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Chromosomal basis of evolution in the genus *Coix* L. (Maydeae): a critical appraisal

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Abstract Among the nine species of the genus Coix, C. lacrymajobi L. and C. gigantea Koen. have 2n=20 small chromosomes and C. aquatica Roxb. 2n=10 large chromosomes, and are widely distributed in South and South East Asia, the first is cultivated and spread to all warmer parts of the world. The other species have restricted localized distribution. C. aquatica originated from a hypothetical diploid ancestor with 2n=10 small chromosomes, which became longer through accumulation of duplicate segments and heterochromatin and adapted to wet habitats. C. lacrymajobi and C. gigantea originated through the same two small chromosome diploid ancestors, one of which may be common to C. aquatica, through allopolyploidy and adapted to drier conditions. All the three species can survive equally well in wet and dry localities. Aneuploid and polyploid races occurred through chromosome nondisjunction and genome doubling in C. gigantea and C. aquatica, generation of alien-addition and substitution chromosome races appears to be the further course of evolution. Six morphological varieties were differentiated in C. lacrymajobi through accumulation of gene mutations, and chromosome structural changes gave rise to a completely euchromatic chromosome race. Two aneuploid species with 2n=32 chromosomes, both large and small, must have originated through allopolyploidy, with C. aquatica (n=6)race as one of the parents in both, C. lacrymajobi and C. gigantea (n=10) the second parent in Madhya Pradesh form and West Bengal form respectively. From comparison of morphology and their distribution, C. puellarum Balansa, C.

ouwehandii Koord. and *C. poilanei* Mimeur are speculated to have originated from the three established species, through chromosomal changes and gene mutations and adapted to restricted localized areas, and *C. gasteenii* Simon an allopolyploid form of *C. lacrymajobi* and *C. gigantea*. A schematic diagram is presented depicting the origin, evolution and interrelationships in the genus.

Keywords Coix · Chromosomes · Evolution

Introduction

The genus *Coix* L. (tribe Maydeae of Poaceae) comprises nine species distinguished by morphological and chromosomal criteria [36]. They are *C. lacrymajobi* L., *C. gigantea* Koen., *C. aquatica* Roxb., two aneuploid species of *Coix*, one from Madhya Pradesh (M.P. form) and the second from West Bengal (W.B. form), *C. poilanei* Mimeur, *C. ouwehandii* Koord., *C. puellarum* Balansa and *C. gasteenii* Simon. The crucial morphological criteria for species differentiation are shape of false fruit (pyriform vs. ovoid, globose, cylindrical etc.) and habit (rhizomatous vs. nonrhizomatous), and the chromosomal features that are instructive in this regard are their size, number and intragenomic pairing in interspecific hybrids [36].

The three traditionally recognized species (*C. lacrymajobi*, *C. gigantea* and *C. aquatica*) are widely distributed throughout South and South East Asia. *C. lacrymajobi*, also called job's tears, cultivated as a cereal by North Eastern hill tribals of India [1], is spread to Polynesia and some isolated locations in Australia and to all other warmer parts of the world. *C. gigantea* was also reported from a North Eastern location of Australia (under the name *C. lingulata* Hack.).

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The rest of the species are limited to restricted areas within the general region of South East Asia and Australia [14, 25, 35, 36].

Despite the fact that the genus is habitually cross pollinated, interspecific hybrids in nature are hardly encountered, partly because of geographical barriers, since the occurrence of any two species in any one area is extremely rare. However when they are brought together in an experimental garden, they often form hybrids spontaneously, but much more frequently when artificially crossed.

The data obtained in this study, together with published and unpublished observations in this laboratory on many populations of different species along with the data of other investigators on morphological features, pachytene chromosomes, chromosome numerical and structural variations, adaptational aspects, interspecific hybridization and chromosome pairing are summed up and utilized in this presentation to explain comprehensively the chromosomal basis of origin, evolution and interrelationships encompassing all the species in the genus.

Materials and methods

The following populations have been maintained over a period of years in the Experimental Field Station, Andhra University, Visakhapatnam for cytogenetic studies.

C. lacrymajobi: Four populations each from Andhra Pradesh and Assam, two from Dehradun, one each from Tamil Nadu, Meghalaya, Nagaland and from an unknown source.

C. gigantea: Two from Maharashtra, one from Madhya Pradesh.

C. aquatica: Two from Andhra Pradesh, one each from Orissa and Madhya Pradesh.

Acetocarmine squash technique was used for cytological studies.

Results

C. lacrymajobi occurs mostly in dry habitats, perennial through basal suckers, broad and glabrous leaf blades and sheaths, and ovoid, globose, cylindrical, hard or soft false fruits.

Six morphological varieties are recognized on the basis of shape, size, colour and texture of fruit case, a) ovoid, bluish-white (var. *lacrymajobi* Bor = var. *typica* Watt), b) globose, broader than long, brown or brownish-black (var. *monilifer* Watt), c) cylindrical or bottle shaped (var. *stenocarpa* Stapf), d) soft shelled in various colours and shapes (var. *Ma-Yuen* Romanet (Stapf), cultivated as a cereal, e) ovoid, larger upto 3 cm in diameter (var. *major* Mimeur) and f) ovoid, small, occurring in twos (var. *minor* Mimeur).

Except var. *Ma-Yuen*, all others have hard, polished fruit cases, some of which are cultivated for their use as beads in necklaces etc. Barring the vars. *major* and *minor*, in which no chromosome information is available, the others have stable genomes with 2n=20 small chromosomes forming 10 bivalents at meiosis. The varieties interbreed readily when grown together and their identity is quickly lost in a few generations. It is therefore conceivable that such of those features which circumscribe the individuals as different morphological varieties had their origin through accumulation of gene mutations in restricted and spatially isolated populations.

Pachytene chromosomes in the traditional species are terminated by telomeres, and the chromosomes are differentiated into proximal heterochromatic segments flanking the centromere and euchromatic segments, the former occur also interstitially in some chromosomes. Under C. lacrymajobi (2n=20), 16 populations from different parts of India (11 of var. lacrymajobi, two of var. monilifer and three of var. Ma-yuen) were examined. Knobs varied from 0-8 ([25, 62], present studies) and macrochromomeres from 0-11 [16]. The knob number, size or position cannot be correlated with morphological varieties or geographical regions (present studies). One population of var. lacrymajobi from Andhra Pradesh (Anantagiri-1) has undifferentiated euchromatic chromosomes, while another from the same region has the usual differentiated chromosomes, indicating that differentiation into euchromatic chromosome races and morphological differentiation into varieties are not interdependent ([25, 62], present studies).

Spontaneous chromosomal variations in *C. lacrymajobi* are extremely rare, being limited to a fragment, two translocations and a triploid [25, 38, 53, 66]. Induced autotetraploids (4n=8x=40) were obtained and in its progenies 4n=40, 39, 41 plants were produced [25, 27, 65, 67]. Gametes with 21 and 19 chromosomes are functional, but individuals with 4n=42 or 4n=38 were not found in the progeny.

In crosses of tetraploids and diploids, triploids with 3n=6x=30, 29, 31 (one plant with a reciprocal translocation) [25, 28, 29] and in triploid progenies (open pollinated or crossed to diploid) trisomics, double and multiple trisomics were obtained [28, 53]. All these showed the expected homologous chromosome pairing and behaviour at meiosis with some fertility.

Thus in this species, there is a clear cut morphological and cytological diversity between populations and possibly none within a population except that which is normally possible in a cross-pollinated population. This species, therefore, appears to be cytogenetically stable and its evolution may be compared in general to other crosspollinated cultivated species like maize [61].

C. gigantea occurs mostly in mountainous terrains and terrestrial regions but grows successfully in semiaquatic and marshy conditions, perennial through basal suckers, generally with broad leaves, glandular leaf sheaths and blades, and pyriform fruit cases with a gaping mouth.

Occurrence of 2n=4x=20 and 4n=8x=40 chromosomes was reported (see [12]). In populations from Western Ghats, Maharashtra, 2n=18 to 22 small chromosomes and 4n=40were reported [24, 25]. Subsequently other numbers of 2n=16, 23, 24 and 26 as well as adjusted numbers of 2n=20and 19 (monosomic-trisomic and nullisomic-trisomic respectively) were also reported from Purandhar, Maharashtra [40, 41, 44–47, 55]. The numbers of 2n=18 and 4n=40(under the name *C. aquatica*), the former from Maharashtra and the latter from Tamilnadu, Karnataka and Kerala are also on record [5, 11, 16].

All the chromosomal variants were more or less similar in external features. Chromosome pairing occurred as nine bivalents in 2n=18 nullisomics, nine bivalents and one univalent in 2n=19 monosomics, 10 bivalents in 2n=20disomics and corresponding higher associations were found from trisomics (2n=21) to octosomics (2n=26) [40, 41, 45, 55]. In 4n=40 plants, in most of the cells 20 bivalents and in a few one to two quadrivalents were found (see [61]).

The main source of numerical variation in the population is the basic diploid (2n=20), many plants (nearly 75%) of which show irregular meiosis and nondisjunction of the large bivalent in 9.7% to 8.0% of the cells [25, 42]. The large pair often remains as two univalents showing irregular movement at anaphase I and II resulting in gametes with n=9 to n=13 chromosomes. These abnormal gametes obviously are functional and their fusion in combinations produces the different kinds of variations observed. Sapre and Barve [42] considered that the nondisjunction observed is not accidental but possibly controlled genetically to generate variability.

Among the variants of the populations, 2n=18 nullisomics occurred predominantly (more than 50%) which are robust, fertile and more efficient survivors than other constituents [25, 40]. The fact that nullisomics and monosomics are viable and vigorous with high reproductive potential, which are usually nonviable in diploids, suggests that losses of whole chromosomes are tolerated because of the polyploid condition of the genome. Plants with 4n=40 chromosomes have comparatively fewer tillers, narrower leaves, smaller false fruits but the glands on leaf sheaths are similar to diploids, fertile and maintain themselves in nature. The other aneuploids also show partial fertility with some seed set. Rao [25] found that in trisomics and tetrasomics also, some plants consistently show 10 bivalents and one univalent and 11 bivalents respectively, indicating

that genetic factors controlling multivalent formation occur which imply that tendencies of separation into numerically different chromosomal races exist. Clearly established races with 2n=18 and 22 with bivalent formation, arising through nondisjunction, and 4n=40with fewer multivalents, resulting from genome reduplication and differentiation, which mostly breed true are already in existence. Although some of the 2n=22 plants with 11 bivalents bred true for the chromosome number, some others even with higher fertility, than the corresponding occasional quadrivalent forming tetrasomics, did produce in the selfed progeny some individuals with 2n=20 and 21 also. These are considered to be on the verge of differentiation into 2n=22 races ([25], present studies). All the chromosome classes on open pollination vielded variable numbers in the progeny.

Intraplant variation was found in Maharashtra and Madhya Pradesh populations occurring between culms, florets, anthers or between PMCs of an anther, possibly resulting from mitotic nondisjunction [24, 25, 54]. In Madhya Pradesh population, in a plant, wide variation ranging from 2n=7 to 29, with or without fragments occurred forming associations (upto a chain of nine) higher than can be expected from the possible number of homologues in a cell, indicating that chromosomal translocations also occurred. The fact that some seed from the population failed to germinate and some died at seedling stage suggests that even wider variation existed than can be tolerated by an individual, affecting its fitness [23]. In the population from Maharashtra variation was limited to 2n=20 and 4n=40 in one plant, 2n=22 and 4n=44 in another, occurring in PMCs of the same anther forming fewer quadrivalents and in still others 2n=18 to 22 chromosome numbers [31, 54]. Apart from the chromosomal translocations mentioned above two other cases of interchange hybridity were found [22, 43].

In *C. gigantea* (2n=20 and 4n=40), two populations with 2n=18 (nullisomic) from Maharashtra and two with 4n=40 from Tamil Nadu, South India, pachytene chromosomes showed that knobs and macrochromomeres were absent in the former [16, 62] but five terminal knobs and 10 macrochromomeres found in the latter in one and generally small, rounded and terminal knobs in the other [16, 24]. In one 2n=18 population, in several PMCs one of the chromosomes had two intercalary heterochromatic segments, one in each arm, but in other cells of the same anther, this chromosome was missing and two chromosomes each with one intercalary segment were present indicating the phenomenon of chromosome structural changes was underway (present studies).

C. aquatica is perennial, aquatic, stoloniferous, but when grown under field conditions becomes annual growing erect without stolons, leaves generally narrow with glabrous sheaths, pyriform fruit case with neck drawn out into a beak.

It has 2n=2x=10 large chromosomes forming five bivalents [25, 61, 62]. Interchange heterozygotes involving one or the other of all chromosomes are frequently found forming associations of four, six and eight chromosomes [25, 59–61].

Naturally occurring chromosome numerical variations in an open pollinated progeny of populations from Orissa, Andhra Pradesh and Maharashtra, presumably originating from meiotic nondisjunction, such as 2n=10to 13 and 3n=14 to 16 with or without fragments, frequent translocations, desynapsis, extra chromatin bodies, chromosome disintegration and variable number of nucleoli (1, 2 to many) were found. Additionally, naturally occurring and induced autotetraploids (4n=4x=20) with or without interchanges were found [25, 60, 61]. All these variants are morphologically indistinguishable. Among the 2n=11plants, any one of the chromosomes, could be involved as the extra chromosome in the primary, interchange and tertiary trisomics. The aneuploids and polyploids in some individuals show the expected homologous pairing or higher associations when interchanges were present [25].

In all categories of plants, in the population resulting from open pollination for several generations, the occurrence of translocations accounted for 30% of the plants, which is significantly more than 25% recorded in the first generation progeny of Madhya Pradesh and Orissa populations [60] which indicates that they have some adaptive advantage.

Among about 1500 plants studied, 67% showed 2n=10, 17% had 2n=11, 9% were 2n=12 and in 7% the rest of the chromosome numbers were found. Some of the 2n=10plants have five bivalents at meiosis but in some PMCs four bivalents and two univalents were formed, the univalents show precocious movement at anaphase and produce gametes with n=4 and 6 chromosomes. The mating at random of the latter and normal gametes will produce progenies with 2n=11 and 12. The former show five bivalents and one univalent or four bivalents and one trivalent or some univalents as the case may be. The 2n=12category which is tetrasomic shows six bivalents in about 66% of plants, four bivalents and one quadrivalent in some or five bivalents and two univalents in the rest. Some plants even with six bivalents show post-metaphase I irregularities (present studies).

When the univalents were formed in any chromosome category, they sometimes show interarm pairing and ring univalents at diakinesis and metaphase I indicating that intrachromosomal duplicate homologous segments were present. In bivalent forming 2n=12 plants also, in some of them, even on self-pollination, progenies with variable chromosome numbers are produced signifying that these 2n=12 plants are still in the process of getting established as a

race. Established bivalent forming populations of 2n=12 were studied from Tamil Nadu and Kerala (in which a pair of the existing chromosomes of 2n=10 populations was duplicated, a different one in each case, with loss of some of the segments) from the rice fields [5, 9, 16, 17], which were considered as two ecological races because of some morphological and chromosomal structural differences between the two, and the temperature differences in which the populations exist. Unlike the monosomics and nullisomics of C. gigantea all of which are devoid of one or a pair of large chromosomes, 2n=11 and 12 plants in C. aquatica are different in that each of them could be a trisomic or tetrasomic for a different chromosome or pair, and its 2n=12 races could be five different types, the extra pair in each belonging to a different chromosome. Some of the plants with consistently five bivalents and two univalents found in this study could be hybrids between two different types. Even 2n=12 categories are bestowed with translocations. These races might diversify into new races through chromosomal repatterning.

Autotriploids have 2–5 trivalents, and autotetraploids have variable number of quadrivalents in different plants ranging from 1–2 to 3–5 per cell [25, 39, 61, 64]. The reduction in the expected frequency of multivalents in polyploids and aneuploids indicates that bivalent chromosome pairing is under some kind of genetic regulation.

The role of translocations in evolution can only be pondered at the present time. Translocation complexes mostly orient at metaphase I, in C.aquatica, in a linear or adjacent manner (the kind of disjunction may be under genetic control), producing gametes with deficiency and duplication of chromosome segments. Even such gametes are functional in many instances indicating such losses or additions of small chromosomal segments are tolerated implying that the genome of C.aquatica has duplicate segments of chromosomes [25]. Their functioning would lead to individuals with deficiencies and duplications having newly organized linkage groups which necessarily alter the recombination potential and pattern, and selection in nature would establish new genotypes. In most cross-pollinated species, these translocations appear to be "floating" as they exist only temporarily in the population. At a later stage, particular chromosomal rearrangements, through acquisition of adaptive gene combinations, may spread widely in a population that can be recognized as distinct races or "prime types". These races occurring in different populations may ultimately bring about barriers to gene exchange through the development of differential accumulation of structural rearrangements. But the populations of C. aquatica under study continue to show random mating and produce fertile progenies indicating that such barriers are not yet developed.

Intraplant variation of chromosome numbers arising from mitotic nondisjunction, chromosome elimination, genome doubling or semigamy occurs in some individuals in different culms, florets, anthers or PMCs of an anther. Variations of n=5 and 2n=10, 9 and 10, 10 and 11, 10 and 12, 12 and 13, 2n=10 and 20 etc. in a plant were found [25].

The pistillate spikelet enclosed in a metamorphosed leaf sheath called spathe, fruit case when ripe, which is the unique feature of the genus, is two-flowered usually only one or rarely both of them function and produce seed so that in some cases twin seedlings are formed from a fruit case. Even triplets have arisen occasionally from one false fruit in which case two embryos must have been borne in one ovule, one of them developed apomictically [37, 63]. Semigamy is a form of fertilization in which the male and female gametes fuse but not their nuclei, the latter divide independently forming the embryo with (i) 2n-n, (ii) 2n-2n or (iii) 2n-aneuploid sectors. In the latter two mosaics, one of them must have resulted from functioning of an unreduced egg and the male nucleus forming a restitution nucleus so that diploid and diploid/aneuploid tissues are formed. In the first category, the egg nucleus is unreduced and the male nucleus is a normal reduced one, the latter which is haploid did not survive beyond seedling stage [30].

In a trisomic (2n=11) with purple seedling base and purple style with a genotype *Ccc* of the triplicated chromosome, the elimination of chromosome carrying *C* occurred predominantly at different times in the ontogeny of the individual producing 2n+1 culms with purple base and purple style and 2n culms with green base and white style. The causes of this selective elimination of chromosome carrying *C*, coming from the male parent, are not known [26]. In two plants some PMCs had 2n=10 and others 20, due to premeiotic doubling, the frequency of quadrivalents in 4n PMCs was different in the two plants [64].

When double, twin and triplet seedlings differing in chromosome number and behaviour arise from a single false fruit/seed in *C. aquatica*, such false fruit/seed is endowed with greater potentialities for wider variation in the offspring. Their significance becomes much more pronounced when the population size is reduced (due to interspecific competition, animal grazing or weed control operations by man when it occurs as a weed in rice fields) since each individual by possessing more than one kind of variation can compensate for the fewer individuals in the population and help in reducing the effects of inbreeding (in an otherwise cross-fertilizing species) and random fixation of genotypes which is likely to shift the population structure in one direction. This variation in *C. aquatica* as well as the intraplant variation in *C. gigantea*

can help not only to maintain but also in enlarging the cytogenetic diversity.

In *C. aquatica* (2n=10), pachytene chromosomes are long and intertwined, and heterochromatic and centromeric associations or stickiness occurred frequently which usually fall apart by diakinesis. These lead to chromosomal exchanges and translocations, which are found in plenty in the populations [25, 59–62]. In two populations from Andhra Pradesh with 2n=10, one showed knobs and macrochromomeres, but the other had none [16, 62]. In two other populations with 2n=12 from South India one was knobless but both had 2–6 macrochromomeres [5, 16].

The average length of pachytene chromosomes in *C. aquatica* is 80.38 μ m, that of *C. lacrymajobi* 58.20 μ m and in *C. gigantea* 54.30 μ m. Thus the chromosomes of *C. aquatica* on average are 40% larger and have 10% more heterochromatin than in the other two species [35, 62]. Even though some chromosomes of *C. lacrymajobi* and *C. gigantea* are longer than the shortest of *C. aquatica* at pachytene, from diakinesis onwards, all the chromosomes of *C. aquatica* appear larger than those of the other two species when present in a common nucleus, as in interspecific hybrids and therefore can be easily recognized.

Chromosome evolution in *C. aquatica* and *C. gigantea* seem to be proceeding in parallel lines in some aspects. Both show intra- and interplant chromosome variation, and tolerance to extra chromosomes, upto 10 in *C. aquatica* and 30 in *C. gigantea* and the latter is tolerant to even loss of upto four chromosomes, as reflected by the morphological stability of all the variants within a species. In *C. aquatica*, individuals with less than 2n=10 and more than 4n=20 and in *C. gigantea*, less than 2n=16 and more than 5n=50 chromosomes were not recorded, may be those outside this range affect the competence and survival ability of the individuals.

In aneuploids and polyploids of these species, nonformation or low frequency of multivalents could be due to some factors, possibly genetic, ensuring bivalent formation more often, which leads to regular meiosis and greater fertility through more regular chromosome disjunction and therefore to greater stability so that aneuploid and polyploid races could be established. But the instability factors present in the parents are passed on to the progeny so that some of the offspring exhibit nondisjunction to produce numerical variation in the subsequent generation. Thus the stability and instability going back and forth seems to be inherent in the genome and therefore is considered to be in a flux.

It has to be pointed out that the factors governing the mechanisms for initiation, stabilization and establishment of chromosomal races have to be assembled by pooling together appropriate tendencies, refined by mutual adjustment, and must be evolved under natural selection like any other function. That propensities for such a strategy are already at work is evidenced by the occurrence of instability factors causing mitotic and meiotic nondisjunction and numerical variation in the progenies, stability factors generating bivalent formation in aneuploids and polyploids, phenotypic constancy even with elimination or addition of chromosomes, viability of gametes with excess or less number of chromosomes even on male side, coupled with sexual reproduction and monoecious condition favouring cross-pollination, ability to reproduce asexually through seed by apomixis and vegetatively through stolons in *C. aquatica* and basal suckers in *C. gigantea*, adaptability to wet and dry habitats etc. ([25], present studies).

The genomes of these two species are conceivably plastic, constantly being evaluated and therefore in a state of continuous evolution. Under conditions of isolation, appropriate ecological niches and development of reproductive barriers between different chromosomal races, it is likely there would be rapid evolution and delimitation of the species into distinct races. Actually well established and self perpetuating different stable races of 2n=12 in *C. aquatica*, 2n=18 and 22, and 4n=40 in *C. gigantea* were in existence. Their development and stabilization could be a strategy for wider adaptation. Further evolution in these could be the establishment of new races with other chromosome numbers.

The genome of *C. lacrymajobi* was considered quite stable, but in the interspecific hybrids and their progenies involving 2n and 4n *C. lacrymajobi* with *C. aquatica* and *C. gigantea*, intraplant variation of chromosome numbers occurred in one to many PMCs of an anther and in all cases the chromosomes lost or gained pertained only to *C. lacrymajobi*. The factors of instability harboured in the genomes of *C. aquatica* and *C. gigantea* destabilized that of *C. lacrymajobi* in the hybrid background, the significance of which is not known [25, 32–34].

C. poilanei is semiaquatic occurring in marshes, nonstoloniferous, endemic to restricted area in Laos, leaves narrow, leaf sheath continuous with blade, brownish crateriform glands on the external part corresponding to leaf sheath, leaf blades tubercled with hairs, single flowered male spikelets, false fruit small, hard, chalky-white and pyriform in shape. Mimeur [20] created this as a new species, very near *C. aquatica* and considered its localized occurrence in Laos as an example of geographic and ecological origin.

Nirodi [24] described plants having 2n=10 large chromosomes (raised from seed obtained from Cuttack, Orissa) and because there were some similarities between them and *C. poilanei* she was inclined to believe them as belonging to *C. poilanei*, but the uncertainty of identity of the species, she designated by a question mark (?).

However, in many other characters they resembled *C. aquatica* more closely, and further, since the endemic *C. poilanei* has not been collected from anywhere else, it is surmised that Nirodi's material actually belonged to *C. aquatica*, therefore, it is inferred that chromosome information on *C. poilanei* is not, in fact, available.

C. ouwehandii is robust, straight growing, endemic to Northern Sumatra, leaves linear, sheaths short, glabrous, upper surface hairy, false fruit pyriform surmounted with a persistent short leaf blade, shining, stony, obscurely brown. Mimeur [20] placed it close to *C. gigantea*. Chromosome studies are not available.

C. puellarum is perennial, with very hard, globose, white or bluish-white false fruits, 4 mm in width [3], comes close to var. *monilifer* of *C. lacrymajobi*, whose false fruits are always more than 4 mm in width, and *C. puellarum* is somewhat smaller in all the morphological features compared to *C. lacrymajobi*. Its occurrence is limited to Indochina, Myanmar and Malaysia. Chromosome studies are not available.

Coix an euploid species (M.P. form) is annual, leaves broad, sheath glabrous, upper surface of leaf blade hairy, false fruit ovoid, hard with a shining shell. It is found in an isolated area in Madhya Pradesh, has an an euploid chromosome number of 2n=6x+2=32 forming six large and 10 small bivalents at meiosis. Except three pairs (two large and one small) which have subterminal centromeres, the rest of the 13 pairs have median or sub-median centromeres, as determined from tapetal cells. The chromosomes are differentiated with proximal hetero-chromatic and distal euchromatic segments. The plants have good fertility and seed set [19].

Coix an euploid species (W.B. Form) has broad, sublanceolate leaves, terminal male spikes long stalked, false fruit pyriform, large, very hard, dark green in colour, occurs in a localized area of Baharampur in the suburbs of Kolkata, West Bengal. It has 2n=6x+2=32 chromosomes with six large and ten small bivalents. Pachytene studies showed that they are differentiated with proximal heterochromatic and distal euchromatic segments and somatic chromosome studies also revealed that they are generally with median or submedian centromeres [7, 8, 16], but two pairs of large and one pair of small chromosomes with subterminal centromeres were reported earlier [6]. There are six terminal knobs and five macrochromomeres [16].

C. gasteenii is endemic to South Eastern part of Cape York Peninsula (CYP), Queensland, Australia [13, 57, 58], semiaquatic, perennial with an elongated rhizome, cane like stems, leaves somewhat narrow and tubercled on the upper surface, leaf sheaths hairy, inflorescences long, hang out like short chains of beads, spathes occur in twos or threes with the rachis between them quite long, false fruits ovoid and extended by well developed long leaf blades [57]. Chromosome studies are not available.

Assessment of origin, evolution and interrelationships The following F_1 interspecific and interracial hybrids were achieved and analyzed.

- i. *C. aquatica* (2n=10) × *C. lacrymajobi* (2n=20): 2n= 15 [25, 34, 35]
- ii. C. lacrymajobi (2n=20) × C. aquatica (2n=12): 2n= 16 [10]
- iii. *C. aquatica* (2n=10) × *C. lacrymajobi* (4n=40) and reciprocals: 3n=25, 24, 26, 27 [25, 34, 35].
- iv. *C. aquatica* (2n=10) × *C. gigantea* (2n=20): 2n=14, 15 [25, 33, 35].
- v. *C. gigantea* (2n=18–24) × *C. aquatica* (2n=10): 2n= 14, 15, 16 [48].
- vi. C. gigantea $(4n=40) \times C$. aquatica (2n=10): 3n=25 [21, 35].
- vii. C. lacrymajobi (2n=20) × C. gigantea (2n=20): 2n= 20 [25, 32, 35].
- viii. *C. lacrymajobi* (4n=40) × *C. gigantea* (2n=20): 3n= 30 [25, 32, 35].
- ix. C. lacrymajobi $(2n=20) \times C$. gigantea (4n=40): 3n=30, suspected reciprocal hybrids [4, 15*]. *(According to Han et al. [15] some aquatic, sterile and vegetatively reproducing populations with 2n=30 chromosomes, forming 10 bivalents and 10 univalents at meiosis, found in Guangxi Zhuang Autonomous Region in China, are hybrids between allooctoploid C. aquatica (2n=40) and C. lacrymajobi (2n=20). This does not seem appropriate because the very existence of octoploid C. aquatica is doubtful as the few South Indian populations in which 2n=40 was recorded, under the name C. aquatica [11, 16], virtually bore the distinctive morphological and chromosome features typical of C. gigantea, and also the contrasting size differences in chromosomes are not present in the hybrid genome of Chinese material (see text for details). It is therefore construed that these populations represent 3n hybrids between C. gigantea (4n race) and C. lacrymajobi (2n)).
- x. Coix an euploid sps. (M.P. form 2n=32) × C. aquatica (2n=10): 2n=21 [18].
- xi. Coix an euploid sps. (W.B. form 2n=32) × C. aquatica (2n=12): 2n=22 [7, 8].
- xii. C. gigantea $(4n=40) \times C$. gigantea (2n=20): 3n=30 [21, 35].

In the hybrids of *C. aquatica* involving the other two traditional species (2n), the parental genomes will be in haploid state, the chromosomes of *C. aquatica* are large and those of the other two species are small, which could be readily identified, so that intra- and intergenomic pairing

could be studied. Even when 4n forms are involved in hybrids, the number and type of multivalents also yield similar further information in these, and those in which the parental genomes cannot be distinguished as in hybrids of 2n and 4n *C. lacrymajobi* with 2n and 4n *C. gigantea* [4, 32, 35]. These studies facilitate an understanding of the origin of genomes, their relationships, mode and further course of evolution in the genus.

The occurrence of up to five heteromorphic (intergenomic) bivalents in both hybrids, involving *C. aquatica*, and some intragenomic pairing in *C. lacrymajobi* and *C. gigantea*, led to the conclusion that the haploid genome of 10 chromosomes in both the latter species comprises two subgenomes of five chromosomes each and that one of them is closely allied to *C. aquatica* genome and also has some homologies with the second subgenome [21, 25, 32–35].

In the hybrids between *C. lacrymajobi* (2n) and *C. gigantea* (2n) mostly bivalents, and one multivalent in some cells, were formed which were all regarded as intergenomic pairing.

From the analysis obtained from the data of Rao [25] and Rao and Nirmala [32–34] it was found that the intergenomic pairing per cell between *C. aquatica* and *C. lacrymajobi* was 2.449, between *C. aquatica* and *C. gigantea* was 3.106 and between *C. lacrymajobi* and *C. gigantea* was 9.183.

In hybrids of *C. lacrymajobi* (4n) with *C. aquatica* and *C. gigantea*, intergenomic pairing was 0.526 and 4.368 per cell respectively, and 4n race of *C. gigantea* and *C. aquatica* hybrid showed 1.784 per cell. In the suspected reciprocal hybrids of *C. lacrymajobi* (2n) and *C. gigantea* (4n), while only bivalents within the latter genome were found by Han et al. [15], bivalents and multivalents involving the genomes of both species were observed by Christopher and Jacob [4], but the extent of inter- or intragenomic pairing was not assessed.

Intragenomic bivalent formation was found to be 0.146, 0.086 and 0.688 per cell in *C. aquatica*, *C. lacrymajobi* and *C. gigantea* respectively. However, Sapre et al. [48] found that intragenomic pairing in *C. gigantea* was rare and absent in *C. aquatica* in the hybrids between them. Christopher et al. [10] found complete lack of intraor intergenomic pairing in hybrids of *C. lacrymajobi* (2n= 20) x *C. aquatica* (2n=12). These and the above mentioned studies [4, 15] indicate that hybrids between different degrees of inter- and intragenomic pairing, and different levels of vigour, fertility, longevity and ecological adaptation.

With five as the basic chromosome number of the genus, *C. aquatica* (2n=10) is a diploid and the intragenomic pairing is an indication of the presence of duplicate homologous segments between chromosomes. Further, the

tolerance of deficiency-duplication gametes in translocation heterozygotes, and the fold back pairing of univalent chromosomes in haploid genome and in aneuploids suggest that duplicate segments are present both within and between chromosomes. It is assumed that C. aquatica originated from a hypothetical ancestral diploid with 2n=10small chromosomes and evolved through chromosomal repatterning involving translocations and accumulation of duplicate segments and heterochromatin (and in the process its chromosomes became longer than in the ancestral form) with preference of adaptation to aquatic habitat. Further evolution in C. aquatica is through differentiation into chromosomal races (2n=12 etc.). Tetraploid races still seem to be in the early formative stages. Two different 2n=12races were already found. Strong tendencies of formation of homozygous translocation races exist.

C. lacrymajobi and C. gigantea (both 2n=20) are apparently tetraploids showing diploid behaviour. The two subgenomes in these species following their origin through hybridization and polyploidy of two closely related hypothetical small chromosome diploid (2n=10) ancestors, have diverged sufficiently to establish the two species, in different pathways, as functional diploids and intragenomic pairing found in C. lacrymajobi and C.gigantea is a reflection of residual homologies between their subgenomes. That one of them in both the species, has considerable homologies with C. aquatica genome, but the one in C. gigantea has more pronounced homologies than the one in C. lacrymajobi is implied by the differences in their intergenomic pairing with C. aquatica (3.106 and 2.449 per cell respectively). These indicate that the same diploid ancestor that gave rise to C. aquatica is also involved in the origin of these two species. In view of the high intergenomic pairing between C. gigantea and C. lacrymajobi (9.183 and 4.368 per cell), it is inferred that these two species are closely related and the two diploid ancestors involved in the origin of both C. lacrymajobi and C. gigantea are the same. However, the two subgenomes in C. gigantea are much less diverged from one another compared to those in C. lacrymajobi as evidenced by the frequent intragenomic pairing in its haploid genome. Thus it is clear that one ancestral diploid species is common to all the three species, and both ancestors are common to C. lacrymajobi and C. gigantea. The higher frequency of intergenomic pairing between C. aquatica and C. gigantea indicates that the ancestral diploid common to all three species did not diversify as much in C. gigantea as it did in C. lacrymajobi.

Subsequent to its origin and stabilization, *C. lacrymajobi* became adapted to drier habitats and is subjected to cultivation. Differentiation leading to six distinct morphological varieties occurred through accumulation of gene mutations, and also differentiation into distinct races with

or without differentiated chromosomes occurred as an independent cytogenetic mechanism. One race with euchromatic chromosomes was found in South Eastern India in aquatic conditions, reproducing apomictically, sexually and vegetatively.

Further evolution in *C. gigantea* is through chromosome racial differentiation into an euploid (2n=16-24) and polyploid (4n=40) races which became adapted to mountainous and terrestrial conditions. Well established races of 2n=18 and 22 and 4n=40 were found in Western Ghats, in some cases even in the same populations. The 4n=40 races seem to have much wider adaptation, and in South India they apparently adapted to wet soils, semifloating in tanks and small streams, floating portions showing aerial roots. There are some indications of structural changes occurring in the genome of diploid *C. gigantea*.

In the open pollinated progenies of hybrids of *C*. *aquatica* and *C*. *gigantea* (presumably backcrossed to either parent), individuals with wide range of chromosome numbers (2n=10-21) with combinations of chromosomes of both the parents occurred and plants with parental genotypes were found more frequently than are expected normally [25, 33, 49, 52]. Sapre and Deshpande [51] found at anaphase I in hybrids, segregation of all chromosomes of one parental genome to one pole and the other to another pole. They explained that a bondage exists within the chromosomes of *C. aquatica* and *C. gigantea* so that they orient nonrandomly and move en bloc to opposite poles.

Sapre and Deshpande [50] recovered some plants having 10 chromosomes of C. aquatica and one of C. gigantea, nine of C. aquatica and two of C. gigantea (both plants have 2n=11), and one plant having nine of C. aquatica and two of C. gigantea, and one fragment of the latter (2n=11+1f). It was concluded [50] that the meiotic behaviour of the one or two C. gigantea chromosomes present in C. aquatica genome was akin to that of B-chromosomes and therefore regarded them as the basis for the origin of Bchromosomes in Coix. Subsequently Barve and Sangeetha [2] isolated a plant with 2n=17 (16 of C. gigantea and one of C. aquatica chromosomes). They felt that presence of one extra chromosome of C. aquatica compensated for the loss of one pair of C. gigantea chromosomes in an otherwise nullisomic (2n=18) C. gigantea. The plant is semifertile and in its progenies, this kind of plants (2n=17)and others with 2n=18 (including one C. aquatica chromosome) were repeatedly found.

While the argument that the extra chromosomes of *C. gigantea* present in *C. aquatica* plants might be the forerunners of B-chromosomes in *Coix* [50] cannot be denied, the possibility that these and other plants with one or more *C. gigantea* chromosomes in *C. aquatica* background and vice versa, represent the early trends in the





origins of alien-addition and substitution chromosome races cannot also be ignored. When appropriate alien-addition and substitution of chromosomes carrying adaptive and productive gene complexes occur in *C. aquatica* or *C. gigantea* backgrounds, well established races may arise in suitable environments which could be the further course of evolution in these species.

Spontaneous hybrids occurred between C. aquatica and C. an euploid species (2n=32) involving the M.P. form [18] and W.B. form [7, 8]. In the hybrids with M.P. form 2n=21and with W.B. form 2n=22 chromosomes (10 small and 11 or 12 large), were present depending on whether n=5 or n=6 race of C. aquatica was involved. In the hybrids, the large chromosomes paired among themselves indicating close relationship between C. aquatica and large chromosome genome of aneuploid species. The small chromosomes in hybrids with M.P. form always remained as univalents, comparable to the meagre intragenomic pairing in the haploid genome of C. lacrymajobi and in the W.B. form, the small chromosomes often formed bivalents among themselves (upto 5 per cell), which is analogous to frequent intragenomic pairing in haploid state of C. gigantea. It is conceivable from these results of intragenomic pairing, that the small chromosomes in M.P. form belong to C. lacrymajobi, and those in W.B. form to C. gigantea, and therefore, inferred that the M.P. form and W.B. form of aneuploid Coix are allotetraploids of C. aquatica (n=6)race) as one parent and C. lacrymajobi and C. gigantea (both n=10) respectively as the other parent, inspite of some comparable chromosome morphology between the two forms. In the origin of the two forms, the 2n=12 race of C. aquatica involved may or may not be the same.

The frequent spontaneous occurrence of interspecific hybrids between *C. aquatica* and *C. gigantea* [25, 48] and isolation of a spontaneous allotetraploid [56] of this hybrid are but a few favourable evidences in support of the present hypothesis.

Further, the fruit case in M.P. form is ovoid, and in W.B. form pyriform in shape, which is one of the two most significant and consistent morphological characters used in species delimitation in *Coix*. Therefore, it is imperative that these two forms should be recognized as two distinct species [36].

Rest of the four species in the genus have no chromosome information, their origin can only be conjectured. Some of them were placed close to the traditional species, like *C. poilanei* to *C. aquatica*, *C. ouwehandii* to *C. gigantea* [20] and *C. puellarum* to *C. lacrymajobi* [3], each of which occurring in the same geographical range of the corresponding closely placed species. These species are postulated to have arisen as well adapted and differentiated forms to specific ecological and geographical factors that are particularly suitable for them. These spontaneous forms originating from their respective closely placed species, possibly through chromosomal changes, gene mutations or both, are endemic and adapted to rather restricted areas, as *C. poilanei* to localized area of 100 mts altitude in Laos, *C. ouwehandii* to Taponoeli, near Lake Toba, 900 mts altitude in Northern Sumatra, and *C. puellarum*, however, is comparatively of wider occurrence in Myanmar, Malaysia and Indochina.

C. gasteenii is endemic to South Eastern location of CYP, Queensland, Australia, occurring as small populations, apparently has a different origin. It shows characters of both C. lacrymajobi and C. gigantea like ovoid fruit case and tubercled leaves respectively. Also some of the plants of C. gigantea often have spathes extended by a reduced leaf blade [25], and var. minor of C. lacrymajobi has spathes occurring in twos [20] are further features of sharing, which features occur in an exaggerated manner in C. gasteenii. In Australia, other species of Coix present are C. lacrymajobi and C. gigantea (the latter reported under the name C. lingulata, which was treated by both Watt [68] and Bor [3] as *C.gigantea*) that occur in small isolated pockets, C. lacrymajobi, besides other locations, also occurs in the northern tip of CYP and C. gigantea in a localized area in South Western CYP [14]. It is now postulated that C. gasteenii has originated as an allopolyploid (4n=8x=40) of C. gigantea (2n=20) and C. lacrymajobi (2n=20) in CYP.

The occasional occurrence of spontaneous vigorous F₁ hybrids between the two species, the rampant manner in which 3n reciprocal hybrids were produced spontaneously between 4n C. lacrymajobi and 2n C. gigantea [25, 32] and between 2n C. lacrymajobi and 4n C. gigantea [4, 15] whenever opportunities were available, indicate that hybridization between them occurs frequently enough, and further the survival and spread of 3n sterile hybrid populations through vegetative reproduction in China, all provide presumptive evidence in support of the present hypothesis of the allopolyploid origin of C. gasteenii. However, it should be pointed out here that Simon [57] mentions that some of the Queensland herbarium specimens of cultivated or naturalized taxa in Queensland under the name C. lacrymajobi have male raceme dimensions of C. gigantea and for this reason they are more appropriately placed under C. gigantea. If this is so, the very presence of C. lacrymajobi in CYP remains in doubt.

The hybridization between *C. lacrymajobi* and *C. gigantea* could have occurred elsewhere also, from Peninsular India to Indonesia and Japan, where both species exist. It is possible that the hybrid polyploid was not successful in those areas because it may be very specific in its habitat requirements. But it is felt likely that the false fruit of hybrid polyploid was excreted by a migrating bird (from a nearby place like Indonesia) at the site in South Eastern location of CYP where it fortuitously grew and succeeded (see [13]), its genome

became differentiated and it flourished as a distinct species because of the ecological and environmental conditions most appropriate for its effective growth and perpetuation in clay loamy soils of Canozoic origin, along drainage lines with very little flow of water such as the back wash channels of creeks (see [58]).

Ability to resort to vegetative propagation, in response to aquatic conditions, as an additional strategy for reproduction, exhibited by some varieties and populations of *C. lacrymajobi* [25, 36] and *C. gigantea* [11, 16] and by the hybrids between these two species [15, 25, 32] may have contributed to the ultimate successful development of rhizome in the allopolyploid in its new abode as an adaptive measure.

The hypotheses proposed above regarding the origin and evolution of the three traditional and the two aneuploid species, also considered the earlier hypotheses proposed by others and explained why they are not acceptable [35]. The present hypotheses about the rest of the species are speculative, which can be decisively verified only when their chromosome analysis becomes available.

A comprehensive schematic diagram depicting the chromosomal basis of origin, evolution and interrelationships of various species of *Coix* is given (Fig. 1).

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