

2. SPECIOLOGY

[EVOLUTION, DESCRIPTION, CLASSIFICATION AND
PHYLOGENY]

ON THE KARYO-SYSTEMATICS OF THE SUB-GENUS AJAX SPACH OF THE GENUS NARCISSUS L.

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[Translated from the French into English by Thomas W. Whitaker.]

INTRODUCTION

The history of the taxonomy of the group *Ajax* Spach of the genus *Narcissus* L. has been recorded in a very satisfactory fashion by Pugsley (1933). According to this author, we learn that the number of species attributed to this group varies a great deal, and that this variation has been due not only to the different numbers of forms known during a certain epoch, but also to differences of opinion among authors concerning the delimitation of specific groups. Thus Linné (1753), in the first edition of the "Species Plantarum," distinguished only one species. Later (1762), in the second edition of the same work, he distinguished two. Salisbury (1812) distinguished 10, Haworth (1831) 29, Herbert (1837) 9, Barr (1884) 8, Baker (1888) one, with 6 sub-species and one variety, and Jordan (1903) 13. Rugsley (1933) finally established two.

Cytological studies in this group have been made by de Mol (1922, 1928), Nagao (1929, 1930, 1933), Fernandes (1931, 1933, 1934), Collins (1933), Philip (1933, 1934) and Sikka (1940). However, in spite of these studies, we can state that we do not yet know the idiograms¹ of these species, since the morphology of the chromosomes has not been studied in detail. Accordingly, having procured material of a great many forms, we have resolved to establish their idiograms with the aim of illuminating the following two questions: (1) Can the data concerning the number and morphology of the somatic chromosomes be of aid in resolving the problem of the delimitation of species?; (2) What are the processes that have been active in the evolution of this group?

Apart from the forms corresponding to the wild species distinguished by Pugsley (1933), we are also presenting in this study, the results of our observations on the cytology of some forms obtained in cultivation, whose origin we have tried to clear up.

¹ [Translator's note. According to Sharp, (Introduction to Cytology) **idiogram** is defined as the diagrammatic representation of a karyotype: **Karyotype** is the chromosome complement characteristic of an individual or group of allied forms.]

MATERIALS AND TECHNIQUE

Some plants have been collected in Portugal in the wild state, others have been furnished by Botanical Gardens, and still others by Maison Barr & Sons², Reginald Kaye, Cayeux-LeClerc & C.ie, Vilmorin-Andrieux & C.ie and Fernandes Coimbra, Valardares (Gaia).

The following list indicates the name of the forms³, as well as their origin:

<i>N. cyclamineus</i> DC.	bed, left bank of the Ferreira River, (fig. 154) near Pôrto.
<i>N. Johnstonii</i> Pugsley	Fernandes Coimbra.
<i>N. Johnstonii</i> Pugsley (<i>N. Queen of Spain</i>)	Barr & Sons.
<i>N. asturiensis</i> (Jord.) Pugsley	Serra da Estrêla.
<i>N. minor</i> L. (<i>nanus</i>)	Barr & Sons; Reginald Kaye.
<i>N. pumilus</i> Salisb. (<i>minor</i>)	Barr & Sons.
<i>N. nanus</i> Spach (<i>lobularis</i>)	Barr & Sons.
<i>N. hispanicus</i> Gouan (<i>maximus superbus</i>)....	Barr & Sons.
<i>N. obvallaris</i> Salisb. (<i>obvallaris</i>)	Barr & Sons.
<i>N. portensis</i> Pugsley? (<i>N. Pseudo-Narcissus</i> L. var. <i>concolor</i> in Coutinho, Flora of Portugal)	Origin uncertain; collected in the wild and cultivated in the Coimbra Botanical Garden.
<i>N. pseudo-narcissus</i> L. (Lent Lily)	Barr & Sons.
<i>N. pseudo-narcissus</i> L.?	Leca do Bailio.
<i>N. Gayi</i> (Hénon) Pugsley (<i>Princeps Maximus</i>)	Barr & Sons.
<i>N. moschatus</i> L. (<i>cernuus</i>)	Barr & Sons.
<i>N. tortuosus</i> Haworth (<i>N. longiflorus</i> Willd.)	Hortus Botanicus Petropolitano in Leningrad.
<i>N. pseudo-narcissus</i> L. var. <i>bicolor</i> (L.), in Coutinho, Flora of Portugal	Serra da Estrêla.
<i>N. pseudo-narcissus</i> L. (double flowered)....	Avelar.
<i>N. yellow double</i> Van Sion	Vilmorin-Andrieux & C.ie
<i>N. trumpet</i> Impératrice	Vilmorin-Andrieux & C.ie

² We are greatly indebted to Maison Barr & Sons, London, who have furnished us bulbs of the forms we have requested.

³ We are extremely thankful to M. H. W. Pugsley, who has communicated to us the equivalence between the names in the Catalogue of Maison Barr & Sons, and those species he has established in his monograph.

<i>N. trumpet</i> Emperor	Cayeux-LeClere & C.ie
<i>N. trumpet</i> Major	Cayeux-LeClere & C.ie
<i>N. trumpet</i> , King of the yellows	Vilmorin-Andrieux & C.ie
<i>N. trumpet</i> , M.me de Graff	Cayeux-LeClere & C.ie
<i>N. King</i> Alfred	Fernandes Coimbra.



Fig. 154. *N. cyclamineus* DC. in its classic locality near Pôrto (bed, left bank of the Ferreira River). Dr. A. Rozeira photo.

Our observations have been carried out principally with root meristems. In only one case have we studied the first division of the nucleus in the pollen grains (*N. pseudo-narcissus* L. var. *concolor*), and meiosis has been studied in *N. hispanicus* (Maximus superbus) and *N. Johnstonii*. For obtaining preparations of root meristems, we have used Navashin's fluid, (Brunn's modification) and staining with gentian violet.

For the study of meiosis, in the pollen mother cells, we have utilized two types of preparations:

(a) Preparations obtained by fixation with La Cour 2BE and stained with gentian violet.

(b) Preparations obtained by fixation in acetic-alcohol (3 parts absolute alcohol: 1 part crystalizable acetic-acid) and stained in acetocarmine. These preparations have been made permanent by employing the technique described by La Cour (1937).

Mitosis in the pollen grains has been studied in non-permanent preparations, obtained by the maceration of anthers in a drop of acetocarmine.

OBSERVATIONS

By examination of the equatorial plates in the cells of the root meristems we have established the existence of 14 chromosomes in the following forms: *N. cyclamineus* DC., *N. asturiensis* (Jord.) Pugsley, *N. minor* L., *N. pumilus* Salisb., *N. nanus* Spach, *N. obvallaris* Salisb., *N. portensis* Pugsley? (*N. pseudo-narcissus* L. var. *concolor* in Coutinho, Flora of Portugal), *N. pseudo-narcissus* L., *N. Gayi* (Hénon) Pugsley,

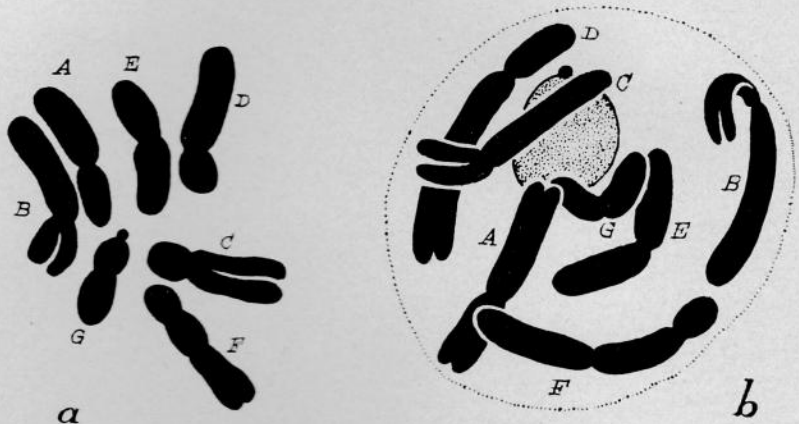


Fig. 155. *N. pseudo-narcissus* L. var. *concolor* (Coutinho, Flora of Portugal). *a*, Equatorial plate of the first division in the pollen grain; the chromosomes are marked by the letters A - G. *b*, Pro-phase of the same division; note chromosome G attached to the nucleolus by its satellite, X 2200.

N. moschatus L., *N. yellow double* Van Sion, *N. double flower* of Avelar, *N. trumpet major* and *N. trumpet*, King of the yellows.

An analysis of metaphase in the first division of the nucleus in the pollen mother cells has permitted us to identify the following 7 types of chromosomes (fig. 155a):

- A—heterobrachial chromosome li;
- B—heterobrachial chromosome Lm;
- C—Chromosome similar to the preceding type, but with the long arm slightly shorter;
- D—Chromosome Lp, resembles the two preceding types but with the short arm less than half the length of the longer one;
- E—Chromosome li similar to type A, but the two arms are shorter and the secondary constriction is located near the extremity of the long arm;
- F—Chromosome Lp similar to type D, but short arm not as long; a submedian constriction on the arm L has been observed in a majority of the figures;
- G—Satellited chromosome Pp'. This chromosome is the only nucleolar one, as indicated in prophase figures, in which this chromosome and its satellite is found attached to the nucleolus (fig. 155b).

In the diploid plates of all the forms mentioned above (Plate 294, fig. 156, 157, 158a and 159a), we have established the existence of 7 pairs of homologues, corresponding to the types described for the pollen grains. We have never found differences between elements of a pair which might indicate structural hybridity. So that, we can say that all these forms possess the same idiogram, expressed by the general formula:

$$2n = 14 = 4 : Lm + 4 : Lp + 4 : li + 2 : Pp'$$

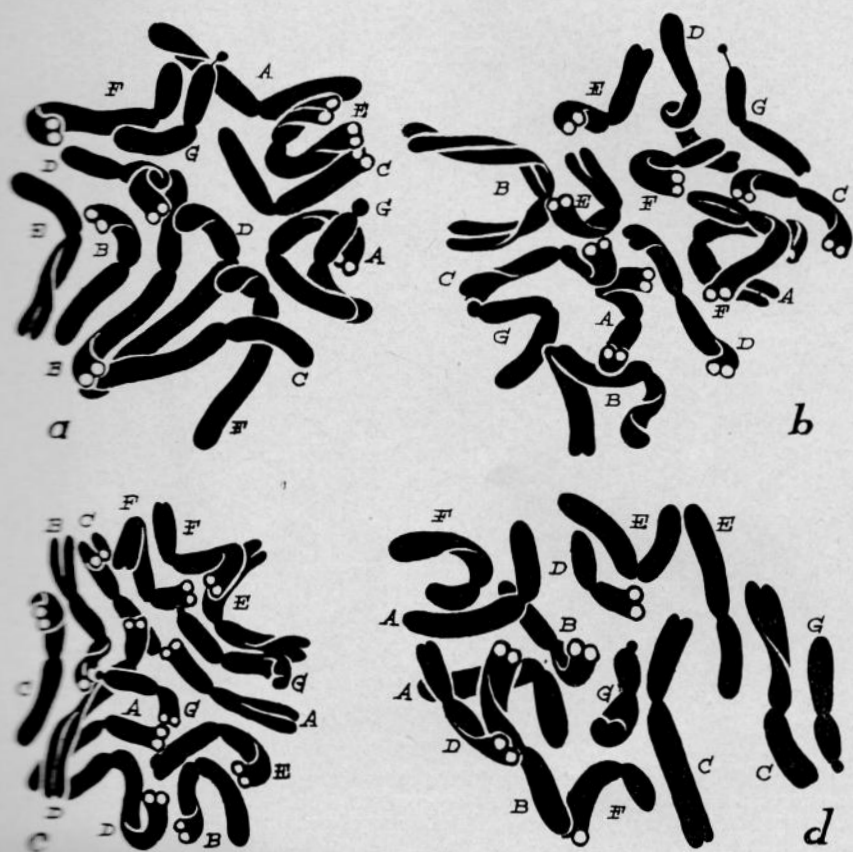


Fig. 156. Equatorial plates from the cells of the root meristem. a, b, *N. pseudo-narcissus* L. (Lent Lily). c, *N. Gayi* (Hénon) Pugsley. d, *N. moschatatus* L. The chromosome pairs are indicated by the letters A - G. X 3250.

In the root meristem of *N. minor*, we have found, apart from the normal diploid plates (fig. 158a), some plates having 28 chromosomes (fig. 158a). This is a question of mixoploidy, a well known phenomenon, and one which has already been described by Fernandes (1936) with another species of the genus (*N. reflexus* Brot.).

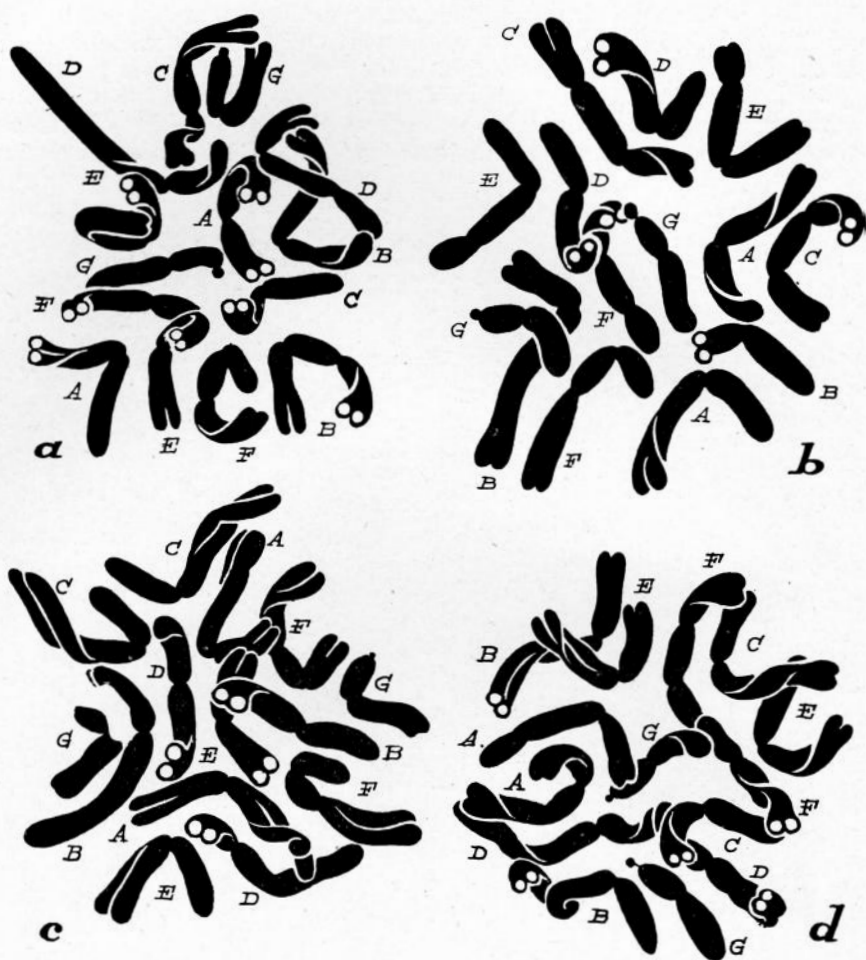


Fig. 157. Equatorial plates from the cells of the root meristem. *a*, *N. pseudo-narcissus* L. (Avelar). *b*, *N. yellow double Van Sion*. *c*, *N. trumpet major*. *d*, *N. trumpet King of the yellows*. The chromosome pairs are indicated by the letters A - G. X 3250.

Plate 294. (See opposite page for plate.) Equatorial plates from the cells of the root meristems. *a*, *N. cyclamineus* DC. *b*, *N. pumilus* Salisb. *c*, *N. nanus* Spach; note that the G chromosomes carry a "tandem satellite." *d*, *N. obvallaris* Salisb. *e*, *N. portensis* Pugsl.? (*N. pseudo-narcissus* L. var. *concolor*). *f*, *N. pseudo-narcissus* L. (Leca do Bailio). The chromosome pairs are indicated by the letters A - G. X 3250.

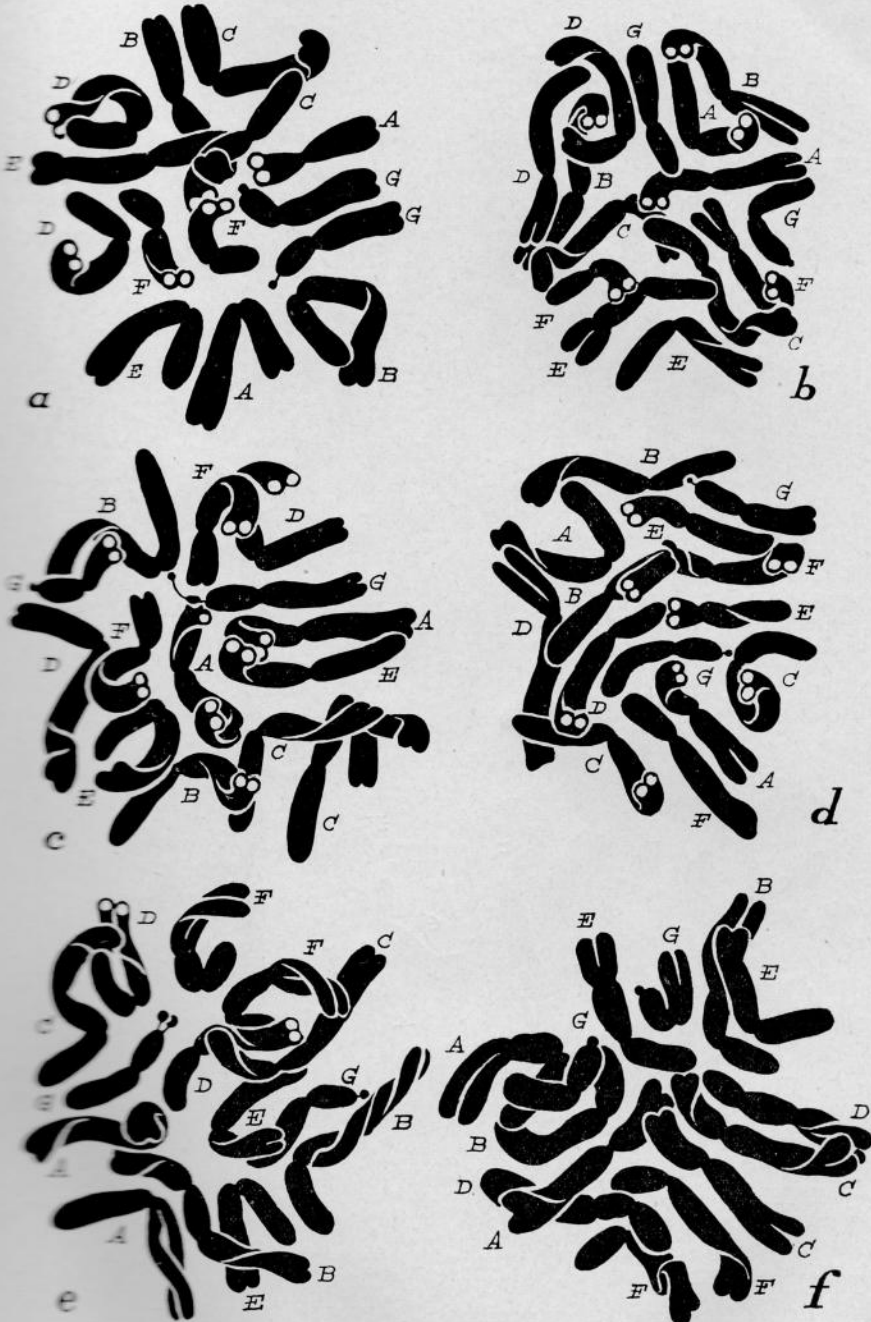


Plate 294 (see opposite page for details.)

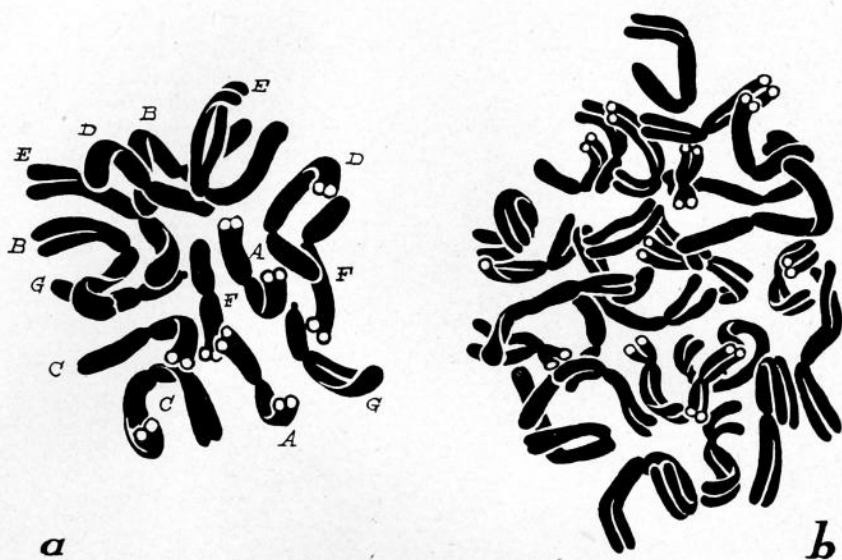


Fig. 158. *N. minor* L. *a*, Equatorial plate in the diploid cells of the root meristems. *b*, Tetraploid plate in another cell of the same root. X 3250.



Fig. 159. *N. asturiensis* (Jord.) Pugsley. *a*, Equatorial plate with 14 chromosomes. *b*, Plate provided with a heterochromatic, supernumerary chromosome (S). X 3250.

In *N. asturiensis* we find some plants with 14 chromosomes (fig. 159a) and others with 15 (fig. 159b). The idiogram of the plants with 14 chromosomes is entirely similar to the previously mentioned forms. The plants with 15, present, aside from the normal complement, a

heterobrachial chromosome, simulating a Pp chromosome with shortened arms. The interphase nucleus shows a small chromocenter, disclosing that it is a question of a supernumerary, heterochromatic chromosome of the type of those that Fernandes (1939, 1943) described in *N. junceifolius* Lag. and *N. bulbocodium* L. Collins (1933) reports the existence

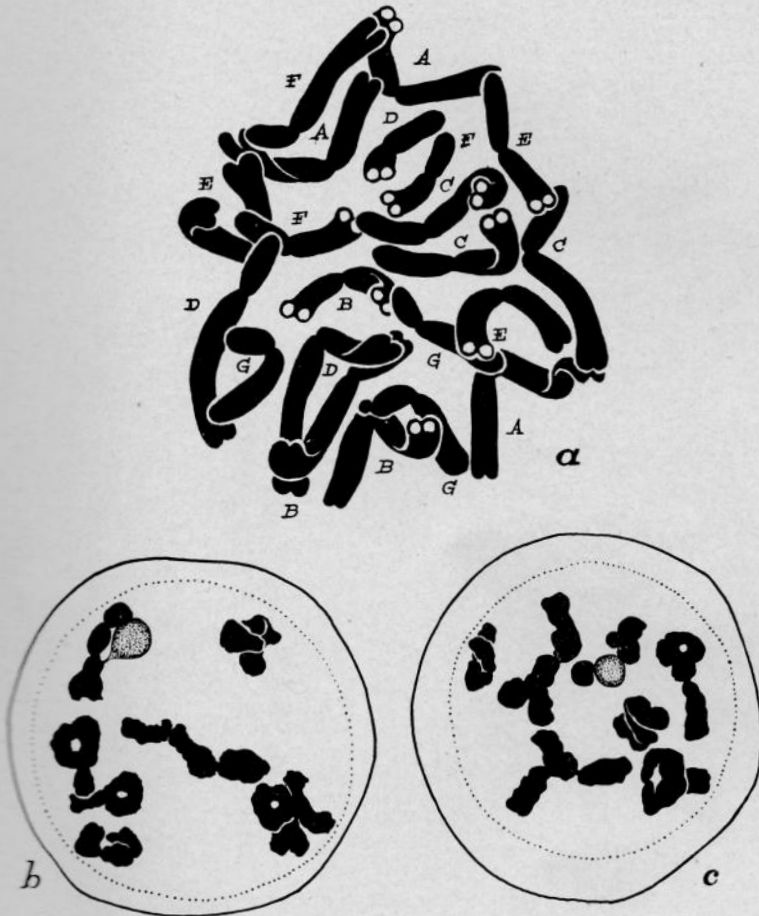


Fig. 160. *N. hispanicus* Gouan. *a*, Equatorial plate with 21 chromosomes in the root meristem cells. X 3250. *b*, Diakinesis showing 7 trivalents, a nucleolar bivalent and a univalent. X 1050. *c*, The same showing 6 trivalents, a nucleolar bivalent and a univalent, also nucleolar. X 1050.

of 15 chromosomes in *N. minor* L. and *N. pumilus* Salisb., and Philp (1933, 1934) mentions the same number in *N. pallidiflorus* Pugsley. Our observations indicate that the normal chromosome complement of *N. minor* and *N. pumilus* is composed of 14 chromosomes. Accordingly, it is almost certain that *N. pallidiflorus* also has 14 chromosomes and that

the supernumerary elements found by Collins and Philp, are of the same nature as those we have found in *N. asturiensis*.

In *N. hispanicus*, we have found 21 somatic chromosomes, and the analysis of the plate reveals the existence of three chromosomes each, of the type distinguished in the haploid complement (fig. 160a). The chromosome complement is therefore expressed by the formula:

$$2n = 21 = 6 : Lm + 6 : Lp + 6 : li + 3 : Pp'$$

Diakinesis and metaphase of the heterotypic division almost always show 7 trivalents. The almost constant formation of this association (fig. 160b) indicates that the form studied is autotriploid.

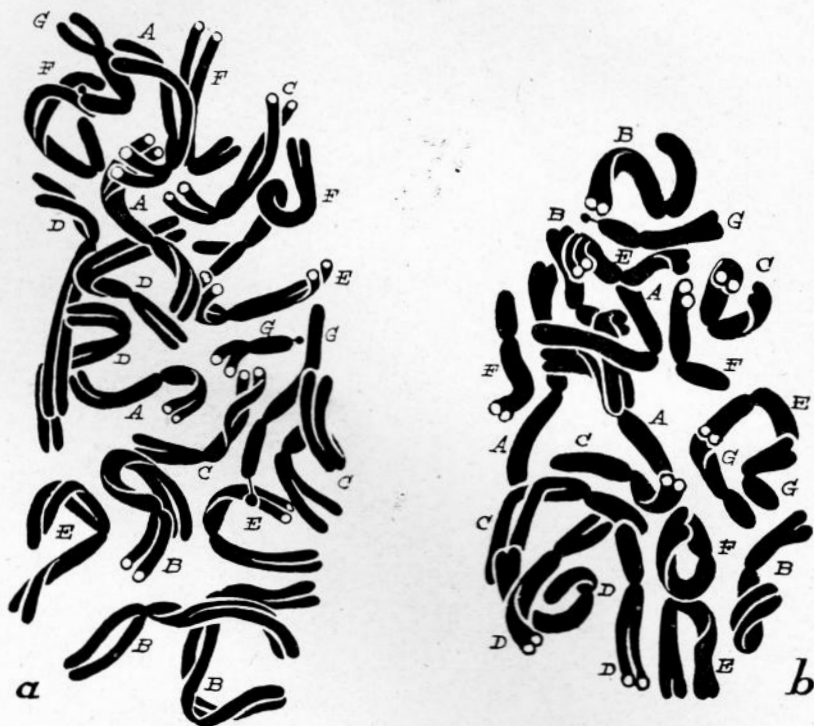


Fig. 161. Equatorial plate from the root meristem cells. *a*, *N. tortuosus* Haw. *b*, *N. trumpet* Emperor. Note the presence of three chromosomes each of the type A - G. X 3250.

Plate 295. (See opposite page for plate.) *N. hispanicus* Gouan. *a, b, c*, Anaphases of the heterotypic division showing some lagging. *d*, Telophase I showing laggards thrown out into the cytoplasm where they become micronuclei. *e*, Metaphase of the homotypic division showing 21 chromosomes; this cell produced 2 pollen grains with 21 chromosomes each. *f*, Telophase I, where such irregularity is not visible. X approx. 1400.

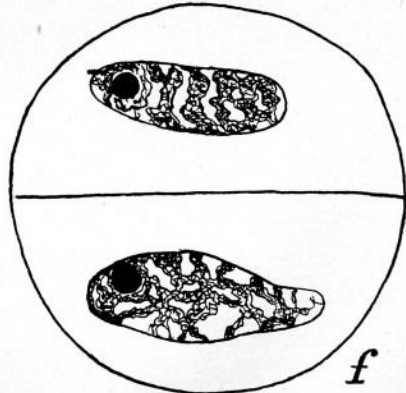
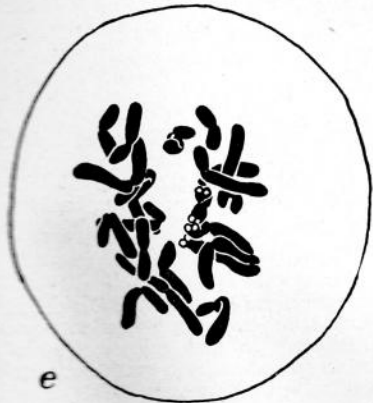
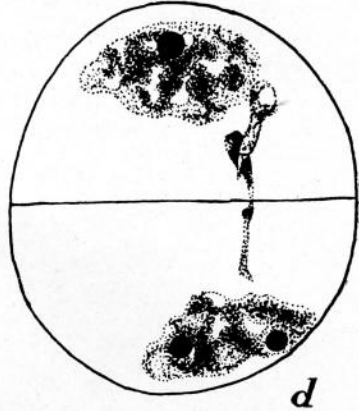
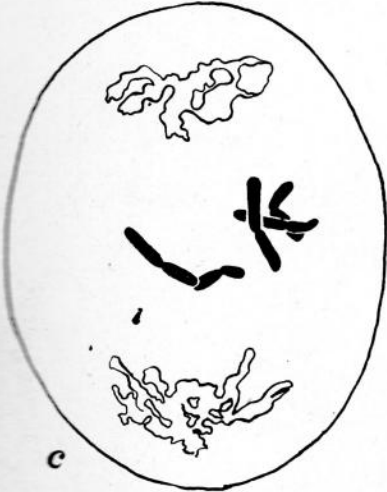
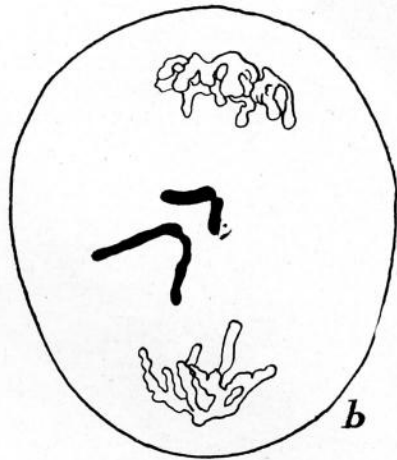
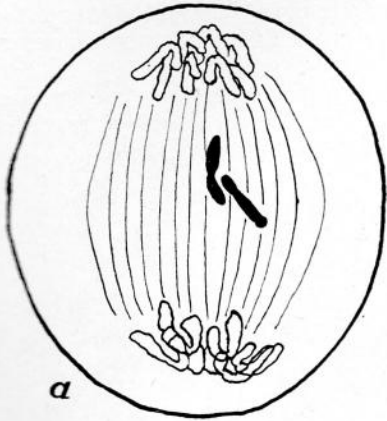


Plate 295 (see opposite page for details.)

Anaphase I (Plate 295a, b, c), telophase I (Plate 295d) and the second division indicate that the irregularities described are characteristic of autotriploid plants.

Philp (1933, 1934) also finds 21 chromosomes in the type species. However, in studying the variety *propinquus* (Herb.) Pugsley, he states

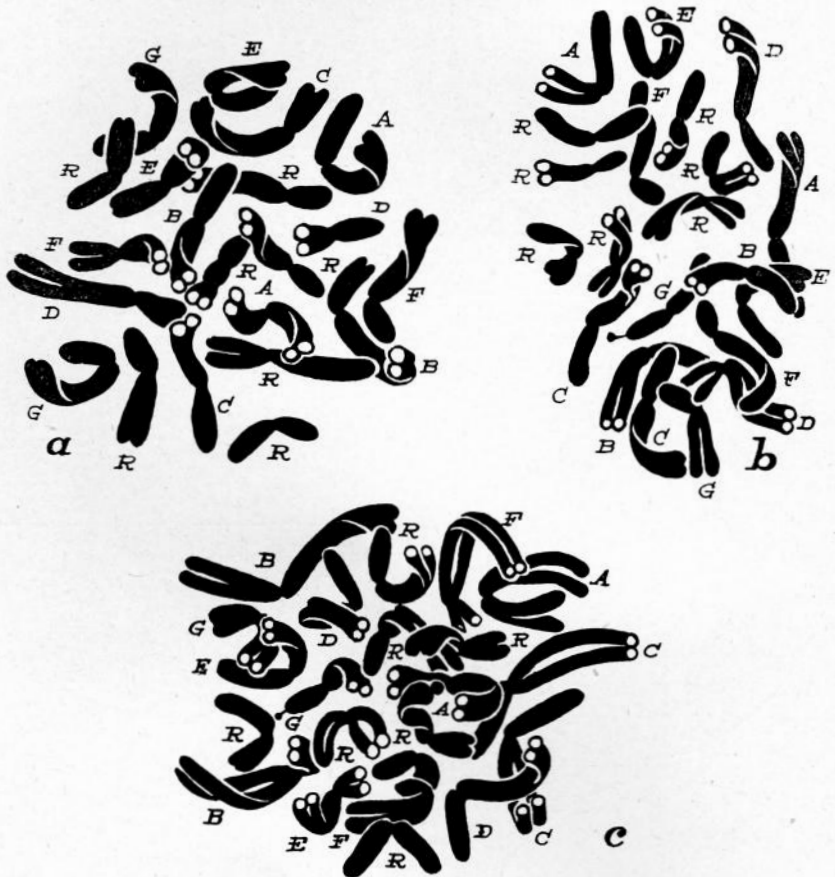


Fig. 162. *N. Johnstonii* Pugsley. *a, b*, Equatorial plate from the cells of a root meristem of a plant furnished by Fernandes Coimbra. *c*, The same, in another plant furnished by Maison Barr & Sons. In these figures the letters A - G indicate the chromosomes of a diploid complement of an *Ajax* and R the haploid complement of *N. reflexus* Brot. X 3250.

Plate 296. (See opposite page for plate.) *N. Johnstonii* Pugsley. *a*, Diakinesis showing 7 bivalents and 7 univalents. *b*, Metaphase I also showing 7 bivalents and 7 univalents. *c* and *d*, Metaphase I showing a trivalent in each cell. *e*, End of anaphase, showing two micronuclei and a bridge, accompanied by a small spherical fragment. *f*, Telophase I showing the same irregularities. X approx. 1400.

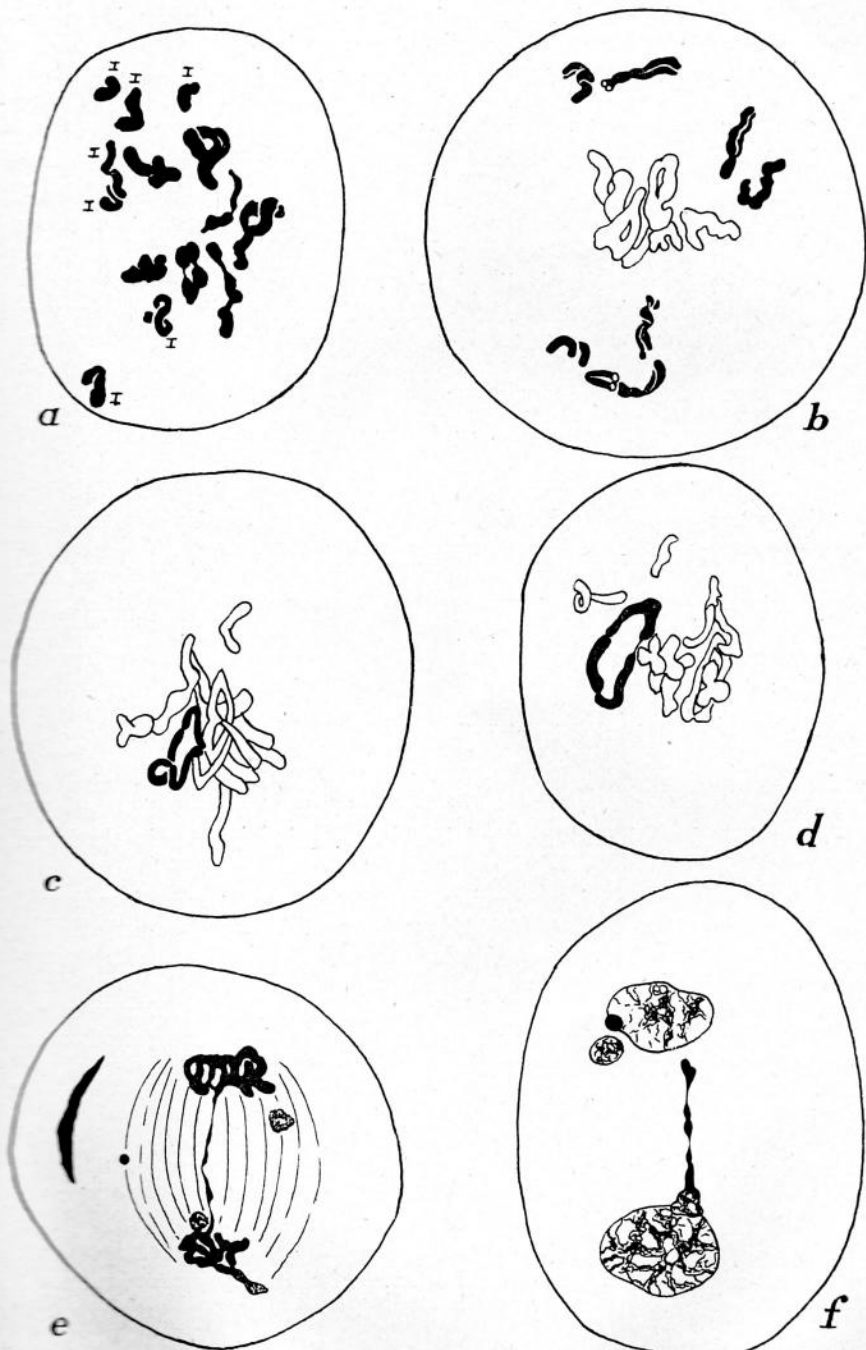


Plate 296 (see opposite page for details.)

that it is a diploid. There is thus in *N. hispanicus* intraspecific polyploidy, the variety being diploid and the type triploid.

Narcissus tortuosus and *N. trumpet Emperor* have also proven to be triploid. Figures 161a and 161b show that in this form the haploid complement is found repeated three times.

In *N. Johnstonii*, we find 21 chromosomes, either in the material furnished by Fernandes Coimbra (figs. 162a, 162b), or in that which was sent by Maison Barr & Sons (fig. 162c). Our observations confirm those of Philp (1933, 1934), concerning chromosome number.

From the fact that spiralization is greatly extended, the chromosomes at metaphase become so long, that to study their morphology is difficult. However, we have succeeded in obtaining some plates where the chromosomes are much shortened, which permitted us to establish the following chromosome formula:

$$2n = 21 = 4 : Lm + 7 : Lp + 4 : li + 1 : lm + 2 : PP + 3 : Pp$$

An analysis of this formula leads us to the conclusion that *N. Johnstonii* is not an autotriploid, since in its idiogram, we do not find the haploid complement represented three times. The presence of 4 chromosomes of the type *Lm* and 4 of the type *li* shows at once that this species possess the haploid complement of *Ajax* repeated two times. In making this withdrawal in the formula below, of the diploid complement, there remains a complement of 7 chromosomes expressed by the formula:

$$n = 3 : Lp + 1 : lm + 2 : PP + 1 : Pp$$

which corresponds exactly to that found in *N. reflexus* Brot., *N. triandrus* L. and *N. bulbocodium* L. The conclusion is reached therefore, that *N. Johnstonii* is a hybrid of a tetraploid form of *Ajax* and a diploid form, of either a *Ganymedes*, or a *Corbularia*.

A study of meiosis in the pollen mother cells confirms this point of view. Indeed, diakinesis (Plate 296a) show 7 bivalents, corresponding to the 14 elements of the *Ajax* complement, and 7 univalents, corresponding to the haploid complement of the other species. The same conformation has been observed in metaphase I, when the bivalents are disposed on an equatorial plane, while the univalents are found, most frequently, in an irregular fashion, on one part or the other of the equatorial plane (Plate 296b). Trivalents have been observed several times (Plate 296c, d).

Plate 297. (See opposite page for plate.) Equatorial plate in the cells of the root meristem. *a*, *N. trumpet Impératrice* ($2n = 22$); note the presence of four B chromosomes. *b*, *N. pseudo-narcissus* L. var. *bicolor* (L) ($2n = 28$). *c*, *N. King Alfred* ($2n = 28$). X 3250.

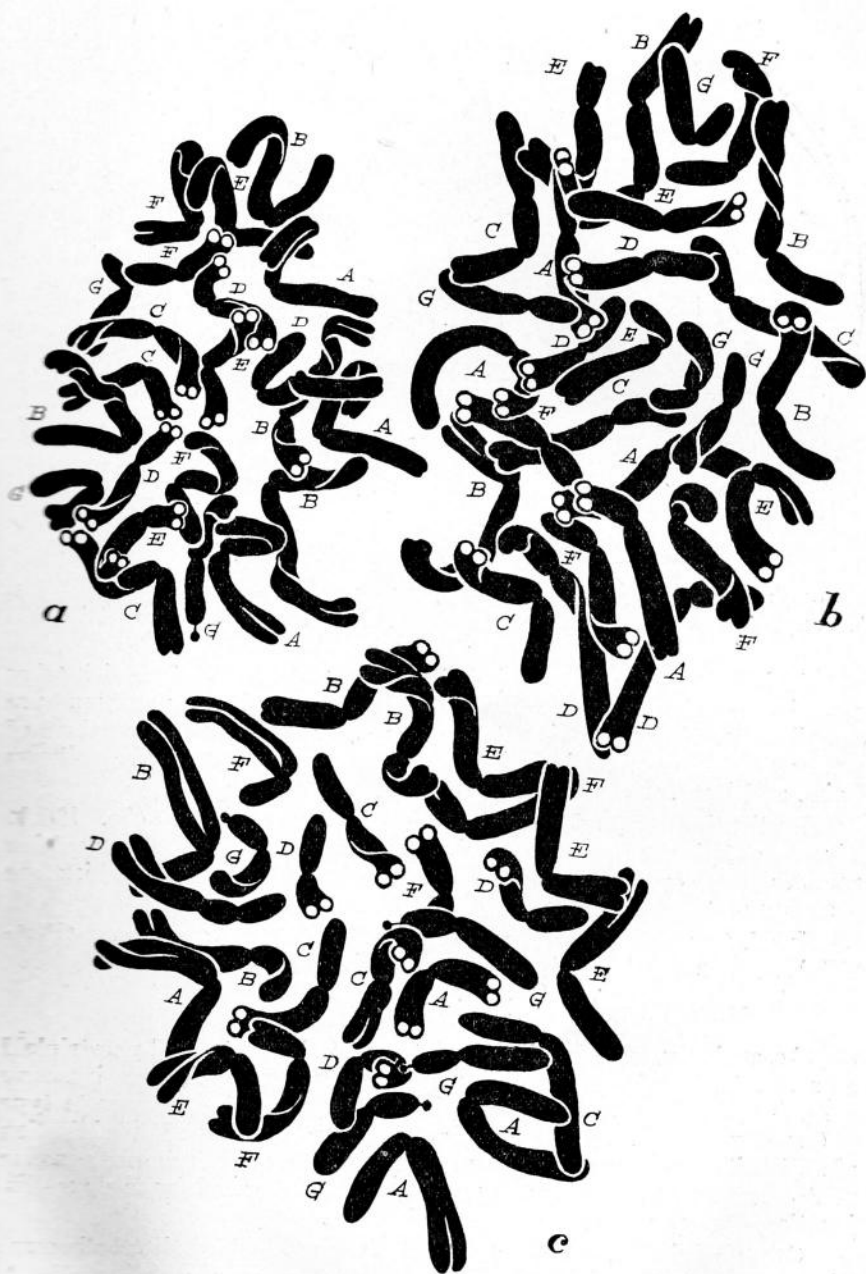


Plate 297 (see opposite page for details.)

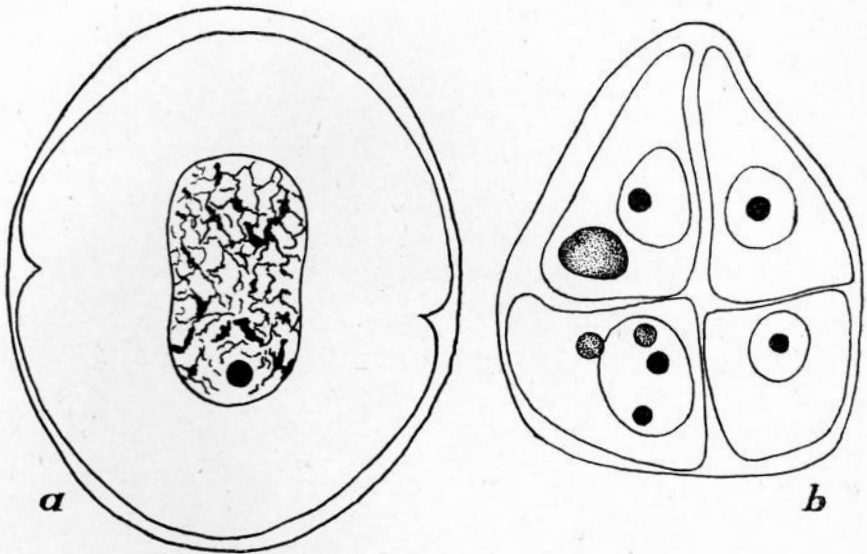


Fig. 163. *N. Johnstonii* Pugsley. *a*, Restitution nucleus formed at telophase I. *b*, Tetrad showing micronuclei in two of the 4 microspores. X approx. 1450.

As is to be expected meiosis is irregular (Plate 296e, f) and it takes place in a fashion entirely comparable with that which the authors have described for *N. jonquilloides* Willk., a species which is also a hybrid of a tetraploid form of *N. jonquilla* L. and a diploid form of *N. gaditanus* Boiss. and Reut.

Restitution nuclei sometimes have been observed (fig. 163a): These nuclei produce dyads of 21 chromosomes, and the resulting gametes are able, by fusion to give origin to hexaploids, which are probably stable and fertile.

In *N. trumpet* Impératrice, we have found 22 chromosomes (Plate 297a) whose morphology is expressed by the formula:

$$2n = 22 = 7 : Lm + 6 : Lp + 6 : li + 3 : Pp$$

Accordingly, this horticultural form is shown to be hypertriploid on account of having a chromosome of the type Lm. A more intensive analysis indicates that the supernumerary chromosome belongs to type B (see Plate 297a). This form has originated by means of hybridization of a diploid gamete, produced by a tetraploid form, with another gamete of 8 chromosomes ($3 : Lm + 2 : Lp + 2 : li + 1 : Pp$), formed by the non-disjunction of the bivalent Lm in a diploid form.

Other horticultural forms with 22 chromosomes are already known: Bicolor Victoria (de Mol, 1922; Philp, 1934), Buttonhole (de Mol, 1922), Empress (Nagao, 1929, 1933; Philp, 1934), Grandee (Nagao, 1929, 1933), Bicolor Horsfieldii (Philp, 1934) and Victoria (Sikka, 1940).

Meiosis has been studied by Nagao (1933) in Grandee and Empress and the results indicate that these forms are hypertriploids, like *N. trumpet* Impératrice.

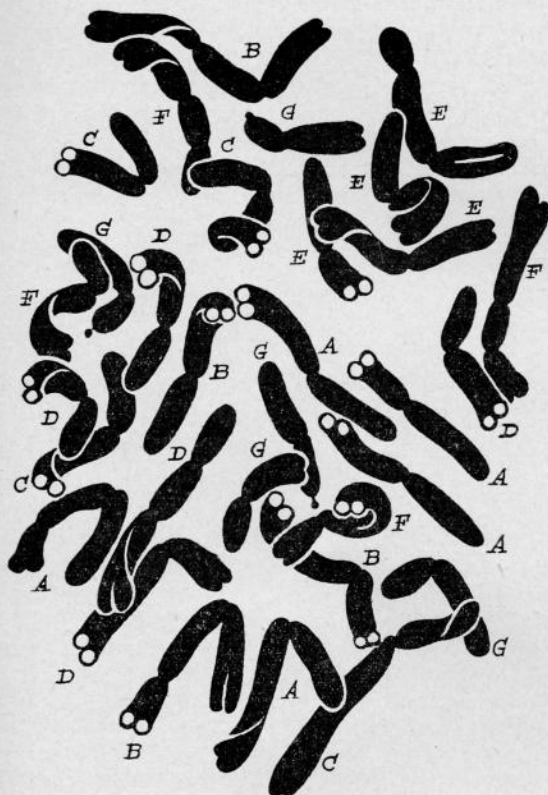


Fig. 164. *N. trumpet* M.me de Graff. Equatorial plate in the cells of the root meristems showing 31 chromosomes. X 3250.

Narcissus pseudo-narcissus L. var *bicolor* (L.) and the horticultural form, "King Alfred" show 28 chromosomes in the cells of the root meristem. The idiogram is represented by the formula:

$$2n = 28 = 8 : Lm + 8 : Lp + 8 : li + 4 : Pp'$$

These two forms present therefore chromosomes of the haploid complement A—G repeated 4 times. Consequently, it is a question of autotetraploidy.

Narcissus trumpet M.me de Graff shows 31 chromosomes in the cells

of the root meristems (fig. 164). The formula indicating the morphology of its chromosomes is the following:

$$2n = 8:Lm + 9:Lp + 9:li + 5:Pp$$

M.me de Graff is therefore a hypertetraploid, in the complement of which, one finds repeated five times, a chromosome of type Lp (D), another li (A) and another Pp (G). This horticultural form, from this fact, was produced by hybridization of a tetraploid gamete of the form (4:Lm+4:Lp+4:li+2:Pp'), that is to say, 2A+2B+2C+2D+2E+2F+2G) with a gamete of 17 chromosomes (4:Lm+5:Lp+5:li+3:Pp') that is to say, 3A+2B+2C+3D+2E+2F+3G), produced by a pentaploid form. However, pentaploid forms have not been found up to the present in *Ajar*.

DISCUSSION

In the list given below, where the species are found arranged according to the classification of Pugsley (1933), we have assembled the number of chromosome counts which have been made up to the present in each of these species.

Section I

- | | |
|---------------------------------|--|
| 1. <i>N. cyclamineus</i> DC. | 14 de Mol (1922); Collins (1933);
Fernandes (1933); Fernandes
& Fernandes (<i>hic</i>).* |
| 2. <i>N. Johnstonii</i> Pugsley | 21 Philp (1933, 1934); Fernandes
& Fernandes (<i>hic</i>). |

Section II

Series I Minores

- | | |
|--|--|
| 3. <i>N. asturiensis</i> (Jord.) Pugsley | 14 Fernandes (1931); Collins
(1933); Fernandes & Fernan-
des (<i>hic</i>).
15 Fernandes & Fernandes (<i>hic</i>). |
| 4. <i>N. Lagoi</i> Merino | |
| 5. <i>N. minor</i> L. | 14 de Mol (1922); Fernandes &
Fernandes (<i>hic</i>).
15 Collins (1933). |
| 6. <i>N. pumilus</i> Salisb. | 14 Fernandes & Fernandes (<i>hic</i>).
15 Collins (1933). |
| 7. <i>N. nanus</i> Spach | 14 de Mol (1922); Philp (1933,
1934); Fernandes & Fernandes
(<i>hic</i>). |
| 8. <i>N. parviflorus</i> (Jord) Pugsley | |

*[Translator's note: "*hic*" is the Latin equivalent of "this." Thus reference is made to the work reported in the present paper.]

Series II Lutei

9. *N. hispanicus* Gouan
 var. *propinquus* (Herb.)
 Pugsley
 10. *N. longispathus* Pugsley
 11. *N. obrvallaris* Salisb.
 12. *N. pisanus* Pugsley
 13. *N. confusus* Pugsley
 14. *N. portensis* Pugsley? (*N. Pseudo-Narcissus* L. var. *concolor* in Coutinho, Flora of Portugal.
 15. *N. nevadensis* Pugsley

21 de Mol (1922); Philp (1933, 1934); Fernandes & Fernandes (*hic*).

14 Philp (1933).

14 Philp (1933, 1934); Fernandes & Fernandes (*hic*).

14 Fernandes & Fernandes (*hic*).

Series III Vulgares

16. *N. pseudo-narcissus* L.
 17. *N. pallidiflorus* Pugsley
 18. *N. macrolobus* (Jord.)
 Pugsley

14 Philp (1933, 1934); Fernandes & Fernandes (*hic*).

15 Philp (1933, 1934).

Series IV Nobiles

19. *N. Gayi* (Hénon) Pugsley
 20. *N. nobilis* Schultes f.
 21. *N. leonensis* Pugsley

14 Philp (1933, 1934); Nagao (1929, 1933); Fernandes & Fernandes (*hic*).

Series V Albiflori.

22. *N. moschatus* L.
 23. *N. alpestris* Pugsley
 24. *N. tortuosus* Haw.
 25. *N. albescens* Pugsley
 26. *N. bicolor* L.? (*N. pseudo-narcissus* L. var. *bicolor* (L.) in Coutinho, Flora of Portugal.
 27. *N. absissus* Schultes f.

14 Philp (1933, 1934); Fernandes & Fernandes (*hic*).

14 Collins (1933).

21 Fernandes & Fernandes (*hic*).

14 Nagao (1929).

28 Fernandes (1933); Fernandes & Fernandes (*hic*).

14 de Mol (1922).

An analysis of this list shows that we do not possess data on the karyology of 9 of these species. However, by the fact that all of the series established by Pugsley (1933), possess representatives which have been studied, we can state that probably all of the species, with the ex-

ception of *N. Johnstonii*, have the same fundamental chromosome complement, represented by the formula:

$$b=7=2:Lm+2:Lp+2:li+1:PP'$$

Apart from *N. Johnstonii*, *N. hispanicus*, *N. tortuosus* and probably *N. bicolor*, all the other species are diploids with 14 chromosomes. From the fact that their idiograms are similar, their karyology does not contribute any data which would permit us to separate these species. In considering even the most distinct forms, *N. cyclamineus* and *N. asturiensis*, we can state that their separation is not possible.

With respect to *N. hispanicus*, we have established in this species diploid forms (the var. *propinquus*) and autotriploids (the type). Here we have intraspecific polyploidy and triploidy which does not permit the effective separation of these forms as distinct species.

With respect to *N. tortuosus* and *N. bicolor*, we have studied only a few examples. We can assume that diploids also exist in these species. Accordingly, we can for the moment consider that triploidy and tetraploidy permit us to separate them.

In summary, we can say that the sub-genus *Ajax*, appears to be a homogenous group, where the Jordonian species established by Pugsley (1933) belong to the same karyotype; there is some polyploidy; but we know that it is not sufficient to permit the separation of the species. The karyology is found to be in accord with the ideas of Baker (1888), who considered this group constituted a single, very polymorphic, Linnéan species.

With regard to *N. Johnstonii*, Henriques (1887) has been the first to suggest that this form could be a hybrid. Barr (1888) considered this plant originated by crossing of *N. pseudo-narcissus* and *N. triandrus albus* (*N. reflexus* Brot.). Peter R. Barr (1929) showed that in harmony with the opinion of his father, the plants concerned either were found by someone in Spain, or were those received by A. W. Tait from Portugal (in the vicinity of Pôrto). In his catalogue of 1937, Barr also considered *N. Queen of Spain* as a hybrid of *N. pseudo-narcissus* and *N. triandrus albus*. Jacob and Calvert (1929) also considered *N. Johnstonii* as a hybrid of *Ajax* and a *triandrus*. Bowles (1934) is of the same opinion, and has substantiated it with the statement that "similar forms have been raised frequently in gardens by crossing *N. triandrus* with a yellow *Ajax*."

Baker (1888) did not attribute its origin to hybridization and considered it as a variety of *N. pseudo-narcissus*. Pugsley (1933) did not believe either in the hybridity of *N. Johnstonii*. In fact, he said, "If *N. Johnstonii* is a *triandrus* hybrid, it is remarkable that it uniformly possesses the equal stamens with linear, sub-basifixed anthers of an *Ajax*; and if a *bulbocodium* cross some curvature of the stamens and style would be expected. The lack of these peculiarities tends to show that no *triandrus* or *bulbocodium* element is present, as does also the relatively broad and flat foliage; and these features seem to outweigh the somewhat *triandrus*-like corona, and the narrow perianth-tube and

segments recalling *bulbocodium*. Moreover, at least in French gardens, the plant produces fully developed capsules and might perfect seeds under favorable conditions. Another fact that tells against hybridity is the plant's abundance. For nearly forty years it must have been collected annually for export in considerable quantity, for wild bulbs have been almost continuously on sale since the early nineties, in some years being offered by the thousand. It is difficult to believe that a *Narcissus* of hybrid could have multiplied to such an extent and remained so uniform. The "Queen of Spain" is therefore treated as an *Ajax*, and, in view of its very distinct features, has been raised to specific rank." (Pugsley, 1933, pg. 38-39).

As we have noted, the karyological data, suggested from all evidence, that *N. Johnstonii* is a hybrid of a tetraploid form of an *Ajax* and a diploid form of a *Ganymedes* or a *Bulbocodium* (these two groups possess similar complements, as Fernandes, 1935, 1936b, has shown). The data of external morphology—flowers more or less inclined, segments of the perianth reflexed, form of the corona, tube of the perianth "more narrowly funnel-shaped than in the other species of *Ajax*," (Pugsley, 1933), color of the flower, the exceptional existence of two flowers, etc.—show, at once, that the diploid parent is *N. reflexus* Brot. We are therefore in accord with the point of view of Henriques, Barr, P. R. Barr, Jacob, Calvert and Bowles.

However, the arguments of Pugsley, which speak against the idea of considering *N. Johnstonii* as a hybrid, are quite justified. How can we explain the facts mentioned by this author? In our opinion, the explanation is to be found in the fact that *N. Johnstonii* is not a hybrid of two diploid forms, but a hybrid of a tetraploid form of *Ajax* and a diploid one of *N. reflexus* Brot. Given this constitution, a greater predominance of the *Ajax* parent is to be expected. Thus it is explained how the leaves can be relatively large and flat, that the stamens are equal, with linear, sub-basifixed anthers.

The fact that *N. Johnstonii* can, especially in French gardens, produce capsules with fertile seed, is explained thus:—the diploid complement of the *Ajax* parent forms 7 bivalents, which disjoin regularly. The univalents of the *N. reflexus* are frequently eliminated in the cytoplasm and gametes with the *Ajax* complement can be formed. Fertilization of these gametes produces fertile seed. This seed evidently produces some *Ajax* and it should be interesting to study plants obtained by germination of this seed.

The extraordinary abundance of plants can be explained by assuming that *N. Johnstonii* possesses a great capacity to multiply vegetatively. The case of *N. jonquilloides*, which as we have shown, possess a constitution comparable to that of *N. Johnstonii*, is entirely worthy of being connected with this fact. *Narcissus jonquilloides* Willk. never develops, at least in our cultures, fertile seed. In spite of this, it multiplies vegetatively in a truly extraordinary fashion, since some pots which have been planted with bulbs were found completely filled in several years.

Among the plants collected by Johnston and A. W. Tait in the

vicinity of Pôrto, Henriques (1887) found one to which he gave the name *N. Taiti* and which he considered, in harmony with its external morphological characters, to be a hybrid of *N. pseudo-narcissus* and *N. reflexus* Brot. (*N. calathinus* L.). In mentioning this plant, Pugsley (1933) says that the exceptional solitary flower figured by Henriques resembles *N. Johnstonii*. However, he noted that the segments of the perianth are not reflexed and that the stamens are unequal. It would have been interesting to study this plant. Is it a question of a true *N. Johnstonii* or a hybrid between the diploid forms of *N. pseudo-narcissus* and *N. reflexus*? According to the characters of external morphology, which shows a greater predominance of the characters of *N. reflexus* than those of *N. Johnstonii*, the second hypothesis seems very probable. Unfortunately, in spite of our efforts we have not succeeded in procuring this plant.

According to the data of Tait (1886), *N. pseudo-narcissus* is present in the same locality in the vicinity of Pôrto, in two forms, one of which is more robust than the other. It is probable that the large, high form is tetraploid and the other diploid. Given these facts, that *N. reflexus* Brot. growing with these forms of *N. pseudo-narcissus* and that the two species are able to flower simultaneously, it seems probable that the two hybrids, *N. Taiti* and *N. Johnstonii*, have originated in the neighborhood of Pôrto, by crossing, respectively, the diploid and tetraploid forms of *N. pseudo-narcissus* with diploid forms of *N. reflexus*.

From the fact that a great majority of these species possess similar idiograms, constituted of 14 chromosomes, we can say that the evolution of *Ajax* in the wild state has taken place particularly by single gene mutations, or accompanied by structural alterations which do not effect the morphology of the chromosomes in such a fashion that they are evident by comparison of somatic plates. Polyploidy has also played its role, but its importance, from the point of view of the formation of new species, cannot yet be estimated. Tetraploidy and hybridization have been responsible for the differentiation of *N. Johnstonii*.

Very numerous forms of *Ajax* are obtained in culture. However, we only know the karyology of the forms mentioned in the following list:

<i>N.</i> yellow double Van Sion	14	de Mol (1922, 1928); Fernandes & Fernandes (<i>hic</i>).
<i>N.</i> trumpet major	14	Philip (1934); Fernandes & Fernandes (<i>hic</i>).
<i>N.</i> trumpet King of the Yellows	14	Fernandes & Fernandes (<i>hic</i>).
<i>N.</i> Henry Irving	14	Philp (1934).
Victoria	14	Nagao (1929).
Victoria	22	Sikka (1940).
Golden Spur	21	de Mol (1922).
Golden Spur	30	Nagao (1929).
<i>N.</i> trumpet Emperor	21	Nagao (1933); Fernandes & Fernandes (<i>hic</i>).

Bicolor Victoria	22	de Mol (1922); Philp (1934).
Buttonhole	22	de Mol (1922).
Empress	22	Nagao (1929, 1933); Philp (1934).
Grandee	22	Nagao (1929, 1930, 1933).
<i>N. bicolor</i> Horsfieldii	22	Philp (1934).
<i>N. trumpet</i> Impératrice	22	Fernandes & Fernandes (<i>hic</i>).
King Alfred	28	de Mol (1922); Nagao (1929, 1933); Sikka (1940); Fernandes & Fernandes (<i>hic</i>).
Van Waveren's Giant	28	de Mol (1922).
Olympia	28	Nagao (1929, 1933).
<i>N. trumpet</i> M.me de Graff	31	Fernandes & Fernandes (<i>hic</i>).

An analysis of this list shows that 5 forms are diploids, 2 triploids, 7 hypertriploids with $2n=22$, 3 tetraploids and 2 hypertetraploids. This data reveals therefore that polyploidy alone, and polyploidy associated with polysomy have played an important role in the differentiation of horticultural forms.

Bowles (1934) states that the form Emperor has been produced by means of hybridization of *N. bicolor* and *N. pseudo-narcissus*. Assuming that the first species is a tetraploid and second a diploid, the chromosome number, $2n=21$, is in accord with this supposition.

From the fact that he did not succeed in identifying the existence of 4 similar haploid chromosome complements in the idiogram of King Arthur and also based on the morphology of the satellited chromosome Sikka, (1940) was led to the conclusion that his form is an allotetraploid. Our observations, on the contrary, show that the complement of King Alfred possesses exactly 4 chromosomes each of the 7 types A—G identified in the haploid complement, even as far as concerns the satellited chromosomes. King Alfred should therefore be considered as an auto-tetraploid, this is in accord with the observations of Nagao (1933), who showed a very frequent formation of tetravalents during the reduction divisions. The figures observed by Sikka (1940), have been produced by interesting translocations in the nucleogenio regions and, from this fact, they cannot be used to show the existence of diploid complements of two different species reunited in the idiogram of King Alfred.

Sikka (1940) expresses the supposition that King Alfred must have originated by fertilization of a non-reduced female gamete of the form Emperor with a haploid gamete of *N. hispanicus* var. *maximus* (*N. major*). This hypothesis is not in harmony with the facts, since *N. hispanicus* (*N. major*) is a triploid like Emperor. It is therefore, most probable that King Alfred has originated by fertilization of two non-reduced gametes produced by a diploid form of *N. hispanicus*, such as the variety *propinquus*.

SUMMARY AND CONCLUSIONS

1. With the exception of *N. Johnstonii*, all the species established by Pugsley (1933) in the sub-genus *Ajax* present the same fundamental chromosome complement, expressed by the formula $n=7=2:Lm+2:Lp+2:li+1:Pp'$. Of 18 of these species, 14 are shown to be diploids, and one, *N. hispanicus*, is shown to be constituted of diploid and triploid forms. *N. tortuosus* is shown to be a triploid, and *N. bicolor* tetraploid. However, we have not established that these last two species do not have diploid forms.
2. The appearance in *N. asturiensis*, *N. minor*, *N. pumilus* and *N. pallidiflorus* of plants with 15 somatic chromosomes should be attributed in these species, to supernumerary, heterochromatic chromosomes of the type of those which have been found in *N. juncifolius* Lag. and *N. bulbocodium* L.
3. *Narcissus Johnstonii* possesses an idiogram represented by the formula:

$$2n=21=4:Lm+7:Lp+4:li+1:lm+2:PP+3:Pp$$

This formula corresponds exactly to the addition of a diploid complement of *Ajax* with a haploid complement of *N. reflexus* Brot.

<i>Ajax</i>	$2n=4:Lm+4:Lp+4:li+$	$2:Pp$
<i>N. reflexus</i>	$n=3:Lp+$	$1:lm+2:PP+1:Pp$
	$3n=4:Lm+7:Lp+4:li+1:lm+2:PP+3:Pp$	

4. By the fact that *N. Taiti* Henriq. presents some characters of *N. reflexus* in a more manifest degree than *N. Johnstonii*, we have suggested that this hybrid originated by means of crossing two diploid forms of *N. Pseudo-Narcissus* L. and *N. reflexus* Brot.
5. The karyological data, except perhaps for *N. tortuosus* and *N. bicolor*, does not furnish any elements which would permit us to separate the Jordonian species established by Pugsley (1933). The fact that all the species possess the same karyotype is in harmony with the ideas of Baker (1888), who considered the group to be a single, very polymorphic, linnean species. The most distinct species, *N. cyclamineus* and *N. asturiensis* cannot be distinguished from the others, from a karyological point of view.
6. The principal role in the evolution of the group in the wild state has been played by gene mutation, alone or accompanied by structural alterations not modifying in a visible fashion the chromosome complement. Polyploidy has also played a role, however its importance cannot yet be estimated, because of the fact that we have not been able to effectuate an intensive study of *N. tortuosus* and *N. bicolor*, and some species (9) yet remain to be investigated.
7. A list of the chromosome numbers of horticultural forms studied up to the present is given. This list indicates that the origin of some

forms can be attributed to gene mutation, but the principal role has been played by polyploidy alone, or polyploidy associated with polysomy.

8. The karyological data is found to be in accord with the question of considering the form Emperor ($2n=21$) as having originated by fertilization of *N. bicolor* ($2n=28$) with *N. pseudo-narcissus* ($2n=14$).
9. King Alfred should be considered an autotetraploid form and M.me de Graff could have been produced by fertilization of a tetraploid form with a pentaploid one.

BIBLIOGRAPHY

- BAKER, J. G.
1888 *Handbook of the Amaryllideae*. London.
- BARR, Peter
1884 *Ye Narcissus or Daffodil Flower, etc.* London. (Citation from PUGSLEY).
- BARR, Peter
1888 Citation from BARR, P. R. 1929, Species and wild forms of *Narcissi*, in CALVERT, A. F. *Daffodil growing for pleasure and profit*, London, 340-358.
- BARR, P. R.
1929 Species and wild forms of *Narcissi*, in CALVERT, A. F. *Daffodil growing for pleasure and profit*, London, 340-358.
- BOWLES, E. A.
1934 *A handbook of Narcissus*. London.
- CALVERT, A. F.
1929 *Daffodil growing for pleasure and profit*. London.
- COLLINS
1933 in PUGSLEY, H. W.
A monograph of *Narcissus*, subgenus *Ajax*. *J. Roy. Hort. Soc.*, 58, 17-93.
- COUTINHO, A. X. P.
1939 *A Flora de Portugal*. Lisboa.
- FERNANDES, A.
1931 Estudos nos cromosomas das Liliáceas e Amarilidáceas. *Bot. Soc. Broteriana*, 7 (2.a série), 3-110.
- FERNANDES, A.
1933 Novos estudos cariologicos no género *Narcissus* L. *Rev. Fac. Ciênc. Univ. Coimbra*, 3, 53.
1934 Nouvelles études caryologiques sur le genre *Narcissus* L. *Bol. Soc. Broteriana*, 9 (2.a série), 3-195.
1935 Les satellites chez *Narcissus reflexus* Brot. et *N. triandrus* L. I. Les satellites des métaphases somatiques. *Bol. Soc. Broteriana*, 10 (2.a série), 247-277.
1936 La mixoploide chez *Narcissus reflexus* Brot. *Bol. Soc. Broteriana*, 11 (2.a série), 27-42.
1937a Sur l'origine du *Narcissus jonquilloides* Willk. *Scientia Genetica*, 1, 16-61.
1937b Sur le comportement d'un chromosome surnuméraire pendant la mitose. *Scientia Genetica*, 1, 141-167.
1943 Sur l'origine des chromosomes surnuméraires hétérochromatiques chez *Narcissus Bulbocodium* L. *Bol. Soc. Broteriana*, 17 (2.a série), 251-256.
- HAWORTH, A. H.
1831 *Narcissinearum Monograph* (ed. 2 praec.). Chelsea. (Citation from PUGSLEY, 1933).

- HENRIQUES, J. A.
1887 *Amaryllideas de Portugal*. *Bol. Soc. Broteriana*, 5 (2a série), 159-174.
- HERBERT, W.
1837 *Amaryllidaceae*. London. (Citation from PUGSLEY, 1933).
- JACOB, Rev. J.
Daffodils. London.
- JORDAN, A.
1903 *Icones ad Floram Europae*, 3. Parisii (Citation from PUGSLEY, 1933).
- LA COUR, L.
1937 Improvements in plant cytological technique. *Bot. Rev.*, 3, 241-258.
- LINNE, C.
1753 *Species plantarum*. Ed. I, Holmiae (Citation from PUGSLEY, 1933).
1762 *Species plantarum*. Ed. II, Holmiae (Citation from PUGSLEY, 1933).
- de MOL, W. E.
1922 The disappearance of the diploid and triploid and triploid magnicoronate *Narcissi* from the larger cultures and the appearance in their place of tetraploid forms. *Proc. Kon. Akad. van Wetenschappen Amsterdam*, 25.
1928 Een nieuwe veredelingsmethode. Het doelbewnst benutten van meerchromo-omige bevruchtungskernen. *Weekbald voor Bloembollencultuur*, July 20 (Citation from NAGAO, 1933).
- NAGAO, S.
1929 Karyological studies of the *Narcissus* plant. I Somatic chromosome numbers of some garden varieties and some meiotic phases of a triploid variety. *Mem. Coll. Sci. Kyoto Imperial Univ. S. B.* 4, 177-178.
1930 On the meiosis in the polyanthus narcissus, *Narcissus tazetta* L. Preliminary note. (Karyological studies of the narcissus plant II). *Jap. Jour. Genetics*, 5.
1933 Number and behaviour of chromosomes in the genus *Narcissus*. *Mem. Coll. Sci. Kyoto Imp. Univ.*, 8, 81-200.
- PHILP, J.
1933 in PUGSLEY, H. W.
A monograph of *Narcissus*, subgenus *Ajax*. *J. Roy. Hort. Soc.*, 58, 17-93.
- PHILP, J.
1934 *Narcissus* chromosome numbers. *R. H. S. Daffodil Year-book*, 52-53.
- PUGSLEY, H. W.
1933 A monograph of *Narcissus*, subgenus *Ajax*. *J. Roy. Hort. Soc.*, 58, 17-93.
- SALISBURY, R. A.
1812 On the cultivation of rare plants. *Trsns. Hort. Soc.*, 1, 343 (Citation from PUGSLEY, 1933).
- SIKKA, S. M.
1940 Study of the somatic chromosomes in *Narcissus*. *Ann. Bot.*, 4 (New Series), 427-464.
- TAIT, A. W.
1886 *Notes on the Narcissi of Portugal*. Pôrto.