Hybridisation Studies in the *Hyparrhenia involucrata*— *H. subplumosa* (Gramineae) complex in Nigeria

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In Nigeria, *Hyparrhenia involucrata* Stapf and *H. subplumosa* Stapf along with speices of *Andropogon*, *Hyperthelia*, *Pennisetum* and other species of *Hyparrhenia* form a major component of tall-grass savanna: i.e. Guinea savanna and Sudan savanna (Keay 1949, Clayton 1962, Nielsen 1965, Stanfield 1970). Both *Hyparrhenia involucrata* and *H. subplumosa* are major flora of relatively shallow and impoverished soils and they appear to have considerable colonising potential (Olorode and Baquar 1976).

From seven collections of *H. involucrata* and five of *H. subplumosa* originating from diverse geographical locations in Nigeria, Olorode and Baquar (1976) provided a synthesis of the cytology and morphology of this complex of Gramineae. The morphological and cytological characterisation showed that chromosome number in the complex consists of euploid series of $2n=20$, $2n=40$ and $2n=60$ and aneuploid numbers of 21, 22, and 23. While the annuals (*H. involucrata*) are aneuploid, diploid or polyploid, diploid numbers have not been reported for the perennials (*H. subplumosa*).

Preliminary observation showed that interesting evolutionary information should be obtained from interspecific and intraspecific hybridisation. Segregation patterns intraspecific F_2 hybrids of *H. subplumosa* also confirm the long-suspected occurrence of hybrid swarms in natural populations. In this paper, morphological and cytogenetic observations from hybridisation are presented. From the observations we hazard a number of evolutionary interpretations and speculations.

Materials and methods

The sources of the materials used have been discussed in previous papers (Olorode 1973, Olorode and Baquar 1976). The accession numbers used in Table 1 and Table 2 (012, 013, 250/3, 025, 026) are the same as in the previous papers referred to and they are the collection numbers of the senior author (Olorode).

Controlled crosses were made in the screen house. Raceme pairs to be crossed were bagged to exclude unwanted pollen so that pollen from appropriate parents can be dispensed everyday until all the spikelets have opened. No attempts were made to emasculate the spikelets of female parents. Hybridity was easily ascertained either because of differences in chromosome numbers between parents or because of the parental morphological differences that make hybridity obvious.

Diakinesis studies were carried out from pollen mother cells obtained from young raceme pairs fixed at appropriate stages, in acetic acid-95% ethanol. The fixed material provided anthers which were squashed in a modified orcein stain (Olorode 1974). Photomicrographs were taken from good preparations with a phase-contrast microscope.

Observations and discussion

Morphology

In this section observation on growth habit, vegetative features and characteristics of inflorescences are presented and analysed. These attributes are displayed in Table 1.

As we reported before, annuality and perenniality are the main features used routinely to demarcate the two species (Clayton 1962, Stanfield 1970) but Olorode and Baquar (1976) speculated that in view of the possibility of natural interspecific hybridisation, growth habit will not be a practical taxonomic attribute in this complex. As Table 1 shows, the results of hybridisation between annual and perennial accessions point to the fact that annuality is basically a recessive trait. However, the annual segregants in F_2 's of perennial-perennial crosses complicate that simple explanation unless we are willing to postulate that recessive genes for annuality occur in the perennial species. Since we have never recovered annual plants from perennial parents (i.e. 013, 025 and 026 in Table 1) it may well be that hybridity enhances the expression of such genes in some way that we cannot now explain. In any case the expression of annuality in perennial-perennial crosses underscores the undesirability of growth habit for species delimitation.

All the characteristics of the leaves and culms discussed are continuously-varying attributes. Table 2 shows that the F_1 hybrids are, as will be expected, intermediate with respect to these attributes while the F_2 's show a wide spectrum of each attribute.

The expression of the number of main awns in the hybrids and parents is of particular interest. In crosses of annual accessions with perennial ones (012×013 ; $250/2 \times 013$) where both parents have four main awns, the spectrum of awn number enlarged (with up to 6) while the modal number remained four (Table 1). In the F_1 between a perennial with six main awns (026 in Table 1) and another with up to 30 main awns (025), awn number was expressed near the lower limit (i.e. between six and eight). In the F_2 of 025×026 crosses however, the whole spectrum between the few-awned parent and the many-awned parent was not only spanned, transgressive awn numbers (as low as zero) below that of few-awned parent were expressed. While the increased variability of the awn number in F_2 's of 025×026 cross is expected, the transgressive awn numbers deserve special attention. Firstly, it should be pointed out that all the annual segregants of the 025×026 F_2 's also have 4 main awns almost invariably. When we examine the parental annual species or accessions we observe that annuality and awn numbers of four are closely correlated; the causal relationships of these two factors are not clear. Secondly even though the homologues of hermaphroditic spikelets are ascertainable on raceme-

Table 1. Morphology of parents and hybrids in the *Hypparrhenia involucreata*-*H. subplumosa*

Parent of hybrid	Growth habit	Leaves	Culms	No. of main awns ¹	
				Non-hermaphroditic spikelets	Pedicellate
<i>Hypparrhenia involucreata</i>					
012 ($2n = 20$; $2n + 1 = 21$)	annual	hairy; with or without bloom; to 15 mm wide	to 15 mm ² ; with or without bloom, hairy sheath	4	all staminate
250/2 ($2n = 40$)	annual	hairless or sparsely hairy; without or with sparse bloom; to 15 mm wide	to 15 mm; sparse bloom; glabrous sheath, reddish	4,5	all staminate
<i>Hypparrhenia subplumosa</i>					
013 ($2n = 40$)	perennial	hairless except when young; heavy bloom; to 7 mm wide	to 7 mm; sparse bloom; glabrous sheath; reddish	4	all staminate
025 ($2n = 40$)	perennial	hairy at base; sparse bloom; to 13 mm wide	to 10 mm; sparse bloom; hairy sheath	up to 30	all neuter
026 ($2n = 40$)	perennial	hairless; sparse bloom; to 13 mm wide	to 10 mm; sparse bloom; glabrous sheath	6	rudimentary neuter or rudimentary
<i>Hybrids</i>					
012 ($2n = 20$) $\times$ 013(F_1)	perennial	hairy; with bloom; to 14 mm wide	to 10 mm; sparse bloom; hairy sheath	4 (to 6)	all staminate
012 ($2n + 1 = 21$) $\times$ 013(F_1) ³	perennial	hairy; with bloom; to 10 mm wide	to 8 mm; with bloom; hairy sheath	6	all staminate
250/2 $\times$ 013(F_1)	perennial	hairless or sparsely hairy at base, with sparse bloom; to 15 mm wide	to 15 mm; with bloom; glabrous sheath; reddish or purplish	4 (to 6)	all staminate
026 $\times$ 025(F_1)	perennial	sparsely hairy at base; sparse bloom; to 15 mm wide	to 10 mm; sparse bloom; sparsely hairy sheath	6 (to 8)	rudimentary
026 $\times$ 025(F_2)	annual or perennial	hairy; sparsely hairy or hairless; with or without bloom; 8–15 mm wide	7–15 mm; with or without bloom; glabrous or hairy sheath	0–30	all staminate, all rudimentary, all neuter or a combination of these

¹ per raceme pair   ² culm diameter   ³ $4n+1=41$ chromosomes.

Table 2. Diakinesis chromosome associations and pollen fertility in parents and hybrids of the materials¹ studied

Species and hybrids	Diakinesis configuration						% pollen Stainability
	I	Ring II	Rod II	Rod III	Y-III	Ring IV	Rod IV
<i>Hyparrhenia involucreata</i>							
012 (2n=20)		1.866 (1-5)	8.1333 (5-10)				99.61 (255)
012 (2n+1=21)	1.00	1.33 (0-5)	8.206 (5-10)				99.64 (305)
250/2 (2n=40)	0.13 (0-2)	2.43 (0-4)	16.40 (14-18)	0.07 (0-1)		0.37 (0-2)	0.13 (0-1)
<i>Hyparrhenia subplumosa</i>							
013 (2n=40)		3.83 (0-7)	14.21 (13-18)			0.31 (0-2)	0.20 (0-2)
025 (2n=40)	0.40 (0-2)	3.37 (1-7)	13.07 (10-16)	0.13 (0-1)	0.07 (0-1)	1.03 (0-2)	0.33 (0-2)
026 (2n=40)	0.13 (0-3)	0.5 (0-4)	12.77 (10-16)	0.13 (0-3)		3.0 (1-4)	0.17 (0-2)
<i>Hybrids</i>							
012 (2n=20) × 013 ²	2.53 (0-9)		2.53 (0-9)	7.47 (1-10)			0.46 (2,389)
012 (2n+1=21) × 013 ³	1.3 (1-5)	0.53 (0-4)	12.07 (10-15)	0.27 (0-4)	0.03 (0-1)	3.50 (0-5)	0.23 (0-2)
026 × 025	0.07 (0-3)	2.05 (0-5)	16.33 (14-19)	0.10 (0-1)		0.30 (0-2)	0.33 (0-2)
250/2 × 013	0.10 (0-2)	1.27 (0-4)	17.10 (16-20)	0.03 (0-1)		0.17 (0-2)	0.37 (0-2)

¹ Diakinesis scores are averages based on 30 cells (ranges are given in brackets); number of pollen grains on which estimates were based are given in brackets.

² 2n=30 triploid hybrids

³ 2n+1=41 tetraploid hybrids

pairs showing total absence of main awns, absence of main awns invariably leads to absence of caryopses, contrary to expectation, in hermaphroditic spikelets. It appears therefore that there might be a genetic or morphogenetic relationship between formation of main awns and caryopsis development.

Attempts have been made in the past (Clayton 1962, Stanfield 1970) to use number of main awns for species delimitation. The difficulties of using this criterion should be obvious from the discussion in the last paragraph. It was noticed under green house conditions that the number of main awns (which is equal to number of caryopses) per raceme pair is heavily dependent on the amount of vegetation which is in turn dependent on the status of nutrition of the plant. Apart from the genetic basis of awn number variation, its plasticity in relation to optimality or nonoptimality of growth conditions is certainly another potent factor determining the number of propagules per raceme-pair. One thing that appears obvious from the nature of expression of number of awns (and therefore, number of propagules) is the wide variation that is possible in response to colonizable environment—a hallmark of most successful colonising or invading species (Zohary 1965, Baker 1965, Levins 1968, Stebbins 1970).

The production of functional or non-functional anthers in non-hermaphroditic spikelets appears to be under definite genetic control (Table 1). The staminate condition appears to be dominant to the neuter and rudimentary conditions while the rudimentary condition appears to be dominant to the neuter condition. In previous speculations on the significance of non-functional non-hermaphroditic spikelets in *Hyparrhenia involucrata*-*H. subplumosa* complex, Olorode and Baquar (1976) suggested that no advantage or disadvantage could be demonstrated for the existence or absence of staminate non-hermaphroditic spikelets. Even a more intensive consideration of the structure of 'andropogonic' spikelets as a whole does not permit us in the present paper to contend that position. In terms of evolutionary 'information' however, progressive sterilization of spikelets may be a derived character state (Davies and Heywood 1965).

Meiotic studies and pollen-fertility estimates

Table 2 shows the various average diakinesis configurations for the parents and hybrids. Only meiotic observations as related to parents and F_1 hybrids are discussed in this paper. However, Morakinyo (1975) accumulated considerable meiotic and related data on F_2 's of $2n=30$ (012×013) hybrids; his data showed massive pollen sterility among the F_2 and zygotic chromosome numbers that range between 27 and 32. Although the meiotic data for the parents are included in Table 2, they are expected to serve only reference purposes since they have been discussed by Olorode and Baquar (1976).

Table 2 shows that the triploid hybrids obtained from 012 ($2n=20$) $\times$ 013 hybrids show a high degree of trivalent associations along with some univalents and bivalents (Fig. 1A). Asynchronous migration of chromosomes to the poles were also quite common around first metaphase of meiosis (Fig. 1B). Anaphase cells were replete with considerable migrational irregularities which include varying number of lagging chromosomes and non-disjunction bridges (Fig. 1C). The amount of

meiotic irregularities observed will easily explain the high pollen sterility indicated in Table 2.

In $012 (2n+1=21) \times 013$ crosses, only $2n+1=41$ hybrids were recovered; extensive search among the progeny of the 21-chromosome race of *H. involucrata* however yielded no plants with such a high number (Olorode 1973, Olorode and Baquar 1976). It appears then that although 21-chromosome embryo-sacs are formed in the 21-chromosome race of *U. involucrata*, pollen grains of tetraploid

Figs. 1A-F. Meiotic chromosomes in parents and hybrids of the *Hyparrhenia involucrata*-*H. subplumosa* complex. Scale lines represent 1μ . A, metaphase I in $2n=30$ hybrids from $2n=20 \times 2n=40$ interspecific hybrids ($7_{III}+3_{II}+3_I$). B, asynchronous chromosome movement around metaphase I in triploid interspecific hybrid. C, late anaphase I in triploid interspecific hybrid showing lagging chromosomes and a nondisjunction bridge. D, diakinesis in 41-chromosome interspecific hybrid from $012 (2n+1=21) \times 013 (2n=40)$ crosses ($2_{IV}+16_{II}+1_I$); arrow shows the extra chromosome. E, anaphase I in 41-chromosome interspecific hybrid. Arrow shows the heteropycnotic extra chromosome. F, diakinesis in 026×025 intraspecific hybrids of *H. subplumosa* ($4_{IV}+12_{II}$).

plants (i.e 20-chromosome pollen grains) are required to fertilise such embryo sacs. The mechanics of formation of such embryo sacs has not been determined in spite of our efforts up to date. However, one possibility is that preferential migration of the extra chromosome (in the $2n+1=21$ chromosome race) to one pole of the pollen mother cell simulates first meiotic division in megasporogenesis. A subsequent equational division of the remaining 21 chromosomes (simulating second meiotic

division) will produce 20-chromosome and 21-chromosome embryo sacs: a similar mechanism was reported for *Rosa canina* (see Swanson 1957).

Diakinesis in the 41-chromosome hybrids show considerable amount of quadrivalent associations (Table 2, Fig. 1D). A comparison of the amount of trivalent associations in 012 ($2n=20$) $\times$ 013 and quadrivalent associations in 012 ($2n+1=21$) $\times$ 013 on one hand and the tetraploid parents on the other indicates the high level of homology between the *H. involucrata* genome and *H. subplumosa* genome. Figure 1E shows the extra chromosome of the 41-chromosome hybrids as the heteropycnotic element (arrow). The comparability of the pollen fertility of the 41-chromosome hybrids with that of the tetraploid parents is obvious.

The fact that 250/2 $\times$ 013 produce viable hybrids which are seed- and pollen-fertile indicates, indirectly, genomic relationship between 250/2 and 012 since the latter also crosses with 013 and their chromosomes pair intimately. However, the relatively low level of quadrivalent associations in 250/2 $\times$ 013 crosses (Table 2) demonstrates that the chromosomes of 250/2 may have undergone changes which make them qualitatively different from those of 012.

Table 2 shows that in spite of the relatively high incidence of bivalent associations in 026 $\times$ 025 hybrids (Fig. 1F is one of the cells with relatively high incidence of quadrivalent associations), pollen fertility level was lower than in one of the parents (025) which shows a comparable amount of diakinesis bivalent associations. The higher level of pollen fertility in 026 $\times$ 025 hybrids when compared with 026 could be explained on the basis of the relatively high incidence of tetravalents in the latter. It is worth emphasising again that seed set in the 026 $\times$ 025 hybrids is comparable with that in the parents.

Conclusion

The observations presented in this paper and their interpretations makes the fact of genetic affinity of the taxa considered obvious; namely there is considerable genomic identity between the annual species of the complex and the perennial component.

The unsuccessful search for a perennial diploid in the complex continues to creat problems as to the origin of perennial habit in the tetraploids. Put together, the diakinesis data, the growth habit data and other morphological data (Table 2) suggest that the tetraploid taxa in this complex may be of autopolyploid origin and that perenniality was a mutation that was quickly selected for along with essentially diploid-like chromosome-pairing behaviour. However, we see two other possibilities both of which will explain the tetraploids as being of allopolyploid origin. Firstly, an unidentified (or extinct) diploid annual with a structurally differentiated genome may have hybridised with 012 ($2n=20$) or a plant of similar genome; polyploidisation of such a hybrid and evolution of perennial habit would have produced *H. subplumosa*. Secondly, an unidentified (or extinct) diploid perennial may have been involved in a similar hybridisation followed by polyploidisation.

The pattern of inheritance of the number of propagules (number of main awns) in this complex throws considerable light on the evolution of the 'andropogonic'

inflorescence in general and 'hyparrhenoid' inflorescence in particular. The relationship of awn formation to development of caryopsis and of transgressive awn numbers to growth habit should be investigated. Similarly, the nature of the interaction of different environmental factors with genetic factors in expression of awn numbers should be investigated with a view to predicting levels of 'seed' production and related capabilities of different genotypes in this complex.

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