study of the herbarium sheets obtained from Indo-China, China (Yunnan), Java and Australia in comparison with the type specimens of the polyploids collected from India showed that only polyploids occur in those regions also. It must, however, be admitted that the inference of the chromosome number based on the external morphology alone is extremely unreliable; but the general tendency towards
gigantism exhibited by all parts of the plants collected from different localities of S.E. Asia provided a clue for such an inference, which in all probability appears to be true. Hence, a generalised statement, namely that polyploid forms of *Ottelia alismoides*, particularly hexaploids, are widespread in all parts of S.E. Asia and that diploids are extremely restricted to certain parts of India, may be hazarded. It may here be pointed out that the tendency towards wide distribution is not only determined by the duplication of the genomes, as pointed out earlier, but also perhaps on the store of variability in characters of great adaptive value from which plants with new and different combination of characters may be formed and selected in order to suit the new territories. A logical deduction from this is that wider distribution is concomitant on the wider variability of plants, which in its turn is conferred by polyploidy. In contrast to this, diploids, which are characterised by only two sets of chromosomes cannot stand the environmental selective factors other than those in which they live. Hence, they necessarily become restricted to particular and stabler localities within the range of distribution. Evidence from the geology of India that will be presented in the subsequent pages of this paper goes to prove the correctness of the later part of the statement in connection with *O. alismoides*. This is somewhat similar to the situation in *Grindelia*, where the diploid species from a phylogenetic standpoint show ancient dispersal in geologically older territory, while the tetraploids show
recent dispersal on geologically youthful regions of N. America (Whitaker and Steyermark, 1935).

That the chromosome races have a geographic range of their own and it is always wider for the polyploids when compared with their diploid progenitors has been demonstrated though not consistently, in a number of cases of intraspecific and interspecific polyploidy. For example, Münsting (1936) showed that in 30 out of 38 cases for which information was available, the intraspecific chromosome races showed differences in ecological properties and hence in the geographic distribution. Manton (1934) found the diploid relicts of Biscutella lavicata were confined to narrowly restricted areas, whereas the polyploid forms are widely distributed in regions that were covered by the ice sheet. Anderson and Sax (1936) studying the American species of Tradescantia allied to T. virginiana, found that the tetraploid races and species have a much wider range of distribution than their diploid relatives. The average "diameter" of the distribution area of the tetraploids were 613 miles in contrast to 289 miles of the diploids. Giles (1940) in Tradescantia rosea var. graminea and Baldwin (1942) in Sedum ternatum also proved that polyploid forms have a wider distribution than the diploids.

The postulate that the polyploids are wider spread than the diploids is by no means universal and that the reverse can happen has been shown again by certain instances of intraspecific and interspecific polyploidy.
For instance, in *Nasturtium officinale*, the diploid forms have a wider distribution than the tetraploids, which are limited to certain localised northern parts of the range of the species (Manton, 1935). Similarly, Tschermak-Woess (1947) discovered no significant differences in the geographical and ecological distribution of diploid and tetraploid forms of *Allium carinatum*. In the genus *Acacia*, Aitchison (1948) found no positive correlation between polyploidy and hardiness nor a wider dispersal than the diploids and therefore concluded that polyploidy had provided no greater degree of adaptability than gene mutations. In this relationship between polyploidy and geographic distribution, it is sometimes remarkable to note that different sections of the same genus may show opposite tendencies. Erlanson (1929) studying *Kose*, observed that the diploid and the tetraploid species of the section Carolinae had essentially the same distribution, while in the section Cinamomeae, the tetraploids were most limited and the diploids widely distributed.

The foregoing review, though incomplete in itself, nevertheless indicates that the data at hand may not warrant any generalised statement regarding the relationship between polyploidy and geographic distribution. However, it may be pointed out that all those who upheld the view that polyploidy extends the range of geographic distribution are of the opinion that a change in the chromosome number brings about a greater degree of adaptability and tolerance to
varied environmental ecological conditions and extremes of climates. This idea is in keeping with the theory of specific tolerance propounded by Godd (1931), as changed physiological conditions set up in the polyploid will result in a change in the specific tolerance, which will enable them to occupy new ecological niches or endure climatic conditions, unsuitable for the related diploid.

This has been consistently shown by a number of cytologists. Hägerup (1932) was first to demonstrate it in the case of the desert flora of Timbuktu and very soon he was followed by Shimotomai (1933) and Tischler (1933, 1946) who worked on *Chrysanthemum* occurring along the coastal regions of Japan and on the flora of Schleswig-Holstein respectively. Müntzing (1936) applied the same principle in order to explain the wide geographic distribution of the polyploid chromosome races. Tischler (1937) and Wulff (1937) working on the halophytic flora, Sokolovskaya and Sterelkova (1940) on the flora of the Caucasus and the corresponding flora of the Pamirs and Altai supported Hägerup's conclusion (*loc. cit.*). Flövik (1940) brought forward evidence to show that a tendency towards polyploidy increases the adaptability of the plants in the Arctic regions and that polyploidy played an important part in the origin of new forms and species that colonised such regions. Löve and Löve (1943) by close statistical investigations on the flora of Europe and other regions explained the greater frequency of polyploids in higher latitudes and higher altitudes as due to their greater hardiness and
according to those authors, the greater tolerance of autopolyploids to photoperiod conditions of long days may be an additional factor. Sax (1937), on the other hand, maintained that although extremes of temperature are responsible for the induction of polyploidy in diploid races, it is was probable that polyploids originated prior to the extension of the range of the diploids and that they survived in extremes of climates on account of greater hardiness.

Whatever may be the case, Sax (1937) in all essentials supported the hypothesis of all the previous workers. Cain (1944) discussed the subject at length and Löve and Löve (1949) gave full list of references in this connection.

However, the thesis of better adaptability of polyploids to extremes of environmental climatic conditions has not received general acceptance. This hypothesis, that was mainly based on the geobotanical studies of the flora of temperate Europe, which suffered extensive glaciation in the Pleistocene period, has not been demonstrated so far to be correct in the unglaciated tropical areas. Every piece of work mentioned in the preceding paragraph, conducted with reference to a particular type of flora has its own exception showing thereby that the thesis is by no means universally applicable (Stebbins, 1950). The main tenet has been consistently disputed by a number of workers. For instance, Bowden (1940) failed to find any correlation between hardiness and polyploidy but ascribed the variation in the degree of hardiness to genic differences. According to him, although
the latter is effected by polyploidy, it by itself cannot induce hardiness in genera, which are devoid of such genes. Clausen, Keck and Heisey (1940, 1945) arrived at the same conclusion with reference to certain Western American genera. Nielson (1947) discovered no correlation between hardiness and polyploidy in *Punica*.

One of those, who challenged the view that polyploidy increases hardiness and therefore extends the geographic distribution, is Gustafsson (1948). Presenting evidence from the genus *Carex*, certain monocotyledons and dicotyledons of Scandinavia, he came to the conclusion that diploids are in no way inferior to polyploids in regard to their abundance and distribution. After a careful evaluation of the different statements mentioned above with reference to extreme alpine, arctic and halophytic floras, Gustafsson (1948) came to the conclusion that polyploidy is in no way related to the extreme environmental conditions but that it is conditioned by life-form, perennial habit and vegetative reproduction.

Gustafsson (1948) further pointed out the inherent defect in accepting the previous conclusion of Tischler school based on computations and comparisons of relative frequency of diploids and polyploids in entire floras. The percentage of polyploidy varies from family to family and if such families like Rosaceae and Gramineae, which are now
known to be polyploid, predominate in a flora, the percentage of polyploidy in that flora also automatically goes up. Furthermore, since perennial and woody genera tend to be polyploid (Müntzing, 1936; Senn, 1938; Stebbins, 1938; Baldwin, 1940 and Perry, 1943) and if they are fairly well represented in a particular flora, an element of error is introduced into the calculations and the conclusions based on them will be unreliable. Some of these objections were also pointed out by Stebbins (1950).

Notwithstanding this sharp cleavage of opinion, it appears to be true that polyploidy played an important part in the wide geographic distribution of *Ottelia alismoides* in S.E. Asia and elsewhere. All the objections raised by Gustafsson (1943) and Stebbins (1950) are probably not valid in the present case. It will be shown in the later part of this paper that the incidence of polyploidy in *O. alismoides*, at least in India, has nothing to do with glaciation or any other drastic topographic changes in the Pleistocene period. Moreover, we are here concerned with a "collective species", the chromosome races of which differ little in their external morphology. Admitting for the moment that the question of geographic distribution of plants is complicated and many-sided and that it is dependent on some internal factors of plants irrespective of polyploidy, there is every reason to assume that this internal factor is determined by polyploidy in the case of *Ottelia*.
alismoideae. It is true that diploids with a wealth of ecotypes are as efficient as their polyploid relatives in expanding the distribution areas (Stebbins, 1950). The present investigation is enough to show that the diploid O. alismoideae is not poor in forms and types* (see the description under II A(1)); still they are localised in distribution and relatively less abundant than the polyploids. This shows clearly that polyploidy accounts for the wide geographic distribution in O. alismoideae, proving thereby the hypothesis first suggested by Hagerup (1932) and later on supported by Tischler (1935) and Mántzing (1936).

* Whether these types are to be designated as ecotypes or not is doubtful at present; but such a possibility is not excluded, as definite types occur in particular areas.
(d) The region of greatest diversity in India and some considerations regarding the centre of distribution of the chromosome races:

During the present investigation, it has been amply demonstrated that *Ottelia alismoides* contains three distinct physiological elements, which are separable only by cytological means and that the cytological variability of the species is the chief cause of its wide distribution in S.E. Asia and elsewhere. According to Vavilov (1940) certain regions of S.E. Asia are characterised by a great diversity of varieties of endemic species, where initial steps for the process of differentiation of the Linnean species may be followed. The very fact that *O. alismoides* occurring in this region has different chromosome races lends abundant proof to the later part of this statement.

Though the races of *O. alismoides* are morphologically and taxonomically indistinguishable, they have a geographic range of their own. It has been pointed out earlier that if India is taken as a geographic unit in the vast range of distribution, the available evidence tends to show that the diploid forms have a relatively smaller and restricted range of distribution, occurring in a tract between Poona on the west and the Andhra area on the east and also in Junagarh (Saurashtra). On the contrary, the hexaploids are found in almost all parts of India, including the region where the
diploids predominate and it is possible that they will eventually replace all the diploids, which are now coexisting with them.

The occurrence of tetraploids only in Dacca and Travancore, however, is a little anomalous and the precise cause leading to it is at present only a matter for conjecture. It can be presumed that having had their origin from the diploids in the former times they existed along with the diploids but they have become spacially displaced by the more aggressive hexaploids. On account of this constant movement and displacement away from their diploid progenitors, they have now come to occupy only the peripheral areas of India. Some of the changes in the drainage systems of the Peninsula and elsewhere and the fact that the hexaploids are rapidly spreading weeds (cf. chromosomal prime types of Datura stramonium, Blakeslee et. al., 1937) might be the contributory causes for such a distribution. It is also possible that the area once occupied by the tetraploids is being contracted and hence has become rather discontinuous, as they are probably on the road to extinction. All these facts either singly or collectively might be responsible for obscuring the causes leading to the present day distribution of the tetraploids.

This pattern of the present day distribution of the different chromosome races in India described above is based on the tacit assumption that the diploid forms are phylogenetically old and the hexaploids are derived from them. (cf.
Anderson, 1937, in American *Tradescantia*; Baldwin, 1942 in *Sedum terantum*). There is no evidence to prove that the reverse had happened in the history of the species and even if it had happened, the survival value of such derived forms with lower chromosome number is doubtful. Hence, it is safe to assume on cytological and genetical grounds that the former counterparts of the present day diploids and the tetraploids are the progenitors of the hexaploids. If this could be established, it can also be said with a fair degree of certainty that the region occupied by the present day diploids is the centre of migration of the polyploid forms of *O. alismoides*. That the area occupied by the diploid race is the centre form which the species has spread in a perime-tric fashion was also shown by Baldwin (1942) with reference to *Sedum ternatum*, which also exists like *O. alismoides*, in diploid, tetraploid and hexaploid chromosome races. The map of the same author (1941) showing the chromosome races of *Galax aphylla* is also of interest in this connection.

Attention has been drawn elsewhere (Sundar Rao, 1951) that this region of the greatest diversity in India, where the diploids and polyploids occur is the centre of origin of the species itself. Though the evidence presented in this paper also leads to the same conclusion, it is however, safe to assume for the present that it is only the centre of dispersal the polyploid chromosome races of *O. alismoides*. Such a centre may or may not spatially correspond to the original home of the species, especially when it has now a wide range of geographic
distribution. Such species may have more than one secondary
centre of dispersal of the races and all of them may be far
removed from the centre of origin of the species.

The region now occupied by diploid forms, which lies
between Poona and the Andhra districts and which extends
east-west across the Deccan has been a continental area ever
since the post-Vindhyan (Cambrian) epoch, except for some
local and temporary moraine incursions (Wadia, 1939, pp.2,
164, 126; Krishnan, 1949, pp.2, 246, 284, 398; Pascoe, 1950,
pp. 453). This tract is covered by the Deccan trap lavas
(upper, Cretaceous to Eocene) in the western part and by the
Archaean complex in the east. Writing about the river valleys
of the Peninsula, Bora (1935) very significantly remarked,
"The lower portion of the present Godavari is certainly of
great antiquity as it dates back from at least the lower
Gondwana period, while the same cannot be said of the present
westerly portion....". This statement about the age of the
lower portion of the Godavari is of interest, as it is the
region where diploids are especially concentrated. In contrast
to it, the Gangetic alluvial plains, in which the hexaploids
occur is geologically of a very young age (Pleistocene and sub-
recent) (Wadia, 1939; Krishnan, 1949 and Pascoe, 1950). It is
believed that during the pre-Tertiary and early Tertiary times,
the regions now covered by the Indi-Gangetic alluvium, formed
the northern extension of the Peninsula India; it was concomi-
tantly with the orogenic uplift of the lofty Himalayan ranges,
converted into a depression which was rapidly filled up with
the post-Tertiary detrites brought down by the streams. The northern extension of the Peninsular geology is believed to be thus lying buried under the thick Indus-Gangetic alluvium.

Taking stock of the information that is now available regarding the geographic distribution of the chromosome races in India and its geological history, it looks as though phylogenetically older populations of a species tend to occupy geologically older areas. This appears to be true as the origin of the polyploid races in India was in no way directly influenced by the Pleistocene glaciation which prevailed in Kashmir and the Himalayas.

It is conceivable that the diploid forms of *Ottelia alismoides* might have moved considerably since the time of origin of the polyploid forms and therefore the tetraploids and the hexaploids might not have migrated from this region. Neither the diploids have the innate capacity for rapid dispersal when compared with the polyploids nor was there any environmental, climatic or topographical changes in that region of India during the Pleistocene period, which compelled them to move away from this region. That it is the centre of dispersal of the polyploid forms could not be verified by the study of the geographic distribution of the diploid races of the other species of *Ottelia*; but that it is an entirely possible assumption may be shown with reference to the other genera. For example, Balasubrahmanyan et al., (1946) reported three new genes in the certain races of *arboreum* cotton collected in the Godavari District of the 'Cocanadas' tract. After giving a survey of the alleles present in the races *indicum* and *burmanicum,*
they advanced the hypothesis that Coconada tract is probably the primary centre of origin for the *arboreums* on account of their large variability in this region and the presence of large number of genotypes not previously recorded in the Burma-Assam area. Bergner (1943) working the purple and white flowered races of *Datura metel* and their geographic distribution in India observed that both these types occur in a zone connecting Poona on the west and Calcutta on the east. In the northern part of the zone only purple coloured races were collected and in the southern parts only the white ones. These two publications are pertinent from the point of view of *Q. alismoides* in as much as the diploid forms of the latter occur in Coconada and at least a part of the region of great diversity of *Datura metel* corresponds to that of *Q. alismoides*. The writer of this paper has recently found that *Scilla indica* exists as a polyploid race (2 *n* = 58) in the central part of India, while a race with lower chromosome number (2 *n* = 44) is found in certain parts of the Andhra area. Though not exactly comparable it is, however, remarkable to note that the pattern of distribution of the chromosome races of *Scilla indica* is in keeping with that of *Q. alismoides*. The presence of the diploid forms in the Poona-Andhra tract affords positive evidence to show that it was a region of great evolutionary activity wherein an array of new forms of different genera, including the polyploid races of *Q. alismoides* came into existence and migrated since then from that region.
That cytology, though of no assistance in distinguishing the different races, \( \neq \) nevertheless aids in determining the centre of distribution of such races, as in \textit{O. alismoides}, is shown by the extensively documented case of American \textit{Tradescantia} (Anderson and Woodson, 1935; Anderson and Sax, 1936; Anderson, 1937). It was concluded that Edwards Plateau in the central Texas, which is the centre for the diploid strains of polyploid \textit{T. occidentalis}, \textit{T. ozarkana}, \textit{T. canaliculata} and \textit{T. hirsutiflora}, is the immediate centre from which the American \textit{tradescantia} have developed in comparatively recent times. In a more recent publication, Anderson (1948) showed that this plateau was also a great centre for the hybridisation of many related plant species and hence for introgression. \textit{Tradescantia rosea} var. \textit{craminea} is now known to exist in diploid, tetraploid and hexaploid chromosome races having a wide range of geographic distribution in the sand hills along the fall line of the north Carolina. Giles (1940) called attention to the fact that the relatively small area occupied by the diploids is geologically old and the other extensive territory inhabited by the more vigorous tetraploids after its emergence from the sea is demonstrably more recent. Manton (1934) showed that the diploid forms of \textit{Biscutella laevigata}, which are regarded as pre-glacial relics, occur only along the river valleys of the central Europe, while the aggressive tetraploids are found in the floristically youthful territory, which was covered by the alpine ice sheet during the glacial period. The same principal was also proved by Anderson (1936) in \textit{Iris versicolor}, by Hågerup (1928) in
*Empetrurn*, by Münzing (1935) in *Phleum*, by Shimotomai (1933) in *Chrysanthemum* and by Stebbins (1942) in *Ervogenum*.

(e) **Origin, migration and the nature of polyploidy in Ottelia alismoides.**

**Origin.** It is now more or less definitely established in a number of plant genera that polyploidy originates as a result of climatic or other environmental changes. After gaining considerable experience with polyploid plants inhabiting the arctic regions and Sahara desert, Lägerup (1932) postulated that extremes of heat and cold and other unfavourable elements in the environment could induce polyploidy just as heat treatments have done in the laboratory (cf. Randolph, 1932 in *Zea mays*; Dorsey, 1936 in *Secale cereale*; Münzing, Tometrop and Mundt-Peterson, 1936-37 in Barley). A parallel explanation for the origin of polyploidy was given by Tischler (1935 a, b), who thought that polyploidy frequently results from plants being exposed to severely cold climates. Flovik (1940) also came to a similar conclusion in the case of the arctic plants. The basic principle underlying the above concept is that sudden changes in the temperature bring about meiotic irregularities and ultimately lead to polyploidy (Sax, 1936; cf. also Gates, 1925). That changes in the environmental temperature are often reflected in the meiotic abnormalities in plants has been demonstrated in *Ranunculus raptans* by Bocher (1936) and in the Italian hemp by Medwedewa (1935). Gustafsson (1947), however, doubted the general
validity of the hypothesis and concluded that there was a methodological error in Flovik's investigations.

Based on the above idea is the postulate that spectacular climatic changes consequent upon Pleistocene glaciation resulted in the formation of many polyploid plant species in Europe and America. For example, Baldwin (1941, 1942) and Baldwin and Culip (1941) suggested that polyploidy is characteristic of many species of the Appalachian range and in all cases the incidence of polyploidy was attributed to glaciation, climatic and topographical changes (cf. Galax aphylla; Sedum ternatum; and Diospyros virginiana). This fact, however, has not been consistently demonstrated in other species like Oxydendrum arboretum, Gleditsia tricanthos and Mitchellia repens, where intraspecific polyploidy is absent (Mackenzie, 1947). Manton (1950) correlated polyploidy with climatic or geographical upheavals or to vicissitudes effecting the surface of the earth.

As far as the writer is aware, there unfortunately no fossil record of O. alismoides,* which could give a glimpse into its past history and the factors that induced polyploidy in the species during the past geological epoch. However, assuming a contemporaneous existence with Vallisneria, which is now known from the Karewas of Kashmir (Krishnan, 1949), it may be safely assigned to the Pleistocene period.

* Here it may be mentioned that O. Parisiensis has been reported by Saporta from Eocene of Paris (cited from Engler and Prantl, 1889). The geological age of this species is similar to the allied genus Stratiotes, which belongs to Eocene and Pliocene period (Chandler, 1923).
India too, like Europe and America, experienced the effects of Pleistocene glaciation, which unlike in those continents, was confined to the mountain ranges. The subject of glacial geology of India has received a monographic treatment at the hands of de Terra and Peterson (1939), who correlated the first glaciation with the Tettrot stage of Siwaliks (lower Pleistocene) and the second with boulder conglomerate stage (middle Pleistocene). There is unequivocal evidence to show that the glaciers descended to a very low level in the Siwaliks and to the present elevation of 5,500 ft., in Kashmir during the first and the second glaciations. In the central and the eastern Himalayas, the glaciers come down to the present height of 5,500 ft. - 7,500 ft., and these were not the heights that obtained in the Pleistocene period, since the Himalayas have risen considerably in the recent times.

As a consequence of this, N. India experienced a little change in the climate, which was more in the direction of increased precipitation rather than refrigeration. On the other hand, there might have not been any marked or perceptible lowering of the temperature conditions of Peninsula, despite the fact that Auden (1949, p. 334) postulated a mild uplift of its central part, probably in consonance with the orogenic movements that resulted in the uplift of the Himalayas. Brooks (cited from Hora, 1949) expressed the opinion that the temperature in the tropics and the equatorial regions (to which the Indian Peninsula belongs) was not appreciably lower in the Pleistocene period than at present, at least not as much as in the northern latitudes.
Since the upper part of the Peninsula has been considered as a centre of dispersal of the polyploid races of Q. alismoides, there appears to be nothing so sudden and dramatic in the climatic and other environmental conditions which could be considered to have brought about the production of polyploid chromosome races in that region. On the contrary, it is conceivable that polyploidy originated in this species on account of the plasticity of its genetic system. It looks as though the direction in which any species progresses, as determined by the polyploidy, is due not to the action of the external agencies nor to the exigencies of the competition but to the innate nature of the species itself. It is true that to some extent environment determines the path of evolutionary changes but its action is confined to the inexorable limits set by the genetic system. If a species has a prepossession towards polyploidy, environmental action brings it to the threshold of expression. But in certain cases, as in Q. alismoides, even this does not seem necessary as polyploidy originates in a spontaneous way.

Migration. It is known that Q. alismoides is propagated by seeds. The ripened capsules float to the surface of the pond or lake and are drifted in the currents of water. Eventually, the capsules discharge the seed contents, which by themselves may float, thus bringing about the migration of the species. To some extent water birds may bring about the dispersal on account of the fact that the seeds may stick to their
bodies by means of the mucilagenous mass, with which they are usually associated. Wide spread geographic distribution over extensive areas may not be possible by the latter method unless mass migration of birds carrying such seeds is imagined. However, it seems to be an effective method for the dispersal of the species from one pond to the other in a limited area.

Ridley (1930) described another large scale method of dispersal of Ottelia in the form of huge floating masses of detached plants, which are drifted in the rivers. A large mass of aquatic plants which float on the surface of water is called Sudd and according to Ridley (loc. cit.) one of the components of the "Indian sudd" is Q. alismoides. By this means not only can plants migrate in larger numbers but also cover longer distances following the courses of rivers. It is not improbable to think that mass movements of plants might have also been effected in this manner in the past geological times within the Peninsula, a part of which is now considered as the centre of dispersal of Q. alismoides and from there to N. India.

From the foregoing consideration, it is clear that the migration of Q. alismoides is intimately associated with the past and the present river systems in India and with the changes in the drainage that India had witnessed during the Pleistocene period. In the case of the Peninsula, there was a reversal and an alteration of the pattern of the river systems and Vredenburg (1909) attributed all the changes to two kinds
of earth movements, namely those connected with the uplift of
the Himalayas and those due to the scarp-faulting along the
west coast and the general crust movements associated with a
general tilting of the Peninsula from east to west. During
the course of these topographical changes, Vredenburg (loc. cit.)
envisioned a series of captures of one stream by another, as a
consequence of which temporary lakes might have been formed.
Naturally, such lakes might have received their aquatic flora
in the form of sudd, which was carried in the streams of the
capturing rivers. This provides by far one of the most impor-
tant and possible mechanisms, by means of which rapid dis-
persal of O. elismoides was probably effected during the
Pleistocene period in the Peninsular India. Perhaps, the same
mechanism also explains the dispersal of the species in
more recent times.

What had happened in the Peninsula, might have also
taken place in the N. India in connection with the changes in
the drainage systems, thereby enabling the species to spread
rapidly. Probably, the changes in N. India were of a
greater magnitude and more frequent than those of the Penin-
sula. This, at any rate, is the logical conclusion in view
of the fact that "the present valley system of N. India....
has inherited nothing from the old, it being an entirely
superposed system" (Wadia, 1936). All these have left a
permanent impress on the geographic distribution of the species
particularly in N. India.
With the available data of the geographic distribution of the different forms and the geological history, it may not be possible to decipher and predict the possible roots of migration. Nevertheless, it seems to be an entirely possible assumption on cytological grounds that the polyploid forms originated in South India and then migrated to the north. The chief water channels for such a dispersal are probably the Mahanadi and the Godavari rivers. There is enough evidence to show that during the early Pleistocene period the Godavari and the Mahanadi were flowing in a northerly direction as tributaries of the west-ward flowing Narbada-Tapathri river systems. This might have facilitated the migration of *Ottelia* from the lower Godavari to the central part of India. From there the possible root of migration of the forms into N. India is through the Mahanadi into the Ganges. This is borne out by the external similarity of the hexaploid forms of *Ottelia* of Orissa and those of Bengal. Discussing the affinities of the fish-fauna of the Mahanadi and that of the deltaic regions of the Ganges, Menon (1951, p. 493) remarked that "during the Pleistocene epoch, when the Bay of Bengal did not extend as far north as at present, there existed a connection between these rivers at the lower portions, or they had common channel draining into the sea far below the present mouth of the Ganges". This appears to be true in connection with *Ottelia alismoides* also. It is also possible to assume that the central Indian forms migrated through the Narbada river via the Sone into the Ganges (cf. Menon, 1951, p. 495). The external similarity between the M.P.for s and those of
Benares is striking from this point of view.

It is, however, not clear how the dispersal took place from the Godavari southwards. Nothing also can be said with regard to the migration of the hexaploids from India to the other parts of S.E. Asia, assuming for the moment that there was only one centre of dispersal within the range of distribution. However, Gregory's researches (1925) on the evolution of the river systems in S.E. Asia may prove to be of some interest in this connection.

Geographic distribution involves not only the origin and migration of new forms but also their colonisation at a new area, where the environmental conditions may sometimes be totally different from those prevailing at the centre of dispersal. It has been pointed out elsewhere in this paper that N. India suffered a climatic change as a consequence of the glaciation in the Pleistocene period, while Peninsular India experienced no such change. It is possible that some of the diploids might have migrated from the Peninsula to N. India but they might not have survived the then existing climatic conditions and hence might have perished. This explains why diploids are absent in N. India. On the other hand, the hexaploids could successfully colonise in N. India and then migrate from there to the other parts of S.E. Asia.
The nature of polyploid chromosome races of *Ottelia alismoides*.

There has been considerable discussion in recent years in connection with the criteria that are now known to distinguish auto- from allopolyploids. The important outcome of such a discussion has been to prove that none of the criteria are wholly satisfactory. Closely following this is the great diversity of opinion regarding the relative evolutionary importance of the two types of polyploids (Müntzing, 1936; Clausen, Keck and Hesey, 1945; Stebbins, 1947, 1950). The most widely accepted classification of polyploids is based on their mode of origin. Those that arise from homozygous diploids due to doubling are autopolyploids and if doubling takes place in a hybrid it will lead to the formation of allopolyploids. The problem, however, is not so simple, as in between these two extremities, there are a whole array of intermediary forms, which show all gradations in their chromosomal and genic differentiation and hence their assignment to either of the two types of polyploids is a matter of considerable difficulty. This aspect of the problem has received a detailed consideration by Stebbins (1947; 1950, pp. 312.)

In view of these inherent difficulties, it is perhaps not desirable to undertake a discussion on the mode of origin and the nature of polyploidy in *O. alismoides*.
This is particularly so, when there is at present no cytogenetical data, which may enable us to assess the genomic differentiation in the chromosome races of *O. alismoides*. However, a few of the outstanding features characterising these races may be mentioned, whatever may be their usefulness in determining the nature of polyploidy within the species.

As far as our present knowledge goes, there are diploid, tetraploid and hexaploid chromosome races in *Ottelia alismoides* and significantly enough triploids are absent. All the chromosome races are unique in presenting a great external similarity. The similarity is so close that they have successfully escaped the attention of the systematists. A comparative study of the different morphological features of the diploid and the hexaploid races has revealed that polyploids are mere enlarged replicas of the diploids. Nevertheless, the chromosome make-up of the hexaploids does not represent a merely re-duplicated system of that of diploids. It is indeed remarkable to note that while the diploids are characterised by only one pair of long medianly constricted chromosomes, there are only two pairs of such chromosomes in the hexaploids. This is contrary to the expectation, since the latter is a hexaploid based on 11 series. Furthermore, the meiosis of the hexaploid races is marked by great regularity leading to the formation of 33 bivalents. Occasional lagging at
anaphase I has sometimes led to a deviation in the chromosome number at metaphase II. Despite this fact, the fertility of the pollen grains is high. Although direct evidence is lacking at present, there is every reason to assume that fertility and viability of the ovules are in no way impaired on account of polyploidy, which has no doubt reached great heights in the species. Whatever may be the ploidy, all the races of *O. alismoides* are naturally occurring plants, which in all probability propagate themselves mostly by seeds.

The external morphology of the chromosome races strongly suggests autopolyplody*, at least at the tetraploid level. It is possible that tetraploid *O. alismoides* has arisen in nature due to the chromosome doubling in the diploid and ever since its origin considerable differentiation of the genomes might have taken place. Such a possibly and differentiation, which was both genic and chromosomal might have become augmented on account of the fact that all such tetraploids were isolated from their diploid relatives by incompatibility and sterility barriers. Later on, hybridization of the tetraploids and the diploid forms and a subsequent doubling of chromosomes might have resulted in the hexaploid *O. alismoides*. That doubling in the chromosome number took place prior to the establishment

* Stebbins (1950, pp. 312) has called attention to the difficulties residing in such inferences based on external morphology alone.
of the hybrid forms in nature is indicated by the absence of the triploid forms today. Such a hypothesis, though highly speculative at present, and should remain as such, till cytogenetical data become available, appears to be possible and provides adequate explanation of the morphological similarity and differences in the gross morphology of chromosomes of the diploid and hexaploid forms.

However, the point that goes much against the autopolyplloid origin of the tetraploids is the total absence of multivalents in the hexaploids, although the chromosomes are long enough to allow the formation of chiasmata. This difficulty can be obviated by assuming that structural differentiation of the chromosomes at the tetraploid stage might have progressed to such an extreme extent as to permit the formation of multivalents impossible. A parallel instance is perhaps afforded by the experimentally produced autopolyplloid maize, which showed a marked reduction in multivalents during the course of a 10 years' period (Giles and Randolph, 1951). These authors pertinently remarked that "the prevalence of quadrivalents in a natural tetraploid can be interpreted with reasonable assurance as evidence of an autoploid or essentially autoploid origin but the absence of quadrivalents is not acceptable proof of alloplloid origin of the polyploid". Emphasising the same fact, Müntzing and Fraken (1940) have explained the
the formation of a larger proportion of bivalents in
Phleum twins by assuming that the "need" for association
was satisfied by the pairing of two homologues, regardless
of the presence of the others in the nucleus. However this
may be, the postulate may not explain the total absence of
multivalents in certain autoploids, as these authors
noted a few bivalents in their material. Furthermore,
gene mutations are now known to inhibit chiasma formation
(Beadle, 1930) or bring about localisation of chiasmata
(Emsweller and Jones, 1935). All these facts very well explain
the absence of multivalents in these tetraploid forms even at
the formative period of their history.

The other possible objection of the autoploid
origin of the chromosome races of Q. alismoides (at least
in the tetraploid stage) is the formation of fertile pollen
grains. Recently, Müntzing (1951, pp.55) has shown that
the autotetraploid rye is more fertile than diploid and that
the tetraploids are characterised by 90-100% apparently
good pollen grains. If this is so in a polyploid, which
is known to produce quadrivalents, it is not surprising
to expect good pollen in a polyploid like Q. alismoides
(2n=66), which produces only bivalents.

The alternate hypothesis that the tetraploid
race has originated in nature due to its crossing with
another diploid species and that it has attained its
morphological similarity on account of the selective
elimination of the characters of one parent and retaining those of the diploid *O. alismoides* must be excluded at present, as there is no other diploid species of *Ottelia* in India. If future taxonomic research could show the existence of another diploid species such a mode of origin enumerated above becomes highly possible. The allopolyploidy of the chromosome races is also dependent on the status that will be accorded to the different diploid forms referred to in the earlier part of the paper.

On the whole, in the light of the present taxonomic and cytological data, it may be tentatively concluded that in the evolution of the chromosome races of *O. alismoides* both auto- and allopolyploidy might have played a part. One of the predominant tendencies is the structural differentiation of the chromosomes during the course of descent, primarily at the tetraploid stage and perhaps to some extent also at the hexaploid stage. They are neither wholly autoploid nor allopolyploid. It is perhaps safe not to categorically assign them to any of the four types of polyploids enumerated by Stebbins (1950, p. 315), although allopolyploidy is strongly indicated in the hexaploid *O. alismoides*. 
(f) Certain possible evolutionary trends in the genus *Ottelia*.

Any conclusions regarding the evolutionary tendencies in the genus *Ottelia* are probably too premature, especially when a relatively large mass of species remain uninvestigated cytologically. The available morphological and cytological data, however, tend to show that probably gene mutations determine mainly the pattern of evolution in the genus and it looks as though it forms the basis of speciation.

One of those remarkable aspects of evolution of the genus is that though polyploidy has reached great heights, it has brought nothing spectacular and has probably not led to the origin of new species with the genus. This conclusion at present is borne out by the intraspecific chromosome races in *O. alismoides*. In the latter, polyploidy has resulted in an array of forms, which, as far as the present investigation goes, are morphologically more or less similar. A critical appraisal of the characters of these forms with different chromosome numbers has revealed that none of them can be safely assigned to the status of a species. Perhaps, polyploidy has been a factor in checking the morphological divergence of these forms in *O. alismoides*. This is analogous in effect to the American species of *Crepis* (Babcock and Stebbins, 1938), where the causative mechanism is apomixis coupled with polyploidy.

In addition to the variation in the chromosome numbers, structural changes in the chromosomes, as manifested in the reduction of the number of long median chromosomes in the karyo-
karyotypes of polyploids, might have also played a part in the
evolution of the chromosome races. It is difficult at this
juncture to point out clearly the nature of these structural
changes, but it is not improbable to think that they are the
outcome of polyploidy (cf. also Salix, Wilkinson, 1944).
However, such alterations in the karyotypes are also not reflected
in the external morphology of the chromosome races. Hence,
Q. alismoides provides one of the most interesting examples to
demonstrate that the evolution of new forms and speciation are
two different phenomena and one need not accompany the other,
although a distinction between these phenomena is sometimes
difficult to draw. Stebbins (1950) has explicated at length
on this point and it has been reiterated by Gates (1951).
Hyxley (1944) remarked: "Species formation constitutes one
aspect of evolution; but a large fraction of it is in a sense
an accident, a biological luxury, without bearing upon the
major continuing trends of evolutionary progress". This
accident of species-formation is yet to take place in the case
of the intraspecific chromosome races of Q. alismoides.

Though singularly little evidence is available, two
distinct divergent lines of evolution can be clearly visualised
in the phyletic history of the genus: viz. (i) a bisexual line,
some of the species of which like Q. alismoides, with diploid,
tetraploid and hexaploid chromosome races; (ii) a unisexual
or probably polygamo-dioecious line (?), as exemplified by
Boottia sp., which are only hexaploids. Perhaps, the latter
arose as an off-shoot from the former at a hexaploid level. That it is a possible assumption is shown by the lack of diploids, which are unisexual and by the occurrence of bisexual hexaploid forms, which resemble Boottia sp. According to this view, the ancestral forms of Boottia were diploid and hermaphrodite and the unisexuality is a later stage in evolution. At present it is not clear how unisexuality arose in the genus at a polyploid level; however, with the available cytological data it may be postulated that it originated spontaneously in certain hexaploid species of the genus. A parallel instance is provided by the hexaploid Fragaria elatior, where the sex differentiation originated 'de novo' subsequent to the evolution of the species (Kihara, 1930).

This situation in Ottelia is exactly opposite to that found in Emátrum, where diploids are unisexual and polyploids are hermaphrodite (Hägerup, 1927). Significantly, however, Blackburn (1938) discovered a bisexual diploid Emátrum and it is just possible that a unisexual diploid form or species Boottia may be found in future. Even then, the concept of sexual divergence among the species of the genus will not suffer a total change and materially alter the evolutionary picture but on the other hand tends to show that the differentiation of the sex was much earlier event and took place at a time when the genus was at a diploid level and that all the hexaploid species in the genus are polyphyletic or at least diphylectic. At whatever level of ploidy the two lines might have had their differentiation, their very existence cannot be denied.
In the earlier part of the paper, it has been observed that there exist in both hexaploid *O. alismoides* and *Boottia* sp., two different types of plants with flowers having pink and white corollas respectively. As far as the present investigation goes, such a variation in the colour of the petals was not at all encountered in the diploid forms, which are uniformly characterised by white corollas. In order to explain these observed facts, it is perhaps necessary to assume that gene mutations are responsible for such a variation. Therefore, in addition to the major cytological and morphological changes in the two distinct phyletic lines enumerated above the representative species of each of them namely *O. alismoides* and *Boottia* sp., show parallel mutations at a hexaploid level. It is very remarkable to find that mutations brought about a similar variation in two distinct species leading to the formation of flowers with identically coloured corollas. Probably, similar chromosomal changes induce parallel gene mutations and parallel seriation implies closer relationships between *Ottelia* and *Boottia*.

*Oenothera lata* mutations from *Oe. laranchiina* and *Oe. biennis* are the original cases of parallel mutations, which were based on experimental foundation (Gates, 1925). Both were characterised by 15 chromosomes, by the same peculiarities of leaf, habit and sterile pollen but had retained the flower size of the species from which they were derived. In a way these are similar to the mutations of *O. alismoides* and *Boottia* sp., having the same chromosome number, the same type of variation in the colour of the petals but retaining the sex of the species from which
they originated. Two white-eyed mutants and several wild-type characters of closely related species of *Drosophila* are now known to be due not to common descent but to parallelism in evolution (Huxley, 1944, p.395). Huxley (loc. cit.), envisaged a distinction between homologous and non-homologous gene mutations, which induce parallel character-change or a similar phenotypic-change.

Parallel trends as guiding principals in the evolutionary changes were recognised long ago by Darwin (1868). Ever since that time, numerous investigations found the phenomenon to be operating in smaller or larger measure in diverse groups of plants and animals. Canong (1894), E.A.N. Arber (1903), Gates (1917), Bower (1918), Hutchinson (1923) and Vavilov (1922) stressed the importance of the concept in the problems of evolution and Arber (1925) illustrated the phenomenon by giving the example of parallelism of the leaf form in different genera of the group Helobiales to one of the families of which the genus *Ottelia* belongs.

An increasing realisation of this fact that similar end products are of frequent occurrence in certain genera on account of parallel chromosomal changes and gene mutations is of profound importance to all systematists and cyto-taxonomists (cf. Ford, 1939; Sturtevant, 1939; Turrill, 1939). In fact, much of the earlier confusion in the taxonomy of the genus *Ottelia* is in itself a reflection of non-realisation of this
principle and it can now be resolved with a fair degree of certainty by imagining that the evolutionary pattern of the genus to some extent is determined by parallelism.

VI. SUMMARY

1. A detailed cytological study of the diploid (2 n=22) and hexaploid (2 n=66) chromosome races of *Ottelia alismoides* in relation to their morphological variation and geographic distribution has been undertaken with a view to evaluate their taxonomic importance. Since the closely related unisexual forms, which were formerly treated under *Boottia* Wall., have some bearing on the problems of evolution and generic boundary of *Ottelia*, the observations on somatic and meiotic chromosomes of the former are also included in this paper.
2. The somatic chromosome complement of the diploid race (2n=22) of \( Q. \) alismoides is characterised by a pair of SAT-chromosomes and a pair of long median chromosomes along with other types. The meiosis is regular with the formation of 11 bivalents.

3. The chromosomes of the hexaploid (2n=66) \( Q. \) alismoides exhibit no marked size variation, when compared with its diploid relative. However, there is a slender thread of evidence to show that structural changes effecting the gross morphology of the chromosomes are involved in the evolution of the polyploid races. Furthermore, the regular formation of 33 bivalents was consistently observed, but for a single P:G, where a trivalent was noted. The chromosome constitution and behaviour of \( B. \) sp., resemble those of hexaploid \( Q. \) alismoides in every detail, except for the marked reduction in the chiasma frequency of the bivalents.

4. The cytological and morphological data accumulated during the present investigation provide cogent evidence for the amalgamation of the genus \( B. \) Wall, in the much older genus \( O. \) pers., as proposed by Dandy (1934). Apparently, the dicliny and monocliny of these two genera respectively are
not significant in carving out the generic boundary of *Ottelia*.

5. Some mitotic and meiotic abnormalities, like the differential staining behaviour of certain segments of the somatic chromosomes of the diploid races, the stickiness and agglutination of the bivalents of the hexaploid races and the formation of bridges due perhaps to agglutination, were recorded and discussed.

6. A critical evaluation of the morphological characters, particularly those that are alleged to have a bearing on the speculation in the genus, clearly tend to show that none of the intraspecific chromosome races can be safely assigned to the status of a species. Hence, *O. alismoides* is a "collective species" with diploid, tetraploid and hexaploid chromosome races.

7. In all probability, polyploidy has aided the wide spread geographic distribution of *O. alismoides*. Relatively, the diploid races show ancient dispersal in a geologically older region in India and the hexaploids exhibit recent dispersal in a geologically youthful territory.

8. The origin of polyploidy in *O. alismoides* has nothing to do with glaciation or topographical changes in the Pleistocene period of India but
is due to its innate predisposition toward an increase in the chromosome number and to some extent to the action of water, in which the plants live. The present study of the geographic distribution is enough to show that probably S. India is the centre of dispersal of the polyploid races of O. alismoides, at least in India. The dispersal of polyploid forms to N. India is correlated with the changes in the river systems during the Pleistocene and the more recent times.

9. In the light of the present cytological and taxonomic data, it is possible that both auto- and allopolyploidy have played a part, each in its own way, in the origin of the different chromosome races.

10. On the whole, there are two general lines of evolution in the genus Ottozia - a bisexual line and a unisexual or perhaps polygamous line of which the latter represents a higher evolutionary stage finding its origin from the former at a hexaploid level. These two lines are marked by the phenomenon of parallelism.

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Explanation of text figures, illustrating the paper entitled "Karyo-systematic studies in Helobiales. IV. The genus Ottelia".

All the drawings were made at bench level with the aid of a camera lucida. An achromatic objective N.A. 1.3 was used in conjunction with a Zeiss eye-piece K 20X giving an approximate magnification of 3,700 diameters, unless otherwise mentioned. The magnification of the figures 24, 26, 53 and 56-64 is 1,420 and that of the figures 49 and 55 is 1,700. All the drawings were reduced to about \( \frac{1}{4} \) in the photographs.

Figs.1-22. **Ottelia alismoides**: diploid race. Fig.1. Polar view of somatic metaphase, showing 22 chromosomes, of which two are satellited. Fig.2. Idiogram. Fig.3. Somatic metaphase; note the peripheral distribution of 14 chromosomes all round the spindle at equidistant spaces between each other. Fig.4. Somatic telophase, showing two finished nucleoli at each pole. Fig.5. Early stage in the organization of the nucleolus; note the 2 big nucleoli and a number of nucleolar globules of variable sizes. Fig.6. Resuming nucleus with 2 nucleoli. Fig.7. Diplotene stage, showing one bivalent attached to the nucleolus by means of satellites. (The rest of the bivalents are not shown). Fig.8. Same stage; the pairing at the region of attachment is hindered. Fig.9. Diakinesis; out of the 11 bivalents, one is attached to the nucleolus. Fig.10. Metaphase I; p=11. Figs. 11-14. Metaphase I to illustrate the mode of arrangement of the bivalents at the equatorial region with one, two and three bivalents respectively in the centre of the spindle and the rest all round. Fig.15. An exceptional metaphase I with 12 bivalents. Figs.16 and 17. Side views of metaphase I. To show the difference in the chiasma frequency of the large bivalents (third from the left in both the cases). Fig.18. Metaphase II; the morphology of the 11 chromosomes is clear in those that are blackened. Fig.19. Metaphase II (only one plate drawn); p=12-. Fig.20. Telophase I; one nucleolus at each pole. Fig.21. Anaphase II (only one cell drawn); note the bridge; the small fragment (?) already at the pole at the top. Fig.22. Lagging of chromosomes at anaphase II.

Figs.23-26. **Ottelia alismoides**: hexaploid race (winged).

Fig.23. Somatic metaphase, showing 66 chromosomes. Fig.24. A pollen mother-cell with its nucleus in the process of degeneration. Fig.25a. Bivalents at diakinesis stage, showing the stickiness. Fig.25b. A bivalent with its satellites. Fig.26. A diad with three nucleoli in one nucleus and two in the other.
Figs. 27-33. *Ottelia alismoides*: hexaploid race (wingless). Fig. 27-30. Resting nuclei to show the variation in the number of nucleoli from 5 to 7. Fig. 31. Somatic metaphase with 66 chromosomes. Fig. 32. Metaphase I; note the two conspicuously large bivalents and the absence of secondary association among the different bivalents. Fig. 33. Anaphase I to show the normal disjunction. Fig. 34. Metaphase II; n=33; note the 2 long chromosomes with median constrictions.

Figs. 35-38. *Ottelia alismoides*: hexaploid race (winged and wingless mixed). Figs. 35 and 36. Side views of metaphases I; the biggest bivalents showing a maximum number of 5 chiasmata. Fig. 37. Side view of metaphase I to show the stickiness of the bivalents, which are connected with each other by fine processes. Fig. 38. Side view of metaphase I to illustrate the 3-valents.

Figs. 39 and 41. *Ottelia alismoides*: hexaploid bisexual forms resembling *Boottia* species. Fig. 39. Somatic metaphase with 66 chromosomes. Fig. 41. 4 long median chromosomes picked out from a somatic metaphase; all the rest are omitted while drawing.

Figs. 40 and 42-64. *Boottia* species. Fig. 40. Somatic metaphase with 66 chromosomes. Fig. 42. A somatic resting nucleus with 6 nucleoli. Fig. 43. A somatic telophase nucleus with numerous drop-lets of nucleolar material. Figs. 44 and 45. Metaphase I to show the segregation of bivalents into roughly three and two groups. Figs. 46-43. Metaphase I showing but little secondary association. Fig. 49. Side view of metaphase I; note the decreased number of chiasmata even in the long bivalents and an increase in the number of the rod-bivalents. Fig. 50. Metaphase II (only one plate drawn); n=33; note the presence of only 2 long chromosomes with median constrictions. Fig. 51. Metaphase II (only one plate drawn); n=34. Fig. 52. Metaphase II (only one plate drawn); n=38. Fig. 53. A diad showing cytomixis. Fig. 54. A P.M.C., showing syndiploidy.

Fig. 55. Anaphase I showing the two lagging bivalents. Fig. 56. Anaphase II with lagging chromosomes. Fig. 57. Anaphase II; to represent the bridge. Fig. 58. Anaphase II; note the single chromosome lying outside the spindle. Fig. 59. Early telophase II with a single chromatic bridge. Fig. 60. the same but the processes are stout and broken irregularly. Figs. 61-66. Diads and tetrads respectively to show 3 or 4 nucleoli in each nucleus.
8 - 10. Hexaploid form collected from other parts of India. 8. Hexaploid form from Orissa.
9. Hexaploid form from Bengal. Hexaploid bi-sexual form, which resembles Boettia (collected from Banaras).
11 - 13. *Boottia* species: All hexaploid and unisexual; note the similarity between the unisexual (photograph 11) and the bisexual (photograph 10) forms in the external features.