On Interchange Complexes in *Commelina diffusa* Burn F. from South Eastern Nigeria

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Various species of *Commelina* Linn. grow as weeds in many parts of Africa. In South Eastern Nigeria, *C. diffusa* Burn. F. is the most common and widespread. However, very little has been reported on cytogenetics of the African members of this genus. Numerous reports are available, elsewhere, on chromosome counts on *Commelina* species (Sharma 1955, Morton 1956, 1967, Sharma and Sharma 1958, Kammathy and Rolla 1961, Panigrahi and Kammathy 1964, Lewis 1964, Jones and Jopling 1972, Rao *et al.* 1972, Chimphamba 1973, Bhattacharya 1975, Faden and Suda 1980) giving the chromosome number of *C. diffusa* as $2n = 28, 30, 35, 42, 56, 58, 60$ or 120 .

Interchange (translocation) complexes have been described in a large number of other plant species such as *Oenothera*, *Campanula*, *Gaura*, *Secale*, *Rhoeo discolor* and *Hypericum punctatum* (Stebbins 1971, Rees and Jones 1977) but there is little report of these in *C. diffusa*. Sharma (1955) and Panigrahi and Kammathy (1964) observed multivalent chromosome associations in some PMC's of Asian collections of *C. diffusa*. Hossain (1984) observed interchanges in a Nigerian collection of this genus, but the identification of the species used was unsatisfactory.

The present investigation was stimulated by the fact that collections of *C. diffusa* found here, unlike the other species present (*C. congesta*, *C. lagosensis* and *C. erecta*) rarely form seeds. Furthermore, although some workers have reported well-developed and matured seeds (Morton 1956, 1967, Panigrahi and Kammathy 1964, Faden 1974), information is lacking on the viability of such seeds from *C. diffusa* collections. This study, therefore, concentrates on the meiotic behaviour of the chromosomes of *C. diffusa* and the resultant infertility associated with the observed chromosomal associations.

Materials and methods

Five populations of *C. diffusa* were established in the University of Calabar from collections in Calabar and its suburbs. Identification of the species and subspecies were based on Brenan's papers (1966, 1968) and Morton's (1956, 1967); and later confirmed by Dr. Lucas of Kew Garden, U. K. The morphological features of the collections as well as their assigned population numbers are shown in Table 1.

For meiotic studies, young flower buds were collected between 9 a. m. and 10 a. m. fixed in acetic alcohol (1: 3) and squashed in aceto-carmin (Darlington and La Cour 1962). One hundred PMC's were analysed for chromosome pairing behaviour from each of the populations.

The structure of mature anthers and their pollen were also studied in an attempt to estimate

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fertility in the populations. Size indices (length $\times$ breadth) of pollen grains were obtained and for each of the populations, a total of 200 pollen grains for each of three anther types observed, were analysed. Ten anthers were studied per anther type for each population. Seed viability was estimated as percentage germination after soaking for 2–3 minutes in Conc. H_2SO_4 , in order to break seed dormancy.

Table 1. Morphological features of *C. diffusa* populations (areas given in square centimeters)

Population	Identification	Features
83/026	<i>C. diffusa</i> subsp. <i>aquatica</i>	Very deep blue flowers; lanceolate leaves and spathes with acute apex; greenish stems and leaf sheaths. Mean area of leaves = 9.51 ± 0.88 Mean area of spathe = 4.78 ± 0.26 .
83/003	<i>C. diffusa</i> subsp. <i>montana</i>	Deep-blue flowers; ovate leaves and spathes; stems and leaf sheaths with reddish stripes. Mean area of leaves = 4.25 ± 0.463 Mean area of spathe = 2.23 ± 0.236 .
83/025	<i>C. diffusa</i> subsp. <i>montana</i>	Deep-blue flowers, ovate leaves and spathes, stems and leaf sheaths with reddish stripes. Mean area of leaves = 6.85 ± 0.189 . Mean area of spathe = 2.31 ± 0.182 .
83/017	<i>C. diffusa</i> subsp. <i>diffusa</i>	Mottled blue/white flowers; green stems and leaf sheaths; ovatelanceolate leaves and spathes. Mean area of leaves = 6.04 ± 0.347 . Mean area of spathe = 2.25 ± 0.129 .
83/023	<i>C. diffusa</i> subsp. <i>diffusa</i>	Purple flowers; green stems and leaf sheaths; ovatelanceolate leaves and spathes. Mean area of leaves = 7.54 ± 0.422 . Mean area of spathe = 2.31 ± 0.241 .

Results

a) Chromosome pairing behaviour

A summary of the pairing behaviour of chromosomes at diakinesis, in the five populations, is given in Table 2 and in Fig. 1. All five populations showed a chromosome number of $2n=30$ as determined from anaphase plates. Meiosis was characterized by varying degrees of chromosomal associations. The dominant configurations at diakinesis were bivalents (II) and 232 (46.4%) of the 500 PMC's studied showed regular 15 bivalents per cell (Fig. 1e). Multivalent associations were observed in 54 per cent of the cells; 111 (or 22.22%) had 13_{II} and 1_{IV} , while the remaining 147 (representing 31.38%) showed associations higher than one quadrivalent per cell. Higher associations of chromosomes were also observed, involving six (Fig. 1f), eight (Fig. 1 a, b), ten (Fig. 1 i), up to sixteen (Fig. 1 g), twenty-four (Fig. 1 h) or even more (Table 2) chromosomes. Despite these associations, irregularities such as non-disjunction of chromosomes, laggards or bridges were not observed in this study.

b) Anther characteristics, pollen grain and seed viability

All the populations of *C. diffusa* studied had five anthers per flower, which could be classified into three types as shown in Table 3. Pollen grains from all three types differed in their stainability in acetocarmine, as well as in their size indices, and could be grouped into two types which were statistically significant. (The test performed was analysis of variance; ($p < 0.001$)). The first (type I) were larger in size and uniformly stained with a length range of 6–9 μm and breadth of 5–6 μm . Type II grains were smaller in size (6–7 μm by 3–4 μm) and failed to

Fig. 1. /Diakinesis Configurations in *C. diffusa*. a, 83/025 ($11_{II}+1_{VIII}$). b, 83/023 ($9_{II}+1_{IV}+1_{VIII}$). c, 83/023 ($11_{II}+2_{IV}$). d, 83/017 ($13_{II}+1_{IV}$). e, 83/023 (15_{II}). f, 83/023 ($12_{II}+1_{VI}$). g, 83/003 ($1_{II}+1_{XII}+1_{XVI}$). h, 83/026 ($3_{II}+1_{XXIV}$). i, 83/026 ($7_{II}+1_{VI}+1_X$). Scale line for all photographs is as shown on a, = 5 μ m.

pick up the stain. The proportion of the two types of microspores produced by each anther type, is shown in Table 3. Although the rate of seed set was low in these plants compared with those of other species of the genus (eg. *C. erecta* produced 90 ± 1.8 , and *C. lagosensis*, 75 ± 2.1 seeds per plant. Data presented in another paper), the seeds produced showed a high percentage of germination (Table 3). The significance of this is mentioned in the discussion.

Table 2. Diakinesis configurations in the five populations of *C. diffusa* studied

Population	Configurations observed and their frequencies out of 100 PMCs per population																
	15II	13II+ 1IV	12II+ 1VI	11II+ 2IV	11II+ 1VIII	10II+ 1X	9II+ 1IV+ 1VIII	8II+ 1VI+ 1VIII	7II+ 2VIII	7II+ 1VI+ 1X	6II+ 3VI	5II+ 1IV+ 1XVI	5II+ 2IV+ 1XII	3II+ 1VI+ 1XVIII	3II+ 1XXIV	2II+ 1XXVI	1II+ 1XII+ 1XVI
84/026																	
<i>C. diffusa</i> subsp. <i>aquatica</i>	48	20			6		6	6	6	4	8			1	1		
83/003																	
<i>C. diffusa</i> subsp. <i>montana</i>	56	24	6			5	7										2
83/025																	
<i>C. diffusa</i> subsp. <i>montana</i>	54	20	9		6			5		4						2	
83/017																	
<i>C. diffusa</i> subsp. <i>diffusa</i>	38	22	14		12	7	6					1					
83/023																	
<i>C. diffusa</i> subsp. <i>diffusa</i>	36	25	12	10			9						7		1		

Discussion

All the populations of *C. diffusa* studied were diploids with $2n=30$ chromosomes. This conforms with the reports from several workers (Kammathy and Rolla 1961, Lewis 1964, Jones and Jopling 1972, Faden and Suda 1980). It, however, disagrees with the reports of Morton (1956) and Sharma and Sharma (1958) who both gave $2n=28$ and 56 chromosomes for African and Indian populations, respectively.

Although the *C. diffusa* collections studied had diploid chromosome complement, they were characterized by multivalent configurations of varying numbers, suggesting the presence of translocations (Sieber and Murray 1981). Hossain (1984) reported the presence of translocation complexes from Nigerian collections of *Commelina*. He, however, did not give a detailed report of the nature of the associations in the different subspecies. Similar scanty reports on chromosome associations have previously been given for *C. diffusa* by a few workers (Sharma 1955, Panigrahi and Kammathy 1964). Majority of the workers, however, give 15 normal bivalents in all the PMC's investigated (Lewis 1964, Zaman and Ahmed 1972, Zaman and Begum 1974).

Table 3. Percentages of different pollen grain types¹ (I & II) in three anther types (A–C) in five populations of *C. diffusa*. Mean number of seeds per plant and percentage seed germination are also shown for the populations

Anther description	Pollen grain types	83/026 <i>C. diffusa</i> <i>aquatica</i>	83/003 <i>C. diffusa</i> <i>montana</i>	83/025 <i>C. diffusa</i> <i>montana</i>	83/017 <i>C. diffusa</i> <i>diffusa</i>	83/023 <i>C. diffusa</i> <i>diffusa</i>
A (lower 2, small, yellow and cruciform)	I	61.4	94.3	90.6	47.1	60.5
	II	38.6	5.7	9.4	52.9	39.5
B (middle 1, large, bilobed with a combination of black and yellow colours)	I	65.0	86.5	90.0	39.0	27.8
	II	35.0	13.5	10.0	61.0	72.2
C (upper 2, small, black, bilobed anthers)	I	66.5	86.7	92.7	34.9	26.6
	II	33.3	13.3	7.3	65.1	73.4
Number of seeds/plant ($\bar{x} \pm SE$)		35 $\pm$ 2.1	60 $\pm$ 0.8	65 $\pm$ 1.2	25 $\pm$ 0.2	23 $\pm$ 0.4
% seed germination ²		97.0	97.5	97.5	91.5	94.0

1. figures based on 2,000 pollen grains, i.e., 200 pollen each from 10 anthers in a given anther type.

2. % germination is based on 200 seeds per population.

The size of the multivalents in a diploid depends on the number of chromosome pairs involved in the interchange complex (es) and the number of such multivalent complexes depends on independent interchange complexes. However, it is not obligatory that all the chromosomes involved in a particular interchange will always be associated together. Thus, in the present study, different configurations were obtained for a given population. This could either be due to failure of pairing during zygotene/pachytene of the homologous segments of the interchange chromosomes, or due to failure of chiasma formation where there was pairing. Regular ring formation in *Rhoeo discolor*, *Paenonia* spp and some *Oenothera* spp (Swanson *et al.* 1967, Cleland 1972) are perhaps extreme cases where all the chromosomes involved in a large translocation complex usually associate together giving the largest possible multivalent configuration. In such species a single ring or chain multivalent arises as a result of segmental interchange involving all the chromosomes (Belling 1921, Cleland 1972).

Three anther types (A, B, and C) were encountered in the present study. Anther type A has hitherto been described as infertile staminode while B and C types have been said to be fertile (Morton 1956, 1967, Faden 1974). These workers, however did not provide detailed

analysis on pollen fertility to support their claims. The present study indicates that the three anther types contain varying proportions of fertile and infertile pollens in the different sub-species (Table 3). Anther types B and C were relatively infertile in subsp. *diffusa* unlike in subspecies *aquatica* and *montana*, while anther type A appeared to be highly fertile in subspecies *montana* and less so in the other two subspecies.

It is also interesting to note that despite the fact that a good proportion of the pollen grains produced were fertile, the plants formed well developed and matured seeds only sparingly (especially with subsp. *diffusa* and *aquatica*). In most cases, the fruits were found to abort before maturity. The propensity of seed-set was highest in subsp. *montana* which also produced the highest percentage of fertile pollen. Seed-set, therefore, appears to be, as one would expect, positively correlated with the percentage of fertile pollen grains produced. The percentage germination of these seeds, as worked out in the present study, was quite high (Table 3), indicating that even though they are sparingly formed, those that are formed are indeed viable. This suggests that *C. diffusa*, in spite of being highly heterozygous for various interchange complexes which are expected to produce abnormal or unbalanced gametes, selectively eliminates such gametes. This hypothesis for selective elimination of unbalanced (or lethal gametes) is re-inforced by the fact that no irregularities such as non-disjunction of chromosomes, laggards or bridges were observed. In all cases examined, 15 clear chromosomes were counted at each pole during anaphase I and Telophase I stages. Thus, the chromosomes though involved in several translocation complexes, always segregated regularly at anaphase I. Consequently, abnormal gametes that might have been formed are either non-functional at fertilization or if they are functional, the embryo concerned is lethal (zygotic lethality).

Since some degree of infertility is an inevitable consequence of interchange (translocation), interchanges only become established within population when their contribution to fitness more than compensates for the infertility (Bailey *et al.* 1976). For vegetatively propagated plants like *C. diffusa*, fitness in relation to seed fertility does not pose any serious problem as it would do in strictly seed-propagated plants. Furthermore, these plants are able to conserve their apparently advantageous heterozygous gene combinations through their vegetative mode of propagation.

Summary

A study of meiosis in the PMC's of five populations of *C. diffusa* ($2n=2x=30$) belonging to three sub-species, *diffusa diffusa*, *diffusa aquatica* and *diffusa montana*, revealed varying degrees of chromosomal associations suggesting the presence of interchange complexes in these populations. Fertility estimates showed a high proportion of infertile microspores and concomitant reduction in seed set. These could be traced, at least in part, to the presence of interchange heterozygotes in these populations.

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