Theoretical and Applied Genetics 43, 242–251 (1973)

Evolution and Improvement of Cultivated Amaranths

VI. Cytogenetic Relationships in Grain Types

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Summary. This group of amaranths was studied using four domesticated species (*A. hypochondriacus, A. cruentus, A. caudatus, A. caudatus* var. *atropurpureus* and *A. edulis*), two ancestral weedy species (*A. hybridus, A. powellii*) and eight hybrids, namely *A. edulis* × *A. hypochondriacus, A. edulis* × *A. caudatus, A. edulis* × *A. caudatus* var. *atropurpureus, A. caudatus* × *A. hybridus, A. edulis* × *A. hybridus, A. caudatus* × *A. hypochondriacus, A. hybridus* × *A. hypochondriacus* and *A. powellii* × *A. hypochondriacus.*

The parents have perfectly normal meiosis and pollen and seed fertility. Except for *A. powellii* and *A. cruentus* ($n = 17$), the species have $n = 16$. However, the hybrids may be divided into three groups. The first group contains *A. edulis* × *A. cruentus,* involving parents with $n = 16$ and 17, which failed totally, although, under the same conditions, crosses between *A. powellii* ($x = 17$) and *A. hypochondriacus* ($n = 16$) and those between species with $n = 16$ succeeded with ease. The second group is made up of *A. edulis* × *A. hypochondriacus, A. caudatus* × *A. hypochondriacus, A. caudatus* × *A. hybridus, A. edulis* × *A. hybridus* and probably also *A. powellii* × *A. hypochondriacus.* Of these, the two combinations, *A. caudatus* × *A. hybridus* and *A. edulis* × *A. hybridus,* did not proceed beyond the two-leaf stage. At pachytene, the other hybrids showed unmistakable evidence of structural hybridity, with deletions, long or short differentiated segments and inversions. Although bivalents were formed, they possessed a chiasma frequency lower than that of either parent. There was total pollen and seed sterility.

The third group comprises *A. edulis* × *A. caudatus, A. edulis* × *A. caudatus* var. *atropurpureus* and *A. hybridus* × *A. hypochondriacus,* which did not show serious developmental defects, the F_1 being vigorous, with good meiotic pairing associated with a reasonable amount of differentiation in the chromosomes leading to 25–55% fertile pollen and 49 to 66% threshable seed. In the F_2 there were 11–18% unthrifty plants, which disturb the ratios of gene combinations controlling the different characters in the two parents. Plants very near one or both parental phenotypes were recovered, and also those showing different degrees of recombination of characters. Amphidiploids from the F_1 hybrids showed the typical autoploid or segmental alloploid type of meiosis indicating that the parental chromosomes are quite homologous.

In view of the present experimental evidence and possible parallel mutations in different grains and weed amaranths, it is not certain whether the cases of natural hybridization and, in particular, of introgression can be taken as evidence for or against the two hypotheses proposed by Sauer (1967) on the basis of his brilliant ecogeographical, morphological, ethnobotanical and archaeological studies of this group of amaranths.

The only point that can be stated categorically is that *A. caudatus* has given rise to *A. edulis.* The dominance of the characters of *A. caudatus* over those of *A. edulis* strengthens such a view, but the latter is sufficiently differentiated morphologically and genetically to deserve independent status. *A. caudatus* var. *atropurpureus* is a fertile but unstabilized hybrid segregate between *A. caudatus* and *A. edulis.* This is borne out by its morphological, cytogenetic and breeding behaviour, and its hybrids with *A. edulis,* and, above all, by the recovery of plants identical with this variety from the F_2 progeny of *A. edulis* × *A. caudatus.*

Whatever the origin of grain types, at present they exist only in cultivation and appear to have a long history, having been selected for large plant body, huge compound inflorescences, large number of female flowers per glomerule, small and soft bracts and pale coloured seed in a dehiscent utricle. At the same time, there has also been inadvertent selection for higher and correctly balanced amounts of protein, carbohydrate and fat.

Introduction

The available information on the relationships between grain amaranths and their putative wild progenitors is almost exclusively based on morphological data. A large number of natural, totally sterile to almost fertile, interspecific hybrids of presumed origin have been reported in the genus as a whole, and in the Section *Amaranthus* (to which all the grain species belong) in particular (Murray, 1940; Tucker and Sauer, 1958; Thellung, 1914; Priszter, 1958; Sauer, 1955, 1967; Grant, 1959b; Aellen, 1961; Brenan, 1961). There are very few reports of chromosome studies on spontaneous hybrids of no definite origin (Covas, 1950; Grant, 1959b), and these can not be relied upon for drawing precise conclusions about the genetic relationship. So far, neither experimental verification of the natural hybrids nor detailed studies of chromosome behaviour at meiosis have been made with a view to systematically elucidating species relationships on cytogenetic considerations. Morphological aspects, particularly of the aberrant phenotypes obtained in some hybrid combinations, have been discussed in detail elsewhere (Pal and Khoshoo, 1972); in the present work hybrids of known ancestry have been worked out cytologically in order to bring out the different mechanisms underlying speciation and evolution in the grain species and their progenitors.

Material and Methods

Besides the four grain species (*A. hypochondriacus, A. caudatus, A. edulis* and *A. cruentus*), two related wild species [*A. hybridus, A. powellii* (*A. bouchoni*)] and an ornamental cultivar of *A. caudatus* (*A. caudatus* var. *atropurpureus*) have also been included in the present studies. These species have been involved in a systematic hybridization programme and only the following combinations are included here (Work on others is in progress):

A. edulis × *A. hypochondriacus* and reciprocal
A. edulis × *A. caudatus* and reciprocal
A. edulis × *A. caudatus* var. *atropurpureus*
A. caudatus × *A. hybridus*
A. edulis × *A. hybridus* and reciprocal
A. caudatus × *A. hypochondriacus*
A. hybridus × *A. hypochondriacus*
A. powellii (*A. bouchoni*) × *A. hypochondriacus*.

In addition, two colchicine-induced amphidiploids, *A. edulis-caudatus* and *A. hybridus-hypochondriacus*, were included to give a precise understanding of the nature of chromosome pairing in the above interspecific hybrids.

Although amaranths are regarded as very difficult cytological material, the authors obtained reliable preparations by fixing young inflorescences for 24 hours in Carnoy's fluid in which the acetic acid component was saturated with ferric acetate, followed by squashing the young flowers in 1% acetocarmine.

Observations

1. Parents

The details of meiotic behaviour in the parenta species are depicted in Figs. 1—9. Pairing between homologous chromosomes was normal and complete along the entire length in all pairs in all the parental taxa, except that a deletion in one bivalent resulting in an unpaired loop was sometimes observed in *A. edulis* (Fig. 1), and in *A. caudatus* var. *atropurpureus* there was evidence of possible duplication in one pair.

At diakinesis, the sixteen bivalents (Fig. 2) were found in all species except *A. cruentus* and *A. powellii*, both of which have $n = 17$ (Fig. 3). Usually, one pair was associated with the nucleolus, indicating the presence of a pair of nucleolar organisers (Fig. 2).

At metaphase I, chiasmata could be studied with a reasonable degree of accuracy. Usually one or two, but sometimes even three, chiasmata per bivalent were seen (Figs. 4—5). The formation of three chiasmata would suggest a submedian to subterminal position for the centromere in such chromosomes. The distribution of chiasmata is random between the bivalents, they are mostly interstitial and thus give a low coefficient of terminalization (0.127—0.216) in the various species at metaphase I. The mean number of chiasmata (Table 1) ranges from a minimum of 24.32 per cell in *A. hybridus* to a maximum of 27.73 in *A. hypochondriacus*. The differences in the mean frequency of chiasmata between various species appear to be genotypically controlled (unpublished data). However, no significant difference was observed between *A. edulis* and *A. caudatus* or between *A. caudatus* and *A. caudatus* var. *atropurpureus*, suggesting a close genetic relationship between these species which is corroborated by their basic morphological similarity.

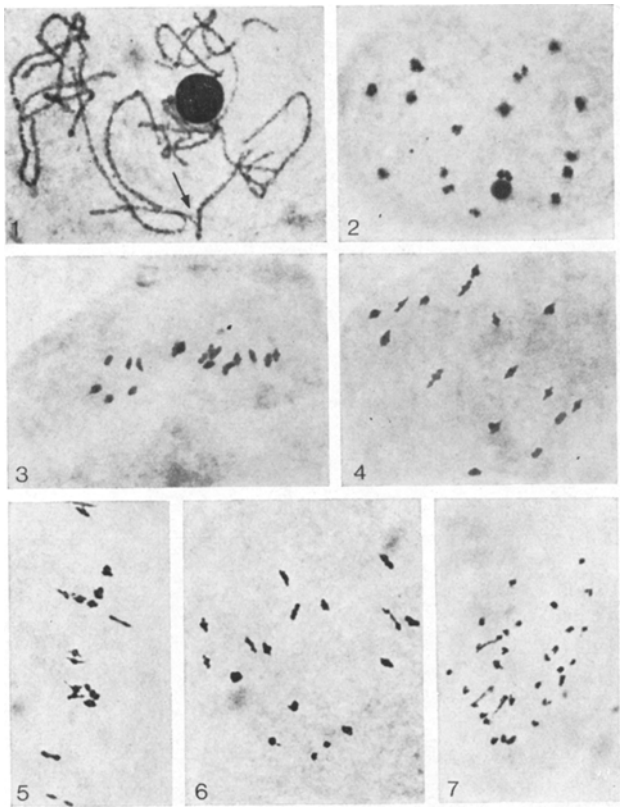

Fig. 1. *A. edulis*, pachytene, note one impaired segment

Fig. 2. *A. hypochondriacus*, Diakinesis, 16 II

Fig. 3. *A. powellii*. Metaphase I, 17 II

Fig. 4. *A. hypochondriacus*. Metaphase I, 16 II, with three chiasmata in one bivalent

Fig. 5. *A. caudatus*, Metaphase I, 16 II with one bivalent showing three chiasmata

Fig. 6. *A. hypochondriacus*. Metaphase I, 15 II + 2 I

Fig. 7. *A. caudatus*. Anaphase I, 16:16 with late disjunction bridges

An interesting feature observed at metaphase I in all the taxa is the precocious disjunction in one or two bivalents (Fig. 6), one of which was usually the smallest bivalent in the complement.

Because of difficulty in terminalization in bivalents with three chiasmata, there were terminalization bridges at anaphase I in all taxa except *A. hypochondriacus*, but otherwise anaphase was perfectly normal. The frequency of such bridges was rather high (0—3 per cell) in *A. caudatus* (Fig. 7). Sometimes, during anaphase separation, chromosomes broke at the point of non-terminalization, which resulted in one of the poles receiving an additional segment carried by the univalent as a tail (Fig. 8). However, all these abnormalities rarely resulted in numerically unequal distribution of chromosomes at anaphase I; this was

Table 1. *Summary of meiosis and pollen fertility in species and hybrids of grain amaranths*

Taxon	No. of cells	No. of bivalents		Average per cell		No. of Xta		Terminalization Coeff.	Pollen fertility %
		Rods	Rings	Rods	Ring	Total	Average per cell		
A. hypochondriacus	15	70	170	4.7	11.3	416	27.73 ± 0.44	0.216	97.15
A. caudatus	25	136	264	5.44	10.56	664	26.56 ± 0.44	0.203	91.96
A. caudatus var. *atropurpureus*	25	138	262	5.52	10.48	659	26.36 ± 0.02	0,127	28.9–83.5
A. edulis	25	159	241	6.36	9.64	641	25.64 ± 0.35	0.127	93.1
A. hybridus	25	187	213	7.48	8.52	608	24.32 ± 0.36	0.202	92.41
A. edulis × *A. hypochondriacus*	25	182	218	7.28	8.72	613	24.52 ± 0.38	0.107	Nil
A. caudatus × *A. hypochondriacus*	25	172	228	6.88	9.12	634	25.36 ± 0.30	0.14	3.4
A. edulis × *A. caudatus*	17	115	157	6.77	9.23	422	24.82 ± 0.37	0.381	24.9
A. edulis × *A. caudatus* var. *atropurpureus*	10	60	100	6.0	10.0	260	26.0 ± 0.64	0.3	56.4
A. hybridus × *A. hypochondriacus*	16	98	158	6.13	9.87	418	26.12 ± 0.58	0.421	55.0

almost always 16:16. Anaphase I irregularities such as lagging and precociously divided univalents were common in *A. caudatus* var. *atropurpureus*, particularly in individuals (Fig. 9) with low pollen fertility which varied from 28.9 to 83.5% (Table 1).

Anaphase II was not studied, but, from the high pollen fertility in the species, it may be concluded that all stages were perfectly normal. In *A. hybridus*, dyads were sometimes observed which resulted in pollen with unreduced number (Fig. 10). Though the frequency of such pollen is very low, such cells may be the source of the few spontaneous triploid and tetraploid plants reported by Grant (1959a) in dioecious species of *Amaranthus*.

2. Hybrids

A resumé of the important cytological characteristics of different hybrids is presented in Tables 1 and 2 and only relevant points will be brought out below:

A. edulis × *A. hypochondriacus*. Little material was available for meiotic study because the anthers were not only small in size but also reduced in number. At pachytene, the chromosomes did not pair along their entire length and a number of unpaired regions were apparent. In some bivalents, terminal differential segments and deletions could also be made out (Fig. 11). Among the hybrids studied in the present investigation, this particular combination revealed the maximum number of such abnormalities at the pachytene, which may be evidence for a good deal of cryptic structural hybridity. In view of the total sterility, this may not be wholly unexpected. However, there was little evidence of these pairing abnormalities at either diakinesis or metaphase I, where invariably 16 bivalents were formed (Fig. 12). There was a drop in the mean chiasma frequency per cell compared with the parents (Table 1). Perhaps the differential regions exerted an influence on the realization of crossovers. At metaphase I, two bivalents were often heteromorphic and, as in the parents, early disjunction was also found (Fig. 12). At anaphase I there was regular disjunction. The further course of meiosis also seems to be regular because micropollen was absent and the pollen was almost uniform in size (20.09–21.32 μ). However, in spite of normal meiosis, there was almost complete pollen sterility (Fig. 13). While all hybrid individuals were totally seed sterile, seed formation was detected in one plant on one of its branches. The size of the seeds tallied with that of the amphidiploids but no cytological confirmation was possible because the plants were completely mature by the time this branch was detected. The size of the pollen grains of flowers in the "fertile" region indicated that they were unreduced, because their size (32.2–40.4 μ) tallied with that of pollen of the induced amphidiploids, and a study of the pollen mitosis, though not conclusive proof of the tetraploid nature of the branch, revealed 32 chromosomes in the pollen (Fig. 14). Fertility of the pollen in this branch was 11.0% and the harvested seeds had translucent centres like *A. edulis*.

No seeds were harvested after either selfing or backcrossing the F_1 hybrid with either parent. Evidently the hybrid is also female sterile. However, late in the season six seeds were harvested from the whole inflorescence. Possibly these were the result of backcrossing with plants of *A. edulis* which were in the proximity. This behaviour suggests a slight improvement of fertility late in the season on the female side, which may be understandable as the vegetative morphology also became comparatively normal in November and early December.

A. edulis × *A. caudatus*. A large number of cells at pachytene indicated that pairing was essentially complete, but a closer analysis revealed some lack

Table 2. *Range and mean of chromosome associations and pollen fertility in amphidiploids and hybrid tetraploids*

Associations	Quadrivalents		Trivalents		Bivalents		Univalents		Pollen fertility (%)
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Amphidiploid *A. edulis-caudatus* ($2n = 64$)	4–8	5.840 ± 0.32	0–5	0.44 ± 0.02	14–24	18.6 ± 0.2	0–6	2.12 ± 0.21	54.1
F_1 *A. edulis* ($4x$) × *A. caudatus* ($4x$)									
Plant 1 ($2n = 64$)	5–12	7.857 ± 0.31	0–1	0.285 ± 0.04	8–12	15.714 ± 0.27	0–1	0.285 ± 0.04	89.63
Plant 2 ($2n = 64$)	2–6	3.5 ± 0.07	–	–	8–19	14.75 ± 0.09	0–2	0.5 ± 0.002	86.67
Plant 3 ($2n = 62$)	2–7	3.666 ± 0.07	0–1	0.25 ± 0.002	16–25	21.916 ± 0.25	1–6	2.75 ± 0.06	82.5
Amphidiploid *A. hybridus-hypochondriacus* ($2n = 64$)	2–11	6.727 ± 0.41	0–2	0.122 ± 0.06	11–28	18.272 ± 0.47	0–3	0.182 ± 0.01	52.6

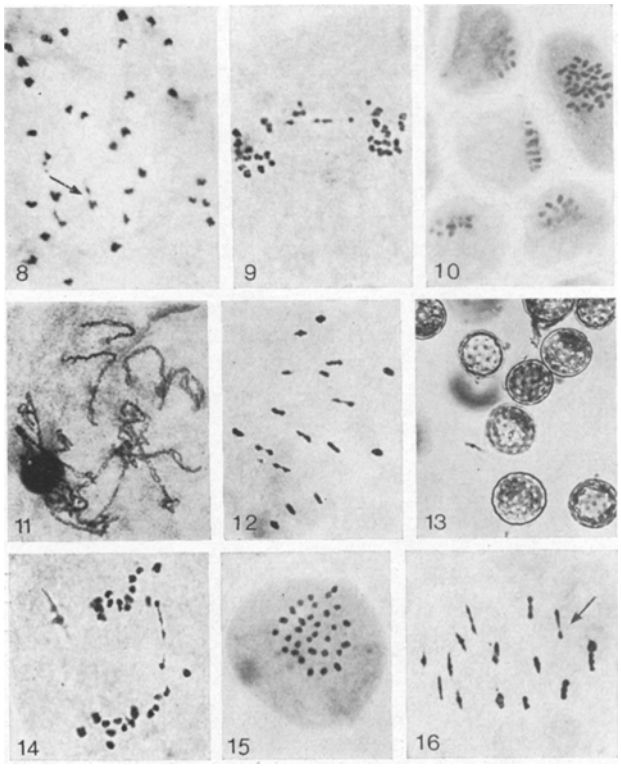

Fig. 8. *A. caudatus*. Ultimate breakage of late disjunction bridges

Fig. 9. *A. caudatus* var. *atropurpureus*. Anaphase I with precociously divided lagging univalents

Fig. 10. *A. hybridus*. Reduced ($n = 16$) and unreduced ($n = 32$) pollen grains undergoing mitosis

Fig. 11. F_1 *A. edulis* × *A. hypochondriacus*. Pachytene showing some differential and unpaired regions

Fig. 12. F_1 *A. edulis* × *A. hypochondriacus*. Metaphase I, 15 II + one precociously divided bivalent. Also note heteromorphic bivalent

Fig. 13. F_1 *A. edulis* × *A. hypochondriacus* sterile pollen grains

Fig. 14. F_1 *A. edulis* × *A. hypochondriacus*. Mitosis in an unreduced pollen grain ($n = 32$) from a spontaneously arisen tetraploid branch

Fig. 15. F_1 *A. edulis* × *A. caudatus*. Metaphase I, 16 II

Fig. 16. F_1 *A. edulis* × *A. caudatus*. Anaphase I, 16:16

of correspondence between the pairing in homologues. This mostly took the form of small unpaired segments and evidence of terminal deletion in one bivalent. Of the 16 bivalents at metaphase I, one or two were heteromorphic (Fig. 15) and another one showed precocious disjunction. The mean chiasma frequency per cell was 24.82 ± 0.37 (Table 1) which is lower than in either parent. The coefficient of terminalization was 0.381. At anaphase I, there were terminalization difficulties which resulted in bridges (Fig. 16) but in most a 16:16 distribution resulted, barring a few cells in which lagging univalents failed to reach

either pole. Such univalents may even undergo precocious separation and may be the source of the 1% of micropollen encountered in the present hybrid. Although meiosis was normal overall, pollen fertility was only 24.9% (Fig. 17). Seed fertility, as determined from the threshable seeds, was 67.92%.

There was considerable mortality in the F_2 at the seedling stage. Even among the 22 plants that survived, four showed retarded growth and ultimately died. The partial sterility in the F_1 and the selective elimination of seedlings in F_2 must affect ratios of recombination between the different characters distinguishing the species.

Pollen fertility in the F_2 varied from 40.4 to 84.8%, though most of the plants fell in the range 60—80%. Plants have not been analyzed for seed fertility so far, but there is evidence that some of the plants, although having high pollen fertility, are completely seed sterile.

An interesting feature of the study of the F_2 population was that some individuals very closely resembling *A. caudatus* var. *atropurpureus* were detected. This indicates the hybrid origin of this cultivar.

Amphidiploids were raised by colchicine treatment of F_1 seedlings, to assess the exact extent and nature of differential/preferential pairing in the F_1 *A. edulis* × *A. caudatus*. The number of quadrivalents ranged from 4 to 8, the average being 5.84 per cell (Fig. 18; Table 2). This is reminiscent of the situation in autotetraploids of the four grain species (Pal and Khoshoo, 1968 and 1973). A very close genetic homology between the two species is indicated. The average univalent frequency of 2.12 per cell bears no relation to the very low frequency (0.44 per cell) of trivalents per cell and the early disjunction of quadrivalents, but these could represent precociously disjuncted bivalents as seen in the parents. Pollen fertility was 54.1 per cent, which was higher than in the F_1 hybrid. There was a high percentage (10.2%) of micropollen, which may be the result of the univalents observed earlier or of segregational irregularities at anaphases I and II.

This amphidiploid was also obtained by crossing the autotetraploids of *A. edulis* and *A. caudatus*. Meiotic studies were carried out on three such individuals. While two plants had the full complement of $2n = 64$ (Fig. 19), the third was hypotetraploid with $2n = 62$ (Fig. 20). Meiosis was typically autoploid in character in all three plants (Table 2). The two plants with tetraploid chromosome number showed a higher quadrivalent frequency than the amphidiploid (Table 2) or the autotetraploids. However, in the hypotetraploid plant the average quadrivalent frequency of 3.66 per cell was accompanied by higher bivalent and univalent frequencies than in the other two plants. Pollen fertility was lower (82.5%) in the plant with $2n = 62$ than in the two

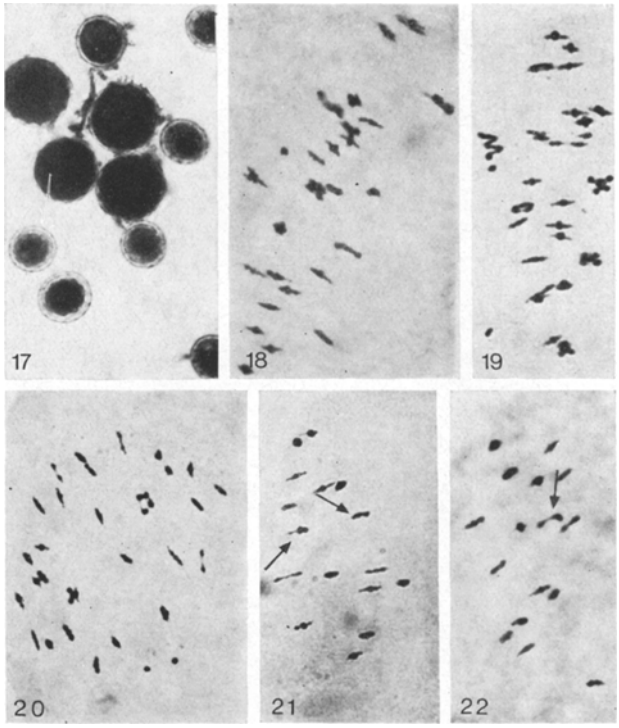

Fig. 17. F_1 *A. edulis* × *A. caudatus*. Pollen grains

Fig. 18. Amphidiploid *A. edulis-caudatus*. Metaphase I, 7 IV + 1 III + 16 II + 1 I

Fig. 19. F_1 *A. edulis* (4 *x*) × *A. caudatus* (4 *x*). Metaphase I, 1 VI + 6 IV + 16 II + 2 I

Fig. 20. Hypotetraploid ($2n = 62$) F_1 *A. edulis* (4 *x*) × *A. caudatus* (4 *x*). Metaphase I, 4 IV + 22 II + 2 I

Fig. 21. F_1 *A. edulis* × *A. caudatus* var. *atropurpureus*. Metaphase I, 16 II

Fig. 22. F_1 *A. caudatus* × *A. hypochondriacus*. Metaphase I. 16 II with some heteromorphic bivalents

tetraploids (89.63 and 86.67%). This was accompanied by a significantly higher percentage (16%) of micropollen in the former than in the latter (6.66 and 4.5%). Perhaps higher univalent frequency, together with their precocious divisions and the existing loss of two chromosomes, resulted in defective pollen grains that failed to stain.

A. edulis × *A. caudatus* var. *atropurpureus*. Pairing at pachytene was essentially complete and only a few differential segments and regions with a change in chromosome pattern were present. Among the 16 bivalents (Fig. 21), two were heteromorphic and some showed precocious disjunction. The mean chiasma frequency per cell was 26.0 ± 0.64 which is nearly intermediate between those of the parents (Table 1). Anaphase I was usually regular with 16:16 distribution, though late disjunction bridges in some cells were also noted. Pollen stainability was 56.4%. Seed fertility, as determined from threshable seed, was

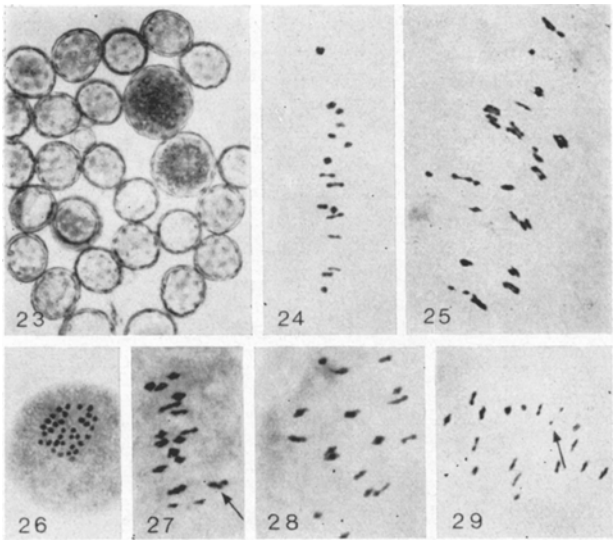

Fig. 23. F_1 *A. caudatus* × *A. hypochondriacus*. Pollen grains

Fig. 24. *A. hybridus* × *A. hypochondriacus*. Metaphase I, 16II

Fig. 25. Amphidiploid *A. hybridus-hypochondriacus* ($2n = 64$). Metaphase I, 9 IV + 1 III + 11 V + 3 I

Fig. 26. Amphidiploid *A. hybridus-hypochondriacus*. Pollen grain mitosis $n = 33$ ($2x + 1$)

Fig. 27. F_1 *A. powellii* × *A. hypochondriacus*. Metaphase I, 15 II + 1 III

Fig. 28. F_1 *A. powellii* × *A. hypochondriacus*. Metaphase I, 16 II + 1 I

Fig. 29. *A. species* (African) Metaphase I, 16 II + 1 very small precociously divided bivalent

61.7%. An interesting feature was that no brown seeds, such as were found in the pollen parent, were recovered from the hybrid.

A. caudatus × *A. hybridus* and *A. edulis* × *A. hybridus*. The first cross was performed in only one direction, i.e. with *A. caudatus* as the female parent, while the latter was performed in both directions. No barriers to crossability were observed and normal seed production was obtained. Seed germination was also normal, though delayed by two days compared with the parents. However, the seedlings did not proceed beyond the first or second leaf stage. The leaves became very thick and distorted. All attempts at making the seedlings grow further failed and the seedlings ultimately died, indicating a strong interspecific barrier due to seedling mortality.

It is pertinent to mention here that Covas (1950, vide Grant, 1959b) reported a semi-fertile natural hybrid between *A. edulis* × *A. hybridus*, which at metaphase I showed 16 II followed by regular meiotic behaviour. The discrepancy between the present results and those of Covas may be due to differential behaviour of the two genotypes of the polymorphic *A. hybridus* involved in the two cases, or may be a case of taxonomic misidentification.

A. caudatus × *A. hypochondriacus*. During pachytene there was no evidence of the extensive asynaptic segments so characteristic of *A. edulis* × *A. hypochondriacus*, but in many cells there was evidence for some unpaired segments and inversions. At diakinesis and metaphase I all the chromosomes associated as bivalents (Fig. 22), among which one was heteromorphic and another disjuncted precociously. Mean chiasma frequency per cell was 25.36 ± 0.30, lower than in either parent. The terminalization coefficient was also lower than in the parents, leading to a rather less orderly disjunction at anaphase I. There were sometimes one or two late disjunction bridges and, rarely, a dicentric bridge with a fragment resulting probably from inversion heterozygosity. Such irregularities resulted in nearly 6—7% of micropollen. The pollen was highly sterile, stainability being as low as 3.4% (Fig. 23).

A. hybridus × *A. hypochondriacus*. Pairing at pachytene was quite orderly without any evidence of unpaired segments. At diakinesis and metaphase I, 16 bivalents were regularly formed and no heteromorphic bivalents were noted (Fig. 24). Precocious disjunction was sometimes observed in one bivalent. The chiasmata frequency of 26.12 per cell was nearly intermediate between the frequencies in the two parents. Anaphase I resulted in a 16:16 distribution, though late disjunction bridges were noted in a few cells. The further course of meiosis also seemed to be regular as evidenced by pollen of uniform size and $n = 16$ in the pollen grains. Pollen stainability was only 55%. Seed fertility, ascertained from threshable seed, was nearly 49%.

Seed germination in the F_2 was normal and there was no mortality at the seedling stage, but six plants out of 54 showed a virus-like syndrome. In two the effect was rather severe, while the remaining four were stunted. Fertility was not affected and there was much variability in the segregating population, no two plants being similar. Some of the plants approached one or other parent in external morphology.

Meiotic studies on the colchicine-induced amphidiploid revealed 2—11 quadrivalents at metaphase I in the various pollen mother cells. However, more than 50% of the cells had 6 to 8 quadrivalents (Table 2; Fig. 25) and the average number per cell was 6.727. The frequency of univalents and trivalents was very low. The amphidiploid showed typical autoploid characteristics during meiosis. The pollen fertility was estimated to be 52.6 per cent. A study of pollen grain mitosis revealed grains with a number higher than the expected diploid number i.e. $n = 33$ (Fig. 26). Seed fertility was very low.

A. powellii (*A. bouchoni*) × *A. hypochondriacus*. This hybrid involves two basic numbers, $x = 17$ and 16. In 62.5% of cells the 33 chromosomes formed 15 II + 1 III (Fig. 27), while in the remaining cells

there were 16 II + 1 I (Fig. 28). The hybrid was totally pollen sterile and there was no fertile seed on selfing but backcrosses with both parents succeeded.

Discussion

Grain amaranths are among the oldest crops, having been cultivated for more than 6,000 years (Agogino, 1957). They arose in the new world, where they were used as "cereals", and may be regarded as one of the counterparts of the true cereals (like wheat and rice) which arose in the old world. With the advent of the true cereals in the new world, grain amaranths have been considerably overshadowed and in most places have been completely ousted from cultivation. At present, the maximum cultivation of grain amaranths is in India, so much so that the erroneous impression has been given that the Indian Subcontinent is their centre of origin (DeCandolle, 1883; Vavilov, 1949—50; see also Darlington, 1963). However, the excellent ethnobotanical studies of Sauer (1950, 1967) have disproved this contention. The present study is the first attempt towards understanding the cytogenetic basis of the evolution of grain species.

Cytogenetic Differentiation

In all the hybrids studied here, almost total bivalent pairing was found at diakinesis and metaphase I. This was, however, accompanied by varying degrees (almost nil in *A. edulis* × *A. hypochondriacus* to 55% in *A. hybridus* × *hypochondriacus*) of pollen fertility. Such a situation is characteristic of a number of hybrids and Stebbins (1950) has explained it on the basis of cryptic structural hybridity. The only way to unravel the extent and nature of such hybridity is through detailed analysis of the pachytene. This approach has proved profitable by Shastry and Misra (1961) and Shastry *et al.* (1960) in *Oryza*, Southern (1967) in *Tulipa*, Ahloowalia (1965) in fescue, Magoon and Tayyab (1967) and Magoon *et al.* (1967) in *Sorghum*, etc.

In amaranths, pachytene pairing of the homologous chromosomes in the parents was found to be regular, except for a possible slight deletion in *A. edulis*. In strong contrast to the parents, pairing was incomplete with evidence of structural hybridity in hybrids such as *A. edulis* × *A. hypochondriacus*, *A. edulis* × *A. caudatus*, *A. edulis* × *A. caudatus* var. *atropurpureus* and *A. caudatus* × *A. hypochondriacus*. The structural changes chiefly involved deletions and differential segments. There were also indications of inversion in *A. caudatus* × *A. hypochondriacus*, corroborated by bridge-fragment configurations at anaphase. The differential segments were rather small in *A. edulis* × *A. caudatus*, *A. edulis* × *A. caudatus* var. *atropurpureus* and *A. caudatus* × *A. hypochondriacus*. Larger segments and terminal deletions were found only in *A. edulis* × *A. hypochondriacus*, indicating greater differentiation of the two genomes. The exact nature of the differentiated segments is not clear at present. However, it appears that genetic differentiation at the interspecific level may have resulted from the accumulation of small structural and genic changes in the chromosomes, facilitated by isolation (even due to cultivation for grain or ornamentation) among these taxa. In *A. hybridus* × *A. hypochondriacus* no visible structural differences were evident. Shastry and Misra (1961), in their studies on the subspecific hybrids of *Oryza*, found a correlation between the general level of abnormalities at pachytene (differential index) and pollen sterility. Though no such index was calculated here, it may be mentioned that the F_1 *A. edulis* × *A. hypochondriacus* showed the maximum abnormalities and was completely sterile. Where pollen abortion was not accompanied by structural differentiation, as in *A. hybridus* × *A. hypochondriacus*, it is probable that, either differentiated segments were not readily visible at pachytene, or the differentiation was at genic level.

Some of the taxa, such as *A. hypochondriacus*, *A. caudatus* and *A. hybridus*, differ in chiasma frequency, which may signify a genetic dissimilarity. No significant differences were found between *A. edulis*, *A. caudatus* and *A. caudatus* var. *atropurpureus*, which may point to their close relationship. The chiasma frequency was usually positively correlated with the frequency of ring bivalents per cell. The chiasma frequencies of hybrids were either lower than those of both parents or were intermediate. The lower chiasma frequency in *A. edulis* × *A. hypochondriacus*, *A. edulis* × *A. caudatus* and *A. caudatus* × *A. hypochondriacus* compared with their parents probably indicates a measure of non-homology in the pairing chromosomes. A similar decrease in chiasma frequency in the hybrid has also been noted in *Sorghum* (Magoon and Tayyab, 1967). Except for the two hybrids *A. edulis* × *A. hypochondriacus* and *A. caudatus* × *A. hypochondriacus* where terminalization was slightly restricted, all the other four hybrid combinations showed a high degree of terminalization.

Among the successful hybrids, differentiation between the parents can be judged from the extent of sterility. On this count the hybrids fall into two groups. One group consists of *A. edulis* × *A. hypochondriacus*, *A. caudatus* × *A. hypochondriacus* and *A. powelli* × *A. hypochondriacus*, which are pollen sterile, do not yield any seed on selfing, and often have deformed anthers which may not dehisce. In the other group, *A. edulis* × *A. caudatus*, *A. edulis* × *A. caudatus* var. *atropurpureus* and *A. hybridus* × *A. hypochondriacus*, fertility ranges from 25 to 56% while threshable seeds range from 49 to 68%. Evidently, species involved in the first group are more strongly differentiated genetically than those in the second group.

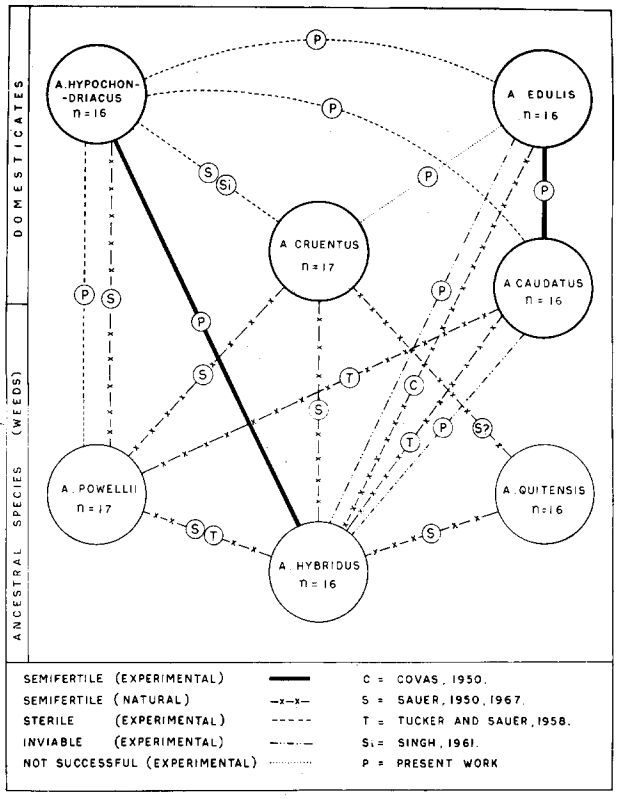

Fig. 30. Crossing polygon depicting interrelationships among seven taxa (4 domesticates and 3 weeds) of the grain amaranth complex. The figure is based on the experimental data obtained in the present studies together with data of other authors on natural hybrids

The nature and extent of cytogenetic differentiation seen here has been correlated with other traits of the genetic system (Khoshoo and Pal, 1972).

The common occurrence of 1 chain of 3 + 15 II in the dibasic hybrid *A. powellii* × *A. hypochondriacus* ($2n = 33$), coupled with the presence of one unusually small bivalent out of 17 present (Fig. 29) in an African species (regarded by Kew authorities as *A. hybridus* $n = 16$), may indicate that $x = 16$ has been derived from $x = 17$ with the complement of the African species representing the transitional stage. In this case, $x = 17$ may have been derived from $x = 8$ and 9, which are commonly found in Amaranthaceae. However, more work is needed before we accept this hypothesis.

Relationships and the Origin of Grain Amaranths

The results of the present hybridization experiments on grain amaranths, together with the spontaneous hybrids recorded earlier by Covas (1950), Tucker and Sauer (1958), Sauer (1950, 1967) and Singh (1961), are summarized in the form of a crossing polygon (Fig. 30). The data are too incomplete for definite conclusions to be drawn but it is apparent that, in general, there is a measure of gene exchange between the three basal species irrespective of differences in chromosome number. According to the data of these authors, these species are also able to exchange genes with their derivative cultivated types, and in fact Sauer (1967) has even reported several possible cases of introgression of characters of the progenitors into grain species and *vice versa*. However, it is clear from Fig. 30 that the present data do not support these observations for *A. powellii* × *A. hypochondriacus*, *A. edulis* × *A. hybridus* or *A. caudatus* × *A. hybridus*. In contrast, there appears to be no gene exchange between the grain types themselves. Furthermore, in case there has been parallel evolution in the different grain amaranths and their progenitors, one may not be sure if the cases of natural hybrids and introgression reported by the above authors can be taken as evidence for or against the two hypotheses for the origin of grain amaranths put forward by Sauer (1967). Experimental confirmation of the presumed natural interspecific hybrids and cases of introgression is necessary, particularly because of the discovery by the authors of a number of interesting genetic-physiologic developmental barriers to crossability (Pal and Khoshoo, 1972).

Sauer (1967) has included *A. edulis* within *A. caudatus*. The genetic differentiation of the two species *vis-a-vis A. hypochondriacus* and *A. hybridus* is at the same level. Tucker and Sauer (1958) reported a semifertile hybrid between *A. caudatus* and *A. hybridus* but the present writers found a very strong barrier in the form of seedling mortality (Pal and Khoshoo, 1972). Whether this reflects differences between the two environments in which the hybrids were raised, or a difference in the genotypes of the parents used, can not be answered with certainty. A more important point is that hybrids between the two taxa, *A. caudatus* and *A. edulis*, are themselves semifertile. In view of the occurrence of dominant characters in *A. caudatus*, it appears that *A. edulis* is a grain derivative of the former, which is essentially an ornamental species. *A. edulis* is cultivated for grain in North West Argentina and Sauer (1967) is also of the opinion that its progenitor is *A. caudatus*. The extent of morphological distinction between *A. edulis* and *A. caudatus* is also about the same as that between other grain species and their wild progenitors. In fact, *A. edulis* possesses the polymerous male flower, which makes the inflorescence determinate, a character peculiar to this species in the whole genus. For these reasons the two taxa have been treated separately in the present studies, although genetically they are fairly closely related. It appears that the end-point of this line is *A. edulis* and the other endpoints are *A. hypochondriacus* and *A. cruentus*. The recovery of plants like *A. caudatus* var. *atropurpureus* from F_2 progeny of the hybrid *A. edulis* × *A. caudatus*, together with morphological and cytogenetic

analysis of this variety and its behaviour when crossed with *A. edulis*, show clearly that it is a fertile but unstabilized hybrid segregate between the two species. Looking at *A. hypochondriacus* and the *A. caudatus-A. edulis* complex, the two latter species appear to be derived in nature because the former shows almost total dominance in hybrids with the latter two species. No cytogenetic data are available about hybrids between *A. cruentus* and the other two species, except for a solitary report by Singh (1961) that a spontaneous hybrid *A. hypochondriacus* × *A. cruentus* is totally sterile. However, the present writers have failed to obtain hybrids between *A. edulis* ($n = 16$) and *A. cruentus* ($n = 17$).

The extent of differentiation between *A. edulis* and *A. hypochondriacus* is the maximum as shown by the ruptured seed coat character of the hybrid seed, reduced germination, abnormal growth and subsequent development, and tumorous stems and roots, together with the highest amount of cryptic structural hybridity. Such restriction on gene exchange between them is understandable because they represent the end-points of long and continued periods of selection by prehistoric American people which allowed not only the selection of useful mutations and recombinants but also the accumulation of considerable genetic differences between the two lines of evolution. Next to *A. edulis*, *A. caudatus* is also differentiated from *A. hypochondriacus*. On the whole, the behaviour of *A. edulis* and *A. caudatus vis-a-vis A. hypochondriacus* and *A. hybridus* is about equal.

The exact mode of origin of the grain amaranths is difficult to unravel, particularly when critical data based on experimental hybrids is not available about the nature of genetic differentiation between the progenitor species on one hand, and between them and grain types on the other, in all the pertinent combinations. Accordingly, it is difficult to comment upon the two hypotheses put forward by Sauer (1967) about the origin of grain amaranths. According to him, each of the 3 grain species has originated from its own progenitor, namely *A. cruentus* ($n = 17$) from *A. hybridus* ($n = 16$), *A. hypochondriacus* ($n = 16$) from *A. powellii* ($n = 17$), and *A. caudatus* ($n = 16$) from *A. quitensis* ($n = 16$). The other hypothesis is that *A. hybridus* ($n = 16$) gave rise to *A. cruentus* ($n = 17$) in the Central American region, *A. cruentus* moved northwards and picked up *A. powellii* ($n = 17$) as its weed, and, by repeated hybridization with it, gave rise to the Mexican grain amaranth *A. hypochondriacus* ($n = 16$). Similarly, *A. cruentus* moved southwards, picked up *A. quitensis* ($n = 16$) and, by continued crossing, may have given rise to *A. caudatus* provided, however, it can be proved that *A. quitensis* and *A. cruentus* cross readily. The second hypothesis has the advantage of postulating a common remote ancestor which might account for the common characters such as the white grain shared by all the grain species. The fact that translucent seeds, so characteristic of *A. edulis*, were obtained in the F_2 segregates from F_1 *A. hybridus* (black seeded) × *A. hypochondriacus* (white opaque seeds) may indicate not only some similarity in genic control in different species but perhaps also a common origin.

Whatever their origin, the grain species arose from a wild progenitor or progenitors by domestication (see also Khoshoo and Pal, 1972). Grain types are not found in the wild condition anywhere in the world. The principles involved during a long selection history resulted, firstly, in the development of rather short and weak bracts, in order to make the inflorescence less prickly when rubbed between the hands while extracting grain. The perfectly dehiscent utricle has been an added advantage in this direction. Secondly, selection has been made for large plant body, particularly large compound inflorescences, so as to give enormous grain yield without an increase in grain size. Thirdly, there has been a decided preference for white seeds with good popping qualities and flavour (Sauer, 1967). During these selection processes, there has been inadvertent selection for the right proportions of protein, carbohydrate and oil components to make it a balanced food (Misra, *et al.*, 1971) with higher calorific value (see Singh, 1961). These features also distinguish the grain types from their wild ancestors.

Acknowledgement

Our thanks are due to Messrs. T. K. Sharma and A. Sen Gupta for help with photographs and Fig. 30 illustrating this paper.

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Received December 27, 1971

Communicated by F. Mechelke

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