Cytogenetics of White *Allium*

I. Translocation heterozygosity

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In many open pollinated varieties of onion, bulbs vary widely from their specific varietal shape and size. The bulbs of the typical Haryana red-round variety of *Allium cepa* var. *cepa* range continuously from flat globes and pyriform bulbs to torpedos. Breeders, in general, have a selection bias towards the large sized round bulbs. Hence, knowledge about the genetic parameters of the bulb traits of onion will be highly useful to breeders in predicting effectiveness of the selection and in determining the choice of the breeding procedure.

During such a study, from the progeny of a population of red onion bulbs, white bulbs segregated. These were further cultured and in the subsequent generation 7 bulbs carrying translocation and 2 bulbs exhibiting desynapsis were detected. Their detection was possible because they were seedless. In these translocation heterozygotes, interchanges involved all the eight non-homologous chromosomes of the *Allium* zygotic complement but in an entirely novel way. In each case, 2 non-homologous chromosomes were involved in a reciprocal exchange, thus resulting in the formation of 4 well organised chromosomal associations and culminating in total seed sterility. Such a translocation system that is so regular in pattern and involves the whole zygotic complement seems to be undocumented so far and is reported for the first time in white onion in the present paper.

Materials and methods

10 mother bulbs were selected from the progeny raised from the seeds of a base population of red onion selection commonly grown locally in Haryana, India. These selected bulbs were planted to yield seeds in an isolated plot where no other onions were grown. 50 seeds of the each mother bulb were sown next year and the resulting progeny got from sowing the seeds of each mother bulb was planted in a separate insect proof pollination cage. The pollination, by honey bees, provided therein, is assumed to have been random within each group of parent plants. Seed was harvested individually from the available plants, thereby providing a series of maternal half sib families. From 50 seeds of each of the 10 families next progeny was raised. This progeny segregated into 417 red bulbed and 79 white bulbed plants. 10 white bulbs were planted the following year and the resulting seeds were sown and the bulbs recovered in 6 families segregated into red and white types but

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in the remaining 4 families no such segregation was observed. These observations indicate the genetic control of bulb colour in these onions.

On the other hand in the nonsegregating population of 114 white bulbed plants, 9 clones though normal morphologically exhibited seed sterility. These sterile clones could be propagated the following year only through bulbs. These were analysed cytologically by staining the flower buds with 0.5 per cent aceto-carmin after being fixed in 1:3 acetic-alcohol. Out of these 7 clones carried reciprocal translocation and 2 desynapsis, the former are described presently.

Observations

At pachytene the chromosomes were tangled and certain cross like configurations were observed in the material. Following pachytene, the chromosomes became shorter and at diplotene began to open out. At diakinesis, rings and chains each formed as a result of the association of 4 chromosomes were observed. These rings and chains were associated with each other by subterminal chiasmata strictly confined to the ends (Figs. 2-3). In some, one arm in the one-three chromosome association of four failed to remain associated and a chain or a string of 4 chromosome configuration resulted (Fig. 2). While in majority of the PMCs, the association of the 4 chromosomes was observed, in 2-5 per cent (3.8 ± 0.7) of PMCs, the arms of one group of 4 chromosomes failed to remain associated and this group then formed two pair like configurations. Probably, an arm remains associated at diakinesis only if there has been a chiasma formation in that arm. Metaphase in these translocation heterozygotes was characterised by 4 quadrivalent formations, each formation comprising of two translocated and two normal chromosomes. These remain associated in a ring or in a chain (Figs. 2, 3) subject to the number and position of the chiasmata. The frequency of ring and chain quadrivalents in the 7 translocation heterozygotes are presented in Table 1. From the table, it is

Table 1. Proportion of rings and chains in 100 meiocytes and pollen fertility percent in translocation heterozygotes of white onion

Set	Bulb number	No. of meiocytes with rings	Total no. of rings per meiocyte	No. of meiocytes with chains	Total no. of chains per meiocyte	Chi-square (1:1 ratio)	Pollen fertility percent (range)
A	1	91	3	9	1	Significant	9-14
	2	87	3	13	1	"	11-17
B	3	22	1	78	3	"	12-16
	4	57	3	43	1	Non-significant	17-20
C	5	48	3	52	1		15-21
	6	46	3	54	1		15-19
	7	45	3	55	1		14-18

evident that while quadrivalent in 4 clones are characterised by a combination of 3 rings and one chain (Fig. 2), each combination being of 4 chromosomes, in 2 other clones, rings and in the remaining one clone a chain of 4 chromosomes (Fig. 3)

are predominantly formed. Chi-square analysis reveals that while in 4 cases, the chains and rings of 4 chromosomes are distributed in a ratio of 3:1, in one only chains and in the other two rings are mainly formed in the PMCs (Table 1). A few

Figs. 1-5. 1, metaphase I of a normal white *Allium cepa* meiocyte showing 7 bivalents and 2 univalents, the latter formed due to the precocious separation. 2-3. Metaphase I of the translocation heterozygote meiocyte of white *Allium cepa*. 2, showing the formation of 3 rings and one chain. 3, showing 4 chains. 4-5. Pollen grains. 4, the normal white onion. 5, the onion of translocation heterozygotes.

chains observed in set A seem to be due to a breakage in the chiasmata or chance opening out of the chiasmata; the breakage could also be due to a defective or a deflected chiasma formation.

Anaphase separation of open rings and chains usually results in adjacent chromosomes passing to the same pole and all the gametes formed following this kind of separation contain chromosomes which have deficiencies and duplications and are therefore lethal. Also an irregular segregation is expected from the rings and chains and naturally some spores with deficient and others with extra chromosomes are formed in these translocation heterozygotes. Furthermore, another possibility exists such that the irregular disjunction may balance each other to produce spores that are deficient and yet have the normal chromosome number of $n=8$. This was observed in 9–14 per cent of spores in set C clones with predominantly 3 chromosomal chains, in which the mechanism of alternate segregation to be operative could be possible for all the interchanged chromosomes in the few PMCs. In the PMCs of the set A clones, that is characterised by predominant ring formations, anaphase bridges at A_1 were observed. These could probably be due to the stickiness of chromosomes at the chiasmata regions leading to their non separation.

Pollen fertility based on the stainability of pollen grains by aceto-carmin was varied between 9–22 per cent (Table 1). Even though some pollen grains appeared normal and fertile, no seed set was obtained in any of the clones. The plants with the interchange complexes were self pollinated, intercrossed among other translocation heterozygotes and also crossed as seed or pollen parents with the plants having normal meiosis. But in all these cases no seeds could be recovered indicating a complete sterility on the ovular and pollen side of these translocation heterozygotes. Similarly direct and reciprocal crossings with red onions yielded no seeds on either side. Furthermore, all the attempts to germinate the pollen grains of these translocation strains failed. Thus even though some pollen grains looked normal in appearance and stained well with aceto-carmin (Fig. 5), they were nonviable.

It was not possible to identify precisely each segment of the chromosomes involved in the formation of rings and chains. Further experiments designated to identify the particular chromosome on cytological or genetical basis are rather difficult because of the compound interchanges involving the whole zygotic complement, absence of cytological markers and the seed sterility.

Discussion

In plants especially perennial in nature, meiotic rings consisting of all members of the zygotic chromosome complement are known e.g. 14 in *Oenothera* ($2n=14$), 10 in *Paonia* ($2n=10$) and 12 in *Rhoeo* ($2n=12$). Evolutionary processes which ultimately lead to the formation of the chromosomal rings are due to the i) gradual accumulation of reciprocal translocations by a plant until all chromosomes are involved in a ring (Watanabe 1973), ii) intercrossing between different reciprocal translocating strains (Cleland 1962) and iii) a cooperation of both these processes. But the reciprocal exchange of chromosomal parts between each of the two non-homologous chromosomes, the exchange being probably extremely specific and pre-

cise and involving in total the whole zygotic complement, is extremely rare and unreported so far. Hence this represents a new type of translocation system existent in the plant kingdom.

The frequency of ring and chain interchange complexes and only bivalent forming PMCs at M_I is considered to indicate the site of breaks in the nonhomologous chromosome and can be deduced from the observed M_I chromosome configuration (Soriano 1957). In general, if the sites of breaks are closer to centromere and the interchange pieces are more or less equal in length, a high frequency of ring interchange complexes can be formed. This could be a possible situation in the set A plants in which the ring formation is predominantly observed and the number of rings exceeds to those of chain configurations significantly. Nevertheless, when the interstitial segment is short, the chance of chiasma occurring within the region is reduced and hence a high proportion of open rings can be formed.

On the other hand, where the exchanged chromosomal piece is relatively short, chain interchanges complexes are formed. Also in the event of a failure of chiasma to form in one of the four arms or of early terminalization of a chiasma in one arm, an open chain of four chromosomes will result. Any one of the situations could be possible in the set B plants in which a predominant chain formation is observed, the number of chain quadrivalents exceeding significantly to those of ring quadrivalents (Table 1).

The situation in set C plants is interesting. In this the site of chromosomal breakage in the three non-homologous chromosomes is close to the centromere and the interchange pieces should more or less be equal in length. This would lead to the formation of three ring interchange complexes as observed presently. In the remaining 2 non-homologous chromosomes, the site of chromosome breakage may be unequal thus leading to the formation of a chain of interchange complex. It is also possible that the chain quadrivalents are formed either by a complete failure of chiasma or by an early disjunction of one terminal chiasma between chromosome 1 and 4 in one case in set C plant and in almost all the cases in set B plants. Further, the possibilities for an early disjunction of the chiasmata in the chain quadrivalents in set A plants seems to be more certain than that of the complete failure of chiasma formation between them. But the predominance of chain configurations in set B plants and their prevalence in equal proportion with that of ring configurations in set C plants points to their genetic significance. If the point of breakage and exchange of heterologous chromosomes are genetically conditioned and controlled, the 'chance factor' operating for the predominance of ring formations in set A, chain formations in set B and an equal proportion of chains and rings formations in set C gets plausibly eliminated.

Genetic control: Since translocations represent mutations in the broad sense, their genetic control cannot be over ruled. Thus in set A it can be visualised that a certain gene 'a' when present in a homozygous state (aa) induces such translocations that lead to the formation of rings. A similar genetic control by the postulated genes 'bb' could result in such translocations leading to chain formations as observed in set B plants. A few number of chains and rings observed in the plants of set A and B, respectively could be due to chance opening out or an early disjunction of

a chiasma between chromosomes 1 and 4. Therefore, their numbers is insignificant at 5 per cent level of probability. In set C, both the genes a and b are present and they are able to induce ring and chain formations; an almost equal number of the two configurations indicates that both are able to act effectively and equally a case similar to that observed in the mutants and recombinants of pea by Gottschalk and Kaul (1974, 1975). That these translocations are genetically controlled is also borne out by the fact that either rings or chains or both are regularly and uniformly formed in sets A, B and C, respectively. Further such a kind of translocation system involving regular and probably specifically 2 heterologues of the entire genome as observed in the present material, are extremely rare thus pointing out to their recessive gene control. However, in the absence of regular and relevant genetic data and segregations, the postulated genetic control for such translocations cannot be finalized at this stage. Nevertheless, a genetic control of alternate segregation has been evidenced by Thompson (1965a, b) and Lawrence (1958) in *Secale*.

Disjunction and lethality: The gametic lethality in a translocation heterozygote is dependent upon the distribution of the 4 chromosomes in a ring or a chain configuration at anaphase I. The chromosome distribution is determined by the centromere orientation that may be either concordant or discordant. In the translocation heterozygote with rings, anaphase separation of open rings usually results in adjacent chromosomes with either homologous or non-homologous centromeres reaching the same pole and all the gametes following this kind of separation contain chromosomes which have deficiencies and these therefore are lethal. Such type of orientation would also naturally be existing in the onion heterozygotes and would account for the observed pollen sterility. Further, since approximate decrease in pollen fertility is about 20 per cent per each one reciprocal translocation, very little pollen fertility is expected in these strains of onion carrying translocations.

Anaphase disjunction of chain quadrivalents on the other hand lead to the formation of nearly 50 percent viable gametes because half of the anaphase separation is of alternate type and leads to a full complement of segments in all the gametes. But since all the 4 sets of non-homologous chromosomes are involved in the translocation, the probability of getting viable gametes gets reduced by $3/4$ th. Thus 9–22 per cent of pollen fertility observed presently for these genotypes exceeds the expected 12.25 per cent by nearly 3–10 per cent if the determined pollen fertility indicates the pollen viability reliably. The reason for this increase could be that the chain quadrivalents orientate alternately more frequently because of the greater flexibility as proposed by Östergren (1951) and Lewis and John (1963).

In many interchange complexes, frequency of different orientation at M_I departs from random expectations considerably. Frequently, an excess of the alternate orientations and deficiency of one or both the adjacent arrangements occurs (Burnham 1956). But unless reasonably high frequency of disjunction is achieved immediately after an interchange event, it is unlikely that interchange heterozygosity will become incorporated into genetic system of the species (Darlington 1956). However, assuming a nonrandom segregation of the four chromosomes in a quadrivalent of a translocation heterozygote, it would be expected under ideal conditions to obtain one third balanced gametes (alternate segregation) and two

thirds unbalanced segregation (adjacent-1 and adjacent-2 segregation). Rickards (1964) on the other hand assumed an expected 1:1 segregation based on the hypothesis that the centromeres behave in twos and not independently. Though several factors influence segregation ratio, the influence favours the frequency of balanced gametes. This influence could explain the increased pollen fertility than expected on theoretical basis especially of set C plants.

Hence, this represents the phenomenon of directed segregation and is usually accentuated in different species. Factors influencing the directed segregation *viz.* centromere and chiasmata position, size of the interstitial segment, type of quadri-valent formation, the genotypic influence on these factors may be operative in the material studied presently. The influence of time available for the centromere to co-orientate, as emphasised by Bauer *et al.* (1961) can also be significant. However, the specific influence of these factors could not be worked out presently.

Disjunctional arrangements tend to be favoured when chromosomes are relatively uniform in length, centromere are median or nearly so and chiasmata are mostly localised to the ends (Burnham 1956). Though all these criteria are met with in the present material, yet in set A plants, with predominant ring formations, disjunction is imperfect, chiasmata are not terminalised fully and frequently anaphase bridges are formed. In other sets, the chiasmata in all the rings and chains are terminalised fully like that in a successful interchange heterozygote. This disjunctional variation between set A and B and between A and C, can be genetically controlled since ring formations of set B and C plants disjunct normally unlike those of set A plants. Further, the influence of postulated gene 'a' alone is unable to induce normal chromosomal disjunction. Thus, if this disjunctional variation is heritable, the defective disjunction could also lead to the observed pollen sterility of set A plants.

Pollen fertility is a common measure of gametic viability in translocation heterozygotes. But the observed pollen fertility in the present material bears no correlation with pollen viability, since even the normal looking pollen grains that stain well in aceto-carmin do not germinate. Also complete male as well as female sterility is realised in all the translocation heterozygotes studied presently, the only method of their propagation is through bulbs. In this connection it is pertinent to mention that Soriano (1957) found that percentage of stainable pollen grains was not a reliable criterion for detecting plants which are heterozygous for the colchicine induced reciprocal translocation.

In interchange heterozygotes, natural selection clearly favours genotypes that lead to a high proportion of disjunctional arrangements and to a relatively high fertility. In fact such selections play important part in populations where interchanges float (some individuals being homozygous, others heterozygous) and where interchanges are fixed in the heterozygous condition as in *Oenothera*, *Rhoeo* etc. Also rings are the visible markers of hybridity and represent a form of big linkage group for conserving a particular genotypic combination by reducing recombination and thereby the variation. In other words, it is a mechanism by which maximum hybridity and minimum cross-over go together. Though it could be possible by institution of balanced lethal system in the interchange heterozygotes of onion,

the interchanges detected in the onion plants are disadvantageous to the population because of the accompanying sterility. Whether the interchange is the cause or consequence of sterility genes is not certain. However, it appears that since the species propagates vegetatively, the rings involving the whole zygotic complement are maintained asexually.

It is possible that white onion may acquire over many generations, a gene system promoting high disjunction frequency guaranteeing gametic fertility but retaining interchange heterozygosity by a process analogous to Fisher's (1931) theory of evolution of dominance. During such long periods, interchanges may become common events and although structural arrangements may get even removed from the populations, their continued occurrence would give rise to a small but persistent selection pressure tending to increase the heterozygosity through enhanced alternate orientations. Since there appears to be a gene system controlling the translocations and subsequently chromosome orientation in the cell, the selection pressure would be larger than that involved in the evolution of dominance. Thus the conclusion that such a genetical adaptation of a species is a product of selection and not a fortitious event, is more in keeping with the known properties of the genetic system.

Summary

During studies over genetic parameters of the local red onion bulbs, certain progenies of maternal half sib families segregated a few white bulbs. Some of these bulbs bred true to the white colour and though they were sexually normal and fertile, a few exhibited seed sterility. These steriles when cultured separately were found to be either translocation heterozygotes or desynaptic strains. In plants a single meiotic ring involving either a few or the entire zygotic chromosome complement is known. But the formation of regular independent rings, each composed of 4 chromosomes, and involving the whole zygotic complement is unknown so far. This has been found in some of these white *Allium cepa* strains and represents a new type of translocation system existent in the plant kingdom. Predominance of either rings or chains or their equal proportion was observed in the various clones, each configuration being specific and constant for each clone. The predominant occurrence of rings in certain clones could be because the sites of chromosomal breakages are closer to centromere and interchange pieces are more or less equal in length. But where the interstitial segment is short, exchanged pieces are relatively small thus resulting in chain configuration formations.

Though pollen fertility ranged from 9–22 per cent, seed sterility was complete in these translocation heterozygotes. When the clones were selfed or intercrossed among each other or crossed as seed or pollen parent with normal fertile *Allium cepa*, no seeds were set, indicating thereby the existence of male and female sterility in these clones. Genetic control of these regular and specific translocations has been postulated. Since such a type of translocations involving regularly 2 heterozygotes, each of the entire genome, are extremely rare and occur in a very low frequency, their control seems under recessive genes.

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References

- Bauer, H. R., Dietz, R. and Robbels, C. 1961. Die Spermatocyteilungen der Tupuliden III. Das Bewegungs Verhalten der Chromosomen in Translokationsheterozygoten von *Tipula oleracea*. *Chromosoma* (Berl.) **12**: 116–189.
- Burnham, C. R. 1956. Chromosome interchange in plants. *Bot. Rev.* **22**: 419–552.
- Cleland, R. E. 1962. The cytogenetics of *Oenothera*. *Adv. Genet.* **11**: 147–237.
- Darlington, C. D. 1956. Chromosome Botany. Allen and Unwin Ltd. London.
- Fisher, R. A. 1931. The evolution of dominance. *Biol. Rev.* **6**: 345–412.
- Gottschalk, W. and Kaul, M. L. H. 1974. Investigation on the cooperation of mutated genes II. Floral structure and seed production of cochleata mutants and recombinants of *Pisum sativum*. *Ber. Deutsch. Bot. Ges. W. Germany.* **68**: 513–524.
- 1975. Genecological investigations in *Pisum* mutants I. The influence of climatic factors upon quantitative and qualitative characters. *Z. Pflanzenzüchtung* **75**: 182–191.
- Lawrence, C. W. 1958. Genotypic control of chromosome behaviour in rye VI. Selection for disjunction frequency. *Heredity* **12**: 127–131.
- 1963. The orientation of multiple associations resulting from interchange heterozygosity. *Genetics* **48**: 347–350.
- Lewis, K. R. and John, B. 1963. Chromosome Marker. J. A., Churchill Ltd., London.
- Östergren, G. 1951. The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. *Hereditas* **37**: 473–494.
- Rickards, G. K. 1964. Some theoretical aspects of selective segregation in interchange complexes. *Chromosoma* (Berl.) **15**: 140–155.
- Soriano, J. 1957. The genus *Collinsia* IV. The cytogenetics of colchicine induced reciprocal translocation in *C. heterophylla*. *Botan. Gaz.* **118**: 139–145.
- Thompson, J. B. 1956a. Genotypic control of chromosome behaviour in rye II. Disjunction at meiosis in interchange heterozygotes. *Heredity* **10**: 99–108.
- 1956b. Genotypic control of chromosome behaviour in rye VI. Selection and disjunction frequency. *Heredity* **10**: 127–131.
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