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THE CYTOLOGY OF DIASCIA (SCROPHULARIACEAE) 1. CHROMOSOME NUMBERS IN SECTION RACEMOSAE

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ABSTRACT. The chromosome numbers of nine perennial species of *Diascia* sect. *Racemosae* and one plant of garden origin (*Diascia barberae* 'Ruby Field') were determined. Eight are diploid with a somatic number of 2n = 18, and two polyploid (*D. fetcaniensis*, 2n = 36; *D. vigilis*, 2n = 54). Among the diploids, the somatic number 2n = 19 in *D. rigescens* is due to the presence of one accessory B-chromosome; the only published chromosome number, 2n = 18 for *D. barberae*, is confirmed. Meiotic irregularity occurs in *D. barberae* 'Ruby Field' (2n = 18) together with a high level of pollen sterility; variation in pollen size and morphology has also been observed, features consistent with the hybrid origin of this plant. It is inferred that x = 9 is the basic number, the only one so far encountered in the genus. The karyotype consists predominantly of metacentric to submetacentric chromosomes. Cytological interrelationships between *Diascia* and other genera in the tribe Hemimerideae are briefly considered.

INTRODUCTION

Diascia Link & Otto in the tribe Hemimerideae Benth. of the Scrophulariaceae is an endemic South African genus of perennial to annual species and is divided into two sections, sect. *Diascia* and sect. *Racemosae* (Benth.) Wettst. After a long period of neglect, interest in the genus has been stimulated by the recent revision of sect. *Racemosae* (Hilliard & Burtt, 1984). This taxonomic framework and the recent introduction of numerous species into cultivation in Britain by its authors (see Benham, 1987) provide a valuable basis for the initiation of a cytological study of the genus. The only recorded chromosome information to date is a diploid number of 2n = 18 for *D. barberae* (Propach, 1934).

Hilliard & Burtt (*op. cit.*) recognize 26 species within sect. *Racemosae*, which they place in seven small alliances or 'Groups'. The present paper is a report on chromosome numbers for nine of these species and one plant of garden origin (*D. barberae* 'Ruby Field'). The taxa examined are distributed among five of the seven 'groups' and are all perennials from the Drakensberg or the eastern parts of South Africa where the largest concentration of perennial species is found.

Although certain aspects of the karyomorphology will be touched upon here, a more detailed treatment of the karyotype and meiosis will be presented in another paper.

MATERIALS AND METHODS

The plants used in this investigation were mostly collected by Dr O. M. Hilliard and B. L. Burtt, and form part of the living collection of the Royal Botanic Garden, Edinburgh. All the species are perennials and, except for *Diascia barberae* 'Ruby Field', are of known wild origin (see Table 1), each being represented by only a single stock. Unfortunately no annual species of sect. *Racemosae* or any of the Cape annuals was available for inclusion in the present survey.

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Chromosome counts were obtained mainly from root tip squashes, with a few from anther squashes. Freshly struck cuttings provide a useful source of actively growing roots; in a peat-based compost, roots appear after two to three weeks if the cuttings are kept under a transparent cover in a warm greenhouse.

Of the several commonly used chemicals tried, pretreatment in a saturated aqueous solution of 1-bromonaphthalene for 2 to $2\frac{1}{2}$ hours at room temperature (c.20°C) yielded a consistently high frequency of well-spread metaphases. Some satisfactory squashes were also obtained after a 4 hour treatment in 0·002M 8-hydroxy-quinolene at 13°C or in saturated aqueous 1,4-dichlorobenzene (paradichlorobenzene) at room temperature. However, after PDB the results were rather erratic, the chromosomes showing a tendency to clump together, as they did very markedly after pretreatment in a range of concentrations of colchicine.

Staining was readily effected in either the Feulgen reagent or lacto-propionic orcein (prepared according to Fox (1969) and Dyer (1963) respectively).

Below is an outline of a preferred schedule for *Diascia* somatic chromosomes:

1. Harvest roots between 10.30 am and 12 noon.
2. Pretreat in a saturated aqueous solution of 1-bromonaphthalene for 2–$2\frac{1}{2}$ hours at room temperature (c.20°C).
3. Fix in Farmer's 3 : 1 (3 parts ethanol to 1 part glacial acetic acid), for 1 hour or overnight.
4. Transfer to distilled water for 2 minutes.
5. Soften in 5M HCl at room temperature for 20 minutes.
6. Transfer to distilled water for 5 minutes.
7. Excise root meristem on a slide, add a drop of 45% lacto-propionic orcein.
8. Tap meristem with a brass tapper, leave for 1 minute before adding a coverslip.
9. Wait for a minute or so before blotting excess stain and applying pressure.
10. Heat gently over a spirit flame.
11. Seal with rubber solution for temporary storage in a deep-freezer or refrigerator.

For meiotic observations, buds were fixed in Farmer's 3 : 1 or Dyer's (1963) modified Carnoy (ethanol : glacial acetic acid : chloroform : formalin—10 : 2 : 2 : 1). They were examined after fixation for 1 hour or overnight, or after storage in a deep-freezer in either the fixative or 70% ethanol. Anther squashes were made in propionic carmine or lacto-propionic orcein. Dyer (1963) recommends very brief fixation (c.5 minutes in his modified Carnoy fixative) before staining in orcein.

Permanent slides were prepared according to a modification of the quick-freeze method of Conger & Fairchild (1953), i.e. using liquid nitrogen instead of dry-ice as a freezing medium. Some slides were also made permanent using the vapour exchange method of Bradley (1948).

Photomicrographs were taken under a Leitz Dialux 20 research microscope equipped with a Wild MPS 45 Photoautomat camera system, using Ilford Pan F or Technical Pan 35mm film.

RESULTS AND DISCUSSION

This survey confirms the single previously published chromosome count of 2n = 18 for *D. barberae* (Propach, 1934); all the other numbers presented in Table 1 below are first reports.

Of the 10 taxa examined, eight are diploid, 2n = 18 (*D. rigescens*, has 2n = 19, but see below), one tetraploid (*D. fetcaniensis*, 2n = 36), and one hexaploid (*D. vigilis*, 2n = 54), all based on a single presumed basic number of x = 9, the only one encountered so far in the genus.

Among the seven 'groups' recognized by Hilliard & Burtt (1984), chromosome counts have been obtained for five; for two of these, 'Groups 5 and 6', only one species each is represented. All species counted are perennials occurring at high elevations mainly in the Drakensberg of eastern South Africa (Table 1). There is as yet no cytological data for any annual species

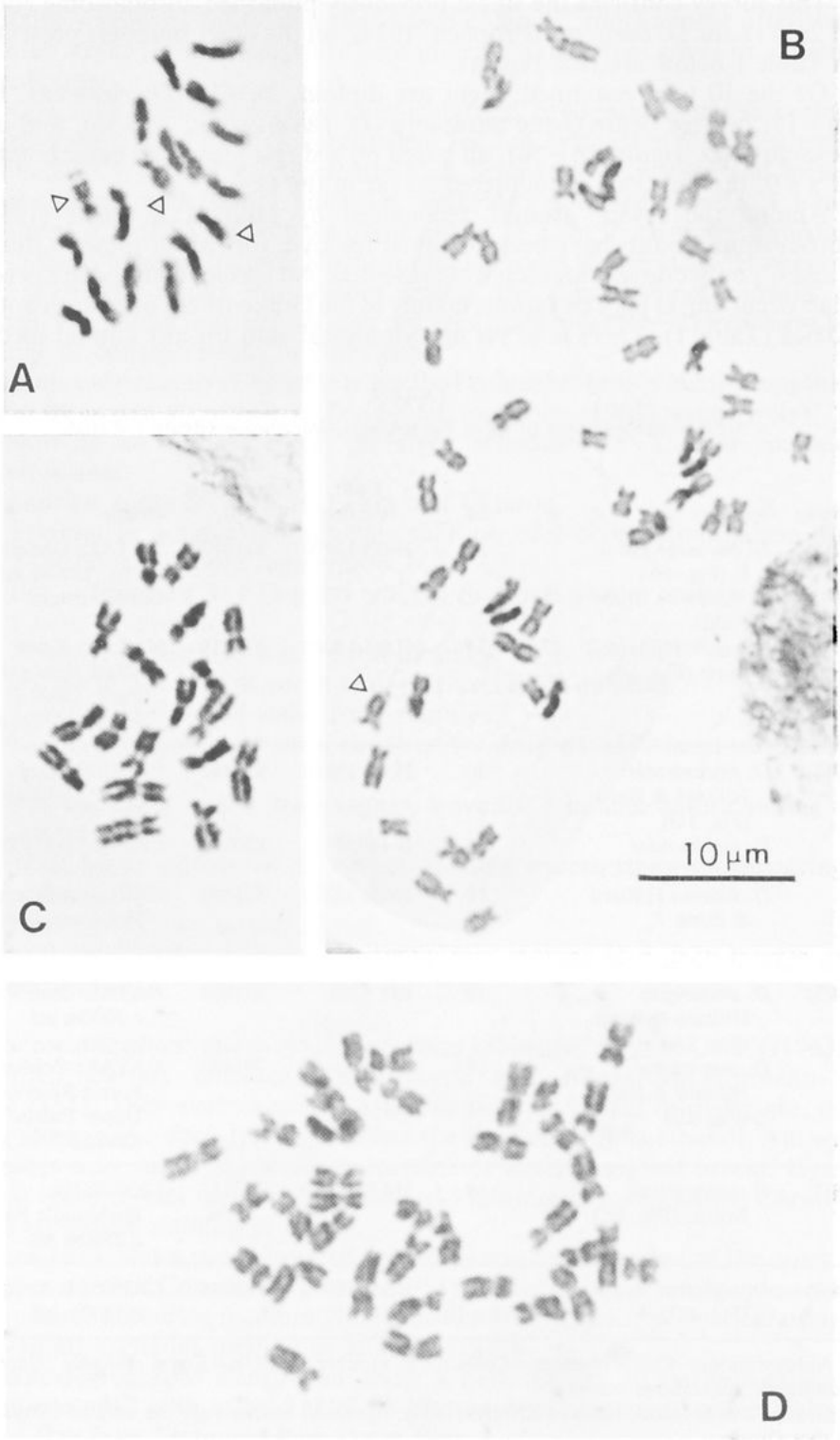
TABLE 1

Chromosome numbers of some *Diascia* sect. *Racemosae* (Benth.) Wettst.

Group	Species	n	2n	Coll. Number	RBGE Accession No.	Origin
2(5)	*D. barberae* Hook. f. (Fig. 1A)		18	H&B 13470	811497	NATAL: Underberg dist. 1890m asl
	D. barberae 'Ruby Field'	9				Garden origin
	D. vigilis Hilliard & Burtt (Fig. 1B)	27	54	H&B 14440	811723	NATAL: Royal Natal National Park, Devil's Hoek, 1675m asl
3(4)	*D. fetcaniensis* Hilliard & Burtt (Fig. 1D)		36	H&B 12318	811494	CAPE: Fetcani Pass, c.2300m asl
	D. stachyoides Hiern (Fig. 1C)		18	B 14554	820243	CAPE: Barkly Pass, 1980m asl
	D. lilacina Hilliard & Burtt		18	H&B 12279	811496	CAPE: Saalboom Nek Pass, 2100m asl
4(3)	*D. anastrepta* Hilliard & Burtt (Fig. 2A)		18	BH 5222	831069	NATAL: Sani Pass, c.3000m asl
	D. megathura Hilliard & Burtt (Fig. 2B)		18	H&B 12465	791405	NATAL: Cobham Forest Reserve, Upper Polela Cave, 2075m asl
5(3)	*D. integerrima* Benth. (Fig. 2C)		18	H&B 12035	790337	LESOTHO: Bushman's Pass, c.2500m asl
6(2)	*D. rigescens* Benth. (Fig. 2D)		19 (18 + 1B)	H&B 11032	780046	CAPE: Mt Kemp, c.1400m asl

Abbreviations: Coll. Number = Collector's number; RBGE = Royal Botanic Garden, Edinburgh; asl = above sea-level.

Groups **2** to **6**—sensu Hilliard & Burtt (1984); figures in brackets are the number of species in that Group.


A
B
C
D
10 μm

either of sect. *Diascia*, whose centre of distribution is the Cape, or of 'Group 7' of sect. *Racemosae*, which are more southerly and westerly than the rest of the section and which are thought to provide a link with sect. *Diascia* (Hilliard & Burtt, 1984).

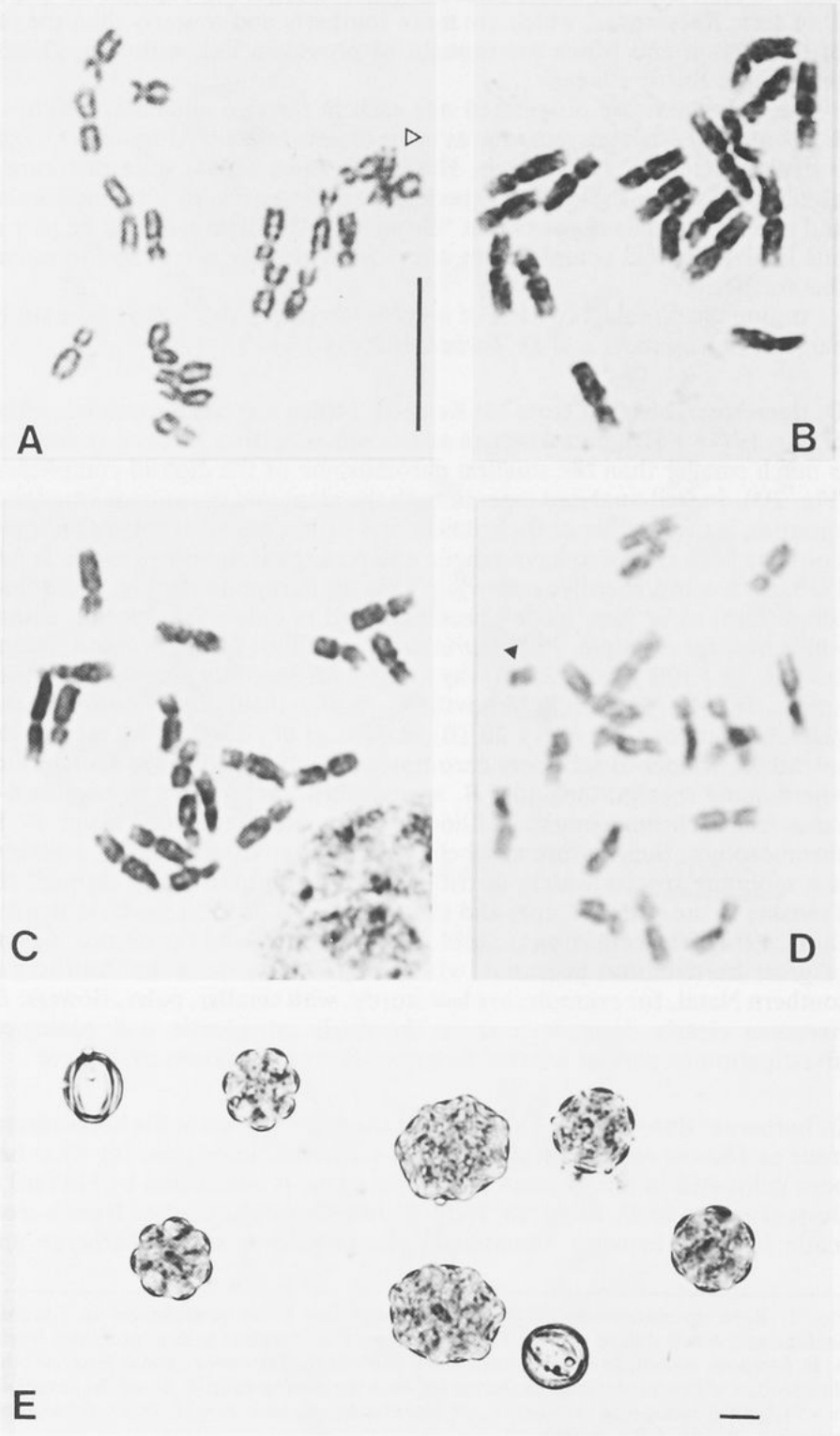
The polyploids are distributed one each in the two alliances, 'Groups 2 & 3'—alliances that are regarded as being closely related. Although *D. vigilis* is distinct from *D. fetcaniensis*, Hilliard & Burtt (1984) state that care is needed to distinguish these two species; their close affinity with one another and related diploids suggests that 'Groups 2 & 3' might possibly be part of one large polyploid complex, but more detailed work is required to pursue this further.

Among the diploids, two are of notable interest at this stage of the study, namely, *D. rigescens* and *D. barberae* 'Ruby Field'.

D. rigescens: The plant from Mt Kemp (c.1400m asl) has a somatic number of 2n = 19 (18 + 1B), possessing an additional, accessory B-chromosome that is much smaller than the smallest chromosome of the diploid complement (Fig. 2D). In well-analysed cases in both the plant and the animal kingdoms, variation in the number of B-chromosomes within as well as between populations has been shown to have genetic and ecological significance (see Jones, 1975, for a comprehensive review). Within the Scrophulariaceae, B-chromosomes seem to be rare, having been observed in only a few species, among which are, for example, *Pedicularis sudetica* Willd. subsp. *interior* Hulten, 2n = 16, 16 + 10B (Dawe & Murray, 1981), *Mecardonia acuminata* (Walt.) Small, 2n = 42 + 2B (in Bohkhovskikh *et al.*, 1969), and *Veronica anagalloides* Gussone, 2n = 18 + 2B (Bjorkqvist *et al.*, 1969). The most widespread occurrence of accessory chromosomes in the family is in *Rhinanthus*, where many species, including *R. major* Ehrh., are known to possess 6–8 extra 'microchromosomes'. Although these are sometimes listed as B-chromosomes, their nature and behaviour are far from clear. *D. rigescens* is a montane species widely distributed from southern Natal through the Transkei to the eastern Cape, and there appear to be local races (Hilliard & Burtt, 1984). The collection from Mt Kemp is robust and floriferous, a plant of great horticultural potential, whereas specimens from the Zuurberg in southern Natal, for example, are less sturdy, with smaller, paler, flowers. *D. rigescens* clearly deserves a more thorough cytogenetic and ecological investigation as part of a fuller biosystematic appreciation of *Diascia*

D. barberae 'Ruby Field': The status of the plant known in the horticultural trade as *Diascia cordata* 'Ruby Field' is somewhat uncertain, for what had been cultivated in recent years under that name is considered by Hilliard & Burtt (1984) to be *D. barberae* 'Ruby Field'. This was obtained from a cross made in 1970 between commercial stocks known as *D. barberae* and

FIG. 1. Root tip metaphases of *Diascia*. All after two hours pretreatment in 1-bromonaphthalene; A & B stained with the Feulgen reagent; C & D stained in lacto-propionic orcein. A, *D. barberae*, diploid, 2n = 18, submetacentric pair satellited (arrowed), and at least one other chromosome with a minute satellite (barely visible in the photograph); B, *D. vigilis*, hexaploid, 2n = 54, SAT-chromosome arrowed; C, *D. stachyoides*, diploid, 2n = 18; D, *D. fetcaniensis*, tetraploid, 2n = 36. (all c. × 2000).


A
B
C
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E

D. cordata (Kelly, 1987). True *D. cordata* N. E. Br., however, has never been in cultivation, and the plant passing under that name is *D. barberae* Hook. f. (Hilliard & Burtt, 1984). Unfortunately it has not been possible to recover the plant grown as *D. barberae*; the nursery concerned reports that it was not hardy and was not retained. It could have been another strain of *D. barberae*, or possibly another species, *D. capsularis* Benth., which had in earlier days been confused with *D. barberae* (Burtt, pers. comm.).

Cytologically, both *D. barberae* and 'Ruby Field' are diploid with 2n = 18. A preliminary examination of meiosis revealed that whereas it is regular in *D. barberae*, and the majority of mature pollen grains fully stained and binucleate (empty aborted grains scarce), much meiotic irregularity has been observed in 'Ruby Field', accompanied not surprisingly by a high proportion of aborted pollen grains (c.25%). Such grains are empty and unstained, and are the smallest among three types of pollen present in this plant (Fig. 2E). The other two types, which are stained by either carmine or cotton blue, differ in size as well as in morphology. The largest type has an angular outline in the polar plane, that is, as seen in optical section, and is elongate to narrow elliptical in side view, with longitudinal colpi. The medium-sized grains are mostly barrel-shaped, with a more scalloped appearance in polar view (Fig. 2E), often only partly stained and these are most probably also sterile, thus enhancing the level of pollen sterility. While the role of these two stained pollen types remains to be elucidated, the above combined features are indicative of hybridity consistent with the hybrid origin of 'Ruby Field'.

KARYOTYPE

The karyotypes of all the species examined are symmetrical, consisting of predominantly metacentric to submetacentric chromosomes which show a gradual gradation in size between the largest and the smallest in the genome. One of the chromosome pairs bears a minute satellite which is often indistinct or sometimes seen only in one member of such a pair, or not at all; in *D. barberae*, up to three SAT-chromosomes have been detected (Fig. 1A). There also appears to be a variation in the degree of heterochromaticity at the extremities of chromosome arms among chromosomes within a genome, for example, in *D. megathura* where this is most pronounced especially at early metaphase (Fig. 2B). Further experiments with different cytological procedures might yield additional information on such variations and possibly others in the comparative karyotype studies now in progress.

FIG. 2. A–D: Root tip metaphases of *Diascia*; all after two hours pretreatment in 1-bromonaphthalene (except *D. rigescens*, after 4 hours in 8-hydroxyquinolene at 13°C), and stained in lacto-propionic orcein. A, *D. anastrepta*, diploid, 2n = 18, one clear satellited submetacentric chromosome (arrowed); B, *D. megathura*, diploid, early metaphase, 2n = 18, note chromosomes with pronounced heterochromatic distal regions; C. *D. integerrima*, diploid, 2n = 18, some chromosomes with heterochromatic distal regions; D. *D. rigescens*, diploid, 2n = 19 (18 + 1B), the B-chromosome indicated by arrow.

E. Fresh mature pollen of *D. barberae* 'Ruby Field' mounted in propionic-carmine: note small empty sterile grains, and stained medium-sized with 6–7 colpi, and two large stained 7-colpate grains.

Scales = 10μm (A–D, c. × 2000; E, c. × 500).

CHROMOSOME NUMBERS IN RELATED GENERA

Diascia belongs with four other allied South African genera, *Nemesia*, *Hemimeris*, *Diclis* and *Colpias*, in the tribe Hemimerideae Benth., which also contains *Angelonia* and *Alonsoa*. The last two are tropical American, and only one of the species is found in South Africa, the little-known *Alonsoa peduncularis* (Kunze) Wettst. For a recent review of affinities of *Diascia*, see Hilliard & Burtt (1984).

The available cytological information, mainly as chromosome counts, is summarized in Table 2. This is based on a survey of the usual sources of published chromosome records, including the most recent Index to Plant Chromosome Numbers (see Goldblatt, 1981, 1985).

TABLE 2

Chromosome numbers of some genera of the tribe Hemimerideae (sensu Bentham, 1836; Bentham & Hooker, 1876)

Genus	n	2n	Distribution
Angelonia Humb. & Bonpl.	10		30 spp. trop. America,
3 spp.		20	West Indies
Alonsoa Ruiz & Pavón, 1 sp.	28		6 spp. trop. America,
3 spp.		24	1 S Africa
Colpias Benth.			
C. mollis Benth.		40	1 S Africa
Diascia Link & Otto	9	18	c.50 S Africa
9 spp. + 1 garden hybrid		36	
(see Table 1)	27	54	
Nemesia Vent.			
c.16 spp.		18	c.65 S Africa
N. strumosa Benth.		18, 20, 36	

Chromosome counts are thus available for five of the genera in the tribe *Hemimerideae* (sensu Bentham, 1836; Bentham & Hooker, 1876), and none yet for the other two, *Hemimeris* L. and *Diclis* Benth. A range of haploid numbers are now known, n = 9, 10, 12, 20 and 28, some of the higher numbers most probably derived. Only *Nemesia* shares with *Diascia* the basic number x = 9, which lends support to the suggested close affinity of the two genera, but this by itself is inadequate as additional karyotype information for *Nemesia* is lacking, a deficiency that needs to be remedied before more meaningful comparisons can be made. It is worth noting, however, that although x = 9 occurs elsewhere in the Scrophulariaceae, among genera of diverse affinities, for example, *Calceolaria* Loefl., *Melampyrum* L., and *Veronica* L., it is a relatively rare basic number in the family. Where it is common to closely allied genera, a fuller cytological scrutiny is clearly worthwhile.

Alonsoa has at least two haploid numbers, n = 28 and n = 12; the first-mentioned (for *A. acutifolia* Ruiz & Pavón) is probably a polyploid number derived from n = 14 or 7. It is too early to deduce what the basic numbers might be, but it is evident that there are more than one. *Colpias mollis*, the only species in its genus, has 2n = 40, probably based on x = 10, a basic number that also occurs in *Angelonia*. *Angelonia* was placed in the separate tribe *Angelonieae* by Pennell (1920), and was later joined there by another American genus, *Basistemon* Turcz. (Barringer, 1985). Barringer thinks they

are related and that they belong to the subfamily *Rhinanthoideae*. It would seem that *Angelonia* is of marginal interest in considering *Diascia*.

It is clear that the published information is far too meagre and incomplete for a proper assessment to be made about cytological interrelationships within the *Hemimerideae*.

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