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Karyo-Morphological Analyses of *Drimia gigantea* And *Drimia Viridula;* Two of the Three Species in *Altissima* Complex of the Family Hyacinthaceae

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Abstract

Drimia is a heterogeneous, poorly understood genus that requires major rearrangement. Chromosome behavior of *Drimia gigantea* and *Drimia viridula;* two species in *D. altissima* complex was studied for karyotypes transmission of extra chromosomes pair and pollen viability. Meristematic cells at the root tips of *D. gigantea* and *D. viridula* were studied by conventional cytogenetic and karyotype analysis. Chromosome numbers were 2n = 22 and 2n = 20 for *D. gigantea* and *D. viridula,* respectively. Extra chromosome pair in *D. gigantea* resulted in 2n = 22. Chromosome length was 55.45 mm for *D. viridula* and 55.50 mm for *D. gigantea*. Bridge formation, laggards, chromosome exclusion and formation of 9 (3.26%), 10 (90.93%) and 11(5.81%) chromosome bodies were observed in *D. gigantea*, *D. viridula* consistently formed 10 bivalents. Pollen viability was 90.63% in *D. gigantea* and 94.95% in *D. viridula*. Karyotypic evidence suggests that an extra chromosome pair in *D. gigantea* evolved from fragmentation of the second-largest chromosome, the extra chromosome is not a B-chromosome because it participated in cell division. High pollen viability and frequent occurrence of ten bivalents, indicated that *D. viridula* is closer to the progenitors of a common genetic system within *D. altissima* complex. Furthermore, the karyo-morphological differences support the separation of the two taxa as distinct species.

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1. Introduction

Drimia (*syn: Urginea*) is a genus of bulbous perennial herbs of the family Hyacinthaceae. They grow in the savannah and semi-arid region of west tropical Africa where they often occur together with *Albuca* species in the Guinea and Sudan zones of tropical Africa and with several species in Eurasia and Far-East (Oyewole, 1987; Goldblatt *et al.,* 2012). *Drimia* representatives in West Africa formed a complex which is resolved into three species: *D. altissima* (L.) Bak. *D. gigantea* (Jacq.) Oyewole, and *D. viridula* Bak. (Oyewole, 1975a) (Figure 1). *Drimia viridula* is used medicinally by local constituencies, whereby the bitter bulbs and dried scales from bulbs are used to cure coughs, dropsy and as an antioxidant (Hutchings & Terblanche, 1989; Ghami, 2003; Kameshwari, 2013). The previous genus *Urginea* is a heterogeneous, poorly understood genus. Hepper (1968) recognized four species in the genus *Urginea* viz: *Urginea indica* (Roxb.) Kunth*, U. ensifolia* (Thonn) Hepper. *U. pauciflora* Baker and *U. altissima* (L.) Baker. Each has its distinguishing features as well as a unique pattern of distribution (Oyewole, 1975a). The genus has been severally reviewed (Oyewole, 1975a; Speta, 1998; Manning *et al.,* 2004; Klopper *et al.,* 2006; Goldblatt *et al*., 2012) and a population of the old genus *Urginea* currently referred to as *Drimia,* in West Africa, consists of three distinct taxa (*D. altissima* , *D. gigantea,* and *D. viridula*) with specific distribution pattern, ecological and soil preferences.

Figure 1: (a) *Drimia altissima*; (b) *Drimia viridula*; (c) *Drimia gigantea*

Though they may overlap in some features, the morphological similarities between the taxa show that the genus has a genealogy in West Africa and many more taxa might evolve with time (Oyewole, 1975a; Oyewole, 1988).

The role of cytogenetics in solving taxonomic problems is of importance and many taxonomic difficulties have been resolved using cytogenetic techniques. A handful of previous studies used cytogenetics to resolve taxonomic problems in higher plants (Naik, 1976; Lackey, 1980; Oyewole, 1987; Speta, 1998). More than a decade ago, Marasek *et al*. (2005) and recently, Luo *et al.* (2013) employed cytogenetics to distinguish some species of lily. Cytogenetic studies on the genus *Drimia* in Africa have been largely confined to plants of Eastern and Southern African origin (Oyewole, 1975b). For instance, Jones & Smith (1967) work on the karyotype of *D. altissima* was based on the cultivated plant from South Africa grown at the Royal Botanic Gardens in Kew. They reported somatic complement of 2n = 20 for the species and also confirmed the basic chromosome number of 5 and 7 earlier reported for the genus (Darlington & Wylie, 1962 cited in Oyewole, 1975b). The reports from these authors may not represent the karyotypic situation in the entire taxon which may be variable in particular among West African taxa.

The *D. altissima* complex, consists of three West African species having a somatic complement of 2n = 20 for *D. viridula* and 2n = 22 for each of *D. gigantea* and *D. altissima* (Oyewole, 1975b). The 2n = 22 is a deviation from the established basic chromosome number of *Drimia* spp. and the extra chromosomes were identified as B-chromosomes, that are different due to chromosomal instability and other features (Jones & Smith, 1967). However, the extra chromosomes identified in *D. altissima* and *D. gigantea*, as reported by Oyewole (1987) were not B-chromosomes in that they stained normally and took part in cell division like other members of the complement. Cytological observation is undertaken not only to detect the occurrence of aberrations but also to understand how they have arisen in a particular karyotype. This gives valuable information on the evolution process.

Cytogenetics of plant species have been performed using chromosome arm length, C-banding, fluorescence banding and $AgNO_3$ staining, among others (Lim *et al*., 2001; Siljak-Yakovlev *et al*., 2003; Marasek *et al*., 2005). Since species in the genus *Drimia* are rather poorly distinguished morphologically, comparative cytological studies of the members would be valuable to describe the patterns and directions of chromosomal evolution and to infer the evolutionary role karyotype changes play in species evolution. The present study, therefore, involved the cytogenetic study of *D. gigantea* and *D. viridula*, two of the three species of the *D. altissima* complex. This article aims to give insights into the origin of chromosomal differences in two *Drimia* species, especially it sought to determine the origin of two extra chromosomes in *D. gigantea*. Efforts were made to determine the outcomes of the extra chromosome on pollen viability of *D. gigantea*.

2. Materials and Methods

2.1 Collection of materials

The population of *Drimia gigantea* was sampled from the wild from Southern and Northern Guinea Savannah Zones of Nigeria. Bulbs were collected from Oyun Riverbank, University of Ilorin, Ilorin, Nigeria where they occurred with ephemerals, perennial herbs and a sparse population of *Dipcadi*. Collections were made from Olalomi areas located about one Kilometre along Offa-Irra Road, Kwara State. Other populations of *D. gigantea* were collected from Gurara town, Kilometre 85, along Minna-Suleja Road. Also, samples were collected from rocky hills where they

occurred with *Albuca* spp along the roadsides near Abattoir at Garki, Abuja.

Further samples were collected from Wusasa in Zaria, Kaduna State where it occurs mainly with *Albuca nigritana*. *Drimia viridula* bulbs were collected from Zaria, Funtua and Malumfashi (Kastina State) in rock crevices with little organic matter, or on soils with laterites where it was sparsely distributed. Some bulb samples were acquired from the botanical garden, University of Ilorin. Figure 2 showed areas of collection of *D. gigantea* and *D. viridula* samples.

Fig 2. Map of Nigeria showing major areas of sampling *D. gigantea* and *D. viridula*.

2.2 Growing of bulbs for cytogenetic studies

The collected plant material was identified using the herbaria of the Department of Plant Biology, University of Ilorin, Ilorin and Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Five bulbs from each population were grown for rooting by placing the condensed stem in a mixture of perlite and peat (1:1) filled beakers placed in a dark enclosure at Plant Biology Laboratory, University of Ilorin. For meiotic studies, the bulbs were grown in planting bags in the screen house facility at Botanical Garden, University of Ilorin (N 080 28′ 53.3″, E 040 40′ 28.9″), Ilorin, Nigeria.

2.3 Mitotic studies

Mitotic slide preparation was carried out as described by Marasek *et al*. (2005) with modifications. Root tips (0.4-1.0 cm long) harvested from the bulbs were rinsed in distilled water and maintained in labelled specimen tubes containing an aqueous solution of 0.1% Paradichlorobenzen for about 4 hrs. The pre-treated root tips were rinsed under tap water and fixed in freshly prepared 3:1 ethanol-glacial acetic acid for 4 hrs. The root tips were hydrolysed in 5-N HCL at room temperature for 10 min. (Adapted from Oyewole, 1972) and squashed in drops of aceto-orcein. The slide was gently covered with a coverslip dehydrated in pre-chilled absolute ethanol, air-dried and stored at 4 ^{o}C until use. The cells of *D. gigantea* and *D. viridula* were observed under an Olympus Microscope at x40 and x100 magnifications power.

Chromosomes' counting was done for about fifty randomly selected cells for each of the taxa. The presence or otherwise of endomitosis and polyteny, laggard formation or chromosome exclusion were noted. Chromosome lengths were measured with micrometre eye-piece graticule. Photomicrographs were taken and the r-value was calculated for each chromosome homologue which was recorded in order of magnitude for the haploid set. Average chromosome lengths, relative chromosome-arm lengths, and the position of the centromere were determined. Chromosome classification was based on chromosome index adapted from Leven *et al*. (1964) and photomicrographs were produced for the chromosome index.

2.4 Meiotic and pollen studies

Young flower buds collected in replicates from the plants maintained in the screen house were fixed in Carnoy's solution (1:3 mixture of acetic acid-ethanol) for 24 hrs. at room temperature. Then, the anthers of the flower buds were dissected in the middle and the pollen mother cells (PMCs) spread on the slide. A drop of 2% acetone-carmine was swiftly added and carefully mixed with the PMCs. The slide was covered with slip and pressed gently with the thumb. About 200 PMCs from 10 slide preparations were viewed under an Olympus microscope. Cell count, chromosome associations, aberrations and the number of chiasmata formed per complement were recorded. Pollen viability was estimated by observing the ability of pollen grains to stain in 1% aceto-camine within two to three minutes. Both viable and unviable pollens were counted and percentage pollen viability determined.

3. Results

3.1 Mitotic chromosomes and karyotype analyses

Twenty-two (22) somatic chromosomes were observed in the cells of *D. gigantea* smaples with no incidence of polyteny or endomitosis (Plate 1a-b). Karyotype studies showed elongation of the centromere in the seventh homologous pair of samples

X4 and a member of the third homologous pair in sample X3 (Plate 1c).

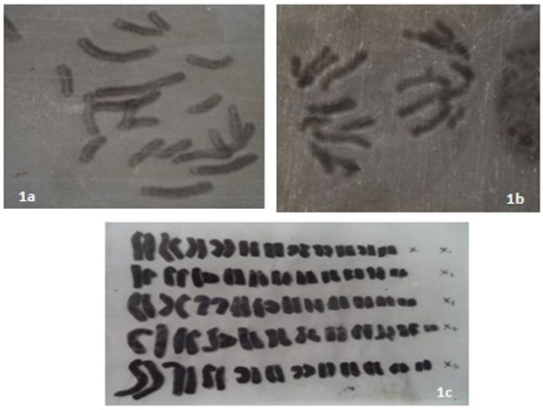


Plate 1: (a) Somatic chromosomes in the cells of *D. gigantea*; (b) *D. gigantea* normal mitotic chromosomes separation at anaphase; (c) (X_1-X_5). The idiograms of each of the representative samples of *D. gigantea.*

The second homologous pair of samples X5 and X1 are unequal in length and differ in centromere position as a member is telocentric while the other is acrocentric (Plate 1c). Secondary constriction was present in the first homologue of sample X5 while the karyotype of sample X2 showed no notable change (Plate 1c). The chromosome in each complement was classified as long (≥ 6.0 mm), medium (4.0-5.9 mm) and short (< 4.0 mm). The somatic complement of *D. gigantea* composed of four long pairs with centromere in the terminal region, and seven short pairs. One of the short pairs has its centromere in the sub-median region, five pairs have sub-terminal centromere and a pair was telocentric (Table 1).

All samples of *Drimia viridula* collected from different locations have a somatic complement of 2n = 20 with no polyteny or endomitosis. Chromosomes movement at anaphase was normal (Plate 2a-b). The karyotype of *D. viridula* comprised four long pairs with centromere in the sub-terminal region, and six short pairs (Plate 2c).

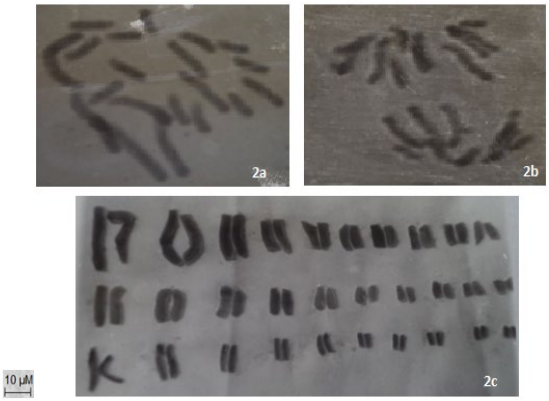


Plate 2: (a) Somatic chromosomes of *D. viridula;* (b) Normal anaphase chromosomes in *D. viridula*; (c) Karyotype of the representative sample of *D. viridula*

Two short pairs have centromere in the median region, while two have centromere in the sub-median region and the other two have centromere in the sub-terminal region (Table 1). Differences were observed in some chromosomes with centromere in the terminal region as two pairs were observed in *D. viridula* while five pairs were observed in *D. gigantea.* Also, two pairs of metacentric chromosomes were found in *D. viridula,* whereas none exist in *D. gigantea* (Table 1).

Table 1. Summary of karyotype data of *Drimia gigantea* and *Drimia viridula*

Taxa	Chromosome homologues (*D. gigantea*)											TCL (mm)
	1	2	3	4	5	6	7	8	9	10	11	
CL (mm)	11.0	9.0	7.5	5.0	3.8	3.8	3.7	3.5	3.2	3.0	1.95	55.45
r-value	5.65	7.75	7.7	8.1	6.0	6.1	6.21	4.2	9.5	9.2	0.0	
CP	st	t	t	t	st	st	st	st	t	t	t	
Chromosome homologues (*D. viridula)*												
CL (mm)	10.8	10.5	7.4	4.8	4.3	4.1	3.9	3.6	3.5	2.5		55.50
r-value	6.5	8.88	7.0	5.3	1.0	1.0	2.6	4.0	2.5	5.0		
CP	st	t	t	st	m	m	sm	st	sm	st		

Key: TCL- Total chromosome length; CL- Chromosome length; CP – Centromere Position; st = sub-terminal; t = terminal; m = median; sm = sub-median

Table 2. Average chromosome length measured for meiotic chromosome behavour in *Drimia altissima* and *Drimia viridula*

		Length of meiotic chromosome (mm)										
Meiotic behaviour		1	2	3	4	5	6	7	8	9	10	11
(*D. gigantea*)	9	11.10	9.01	8.95*	7.51	5.01	3.90	3.81	3.60	3.10	-	-
	10	11.01	10.9*9.	7.61	5.01	3.81	3.80	3.71	3.51	3.30	3.01	-
	11	11.10	01*	7.60	5.01	3.81	3.80	3.71	3.50	3.20	3.00	1.95
(*D. viridula*)	10	9.70	9.10	6.70	6.10	4.15	3.20	3.15	3.10	3.05	3.02	-

*Length measured for the hexavalent

Table 3: Chiasmata frequency of the meiotic behaviours of *Drimia gigantea* and *Drimia viridula*.

Meiotic behaviour (NB)	Number of chiasmata observed			TC/C	AC/C
	Long	medium	Short		
		Drimia gigantea			
9	5	6	8	17	1.81
10	6	3	11	20	2.00
11	3	5	12	20	1.81
		Drimia viridula			
10	6	7	4	17	1.7

Key: NB -Number of bodies; TC/C-Total chiasmata per cell; AC/C -Average chiasmata per cell

Table 4: Summary of meiotic behaviour and pollen viability in *Drimia gigantea* and *Drimia viridula*.

Meiotic studies	Observation	Frequency	Percentage %
Chromosome formed at diplonema-I	9 bodies	28	3.26
	10 bodies	782	90.93
	11 bodies	50	5.81
Total No. of PMC Examined	860		
Chromosome behaviour at Anaphase I - Telophase I	Normal movement	644	92.0
	Bridge formation	19	2.71
	Telophase-I laggard and excluded chromosome	37	5.29
Total No. of PMC Examined		700	
Chromosome behaviour at Anaphase II-Telophase II	Normal movement	181	90.5
	Bridge formation	19	9.5
Total No. of PMC Examined		200	
	Drimia gigantea		
Pollen viability	Viable pollen	1, 858	90.63
	Aborted pollen	192	9.37
Total No. of pollen examined		2, 050	
	Drimia viridula		
Pollen viability	Viable pollen	1,138	94.95
	Aborted pollen	62	5.05
Total No. of pollen examined		1,200	

Key: PMC = Pollen Mother Cell

3.2 Meiotic studies

Chromosome pairing of 11 bivalents occurred in about 5.81% for *D. gigantea*. The chromosome bodies consisted of one large, two medium and eight small bivalents with the medium and small bodies more prevalent (Plate 3a). The formation of 10 chromosome bodies was most frequent (90.93%). These consists of a quadrivalent, a large body, a medium while the remaining seven are small bodies (Plate 3b). Besides, 9 bodies formation was observed. The nine bodies were least frequent (3.26%) consisting of one hexavalent and eight bivalents (Plate 3c). The eight bivalents in turn composed of; one large, two medium and five small bivalents.

In *D. viridula,* all the chromosomes paired up consistently to form ten bivalents without the formation of bridge or chromosome exclusion at either anaphase or telophase. Two large pairs, three medium and five small size bivalents made up the ten bivalents (Plate 3d). However, one of the large bivalents showed evidence of duplication in one of its chromosomes (Plate 3d).

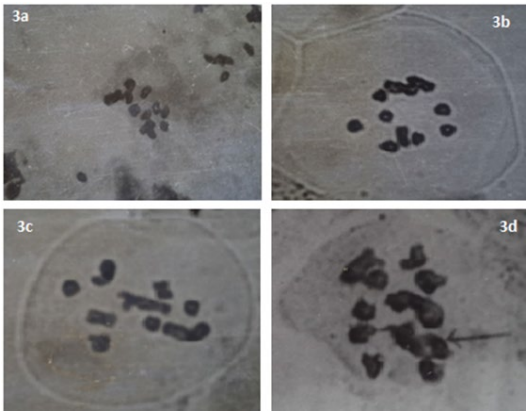


Plate 3: Chromosome bodies formed at diplonema-I of *D. gigantea* and *D. viridula.* (a) Chromosome bodies formed at diplonema- I; (b) Ten chromosome bodies found at diplonema-I; (c) Nine chromosome bodies formed at diplonema-I; (d) Ten bivalents at meiotic metaphase-I of *D. viridula* with duplication of chromosome shown by the arrow.

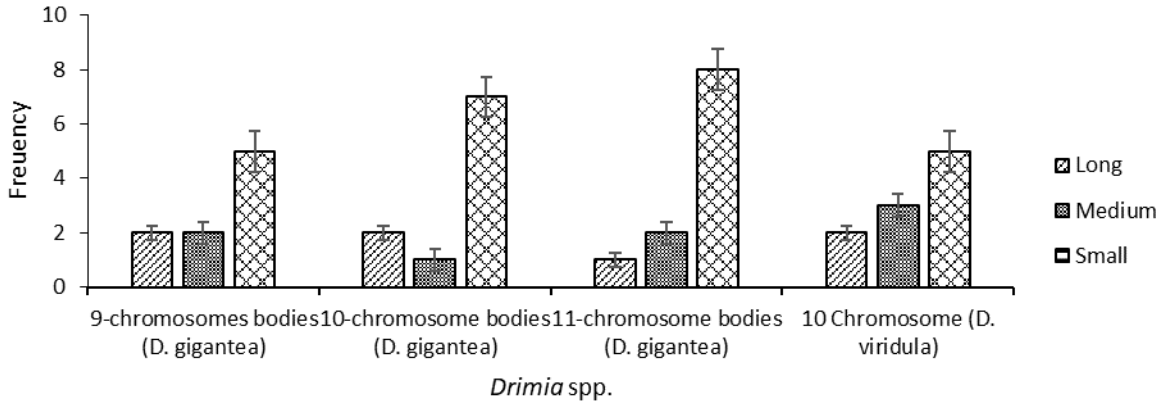
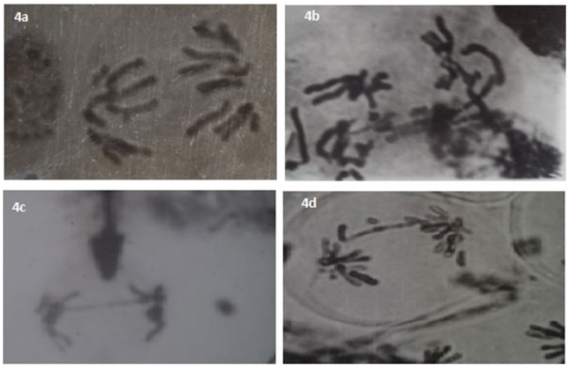


Figure 3: Frequency of occurrence of different chromosome sizes at meiotic metaphase-I in *D. gigantea* and *D. viridula*

The frequency of occurrence of different sizes of chromosomes at meiotic metaphase in *D. gigantea* and *D. viridula* is shown in Fig 3 and the average length measured for each of the meiotic chromosomes is summarized in Table 2. Chiasmata formation for *D. gigantea* in the 11 bivalents showed that largest bivalent has three chiasmata, the medium pair formed two chiasmata. One of the small size bivalents formed three chiasmata, three others formed two each, while another set of three pairs formed one chiasma each. But no chiasma formation was seen in the remaining one (Table 3). The total numbers of chiasmata observed per complement were 20 and the average chiasmata per cell was 1.82 in *D. gigantea*.

At diplonema, two large bivalents of *D. viridula,* formed three chiasmata each. One of the medium bivalents chromosomes had three chiasmata while the other one had two which were acrocentric. The third member of the medium size bivalents showed two chiasmata and they are sub-median chromosomes (Table 3). Furthermore, the number and position of chiasma varied in the small bivalents except in two which did not form chiasma but were effectively paired. The number of chiasmata per complement in *D. viridula* was 17 with an average of 1.7 per bivalence (Table 3). Normal chromosome movement to the poles was

observed at anaphase-I in *D. viridula* while bridge formation and excluded chromosome occurred at telophase-I in *D. gigantea* (Plate 4a-d). Also, bridge formation and excluded chromosomes were seen at telophase-II of *D. gigantea* (Plates5a-c).



Plates 4: Chromosome behaviour at anaphase I and telophase I in *U. gigantea* and *D. viridula* (a) Normal anaphase-I movement in *D. viridula;* (b) Anaphase-I bridges in *D. gigantea;* (c) Telophase-I excluded chromosome observed in D. *gigantea;* (d) Adicentric bridge and an excluded fragment in *D. gigantea*.

3.3 Pollen formation and pollen viability

Dyad, tetrad and mature pollen grains were observed (Plates 6a-c). The occurrence of 9, 10 and 11 bodies was concurrent to Anaphase I bridges, normal movement, lagging and excluded chromosome at Telophase-I in *D. gigantea* (Table 4). The formation of nine bodies per PMC is proportional to the frequency of occurrence of Anaphase I bridges etc. Table 4 shows the summary of meiotic behaviors with pollen viability in *D. gigantea* and *D. viridula.* In *U. viridula,* about 95% pollen viability was recorded and 90.63% in *D. gigantea* (Table 4).

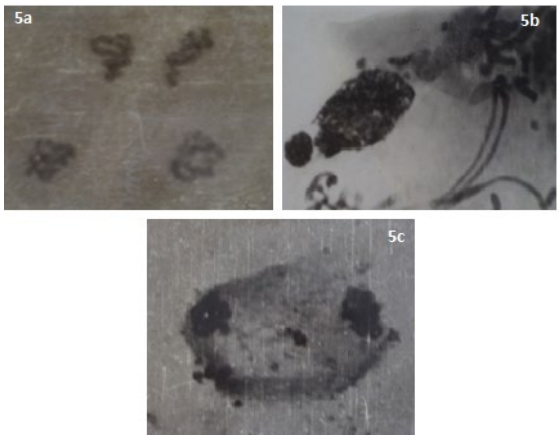


Plate 5: Chromosome behaviour at anaphase II - telophase II in *D. gigantea* (a) Normal anaphase II movement; (b) Anaphase bridges; (c) Telophase II excluded chromosome.

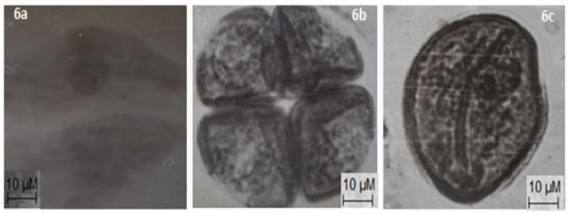


Plate 6: Pollen grain formation in *D. gigantea* and *D. viridula* (a) Dyad formation feature observed after first meiotic division; (b) Tetrad formation feature observed after the second meiotic division; (c) Matured pollen grain of *D. gigantea*

4. Discussion

The results of the somatic metaphase complement of *Drimia gigantea* and *D. viridula* revealed 2n = 22 and 2n = 20, respectively. This observation corroborated earlier work on the two taxa (Oyewole, 1975b). Also, the extra two chromosomes in *D. gigantea* observed in this study were small and telocentric which confirm the report of Oyewole (1987). In contrast, the extra chromosomes differ from B-chromosomes identified by Jones & Smith (1967) because the extra chromosomes stained normally and took part in the mitotic division as all other members of the complement. Though some representatives of the family Hyacinthaceae have B-chromosomes and secondary constrictions which is useful in delimiting the population of such groups (Jones & Smith, 1967, Kameshwari *et al*., 2013), accessory chromosomes were rarely reported in any of the West Africa *Drimia,* and they are known to be different from the autosomal members of their complement. Apart from the difference in chromosome number, karyo-morphological differences obtained in the present study indicated the two taxa as distinct species within the complex as earlier suggested by Oyewole (1975b). The formation of a hexavalent involving the extra pair of chromosomes may indicate some genetic affinities between the pairs involved or show the probable origin of the extra pair.

The result revealed chromosome length for the largest and second-largest pairs in *D. gigantea* were 11.0 mm and 9.0 mm respectively. A summation of the length of the extra pair (1.95 mm) and the second-largest pair observed in *D. gigantea* would give 10.95 mm which is approximately equal to the length of the longest pair. This implies the extra chromosome pair possibly originated from the breakage of the second-largest pair in the karyotype. The extra chromosomes behaved normally like other members of the complement, thus suggesting the

point of breakage of the otherwise acentric chromosome was along the heterochromatic region. It is, therefore, reasonable to infer that the species *D. gigantea* probably evolved from *D. viridula* through chromosome breakage or the two species have evolved from a common ancestor. A similar scenario was observed among lily genotypes studied by Marasak *et al.* (2005).

The karyomorphological similarities and chromosome arm length among the two taxa predicts a common ancestor. On this basis, Oyewole (1975b) suggested that "whatever changes have occurred did not involve substantial increase or decrease in the volume of the genetic material in the whole complex, but a rearrangement of gene and/or gene block". Loss of chromosome segments from certain members of the complements might have rendered homologous members dissimilar in the length of *D. gigantea* chromosomes. However, the identification of chromosomes based on morphology may not be effective for taxa classification. Molecular cytogenetics and chromosome-band staining techniques would provide a better insight into chromosome classification (Friebe *et al.,* 1996; Marasek & Orlikowska, 2003).

Meiotic studies revealed irregularities such as bridge and the laggard formation and chromosome exclusion attributable to the erratic behavior of the extra pair in *D. gigantea.* Similarly, Luo *et al*. (2013) reported the formation of bridges, paracentric inversion and other chromosome irregular behaviors in lily which was likely due to the presence of extra chromosome bodies. The extra pair of chromosomes in *D. gigantea* was similar to other pairs in structure and behavior, thus they merely increased chromosome number which is favored by the environment. This agreed with the findings of Mooring (1960) and Wedberg *et al*. (1968) who worked on cytogenetics of *Clarkia unguiculata* and *C. williamsonia,* respectively. Furthermore, chiasma formation at diplonema for 10 and 11 chromosome bodies was similar. The average chiasmata per cell were however higher in the 10 chromosome bodies; this shows that a higher genetic affinity exists among the chromosome when 10 chromosome bodies are formed. *D. viridula* regularly formed 10 bivalents with a high frequency of chiasmata formation. The meiotic stability observed in *D. viridula* suggested its closeness to the basic progenitor of the genetic systems within the *altissima* complex, having not undergone any noticeable chromosomal changes.

The incidence of bivalent fates involving extra pair of chromosomes and the second-largest pair (10 bivalents) indicates high pollen viability in the species, while the percentage of pollen abortion also interrelated with the total frequency of nine chromosome bodies and 11 bivalent formations. Khan *et al.* (2009) reported that a high frequency of aneuploidy could result in low viability among lily hybrids. The involvement of the extra pair of chromosomes in *D. gigantea* in a specific association may lead to the formation of viable pollen. For instance, if the extra chromosome is associated in pairing with its parental part, as is the case in 10-body formation, this could result in normal anaphase movement and a high percentage of viable pollen. Any other form of possible association would result in the production of nonviable pollen.

In *D. gigantea*, the high frequencies of 10-body formation, leading to a successive meiotic product show that the process is guided by a genetic mechanism. According to Oyewole (1987), the mechanism of transmission of the extra pair chromosomes might involve protected regions of the chromosomes which contain genes that interact in an epistatic fashion resulting in high adaptive value. The infrequent occurrence of dicentric chromosome and excluded fragment at Anaphase-I in the pollen mother cells of *D. gigantea* also suggests possible heterozygote inversion in the chromosome complement as part of the evolution of the genetic system.

5. Conclusion

The present study evaluated cytogenetics of *D. gigantea* and *D. viridula* of the *D. altissima c*omplex. Somatic chromosome number of 2n = 20 and 2n = 22 were observed for *D. viridula* and *D. gigantea,* respectively. The extra chromosome pair in *D. gigantea* was probably derived from fragmentation of member of a pair in the karyotype and the extra chromosomes differed from B-chromosomes as it partook in cell division, but chiasma formation and pollen viability were affected by the extra chromosome pair. Therefore, the extra chromosomes could be of evolutionary importance for the success of *D. gigantea* genetic system. The study concluded that by fragmentation or otherwise, somatic number of 2n = 20

transformed to 2n = 22, which should be seen as 2n = 20 + 2 in *D. gigantea*, indicating the two taxa are possibly distinct species within the complex.

Author's Contributions

SBA and SOO conceived and designed the study. SBA and DAA performed the analyses. SBA, DAA, OTM and SOO contributed to data acquisition and interpretation. SBA and DAA wrote the manuscript with the help of OTM and SOO. All authors contributed to the discussion, revision and approval of the final manuscript.

Competing Interests

The authors declare no conflicts of interest.

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